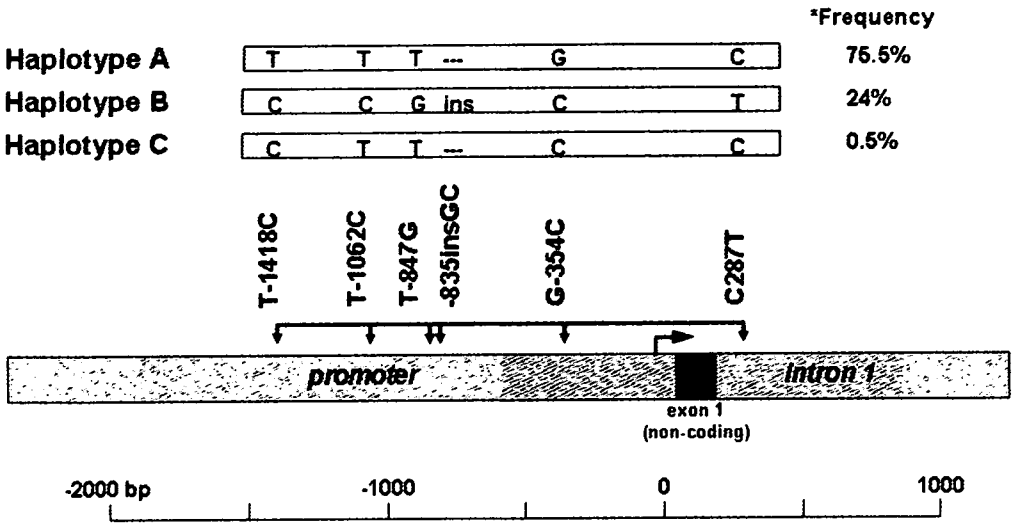


Fig 7. Haplotypes identified within the proximal promoter region of *SCN5A*, a cardiac sodium-channel gene. The 6 polymorphisms are in near-complete linkage disequilibrium. Haplotype A is designated as containing all common alleles, and Haplotype B as containing all minor alleles. The discordant haplotype is designated Haplotype C. *Frequency in the Japanese (control) population (Modified from *Circulation* 2006; **113:** 338–344 with permission).

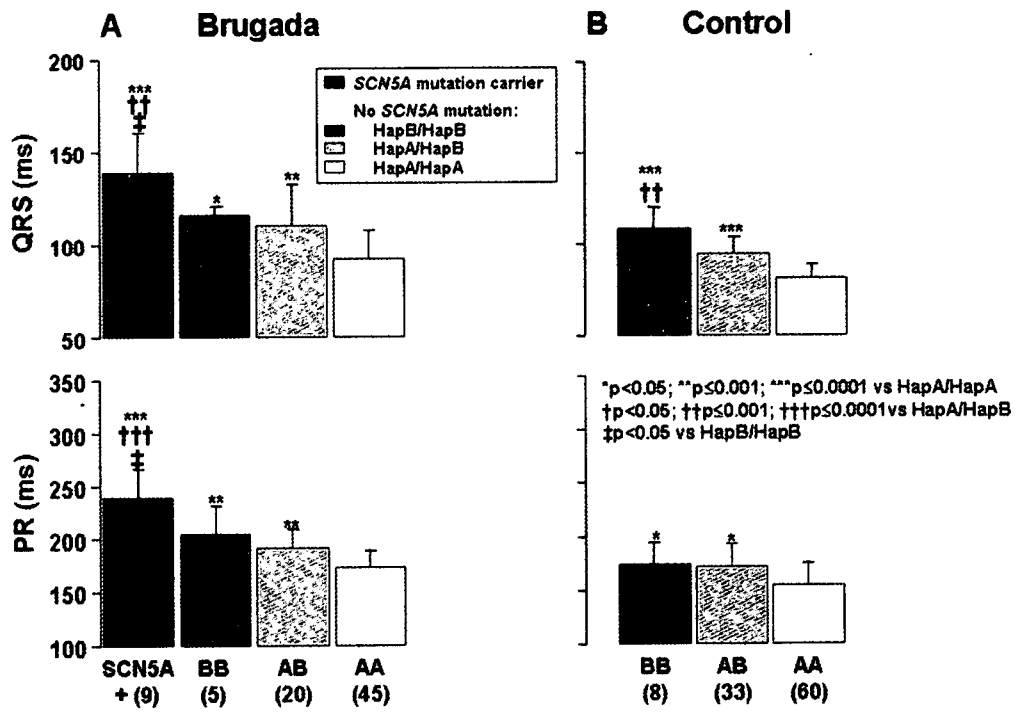


Fig 8. *SCN5A* promoter haplotype pair effects on QRS duration in lead $V_6$ and PR duration in lead II in patients with Brugada syndrome and in control subjects. In the Brugada patients without *SCN5A* mutations and in the control subjects, both QRS and PR duration show a gene–dose effect, being longest in Haplotype B homozygotes (BB), intermediate in Haplotype A/Haplotype B heterozygotes (AB) and shortest in Haplotype A homozygotes (AA). The Brugada patients with *SCN5A* mutations show the longer duration of both QRS and PR than do those without *SCN5A* mutations. Patient numbers are indicated between parentheses. Data mean±SD (Modified from *Circulation* 2006; **113:** 338–344 with permission).

conduction, the relationship between the *SCN5A* promoter haplotype and indices of conduction velocity (ie, PR and QRS durations) was analyzed in a cohort of 71 Japanese BS subjects without *SCN5A* mutations and in 102 Japanese controls. In both groups, PR and QRS durations were significantly longer in Haplotype B individuals, with a gene–dose effect (Fig 8). Moreover, increases in both the PR and QRS duration with sodium channel blockers, which are known to be arrhythmogenic in BS, were genotype-dependent and a gene–dose effect was also observed. These data demonstrate that the Haplotype B within the *SCN5A* promoter region alone does not give rise to BS, but that it likely contributes to a higher incidence of BS in Asian population in combination with other yet unknown (genetic) factors.

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EDITORIAL COMMENTARY

# Proarrhythmic effect of altered ventricular activation sequence in patients with permanent pacemaker

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Cardiac events including syncope or sudden cardiac death in patients with a permanent ventricular pacemaker are mainly caused by ventricular standstill or polymorphic ventricular tachycardia known as Torsade de Pointes (TdP), which are usually associated with a malfunction of ventricular pacing. Bradycardia-induced TdP in patients with pacemaker malfunction is usually accompanied by marked QT prolongation, the so-called acquired form of long QT syndrome (LQTS). It was previously reported that a short-long-short initiating sequence before the onset of TdP has been observed in the majority of patients with bradycardia-induced TdP,[1–5] and the mechanism of initiating premature beat triggering for TdP is thought to be bradycardia (pause)-dependent early afterdepolarization.[6–12] More recently, the short-long-short initiating sequence was reported to be specifically observed in the LQT2 form of congenital LQTS among genotyped patients with congenital LQTS.[13]

However, an exception exists in the initiating sequence of TdP in patients with a permanent ventricular pacemaker.[4,5,8] Kurita et al reported a patient with a permanent VVI pacemaker because of sick sinus syndrome, who developed TdP without bradycardia due to pacemaker malfunction.[14] TdP was initiated at a junctional beat, which was associated with marked QT prolongation and a bizarre (giant negative) T wave despite shorter preceding RR interval than that during paced beats. In that case, remarkable QT prolongation and subsequent TdP were closely related to the abrupt alteration of the ventricular activation sequence (from right ventricular pacing to junctional normal conduction). Constant ventricular pacing by increasing the lower pacing rate to 80 beats/min suppressed the junctional beats and TdP. A similar initiating pattern of TdP following altered ventricular activation sequence has also been reported in patients with a congenital form of LQTS, in whom a permanent pacemaker was not implanted. Noda and coworkers retrospectively analyzed the initiating mode of 111 episodes of TdP recorded from 24 patients with congenital LQTS.[15] Eleven episodes (10%) of TdP developed following altered ventricular activation sequence due to ventricular premature contraction or fusion beat, which was associated with QT prolongation.

Long-lasting atypical ventricular activation such as permanent right ventricular pacing produces a new ventricular gradient (a significant inverse relationship between the activation time and the action potential duration (APD)) to the new activation sequence.[16,17] Under these conditions, abrupt alteration of the normal activation sequence (e.g. spontaneous or junctional beat) may induce a change in T wave polarity, which is concordant with the previous paced R wave ("cardiac memory").[16–18] Moreover, the QT interval (repolarization duration) is prolonged and the dispersion of repolarization may be increased, since the ventricular site with longer APD activates later and that with shorter APD activates earlier due to the abrupt alteration of the activation sequence.[17] The increased dispersion of repolarization as well as QT prolongation may provide an ideal substrate for reentrant arrhythmia, thus inducing serious ventricular arrhythmias such as TdP.[2,19,20] Therefore, in such a patient with a permanent pacemaker and TdP, which is related to the altered ventricular activation sequence, keeping the activation sequence with constant pacing using the proper pacemaker functions (e.g. rate response and/or A-V interval adaptation to heart rate) is expected to be effective in preventing recurrence of TdP.

