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Mutation Site-Specific Differences in Arrhythmic Risk and Sensitivity to Sympathetic Stimulation in the LQT1 Form of Congenital Long QT Syndrome

Multicenter Study in Japan

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OBJECTIVES We sought to compare the arrhythmic risk and sensitivity to sympathetic stimulation of mutations located in transmembrane regions and C-terminal regions of the *KCNQ1* channel in the LQT1 form of congenital long QT syndrome (LQTS).

BACKGROUND The LQT1 syndrome is frequently manifested with variable expressivity and incomplete penetrance and is much more sensitive to sympathetic stimulation than the other forms.

METHODS Sixty-six LQT1 patients (27 families) with a total of 19 transmembrane mutations and 29 patients (10 families) with 8 C-terminal mutations were enrolled from five Japanese institutes.

RESULTS Patients with transmembrane mutations were more frequently affected based on electrocardiographic (ECG) diagnostic criteria (82% vs. 24%, $p < 0.0001$) and had more frequent LQTS-related cardiac events (all cardiac events: 55% vs. 21%, $p = 0.002$; syncope: 55% vs. 21%, $p = 0.002$; aborted cardiac arrest or unexpected sudden cardiac death: 15% vs. 0%, $p = 0.03$) than those with C-terminal mutations. Patients with transmembrane mutations had a greater risk of first cardiac events occurring at an earlier age, with a hazard ratio of 3.4 ($p = 0.006$) and with an 8% increase in risk per 10-ms increase in corrected Q-Tend. The baseline ECG parameters, including Q-Tend, Q-Tpeak, and Tpeak-end intervals, were significantly greater in patients with transmembrane mutations than in those with C-terminal mutations ($p < 0.005$). Moreover, the corrected Q-Tend and Tpeak-end were more prominently increased with exercise in patients with transmembrane mutations ($p < 0.005$).

CONCLUSIONS In this multicenter Japanese population, LQT1 patients with transmembrane mutations are at higher risk of congenital LQTS-related cardiac events and have greater sensitivity to sympathetic stimulation, as compared with patients with C-terminal mutations. (J Am Coll Cardiol 2004;44:117–25) © 2004 by the American College of Cardiology Foundation

Congenital long QT syndrome (LQTS) is a hereditary disorder characterized by a prolonged QT interval on the electrocardiogram (ECG), commonly associated with polymorphic ventricular tachycardia known as torsade de pointes (TdP), often leading to severe symptoms, such as syncope and sudden cardiac death (1,2). Genetic studies have so far identified seven forms of congenital LQTS caused by mutations in genes of the potassium and sodium channels or the membrane adapter located on chromosomes 3, 4, 7, 11, 17, and 21 (3–5). Among the seven forms, LQT1 syndrome is one of the two most common genetic variants of LQTS and accounts for approximately 25% of genotyped patients (6). Mutations in *KCNQ1* are responsible for defects in the slowly activating component of the delayed rectifier potassium current (I_{Ks}) underlying LQT1 syndrome (7). The LQT1 syndrome is frequently manifested with variable expressivity and incomplete penetrance (8–10) and is much more sensitive to sympathetic stimulation than the other forms (11,12).

Examination of the genotype-phenotype correlation is important for the management and treatment of patients with congenital LQTS, especially in the LQT1, LQT2, and LQT3 forms, which constitute approximately two-thirds of genotyped LQTS (13). More recently, mutation site-specific differences in the severity of phenotype have been

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Abbreviations and Acronyms

APD	= action potential duration
DNA	= deoxyribonucleic acid
$I_{Cl(Ca)}$	= Ca^{2+}-activated chloride current
I_{Kr}	= fast component of the delayed rectifier potassium current
I_{Ks}	= slow component of the delayed rectifier potassium current
$I_{Na\text{-}Ca}$	= Na^{+}/Ca^{2+} exchange current
LQTS	= long QT syndrome
LZ	= leucine zipper
PCR	= polymerase chain reaction
TdP	= torsade de pointes
Tpeak-end	= interval between Tpeak and Tend

evaluated in each genotype. Moss et al. (14) have suggested that LQT2 patients with mutations in the pore region of the *KCNH2* gene were at markedly increased risk of arrhythmia-related cardiac events, as compared with patients with non-pore mutations, in the International Long-QT Syndrome Registry. With regard to the LQT1 syndrome, Donger et al. (15) initially suggested that the missense mutation, R555C, located in the C-terminal region of the *KCNQ1* gene was associated with a less severe phenotype than the mutations in the transmembrane regions. Since then, more than 20 mutations located in the C-terminal region of the *KCNQ1* gene have been reported, but neither the severity nor the function of the mutations has been fully determined. In the present study, we compared the arrhythmic risk and sensitivity to sympathetic stimulation with exercise between LQT1 patients with mutations located in the transmembrane regions and those with mutations in the C-terminal regions of the *KCNQ1* gene.