It has been reported that "cardiac memory" reaches a steady-state in the human heart within one week of the onset of the right ventricular endocardial pacing at physiological rates.[21] In this issue of Heart*Rhythm*, Wecke and co-workers used vectorcardiography (VCG) to detect early signs of altered ventricular repolarization associated with "cardiac memory" and investigated the temporal characteristics of these signs.[22] A DDD-R pacemaker was implanted in 20 patients with sick sinus syndrome, who were paced from the right ventricular endocardium. Twelve-lead electrocardiography (ECG) and VCG were recorded before and one day

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after implantation, and then daily for the first week in 6 patients and weekly for 5-8 weeks in the remaining 14 patients, with pacemakers temporarily programmed to AAI mode (normal ventricular activation). VCG analysis allowed early detection of altered ventricular repolarization associated with "cardiac memory", on average one day before they were detected on ECG. An abrupt switch from right ventricular pacing to normal conduction was accompanied by VCG signs of heterogeneous ventricular repolarization such as increased T loop distortion and circularity. However, ventricular repolarization adapted to the new activation sequence with successfully shorter repolarization time over days to weeks during constant ventricular pacing. Observations with VCG analysis by Wecke and coworkers suggested that the new ventricular gradient to the new activation sequence following permanent ventricular pacemaker seems to be established after a few days and is definitely present within one week after pacemaker implantation. These data recommend that careful ECG monitoring and follow-up should be started as soon as the permanent ventricular pacemaker is implanted, because severe ventricular arrhythmias such as TdP, which are related to altered ventricular activation sequence may appear soon after the pacemaker implantation.

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# Clinical Aspects of Type-1 Long-QT Syndrome by Location, Coding Type, and Biophysical Function of Mutations Involving the KCNQ1 Gene

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***Background***—Type-1 long-QT syndrome (LQTS) is caused by loss-of-function mutations in the KCNQ1-encoded $I_{Ks}$ cardiac potassium channel. We evaluated the effect of location, coding type, and biophysical function of KCNQ1 mutations on the clinical phenotype of this disorder.

***Methods and Results***—We investigated the clinical course in 600 patients with 77 different KCNQ1 mutations in 101 proband-identified families derived from the US portion of the International LQTS Registry (n=425), the Netherlands' LQTS Registry (n=93), and the Japanese LQTS Registry (n=82). The Cox proportional hazards survivorship model was used to evaluate the independent contribution of clinical and genetic factors to the first occurrence of time-dependent cardiac events from birth through age 40 years. The clinical characteristics, distribution of mutations, and overall outcome event rates were similar in patients enrolled from the 3 geographic regions. Biophysical function of the mutations was categorized according to dominant-negative (>50%) or haploinsufficiency (≤50%) reduction in cardiac repolarizing $I_{Ks}$ potassium channel current. Patients with transmembrane versus C-terminus mutations (hazard ratio, 2.06; $P<0.001$) and those with mutations having dominant-negative versus haploinsufficiency ion channel effects (hazard ratio, 2.26; $P<0.001$) were at increased risk for cardiac events, and these genetic risks were independent of traditional clinical risk factors.

***Conclusions***—This genotype–phenotype study indicates that in type-1 LQTS, mutations located in the transmembrane portion of the ion channel protein and the degree of ion channel dysfunction caused by the mutations are important independent risk factors influencing the clinical course of this disorder. (***Circulation.*** **2007;115:2481-2489.)**

**Key Words:** electrocardiography ■ genetics ■ long-QT syndrome

The hereditary long-QT syndrome (LQTS) is characterized by prolonged ventricular repolarization on the ECG and arrhythmia-related syncope and sudden death.[1] Mutations in 1 or more of several ion channel genes are known to cause this disorder,[2] with mutations in the KCNQ1 gene causing the type-1 long-QT syndrome.[3,4] The KCNQ1 gene codes for the potassium channel protein responsible for the slow component of the delayed rectifier repolarizing current ($I_{Ks}$). Mutations involving this gene result in reduction of the repolarizing $I_{Ks}$ current and lengthening of the QT interval.[3]

## Clinical Perspective p 2489

Functional $I_{Ks}$ channels result from the coassembly of 4 subunits into a tetrameric protein channel that is transported to the myocyte membrane. Each subunit contains 6 membrane-spanning domains (S1 to S6) flanked by amino (N)- and carboxyl (C)-terminus regions. Two distinct biophysical mechanisms mediate the reduced $I_{Ks}$ current in patients with KCNQ1 mutations: (1) coassembly or trafficking defects in which mutant subunits are not transported

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properly to the cell membrane and fail to incorporate into the tetrameric channel, with the net effect being a ≤50% reduction in channel function (haploinsufficiency)[5]; and (2) formation of defective channels involving mutant subunits with the altered channel protein transported to the cell membrane, resulting in a dysfunctional channel having >50% reduction in channel current (dominant-negative effect).[6]

Limited prior studies involving relatively small numbers of patients with type-1 LQTS have been reported with conflicting results on the relationship between various KCNQ1 mutations and the clinical outcome.[7,8] We hypothesized that the location, coding type, and functional effect of the channel mutation would have important influence on the phenotypic manifestations and clinical course of patients with this disorder. To test this hypothesis, we investigated the clinical aspects of a large cohort of subjects having a spectrum of KCNQ1 mutations categorized by their location, coding type, and type of biophysical ion channel dysfunction.

## Methods

### Study Population

The study population of 600 subjects with genetically confirmed KCNQ1 mutations was derived from 101 proband-identified families with the type-1 LQTS disorder. The proband in each family had QTc prolongation not due to a known cause. The subjects were drawn from the US portion of the International LQTS Registry (n=425), the Netherlands' LQTS Registry (n=93), and the Japanese LQTS Registry (n=82). All subjects or their guardians provided informed consent for the genetic and clinical studies.

### Phenotype Characterization

Routine clinical and ECG parameters were acquired at the time of enrollment in each of the registries. Follow-up was censored at age 41 years to avoid the influence of coronary disease on cardiac events. Measured parameters on the first recorded ECG included QT and R-R intervals in milliseconds, with QT corrected for heart rate by Bazett's formula. The QTc interval was expressed in its continuous form and categorized into 3 levels: <500, 500 to 530, and >530 ms. Clinical data were collected on prospectively designed forms with information on demographic characteristics, personal and family medical history, ECG findings, therapy, and end points during long-term follow-up. LQTS-related cardiac events included syncope, aborted cardiac arrest, or unexpected sudden death without a known cause. Data common to all 3 LQTS registries involving genetically identified patients with type-1 genotype were electronically merged into a common database for the present study.

### Genotype Characterization

The KCNQ1 mutations were identified with the use of standard genetic tests performed in academic molecular-genetic laboratories including the Functional Genomics Center, University of Rochester Medical Center, Rochester, NY; Baylor College of Medicine, Houston, Tex; Mayo Clinic College of Medicine, Rochester, Minn; Boston Children's Hospital, Boston, Mass; Laboratory of Molecular Genetics, National Cardiovascular Center, Suita, Japan; and Department of Clinical Genetics, Academic Medical Center, Amsterdam, Netherlands.

Genetic alterations of the amino acid sequence were characterized by location and by the specific mutation (missense, splice site, in-frame insertions/deletions, nonsense, stop codon, and frameshift). The transmembrane region of the KCNQ1-encoded channel was defined as the coding sequence involving amino acid residues from 120 through 355 (S5-pore-S6 region 285 to 355), with the N-terminus region defined before residue 120 and the C-terminus region after residue 355. Nineteen study patients had intron mutations predicted to disrupt the canonical splice-site domains. Fifty-one subjects died of sudden cardiac death at a young age but did not have genotype studies. These 51 subjects were assumed to have the same KCNQ1 mutation as other affected members of their respective family. Twelve subjects had 2 mutations, one in the KCNQ1 gene and a second mutation in another LQTS ion channel gene; these 12 subjects are described separately and are not included in any of the tables or outcome analyses. Subjects with Jervell and Lange-Nielsen syndrome with deafness and 2 KCNQ1 mutations as well as those with 1 known KCNQ1 mutation and congenital deafness are not included in the present study.

The biophysical function of the mutant channels was classified as having dominant-negative effect (>50% reduction in function) or haploinsufficiency (≤50% reduction in function) on the basis of the following: (1) cellular expression studies for those with missense (n=21) and nonsense (n=2) mutations reported in the literature, with the functional information derived exclusively from heterologous expression studies; and (2) assumed loss of function for identified nonsense, splice site, in-frame deletion, and frameshift mutations (n=10) that have not yet been functionally characterized. Forty-one missense mutations and the 3 intron mutations that have not been functionally reported in cellular expression studies were categorized as unknown in terms of type of functional perturbation.