METHODS

Patient population. The study population consisted of 95 patients from 37 unrelated Japanese LQT1 families enrolled from five institutes in Japan: the National Cardiovascular Center, Kyoto University Graduate School of Medicine, Kanazawa University, Niigata University Graduate School of Medical and Dental Science, and Okayama University Graduate School of Medicine and Dentistry. The *KCNQ1* mutations were confirmed in all patients by using standard genetic tests. Briefly, genomic deoxyribonucleic acid (DNA) was isolated from leukocyte nuclei by conventional methods. Screening for mutations of *KCNQ1, KCNH2, SCN5A, KCNE1,* and *KCNE2* was performed by using polymerase chain reaction (PCR)/single-strand conformation polymorphism or denatured high-performance liquid chromatography analyses. For aberrant PCR products, DNA sequencing was conducted with a DNA sequencer (3700 DNA Analyzer, PE Applied Biosystems, Foster City, California). If the patients had double mutations within the *KCNQ1* gene or accompanying additional mutations in other genes, they were excluded from the present study. Genotyping of LQTS was reviewed and approved according to each Institutional Review Board's guideline, and written, informed consent was obtained from all patients. No patients were taking beta-blockers at the time of the baseline ECG and exercise treadmill test.

Clinical characterization. Routine clinical and ECG parameters were usually obtained at the time of first admission to each institute for evaluation of LQTS, and thereafter at the time of at least yearly follow-up contact.

CLINICAL DIAGNOSIS. We evaluated two major clinical ECG criteria for diagnosing LQTS-affected individuals. The ECG diagnostic criteria of Keating et al. (16), included a corrected QT (QTc) interval ≥470 ms in asymptomatic individuals and a QTc interval >440 ms for males and >460 ms for females, were associated with one or more of the following: 1) stress-related syncope; 2) documented TdP; or 3) a family history of early sudden cardiac death. The LQTS was also diagnosed by the diagnostic criteria (score ≥4) of Schwartz et al. (17).

BASELINE 12-LEAD ECG MEASUREMENTS. Baseline 12-lead ECG parameters included the RR, Q-Tend, Q-Tpeak, and Tpeak-end (Q-Tend − Q-Tpeak) intervals as an index of transmural dispersion of repolarization. The Q-Tend, Q-Tpeak, and Tpeak-end intervals were also corrected using Bazett's method. These parameters were measured manually in three leads (II, V_2, and V_5), with quantitative repolarization values reported for lead V_5, because the measurements were similar in all three leads. Q-Tend was defined as the interval between the QRS onset and the point at which an isoelectric line intersected a tangential line drawn at the minimum first derivative (dV/dt) point of the positive T-wave or at the maximum dV/dt point of the negative T-wave. When a bifurcated or secondary T wave fusing the first component appeared, it was included as part of the measurement of Q-Tend, but a normal U-wave, which was apparently separated from a T-wave, was not included. Q-Tpeak was defined as the interval between the QRS onset and the peak of the positive T-wave or the nadir of the negative T-wave. Measurements were carried out by two investigators who were unaware of the subjects' genetic status. There were no significant differences in the measured data between the two (data not shown). In addition, TdP and T-wave alternans on the ECG were assessed.

CARDIAC EVENTS, THERAPY, AND FOLLOW-UP. Congenital LQTS-related cardiac events were defined as syncope, aborted cardiac arrest, or unexpected sudden cardiac death without a known cause. Cardiac events, which brought the probands to medical attention but were secondary to apparent causes known to prolong repolarization, such as antiarrhythmic drugs, electrolyte abnormalities, or bradycardia, were excluded from the analysis of congenital LQTS-related cardiac events. Such secondary cardiac events were documented in one patient with C-terminal mutation (hypokalemia 1) and one patient with transmembrane mutation

(hypokalemia 1). Therapy, including beta-blockers, pacemakers, sympathectomy, and defibrillator, was also evaluated. Follow-up was censored at age 50 years to avoid the influence of coronary artery disease on cardiac events in the Japanese population.