### Statistical Analysis

Differences in the univariate characteristics by specific groupings were evaluated by standard statistical methods. The primary end point was time to syncope, aborted cardiac arrest, or sudden death, whichever occurred first. The cumulative probability of a first cardiac event was assessed by the Kaplan-Meier method with significance testing by the log-rank statistic. The Cox proportional hazards survivorship model was used to evaluate the independent contribution of clinical and genetic factors to the first occurrence of time-dependent cardiac events from birth through age 40 years.[9] Stratified and unstratified Cox regression models, allowing for time-dependent covariates, were fit to estimate the adjusted hazard ratio of each factor as a predictor of first cardiac events. We observed that sex was not proportional as a function of age with crossover in risk at age 13 years on univariate Kaplan-Meier analysis. To relax the assumption of proportional hazards for sex over the entire age range, separate nonparametric baseline hazard functions were allowed for male and female subjects via the stratified Cox model; then, to summarize the sex effect, sex was modeled in an unstratified Cox model as a time-dependent covariate (via an interaction with time), allowing for different hazard ratios by sex before and after age 13 years.

Because almost all the subjects were first- and second-degree relatives of probands, the effect of lack of independence between subjects was evaluated in the Cox model with grouped jackknife estimates for family membership.[10] All grouped jackknife standard errors for the covariate risk factors fell within 3% of those obtained from the unadjusted Cox model, and therefore only the Cox model findings are reported.

Patients who died suddenly at a young age from suspected LQTS and who did not have an ECG for QTc measurement were identified in the Cox models as "QTc missing." Prespecified covariate interactions were evaluated. The influence of time-dependent β-blocker therapy (the age at which β-blocker therapy was initiated) on outcome was determined by adding this variable to the final Cox model containing the various covariates.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

## Results

### Total Study Population

The spectrum and number of KCNQ1 mutations by location, type of mutation, and functional effect are presented in Table 1, with the location frequency of the mutations presented diagrammatically in Figure 1. A total of 77 different KCNQ1

**TABLE 1. KCNQ1 Mutations by Location and Coding, Type of Mutation, and Functional Effect**

| Location and Coding* | No. of Subjects† | Type of Mutation | Functional Effect‡ |
|---|---|---|---|
| N-terminus | | | |
| M1V | 1 | Missense | Unknown |
| G57V | 1 | Missense | Unknown |
| Transmembrane | | | |
| W120C | 2 | Missense | Unknown |
| T144A | 7 | Missense | Unknown |
| A150fs/133 [del CT 451-452] | 2 | Frameshift | Haploinsufficiency |
| E160K | 3 | Missense | Unknown |
| G168R | 44 | Missense | Unknown |
| Y171X [513 C>G] | 6 | Nonsense | Haploinsufficiency |
| R174H | 2 | Missense | Unknown |
| A178P | 5 | Missense | Dominant-negative effect (a) |
| Y184S | 18 | Missense | Unknown |
| G185S | 10 | Missense | Unknown |
| G189E | 2 | Missense | Unknown |
| G189R | 4 | Missense | Dominant-negative effect (b) |
| R190Q | 4 | Missense | Haploinsufficiency (b, c) |
| L191fs/90 [del TGCGC 572-576] | 8 | Frameshift | Haploinsufficiency |
| R195fs/40 [del G 585] | 2 | Frameshift | Haploinsufficiency |
| S225L | 13 | Missense | Dominant-negative effect (d) |
| A226V | 3 | Missense | Unknown |
| R237P | 1 | Missense | Unknown |
| D242N | 3 | Missense | Unknown |
| R243C | 13 | Missense | Haploinsufficiency (e) |
| V254 mol/L | 59 | Missense | Dominant-negative effect (b, f) |
| R258C | 1 | Missense | Haploinsufficiency |
| R259C | 1 | Missense | Haploinsufficiency (g) |
| L266P | 15 | Missense | Unknown |
| G269D | 35 | Missense | Dominant-negative effect (h) |
| G269S | 25 | Missense | Haploinsufficiency (i) |
| L273F | 6 | Missense | Dominant-negative effect (a) |
| I274V | 1 | Missense | Unknown |
| S277L | 3 | Missense | Unknown |
| Y278H | 2 | Missense | Unknown |
| E284K | 2 | Missense | Unknown |
| G292D | 3 | Missense | Unknown |
| F296S | 2 | Missense | Unknown |
| G306R | 2 | Missense | Dominant-negative effect (b, j) |
| V310I | 1 | Missense | Unknown |
| T312I | 14 | Missense | Dominant-negative effect (a) |
| G314S | 8 | Missense | Dominant-negative effect (h, k, l, m) |
| Y315C | 10 | Missense | Dominant-negative effect (d, n) |
| Y315S | 1 | Missense | Dominant-negative effect (h, m) |
| D317G | 3 | Missense | Unknown |
| P320H | 1 | Missense | Unknown |
| T322 mol/L | 2 | Missense | Unknown |
| G325R | 3 | Missense | Unknown |
| delF340 [del CTT 1017-1019] | 7 | In-frame deletion | Haploinsufficiency |
| A341E | 9 | Missense | Dominant-negative effect (b) |
| A341V | 20 | Missense | Dominant-negative effect (o) |

**TABLE 1. Continued**

| Location and Coding* | No. of Subjects† | Type of Mutation | Functional Effect‡ |
|---|---|---|---|
| P343S | 1 | Missense | Dominant-negative effect (p) |
| A344A/sp [1032 G>A] | 27 | Splice site | Haploinsufficiency |
| A344V | 17 | Missense | Unknown |
| S349W | 15 | Missense | Unknown |
| L353P | 4 | Missense | Unknown |
| C-terminus | | | |
| Q357H | 3 | Missense | Unknown |
| R360G | 3 | Missense | Unknown |
| S373P | 7 | Missense | Unknown |
| K393N | 10 | Missense | Unknown |
| R397W | 5 | Missense | Unknown |
| P400fs/62 [ins C 1201-1022] | 6 | Frameshift | Haploinsufficiency |
| P448fs/13 [ins G 1344-1345] | 11 | Frameshift | Haploinsufficiency |
| I517T | 3 | Missense | Unknown |
| R518X [1552 C>T] | 11 | Nonsense | Haploinsufficiency (q) |
| M520R | 3 | Missense | Unknown |
| V524G | 4 | Missense | Unknown |
| Q530X [1588 C>T] | 13 | Nonsense | Haploinsufficiency (q) |
| R562 mol/L | 2 | Missense | Unknown |
| S566F | 3 | Missense | Unknown |
| I567S | 6 | Missense | Unknown |
| S571fs/20 [del C 1714] | 3 | Frameshift | Haploinsufficiency |
| R591C | 5 | Missense | Unknown |
| R591H | 6 | Missense | Haploinsufficiency (r) |
| R594Q | 11 | Missense | Haploinsufficiency (q) |
| D611Y | 10 | Missense | Haploinsufficiency (s) |
| A636fs/28 [del C 1909] | 2 | Frameshift | Haploinsufficiency |
| Intron | | | |
| IVS2+1 G>A | 2 | Splice site | Unknown |
| IVS4+5 G>A | 2 | Splice site | Unknown |
| IVS7+5 G>A | 15 | Splice site | Unknown |

*The numbers and letters refer to the amino acid coding of the mutant channel protein. The brackets contain the nucleotide code for deletions, frameshift, splice site, and nonsense mutations.

†Included in this table are 52 subjects who died suddenly at a young age. These subjects were from families with a known KCNQ1 mutation and were assumed to have their respective family mutation.

‡Dominant-negative effect is associated with >50% reduction whereas haploinsufficiency is associated with <50% reduction in ion channel repolarizing current. See text for details. Letters in parentheses refer to references that are available in the online-only Data Supplement.

mutations were identified. A majority of the mutations were localized to the S1 to S6 transmembrane domains (66%), and missense (single amino acid substitutions) accounted for 81% of all the mutations.

The phenotypic characteristics of patients enrolled in each of the 3 registries and by location and type of mutation are presented in Table 2. The clinical characteristics of the patients were similar among the 3 registries except for QTc duration and frequency of β-blocker use. The QTc interval was longer and cardiac events and β-blocker use were more frequent in patients with mutations in the transmembrane than in the C-terminus location. β-Blockers were used less frequently in patients from the Japanese registry than in patients from the other 2 registries. The frequency of first cardiac events was higher in those with than without missense mutations. The clinical characteristics of the 19 subjects possessing intron mutations resembled those with transmembrane and missense mutations.

The QTc interval was significantly longer in the 12 patients with 2 mutations than in those with only single KCNQ1 mutations (570±70 versus 480±60 ms; $P<0.01$). All 12 patients with 2 mutations experienced at least 1 cardiac event.