EXERCISE TREADMILL TESTING. Forty-nine of the 95 patients were included for the analysis with exercise treadmill testing. All patients were in sinus rhythm, and none had atrioventricular or bundle branch block during the exercise testing. Exercise treadmill testing was performed using the standard Bruce protocol. Twelve-lead ECGs were recorded every 1 min from the baseline condition through the maximal exercise to the recovery phase for 8 min. The ECG measurements before exercise were obtained in the standing position before exercise, and those after exercise were usually obtained within 2 min after stopping exercise to avoid noise in the measurement.

Genetic characterization. Genetic mutations of the *KCNQ1* amino acid sequence were characterized by a specific location and coding effect (missense, splice site, frameshift, or deletion). The transmembrane regions were defined as six transmembrane segments (S1 to S6, amino acid residues 112 through 354), including cytoplasmic and extracellular linkers as well as the pore region. The pore region of the *KCNQ1* channel was defined as the area extending from S5 to the mid-portion of S6 involving amino acid residues 301 through 320.

Statistical analysis. Data are expressed as the mean value ± SD. Repeated measures two-way analysis of variance (ANOVA), followed by the Scheffé test, was used to compare data between mutations located in the transmembrane regions and those in the C-terminal regions, as well as to compare measurements made before and after exercise (STATISTICA, 1998 edition). Repeated measures one-way ANOVA, followed by the Scheffé test, was used to compare changes (Δ) in the measurements with exercise between the groups. Differences in frequencies were analyzed by the chi-square test. A two-sided p value <0.05 was considered to indicate significance. The cumulative probability of a first cardiac event was assessed by the Kaplan-Meier method and log-rank statistic. The multivariate Cox proportional hazards survivorship model (adjusting for mutation locations, age, and gender) was used to evaluate the independent contribution of clinical and genetic factors to first cardiac events from birth through to age 50 years. Clinical data were also compared between transmembrane mutations and C-terminal mutations for the probands and non-probands, separately. Because the non-probands (family members) in this study were mainly relatives in the first or second degree of the probands, the non-probands in each family were equally handled in the analysis.

RESULTS

Genetic characteristics. Table 1 illustrates the numbers of LQT1 patients by mutation and location (18–24). We identified 27 *KCNQ1* mutations among the 95 LQT1 patients, with 19 mutations located in the transmembrane regions and eight mutations in the C-terminal regions. Four mutations were located at the pore region in the transmembrane domain. Twenty-three of the 27 mutations were missense mutations; 2 were frameshift mutations; 1 was a deletion mutation; and 1 was a splice mutation. Thirteen mutations (seven in the transmembrane regions and six in the C-terminal regions) were novel. Functional effects by cellular electrophysiologic tests have been reported in eight of the 27 mutations (Table 1).

Clinical characteristics. Sixty-six patients from 27 unrelated families had mutations located in the transmembrane regions, and 29 patients from 10 unrelated families had mutations located in the C-terminal regions. Table 2 illustrates the clinical characteristics of the patient population. No significant differences were observed with regard to gender, percentage of proband, and age at baseline ECG recordings. The LQTS-affected individuals were more frequently diagnosed in patients with transmembrane mutations than in those with C-terminal mutations. The LQTS diagnostic score of Schwartz et al. (17) was significantly higher in patients with transmembrane mutations. The Q-Tend, Q-Tpeak, and Tpeak-end intervals, both uncorrected and corrected, were significantly greater in patients with transmembrane mutations than in those with C-terminal mutations (Figs. 1A and 1C). Although the frequency of TdP was no different, that of T-wave alternans was higher in patients with transmembrane mutations. Patients with transmembrane mutations had more frequent LQTS-related cardiac events (all cardiac events, syncope, and aborted cardiac arrest or unexpected sudden cardiac death) than did those with C-terminal mutations. More therapy with beta-blockers for LQTS was initiated in patients with transmembrane mutations.

Clinical course by mutation location. Figure 2A illustrates Kaplan-Meier cumulative cardiac event curves from birth through to age 50 years for a total of 95 patients with mutations located in the transmembrane regions ($n = 66$) and C-terminal regions ($n = 29$). 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. Most of the first cardiac events occurred before age 15 years in LQT1 patients with transmembrane mutations, whereas half of the LQT1 patients with C-terminal mutations had their first cardiac events after age 15 years. Multivariate Cox proportional hazards regression analysis revealed that patients with transmembrane mutations had a greater risk of first cardiac events, with a hazard ratio of 3.4 (95% confidence interval 1.4 to 8.2, $p = 0.006$). The corrected Q-Tend modulated the risk among patients with transmembrane mutations, with an 8% increase in risk per 10-ms increase in corrected Q-Tend, but had no effect on risk among patients with C-terminal mutations. Figures 2B and 2C illustrate Kaplan-