The cumulative probabilities of first cardiac event by location and type of mutation are presented in Figure 2A and 2B, respectively. Significantly higher event rates were found in subjects with transmembrane than C-terminus mutations and in those with than without missense mutations, with the most rapid increase in event rates occurring during ages 7 to

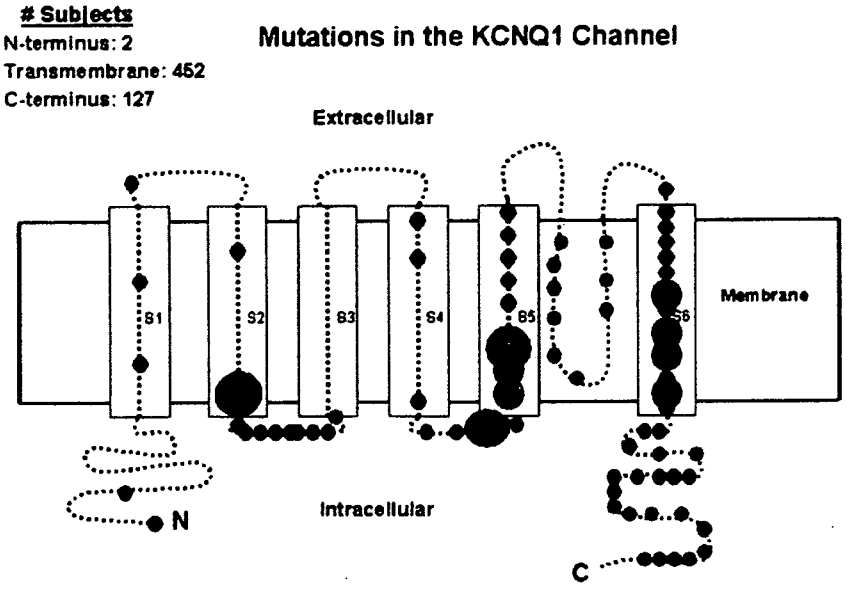


**Figure 1.** Frequency and location of 74 different mutations in the KCNQ1 potassium channel involving 581 subjects. The 19 subjects with 3 intron mutations are not included in this diagram. The $\alpha$ subunit involves the N-terminus (N), 6 membrane-spanning segments, and the C-terminus portion (C). The size of the circles reflect the number of subjects with mutations at the respective locations, with the small circles indicating <15, medium-sized circles 15 to 30, and large circles >30 subjects.

20 years. In patients with transmembrane-localized mutations, the event rates for patients with mutations localized to the pore region (S5-pore-S6) were nearly identical to those with nonpore mutations (data not shown).

The findings from the Cox regression analysis for location and type of mutation are presented in Table 3. The clinical risk factors associated with first cardiac events involved males before age 13 years, females after age 13

**TABLE 2. Phenotypic Characteristics by Source of Subjects, Location of Mutation, and Type of Mutation**

| | Source of Subjects | | | Location of Mutation | | Missense Mutation | | |
|---|---|---|---|---|---|---|---|---|
| Characteristics | United States (n=425) | Netherlands (n=93) | Japan (n=82) | Trans Membrane (n=452) | C-Terminus (n=127) | Yes (n=483) | No (n=98) | Intron Mutation (n=19) |
| Female, % | 57 | 53 | 54 | 57 | 51 | 54 | 62 | 63 |
| ECG at enrollment | | | | | | | | |
| QTc†‡, ms | 488±58 | 450±45 | 472±46 | 485±53 | 460±61 | 481±59 | 471±38 | 478±60 |
| Therapy, % | | | | | | | | |
| β-Blockers†‡ | 45 | 34 | 26 | 45 | 28 | 42 | 38 | 37 |
| Pacemaker | 2.4 | 0 | 0 | 1.5 | 2.4 | 1.4 | 3.1 | 0 |
| Sympathectomy | 0.5 | 0 | 0 | 0.4 | 0 | 0.4 | 0 | 0 |
| Defibrillator | 6.4 | 3.2 | 0 | 5.8 | 3.1 | 5.2 | 5.1 | 0 |
| First cardiac event*‡§, % | 41 | 37 | 38 | 45 | 21 | 43 | 26 | 42 |
| Syncope‡ (n=200) | 35 | 31 | 29 | 38 | 17 | 36 | 21 | 32 |
| Aborted cardiac arrest (n=15) | 1.9 | 1.1 | 7.3 | 2.9 | 0.8 | 2.5 | 2.0 | 5.3 |
| Death (n=23) | 4.0 | 5.5 | 1.2 | 4.0 | 3.1 | 4.2 | 2.0 | 5.3 |
| Ever cardiac event, % | | | | | | | | |
| Syncope‡§ | 35 | 31 | 31 | 39 | 17 | 37 | 21 | 33 |
| Aborted cardiac arrest† | 2.4 | 15 | 8.8 | 5.3 | 3.2 | 5.4 | 2.0 | 11 |
| Death | 11 | 14 | 2.4 | 10 | 6.3 | 11 | 4.1 | 26 |

Plus-minus values are mean±SD. Percentages >10 are rounded to a whole number. The 600 subjects in this table include 51 subjects who died suddenly at a young age, were from families with known KCNQ1 mutation, and were assumed to have the family mutation. Patients with intron mutations are categorized separately and are not included in the location or missense categories. Seven subjects with transmembrane mutations and 1 with C-terminus mutations had missing data about the date of the first cardiac event. Eight subjects with missense mutations had missing data about the date of the first cardiac event. Numbers in parentheses indicate the total number of specific first cardiac events from the 3 sources of patients.

*First cardiac event was syncope, aborted cardiac arrest, or sudden death, whichever occurred first.

†$P<0.01$ for the comparison of characteristics among the 3 sources of subjects.

‡$P<0.01$ for the comparison of characteristics between the 2 locations of the mutations.

§$P<0.01$ for the comparison of characteristics between missense yes and no.

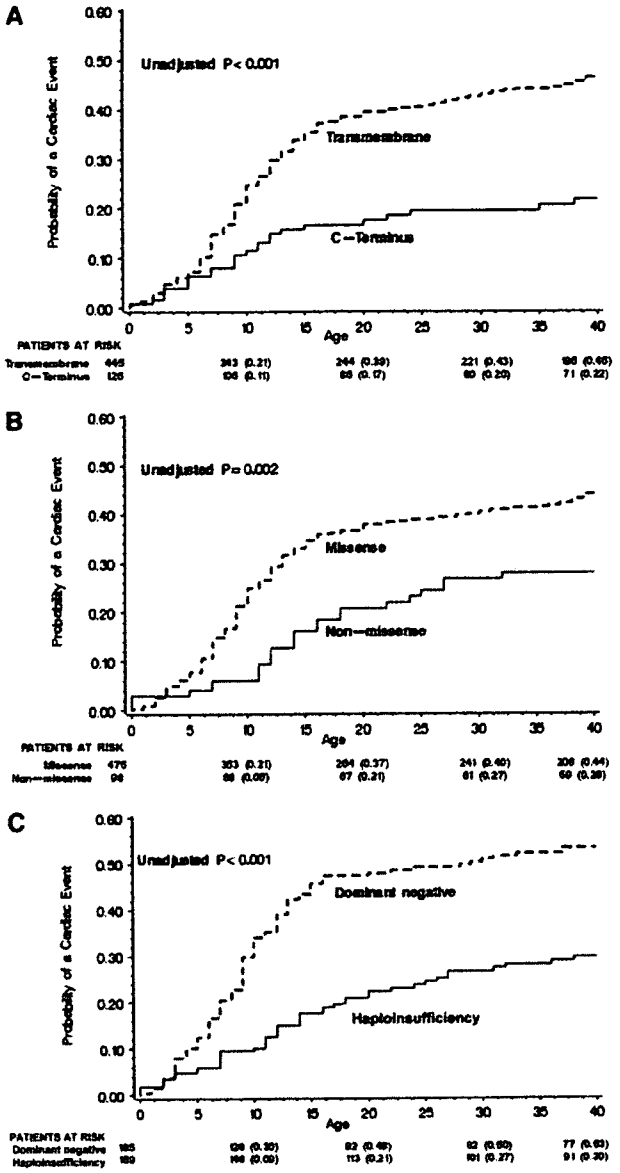


**Figure 2.** Kaplan-Meier estimate of the cumulative probability of a first cardiac event by location (A), type (B), and biophysical function of the mutation (C).

years, and longer QTc intervals. Mutations located in the transmembrane region of the channel made significant and independent contributions to the risk model, but missense mutations were not an independent risk factor. Three different intron mutations were present in 19 subjects from 4 families, and these intron mutations made a meaningful but nonsignificant contribution to the risk model. Prespecified interactions were investigated for their effect on cardiac events, and no significant interactions were found for transmembrane location by type of mutation, transmembrane location by QTc, or mutation type by QTc. Time-dependent β-blocker use was associated with a significant 74% reduction in the risk of first cardiac events ($P<0.001$).

**TABLE 3. Cox Regression With Multiple Predictor Variables Including Location and Type of Mutations for First Cardiac Event**

| Variable | Hazard Ratio | 95% CI | P |
|---|---|---|---|
| Netherlands:United States | 1.15 | 0.74–1.78 | 0.55 |
| Japan:United States | 1.45 | 0.98–2.16 | 0.07 |
| Male <13 y:female <13 y | 1.72 | 1.25–2.38 | <0.001 |
| Female 13–40 y:male 13–40 y | 2.27 | 1.30–3.96 | <0.01 |
| QTc 500–530 ms:QTc <500 ms | 2.04 | 1.41–2.96 | <0.001 |
| QTc >530 ms:QTc <500 ms | 3.25 | 2.25–4.69 | <0.001 |
| QTc missing*:QTC <500 ms | 2.26 | 1.57–3.25 | <0.001 |
| Transmembrane:C-terminus | 2.06 | 1.36–3.12 | <0.001 |
| Missense yes:no | 1.33 | 0.86–2.05 | 0.20 |
| Intron:C-terminus | 2.45 | 0.98–6.11 | 0.06 |
| Time-dependent β-blocker use | 0.26 | 0.14–0.49 | <0.001 |

The Cox analysis involved 592 subjects with 445 transmembrane, 126 C-terminus, 2 N-terminus, and 19 intron mutations; 8 subjects were not included in this Cox analysis because of missing data about the date of their first cardiac event.

*QTc missing category involves 47 subjects who died suddenly at a young age without a prior ECG.

## Biophysical Function and Outcome

The clinical implications of disordered biophysical function of the mutant KCNQ1 channels were investigated in a subset of 356 subjects with known or suspected alteration in ion channel function (see Methods for functional categorization). The clinical characteristics of patients with dominant-negative and haploinsufficiency ion channel dysfunction are presented in Table 4. Patients with mutations having dominant-negative ion current effects had a longer QTc interval and a higher frequency of cardiac events than subjects with mutations resulting in haploinsufficiency. The cumulative probabilities of a first cardiac event by the biophysical function of the mutations are presented in Figure 2C. As shown in Table 5, patients with mutations having

**TABLE 4. Phenotypic Characteristics by Biophysical Function of the KCNQ1 Mutations in 356 Subjects**

| Characteristics | Dominant-Negative Effect (n=187) | Haploinsufficiency (n=169) |
|---|---|---|
| Female, % | 51 | 61 |
| ECG at enrollment | | |
| QTc,* ms | 500±60 | 470±50 |
| Therapy, % | | |
| β-Blockers | 47 | 37 |
| Pacemaker | 1.1 | 4.1 |
| Sympathectomy | 0.5 | 0 |
| Defibrillator | 4.8 | 7.7 |
| First cardiac event*, % | 53 | 27 |
| Syncope | 45 | 22 |
| Aborted cardiac arrest | 2.1 | 3.0 |
| Death | 5.3 | 2.4 |

Percentages >10 are rounded to a whole number. Two subjects had missing data about the date of their first cardiac event.

*$P<0.01$.

**TABLE 5. Cox Regression With Multiple Predictor Variables Including Ion Channel Dysfunction for First Cardiac Events**

| Variable | Hazard Ratio | 95% CI | P |
|---|---|---|---|
| Netherlands:United States | 2.78 | 1.48-5.23 | <0.01 |
| Japan:United States | 1.63 | 1.02-2.63 | 0.04 |
| Male <13 y:female <13 y | 1.94 | 1.29-2.91 | <0.01 |
| Female 13-40 y:male 13-40 y | 1.95 | 0.99-3.87 | 0.06 |
| QTc 500–530 ms:QTc <500 ms | 1.88 | 1.18-2.99 | <0.01 |
| QTc >530 ms:QTc <500 ms | 3.22 | 2.06-5.05 | <0.001 |
| QTc missing*:QTc <500 ms | 2.07 | 1.29-3.33 | <0.01 |
| Dominant-negative:haploinsufficiency | 2.26 | 1.56-3.25 | <0.001 |
| Time-dependent β-blocker use | 0.21 | 0.09-0.48 | <0.001 |

The analysis involved 354 subjects with known or suspected ion channel dysfunction; 2 subjects were not included because of missing data about the date of their first cardiac event.

*The QTc missing category involves 26 patients who died suddenly at a young age without a prior ECG.

dominant-negative functional effects experienced a significantly greater risk for cardiac events than those with haploinsufficiency (hazard ratio, 2.26; 95% CI, 1.56 to 3.25; $P<0.001$) after adjustment for relevant covariates including QTc and gender effects by age group. β-Blocker use was associated with a significant 79% reduction in first cardiac events in this subset of patients. Because substantial colinearity exists for transmembrane mutations, missense mutations, and mutations with dominant-negative biophysical function, the individual effects of these 3 mutation parameters could not be ascertained reliably in the same Cox model.

## Discussion

The main results of the present study from 600 patients having a spectrum of KCNQ1 mutations derived from 3 LQTS registries are significantly higher cardiac event rates in patients with transmembrane mutations and in patients with mutations having a putative dominant-negative effect on the repolarizing $I_{Ks}$ current. The effect of these genetically determined factors is independent of traditional clinical risk factors and of β-blocker therapy.

Since 1995, when the first 2 genes responsible for LQTS were identified,[11,12] molecular genetic studies have revealed a total of 9 forms of congenital LQTS caused by mutations in genes involving potassium channel (LQT-1, -2, -5, -6, and -7), sodium channel (LQT-3, -9), and calcium channel proteins (LQT-8) as well as a membrane-adapter protein (LQT-4).[2,13] Genotype–phenotype studies have enabled us to stratify risk and to treat more specifically patients with LQT-1, LQT-2, and LQT-3 subtypes of this genetic disorder. LQT-1, the most common form of LQTS, accounts for ≈50% of genotyped patients[4,14] and has more variable expressivity and incomplete penetrance than the other forms.[15] Mutation location and knowledge of the functional effects of the mutation provide additional risk information beyond the clinical risk factors and the genotype, at least for LQT-1, and this information should contribute to improved risk stratification and more focused management of these higher-risk patients.

Mutations in KCNQ1 are responsible for defects in the slowly activating component of the delayed rectifier current $I_{Ks}$.[16] This current is the main repolarizing current at increased heart rate and is highly sensitive to catecholamines.[3] We speculate that $I_{Ks}$ channels with transmembrane mutations might have reduced responsiveness to the regulatory β-adrenergic signaling of the ion-conduction pathway with more impairment of shortening of the QTc with exercise-related tachycardia than mutations in the C-terminus region.

Functional $I_{Ks}$ channels result from the coassembly of 4 KCNQ1-encoded subunits. A mutated gene encodes a protein with aberrant function, and the presence of both normal and abnormal proteins in the ion channel contributes to a >50% reduction in ion channel function (dominant-negative effect). An alternative mechanism of reduced repolarizing KCNQ1 $K^+$ current is the inability of mutated subunits to coassemble with normal gene products, such as occurs with a trafficking defect, resulting in a ≤50% reduction in channel function (haploinsufficiency). With only 1 exception,[17] this is the case for all studied truncating mutations leading to incomplete proteins. Our assumption that truncated proteins (based on frameshift nonsense mutations) lead to haploinsufficiency seems justified. The biophysical effect of missense mutations is unpredictable, and both haploinsufficiency and dominant-negative effects have been described. In the absence of reported biophysical studies, missense mutations were classified as unknown.

Previous attempts to identify a genotype–phenotype relationship for KCNQ1 mutations failed to reach consensus on the clinical outcome of the type and site of mutations.[7,8] Relatively small numbers and different ethnic background of the previously reported patients with the LQT-1 genotype might be responsible for the discrepant results. The present larger study allows us to demonstrate for the first time that the biophysical effect clearly affects the clinical outcome (ie, dominant-negative mutations are associated with a more severe phenotype than are mutations conferring haploinsufficiency [Figure 2C], even after adjustment for relevant covariates [Table 5]). The risk observed in 19 subjects with 3 different intron mutations was not quite significant ($P=0.06$), possibly because of small numbers, but the magnitude of the risk effect was similar to the risk accompanying transmembrane mutations. Although these intron mutations produced splice-site alterations predicted to affect the transmembrane portion of the ion channel, we used a separate categorization of intron mutations in view of the limited understanding of the structural alterations and functional effects resulting from these exon-skipping intron mutations.

A few additional findings from this large genotype–phenotype study of type-1 LQTS patients emphasize high risk for first cardiac events during adolescence, a crossover in risk by sex at approximately age 13 years, and a lower rate of first cardiac events in the adult years than in the younger years. These findings are not especially new,[18,19] but the present study highlights their presence in type-1 LQTS.

### Study Limitations

The present study used the biophysical function of mutations reported in the literature in only a portion of the mutations

that were included (see references associated with Table 1 in the online-only Data Supplement). The published studies were from many different laboratories with the use of different cellular heterologous expression systems involving *Xenopus* oocytes and other cells at both room and physiological temperatures. Although such nonuniform testing may have contributed to some inconsistency in the categorized biophysical function, the finding of a significantly higher event rate in mutations with dominant-negative than with haploinsufficient effects (hazard ratio, 2.26; $P<0.001$) is unlikely to have resulted from the nonuniform testing. Unfortunately, we did not have the resources to perform such uniform testing in all 77 mutations presented in the present study.

Once a mutation was identified in KCNQ1, thorough genetic sequencing was not performed routinely in all the ion channel genes to look for second mutations. Thus, some of the patients included in the analysis may have had a second mutation in addition to the identified KCNQ1 mutation. It is estimated that ≈10% of genotype LQTS patients may carry a second mutation, and those with >1 mutation could contribute to some of the findings in our study. In addition, it is possible that some of the reported mutations (Table 1) are simply uncommon sequence mutations, but this is relatively unlikely because all the subjects in the present study were derived from families in which the proband had QTc prolongation not due to a known cause.