Table 1. *KCNQ1* Mutations by Location, Amino-Acid Coding, Type of Mutation, and Reported Functional Effects

Location and Coding	No. of Families	No. of Subjects	Position	Exon	Type	Functional Effect in Expression Studies
Transmembrane domains						
Pore region						
G306R	1	2	Pore	6	Missense	Dominant negative (18)
I313K*	1	1	Pore	7	Missense	
G314A*	1	1	Pore	7	Missense	
G314S	1	1	Pore	7	Missense	Dominant negative (19,20)
Non-pore region						
R174H	1	2	S2/S3	2	Missense	
F193L	1	4	S2/S3	3	Missense	Loss of function (21)
A226V*	1	3	S4	4	Missense	
R237P*	1	1	S4/S5	5	Missense	
R243C	2	4	S4/S5	5	Missense	Loss of function (22)
R243I*	1	2	S4/S5	5	Missense	
V254M	2	3	S4/S5	5	Missense	Dominant negative (18)
R259C	1	1	S4/S5	5	Missense	Loss of function (23)
G269S	3	6	S5	6	Missense	
S277L*	2	4	S5	6	Missense	
G325R	1	4	S6	7	Missense	
delF339	1	2	S6	7	Deletion	
A341V	4	19	S6	7	Missense	Loss of function (18)
A344sp	1	4	S6	7	Splice site	
A344E*	1	2	S6	7	Missense	
C-terminus region						
R451Q*	1	1	C-term.	10	Missense	
I517T*	1	3	C-term.	12	Missense	
A525V*	2	2	C-term.	12	Missense	
L572fs/20*	1	3	C-term.	14	Frameshift	
T587M	2	2	C-term.	15	Missense	Trafficking abnormality (24)
R591H	1	6	C-term.	15	Missense	
D611Y*	1	10	C-term.	16	Missense	
H637fs/28*	1	2	C-term.	16	Frameshift	

*Novel mutation.
del = deletion; sp = last unaffected amino acid before predicted splice mutaion; fs = first amino acid affected by a frameshift (number after fs is number of amino acids before termination); term. = terminus.

Meier cumulative cardiac event curves for 37 probands and 58 non-probands with transmembrane mutations and C-terminal mutations, respectively. The difference in phenotype severity based on mutation location persisted ($p = 0.007$) in the non-probands. There was no significant difference in the clinical course of the probands according to mutation site, although the number of probands was relatively small.

Exercise treadmill testing. Exercise treadmill testing was conducted in 33 patients with transmembrane mutations and 16 patients with C-terminal mutations. Table 3 illustrates the ECG measurements before and after exercise testing in both patient groups. The baseline RR and corrected repolarization parameters before exercise in both groups showed quite similar values to those evaluated in the total patients (Table 2), indicating that these patients who had exercise testing were representative of each group. The RR interval was similarly shortened with exercise between the two groups. Exercise produced a significant prolongation in the corrected Q-Tend interval, but not at all in corrected Q-Tpeak, resulting in a significant increase in corrected Tpeak-end in both groups. These changes were much more pronounced in patients with transmembrane mutations than in those with C-terminal mutations (Figs. 1B and 1D). Therefore, the increases in the corrected Q-Tend and corrected Tpeak-end intervals with exercise were significantly greater in patients with transmembrane mutations (Table 3).

When we re-analyzed the ECG measurements for the probands ($n = 26$) and for the non-probands ($n = 23$) separately, the corrected Q-Tend intervals both before and after exercise were longer in the probands than in the non-probands. However, the magnitude of differences in corrected Q-Tend for the two mutation groups persisted after this re-analysis both in the probands and non-probands (data not shown).

DISCUSSION

The major findings of the present study are: 1) LQT1 patients with mutations located in the transmembrane regions are at a higher risk of congenital LQTS-related cardiac events than are patients with C-terminal mutations; and 2) LQT1 patients with transmembrane mutations had a greater sensitivity to sympathetic stimulation than did patients with C-terminal mutations.