The outcome analyses included subjects from families with a known KCNQ1 mutation who died suddenly and unexpectedly at a young age and were classified as LQTS-related death with the same mutation that was present in the family. It is possible that a few of these subjects could have died from a non-LQTS cause or had an LQTS mutation different from the family mutation, but we think the error rate is likely to be small. The number of deaths and aborted cardiac arrest events is small, and there is insufficient power to evaluate the risk association of the genotype characteristics with these endpoint events in a multivariate time-dependent model.

## Conclusions

The present study confirms that in patients with type-1 LQTS, longer QTc intervals are associated with higher cardiac event rates and that male patients are generally younger than female patients at first cardiac events.[20,21] The new findings from the present study are that transmembrane mutations and mutations with dominant-negative functional effect adversely influence the outcome of this disorder independent of traditional clinical risk factors and β-blocker therapy. The present study was not designed to assess the effectiveness of different therapies in patients with KCNQ1 mutations. The findings presented do not provide justification for using specific genotype characteristics to identify patients for implanted defibrillator therapy.

## Note Added in Proof

After this article was accepted for publication, we noted the recent article by Tsuji et al, in which the A344A/sp [1032G>A] mutation that we categorized as haploinsufficient (Table 1) was reported to have a weak dominant-negative effect.[22] We reran the KCNQ1 data recategorizing the 27 A344A/sp [1032 G>A] mutations as dominant-negative. Negligible changes occurred in the results as presented in Table 5 and Figure 2C; the hazard ratio for dominant-negative:haploinsufficiency (Table 5) was unchanged at 2.26 ($P<0.001$).

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## Disclosures

Dr Ackerman is a consultant for Clinical Data (formerly Genaissance Pharmaceuticals) with respect to the FAMILION genetic test for cardiac ion channel mutations. The other authors report no conflicts.

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## CLINICAL PERSPECTIVE

Type-1 long-QT syndrome is caused by loss-of-function mutations in the KCNQ1-encoded $I_{Ks}$ cardiac potassium channel. In the present study involving 600 patients having a spectrum of KCNQ1 mutations derived from 3 long-QT syndrome registries, we found that cardiac event rates are increased significantly in patients with mutations located in the transmembrane region of the potassium channel and in patients with mutations having a putative dominant-negative effect on the repolarizing $I_{Ks}$ current. The effects of these genetically determined factors are independent of traditional clinical risk factors and of $\beta$-blocker therapy. Mutation location and knowledge of functional effects of the mutation provide additional risk information beyond the clinical risk factors and the genotype, at least for type-1 long-QT syndrome, and this information should contribute to improved risk stratification and more focused management of these higher-risk patients.

# Diagnostic and Prognostic Value of a Type 1 Brugada Electrocardiogram at Higher (Third or Second) $V_1$ to $V_2$ Recording in Men With Brugada Syndrome

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**To evaluate the diagnostic and prognostic value of an electrocardiogram (ECG) recorded at a higher (third or second) intercostal space, 98 men (17 to 76 years of age, mean ± SD 47 ± 13; with documented ventricular fibrillation [VF] in 22 and syncope in 32) were categorized into 3 groups; 68 men had a spontaneous type 1 ECG in standard leads $V_1$ and $V_2$ (S group), 19 had a spontaneous type 1 ECG only in the higher $V_1$ and $V_2$ leads (H group), and 11 had a type 1 ECG only after receiving class Ic sodium channel blockers (Ic group). There were no significant differences in baseline clinical characteristics, including VF episodes, syncope, atrial fibrillation, family history, late potentials, and inducibility of VF during electrophysiologic study across the 3 groups. During prospective follow-up periods (779 ± 525, 442 ± 282, and 573 ± 382 days, respectively), subsequent cardiac events occurred in 11 men (16%) within the S group, in 2 men (11%) in the H group, and in 0 men (0%) in the Ic group (p = NS, S vs H group). In men with previous episodes of VF, subsequent cardiac events occurred in 7 (44%) within the S group and in 2 (50%) in the H group (p = NS). In conclusion, men with a spontaneous type 1 Brugada ECG recorded only at higher leads $V_1$ and $V_2$ showed a prognosis similar to that of men with a type 1 ECG in using standard leads $V_1$ and $V_2$.**  **(Am J Cardiol 2007;99:53–57)**

Brugada syndrome is characterized by a high risk of sudden cardiac death due to ventricular fibrillation (VF) and a specific ST-segment elevation in the right precordial leads ($V_1$ to $V_3$) in the absence or presence of sodium channel blockers.[1,2] Recent consensus reports have proposed 3 types of ST-segment elevation (types 1 to 3) in this syndrome.[3–5] Although the magnitude and pattern of ST-segment elevation differ in each patient and can change even in the same patient,[6–8] documentation of a spontaneous type 1 electrocardiogram (ECG), which is defined as a coved type and a J-point elevation ≥0.2 mV, has been associated with a high risk of sudden cardiac death.[3–5,9–11] Electrocardiographic recording in leads $V_1$ and $V_2$ at a higher (third or second) intercostal space has been reported to unmask or confirm a type 1 Brugada ECG, with a high sensitivity in individuals with suspected Brugada syndrome.[12–14] However, systematic evaluation of recording leads $V_1$ and $V_2$ at a higher space, especially with regard to diagnostic and prognostic values, has not been done. This study evaluated the diagnostic and prognostic value of documentation of a spontaneous type 1 Brugada ECG in leads $V_1$ and $V_2$ recorded at a higher intercostal space.

## Methods

The study population consisted of 98 probands from 98 unrelated families in whom a type 1 Brugada ECG was documented in leads $V_1$ and $V_2$ at a standard (fourth) and/or higher (third or second) intercostal space in the absence or presence of class Ic sodium channel blockers. They were enrolled between October 2000 and September 2004 and were followed prospectively. All 98 patients were men. Their average age at enrollment was 47 ± 13 years (17 to 76). VF had been documented in 22 men and 32 had shown only syncope. An SCN5A coding region mutation was identified in 8 men. Physical examination showed no abnormal findings, and no evidence of structural heart disease was demonstrated by echocardiogram in any subject. Informed consent was obtained from all subjects.

The 98 men were categorized into 3 groups; 68 had a spontaneous type 1 Brugada ECG recorded at a standard (fourth) intercostal space in leads $V_1$ and $V_2$ (S group), 19 had a spontaneous type 1 Brugada ECG recorded only at a higher (third or second) intercostal space in leads $V_1$ and $V_2$ (H group), and 11 had a type 1 Brugada ECG recorded only

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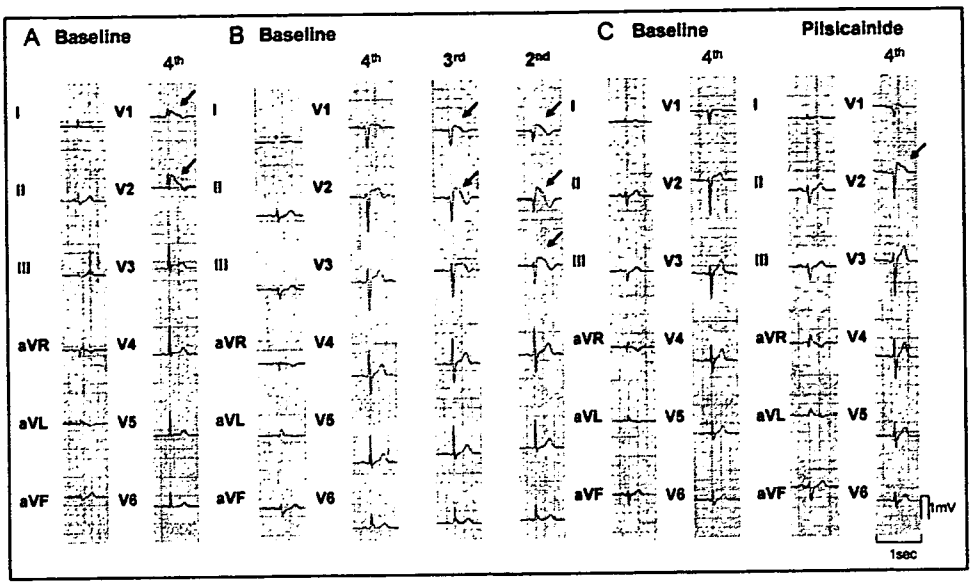


Figure 1. Twelve-lead ECG in representative subjects of the 3 groups. *(A)* S group (spontaneous). A type 1 coved-type ST-segment elevation was seen at a standard (fourth) intercostal space in leads $V_1$ and $V_2$ *(arrows)* at baseline. *(B)* H group (spontaneous). A type 1 Brugada ECG was recorded at higher (third and second) intercostal spaces in leads $V_1$ and $V_2$ ($V_3$) *(arrows)* but not at a standard (fourth) intercostal space in these leads at baseline. *(C)* Ic group. A type 1 Brugada ECG was recorded at a standard (fourth) intercostal space in leads $V_2$ only after injection of 30 mg of pilsicainide.

Table 1
Comparison of clinical, electrocardiographic, and electrophysiologic characteristics across the 3 groups (S group vs H group vs Ic group)

| Variable | S Group (only spontaneous) (n = 68) | H Group (only spontaneous) (n = 19) | Ic Group (n = 11) | p Value |
|---|---|---|---|---|
| Age (yrs) (range) | 48 ± 16 (21–76) | 46 ± 15 (17–72) | 44 ± 16 (17–62) | 0.64 |
| Symptomatic | 40 (59%) | 7 (37%) | 7 (64%) | 0.20 |
| Documented VF | 16 (24%) | 4 (21%) | 2 (18%) | 0.91 |
| Syncope only | 24 (35%) | 3 (16%) | 5 (45%) | 0.17 |
| Inducible VF/ventricular tachycardia | 42 (78%) | 6 (55%) | 7 (70%) | 0.27 |
| Family history | 14 (21%) | 2 (11%) | 3 (27%) | 0.48 |
| Presence of late potential | 46 (74%) | 11 (65%) | 6 (55%) | 0.59 |
| Presence of atrial fibrillation | 16 (24%) | 3 (16%) | 1 (9%) | 0.47 |
| Follow-up (d) | 779 ± 525 | 442 ± 282 | 573 ± 382 | <0.01* |

* S group versus H group.

after receiving class Ic sodium channel blockers at standard and/or higher spaces in leads $V_1$ and $V_2$ (Ic Group) (Figure 1). We compared clinical, electrocardiographic, and electrophysiologic characteristics, and subsequent occurrence of cardiac events across the 3 groups.

Twelve-lead electrocardiographic data were recorded at a paper speed of 25 mm/s during sinus rhythm in a supine state at rest. Leads $V_1$ and $V_2$ were recorded at standard (fourth) and higher (third and second) intercostal spaces. At enrollment and categorization of subjects into 3 groups, ≥3 separate recordings of 12-lead ECGs were reviewed in each subject. If a spontaneous type 1 Brugada ECG was recorded at a standard space in leads $V_1$ and $V_2$ ≥1 time among multiple ECGs, the subject was classified into the S group. Similarly, if a spontaneous type 1 Brugada ECG was recorded at a higher space in leads $V_1$ and $V_2$ ≥1 time but not at all in standard leads $V_1$ and $V_2$, the subject was classified into the H group.

Drug challenge testing was performed with intravenous pilsicainide (1 mg/kg, maximum 50 mg, 5 mg/min) and/or flecainide (2 mg/kg, maximum 100 mg, 10 mg/min). The test result was considered positive if a type 1 Brugada ECG appeared in >1 precordial lead.

Late potential was analyzed using a signal-averaged electrocardiographic system (Arrhythmia Research Technology 1200EPX, Milwaukee, Wisconsin). Three parameters were assessed using a computer algorithm: (1) total filtered QRS duration, (2) root-mean-square voltage of the terminal 40 ms of the filtered QRS complexes, and (3) duration of low-amplitude signals <40 μV of the filtered QRS complex. Late potential was considered present when a root-mean-square voltage <18 μV and a duration >38 ms were present.

An electrophysiologic study was conducted without antiarrhythmic drugs after informed consent was obtained. Programmed electrical stimulation was performed from the right ventricular apex and the right ventricular outflow tract with up to 3 extrastimuli. Induction of VF requiring direct cardioversion or nonsustained polymorphic ventricular tachycardia lasting ≥15 beats was considered a positive result.

All men were followed up at outpatient clinics of the National Cardiovascular Center. The end point was VF

documented in the storage memory of an implantable cardioverter-defibrillator, apparent syncope, or sudden cardiac death.

Quantitative values were expressed as mean ± SD. Statistical significance in differences was analyzed by chi-square test or 1-way analysis of variance across the 3 groups (S vs H vs Ic group). A p value $<0.05$ was considered statistically significant. Survival curves were plotted using Kaplan-Meier methods and analyzed by log-rank test.

## Results

Table 1 presents a comparison of clinical, electrocardiographic, and electrophysiologic characteristics across the S group (spontaneous only), H group (spontaneous only), and Ic group. There were no significant differences in baseline clinical characteristics with respect to gender, age, frequency of documented episodes of VF and syncope, family history (sudden cardiac death or a Brugada ECG), late potential, atrial fibrillation, and inducibility of VF/ventricular tachycardia during the electrophysiologic study across the 3 groups.

In all 68 men in the S group, a spontaneous type 1 Brugada ECG was always seen at a higher space in leads $V_1$ and $V_2$ on all ECGs showing a spontaneous type 1 Brugada ECG at a standard space in leads $V_1$ and $V_2$. Ten of 68 men (15%) in the S group always showed a type 1 Brugada ECG at a standard space in leads $V_1$ and $V_2$ on multiple ECGs. However, the remaining 58 men (85%) did not always show a type 1 Brugada ECG at a standard position, and 30 of these (52%) always showed a type 1 Brugada ECG at a higher space in leads $V_1$ and $V_2$. Of the 19 men in the H group, 7 (37%) always showed a type 1 Brugada ECG at a higher space in leads $V_1$ and $V_2$. In the 11 patients in the Ic group, 8 (73%) showed a type 1 Brugada ECG after class Ic drugs at a standard space in leads $V_1$ and $V_2$, and 3 (27%) showed this only at a higher space in leads $V_1$ and $V_2$.

An implantable cardioverter-defibrillator was implanted in 47 of the 68 subjects (69%) in the S group (VF in 14 of 16, 88%; syncope only in 19 of 24, 79%; asymptomatic in 14 of 28, 50%), in 7 of the 19 subjects (37%) in the H group (VF in 4 of 4, 100%; syncope only in 1 of 3, 33%; asymptomatic in 2 of 12, 17%), and 7 of the 11 subjects (64%) in the Ic group (VF in 2 of 2, 100%; syncope only in 3 of 5, 60%; asymptomatic in 2 of 4, 50%). Three subjects (4%) in the S group and 1 (5%) in the H group were treated with antiarrhythmic drugs only (2 with amiodarone and 1 with disopyramide in the S group and 1 with atenolol in the H group).

The mean prospective follow-up period was 779 ± 525 days in the S group, 442 ± 282 days in the H group, and 573 ± 382 days in the Ic group. The follow-up period was significantly longer in the S group than in the H group ($p<0.01$; Table 1). This difference was explained by the fact that more men were enrolled unintentionally in the S group soon after the prospective study was started.

Kaplan-Meier analysis of subsequent cardiac events during follow-up in the 3 groups is shown in Figure 2.

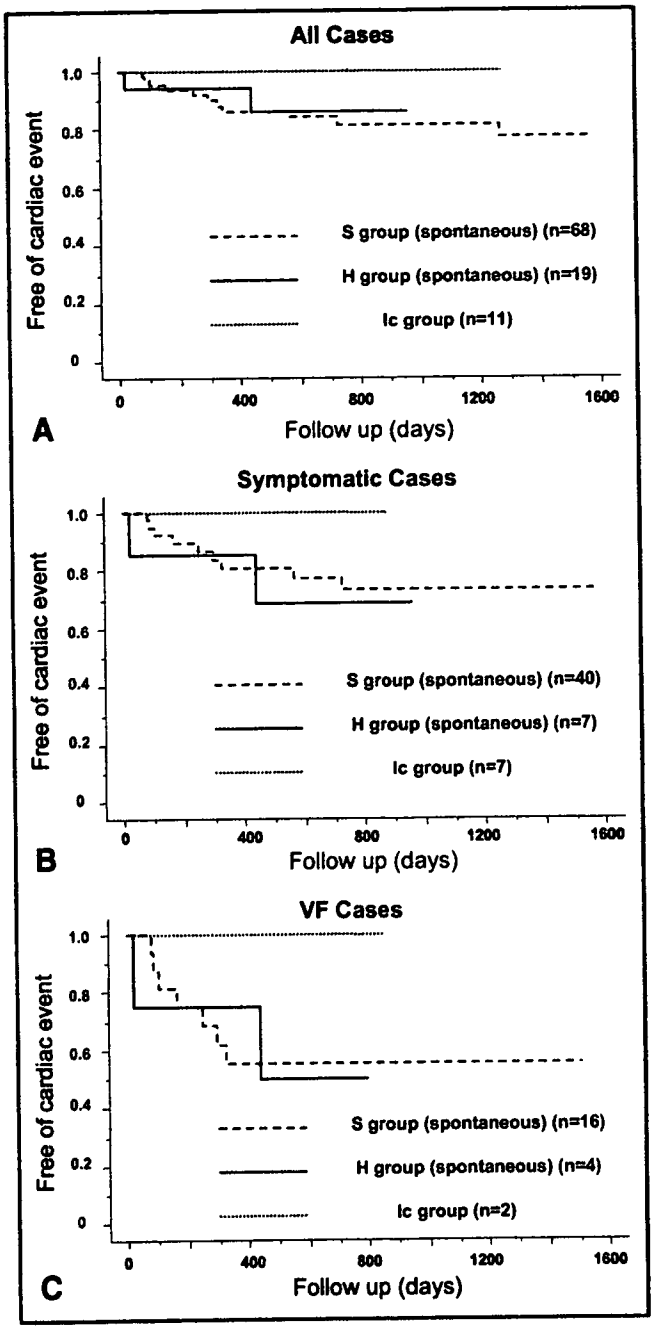


Figure 2. Kaplan-Meier analysis of subsequent cardiac events (VF in implantable cardioverter-defibrillator storage or sudden cardiac death) in the S group (spontaneous) *(dashed line)*, H group (spontaneous) *(solid line)*, and Ic group *(dotted line)* for *(A)* all patients, *(B)* symptomatic patients with previous VF and/or syncope, and *(C)* patients with previously documented VF.

Subsequent cardiac events occurred in 11 of 68 subjects (16%) in the S group (VF in implantable cardioverter-defibrillator storage in 9, sudden cardiac death in 2), 2 of 19 subjects (11%) in the H group (VF in implantable cardioverter-defibrillator storage in 2), but 0 of 11 subjects (0%) in the Ic group (Figure 2). No significant difference was observed in the frequency of cardiac events between the S and H groups.

Of the 13 men with subsequent cardiac events, 9 (69%) had previous VF (7 in the S group, 2 in the H group), 2 (15%) had previous syncope only (2 in the S group), and 2 (15%) were asymptomatic (2 in the S group) at enrollment. Because previous VF and/or syncope are strong indicators of subsequent cardiac events,[9,10,15] the frequency of subsequent cardiac events was evaluated when the subjects were limited to symptomatic patients with previous VF and/or syncope. No significant difference was observed in the frequency of subsequent cardiac events between 40 symptomatic subjects in the S group and 7 symptomatic subjects in the H group (23%, 9 of 40, vs 29%, 2 of 7; Figure 2).

When the subjects were limited to patients with previous VF (16 in the S group, 4 in the H group), there was no significant difference in the frequency of subsequent cardiac events between the 2 groups (44%, 7 of 16, vs 50%, 2 of 4; Figure 2).

Of the 19 subjects in the H group, 14 underwent a drug challenge test, and 2 showed a type 1 Brugada ECG at a standard space in leads $V_1$ and $V_2$ after the test.

## Discussion

The major findings of our study were that (1) recording at a higher space in leads $V_1$ and $V_2$ had higher sensitivity than that at a standard space in these leads in detecting a type 1 Brugada ECG and (2) a type 1 Brugada ECG recorded only at a higher space in leads $V_1$ and $V_2$ showed a similar prognostic value for subsequent cardiac events as that recorded at a standard space in these leads.

Priori et al[9] reported that only 50% of patients with Brugada syndrome in whom repetitive baseline ECGs were recorded had ≥1 positive baseline ECG. In the present study, only 10 of 68 subjects (15%) in the S group always showed a type 1 Brugada ECG at a standard space with leads $V_1$ and $V_2$. Because a region reflecting the potentials of the right ventricular outflow tract includes higher precordial ECGs (second or third in leads $V_1$ and $V_2$), we hypothesized that recordings at a higher space in leads $V_1$ and $V_2$ would detect a type 1 coved-type Brugada ECG more frequently in patients with Brugada syndrome and transient ST-segment elevation. Shimizu et al[12] used body surface potential mapping and examined the body surface distribution of maximum (coved type) ST-segment elevation in patients with Brugada syndrome in whom spontaneous coved type ST-segment elevation was documented ≥1 time in the standard leads $V_1$ and $V_2$. They reported that the maximum ST-segment elevation was distributed at row 5 of the body surface potential mapping, on which leads $V_1$ and $V_2$ on standard ECG were located, in 18 of 25 patients (72%) with Brugada syndrome and at row 6, which was on the level of parasternal second intercostal space, in the remaining 7 patients (28%) with Brugada syndrome. In the latter patients, typical coved type ST-segment elevation was recognized only at a higher (third or second) space in leads $V_1$ and $V_2$ on the standard 12-lead ECG.

In the present study, the remaining 58 of 68 subjects (85%) in the S group did not always show a type 1 Brugada ECG on standard leads $V_1$ and $V_2$; however, 30 of 58 subjects (52%) always showed a type 1 Brugada ECG on the higher leads $V_1$ and $V_2$, suggesting that a higher electrocardiographic recording has higher sensitivity for detecting a type 1 Brugada ECG, as in previous studies.[12–14] Moreover, higher recordings in leads $V_1$ and $V_2$ showed similar prognostic value as standard recordings in these leads. Because recordings of leads $V_1$ and $V_2$ at a higher (third or second) intercostal space are easy and noninvasive procedures, we recommend the higher recordings in leads $V_1$ and $V_2$ as an alternative to drug challenge testing with sodium channel blockers. Only when the result of this procedure is negative should a drug challenge test be considered as a next diagnostic test.


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*MUTATION IN BRIEF*

# Genotype-Phenotype correlations of *KCNJ2* mutations in Japanese patients with Andersen-Tawil syndrome

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Short Title: *KCNJ2* Mutations in Andersen-Tawil Syndrome
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**Andersen-Tawil syndrome (ATS) is a rare inherited disorder characterized by periodic paralysis, mild dysmorphic features, and QT or QU prolongation with ventricular arrhythmias in electrocardiograms (ECGs). Mutations of *KCNJ2*, encoding the human inward rectifying potassium channel Kir 2.1, have been identified in patients with ATS. We aimed to clarify the genotype-phenotype correlations in ATS patients. We screened**

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**23 clinically-diagnosed ATS patients from 13 unrelated Japanese families. Ten *KCNJ2* mutations were identified in ATS patients. Their ECGs showed normal QTc intervals and abnormal U waves with QUc prolongation and a variety of ventricular arrhythmias. Especially, bidirectional ventricular tachycardia (VT) was observed in 13 of 23 patients (57%). Periodic paralysis was seen in 13 of 23 carriers (57%), dysmorphic features in 16 (70%), and seizures during infancy in 4 (17%). Functional assays for the two novel *KCNJ2* mutations (c. 200G>A/p. R67Q and c. 436G>A/p. G146S) displayed no functional inward rectifying currents in a heterologous expression system and showed strong dominant negative effects when co-expressed with wild-type *KCNJ2* channels (91% and 84% reduction at –50 mV respectively compared to wild-type alone). Immunocytochemistry and confocal imaging revealed normal trafficking for mutant channels. In our study, all of the clinically diagnosed ATS patients had *KCNJ2* mutations and showed a high penetrance with regard to the typical cardiac phenotypes: predominant U wave and ventricular arrhythmias, typically bidirectional VT.**



KEY WORDS: Andersen-Tawil syndrome; long QT syndrome; tachyarrhythmia; *KCNJ2*; ion channelopathy; potassium channels; bi-directional ventricular tachycardia; QU prolongation; periodic paralysis; dysmorphic features.

## INTRODUCTION

Andersen-Tawil syndrome (ATS, OMIM 170390) is a rare autosomal dominant disorder characterized by classical triad: (1) ventricular tachyarrythmias associated with QT prolongation in electrocardiograms (ECGs), (2) periodic paralysis, and (3) dysmorphic features (Andersen, et al., 1971; Canun, et al., 1999; Sansone, et al., 1997; Tawil, et al., 1994). Plaster et al. revealed that mutations in *KCNJ2* (OMIM 600681) caused the syndrome in the majority of clinically diagnosed ATS families (Plaster, et al., 2001). *KCNJ2* encodes a pore-forming subunit of inwardly rectifying potassium channels (Kir 2.1), a critical contributor for the $I_{K1}$ current, which maintains normal resting membrane potentials and regulates the final phase of action potential repolarization in various types of cells (Kubo, et al., 1993; Raab-Graham, et al., 1994). To date, more than 30 *KCNJ2* mutations were identified and reported to be responsible for ATS (Ai, et al., 2002; Bendahhou, et al., 2005; Davies, et al., 2005; Donaldson, et al., 2003; Hosaka, et al., 2003; Plaster, et al., 2001; Tristani-Firouzi, et al., 2002; Zhang, et al., 2005). Most mutations in *KCNJ2* showed loss-of-function and dominant negative suppression effects (Tristani-Firouzi, et al., 2002), and a mutation, p.S136F, has been shown to suppress the native $I_{K1}$ in neonatal rat cardiomyocytes (Lange, et al., 2003).

In contrast to the relatively homogenous change in functional outcome as a result of the mutations, ATS patients displayed a wide range of penetrance and severity of clinical phenotypes (Plaster, et al., 2001). This perplexity makes it difficult for physicians to properly diagnose the syndrome. Indeed, some cases have been diagnosed and treated as long QT syndrome (LQTS) and others as periodic paralysis. Tristani-Firouzi et al. performed extensive genetic and phenotypic analyses of ATS patients from 17 families and suggested that ATS might be classified as a new subtype of LQTS (referred to as *KCNJ2*-associated LQTS (LQT7)) (Tristani-Firouzi, et al., 2002). Recently, Zhang et al. reviewed the ECGs of 96 ATS patients with *KCNJ2* mutations and revealed the median QTc interval in ATS patients to be within the normal range (Zhang, et al., 2005). They concluded that *KCNJ2*-associated ATS is not a subtype of LQTS and recommended to have them annotated as ATS1. In a study on guinea pig