Table 2. Clinical Characteristics of the Study Population

	Transmembrane Domain (n = 66)	C-Terminal (n = 29)	p Value
Demographics			
Female gender (%)	41 (62%)	14 (48%)	NS
Proband (%)	29 (44%)	8 (28%)	NS
Age (yrs) at ECG (range)	32 ± 20 (6–83)	28 ± 17 (4–64)	NS
Diagnosis			
Diagnosed LQTS (Keating)	54 (82%)	7 (24%)	< 0.0001
Diagnosed LQTS (Schwartz >4)	43 (65%)	5 (17%)	< 0.0001
Schwartz score	4.4 ± 2.1	2.0 ± 1.5	< 0.0001
Baseline ECG measurements			
RR (ms)	910 ± 127	918 ± 131	NS
Q-T_{end} (ms)	472 ± 54	419 ± 46	< 0.0001
Q-T_{peak} (ms)	382 ± 46	349 ± 44	0.002
$T_{peak-end}$ (ms)	90 ± 20	71 ± 12	< 0.0001
Corrected Q-T_{end} (ms)	496 ± 46	439 ± 38	< 0.0001
Corrected Q-T_{peak} (ms)	402 ± 42	365 ± 39	0.0002
Corrected $T_{peak-end}$ (ms)	95 ± 19	74 ± 11	< 0.0001
Torsade de pointes (%)	10 (15%)	2 (7%)	NS
T-wave alternans (%)	10 (15%)	0	0.03
Cardiac events			
All cardiac events (%)	36 (55%)	6 (21%)	0.002
Age (yrs) at first events (range)	11 ± 8 (3–48)	13 ± 9 (2–25)	NS
Syncope (%)	36 (55%)	6 (21%)	0.002
Aborted cardiac arrest/SCD (%)	10 (15%)	0	0.03
Therapy			
Beta-blockers (%)	30 (45%)	6 (21%)	0.02
Pacemakers (%)	1 (2%)	0	NS
Sympathectomy (%)	0	0	NS
Defibrillator (%)	0	0	NS

Data are presented as the mean value ± SD or number (%) of subjects.
ECG = electrocardiography; LQTS = long QT syndrome; SCD = sudden cardiac death.

Mutation site-specific arrhythmic risk in LQT1 syndrome. Moss et al. (14) have recently reported that the greater risk of arrhythmia-related cardiac events in LQT2 patients with pore mutations of the *KCNH2* gene was consistent with the cellular electrophysiologic effects of the *KCNH2* mutations, with pore mutations showing a greater negative effect on the rapidly activating component of the delayed rectifier potassium current (I_{Kr}) than non-pore mutations. Although the cellular electrophysiologic effects of a small percentage of known *KCNQ1* mutations have been reported to be like those of *KCNH2* mutations, several in vitro electrophysiologic studies have reported missense mutations with dominant-negative effects on I_{Ks} in the transmembrane regions of the *KCNQ1* gene (18,19,25,26). However, Wang et al. (18) have suggested that the degree of I_{Ks} suppression by *KCNQ1* transmembrane mutations evaluated in the heterologous expression system did not correlate with severity in the clinical phenotype in LQT1 patients. There have been fewer reports on the cellular electrophysiologic effects of the C-terminal mutations of the *KCNQ1* gene. To the best of our knowledge, the electrophysiologic effects were examined in seven C-terminal mutations of the *KCNQ1* gene (R555C, R533W, R539W, Δ544, G589D, T587M, and G643S) by French, Finland, and Japanese groups (19,20,24,27–30). All seven C-terminal mutations, except for Δ544 and T587M, when co-expressed with *KCNE1*, could produce functional heteromultimeric channels and showed only a mild reduction of I_{Ks} due to a rightward voltage shift in the activation process and/or acceleration of the deactivation kinetics. Neyroud et al. (28) have reported a homozygous deletion-insertion mutation in the C-terminal region of the *KCNQ1* gene (Δ544), causing a severe phenotype—the Jervell and Lange-Nielsen syndrome. However, the heterozygotes of Δ544 clinically displayed mild or no QT prolongation, with no symptoms, mainly due to the lower dominant-negative effects of the Δ544 mutant. More recently, Larsen et al. (31) have described a severe form of Romano-Ward syndrome associated with compound heterozygosity for two C-terminal mutations (R518X and A525T) in the *KCNQ1* gene. Once again, none of the heterozygotes of the C-terminal mutations (R518X or A525T) had symptoms with minor or no QT prolongation. These previous reports on C-terminal mutations in the *KCNQ1* gene indicate a less severe phenotype in C-terminal mutations than that in transmembrane mutations in LQT1 syndrome, concordant with the findings in the present study.

It is noteworthy that most of the first cardiac events occurred before age 15 years in the LQT1 patients with transmembrane mutations, whereas half of the LQT1 patients with C-terminal mutations had their first cardiac events after age 15 years. This tendency holds up regardless

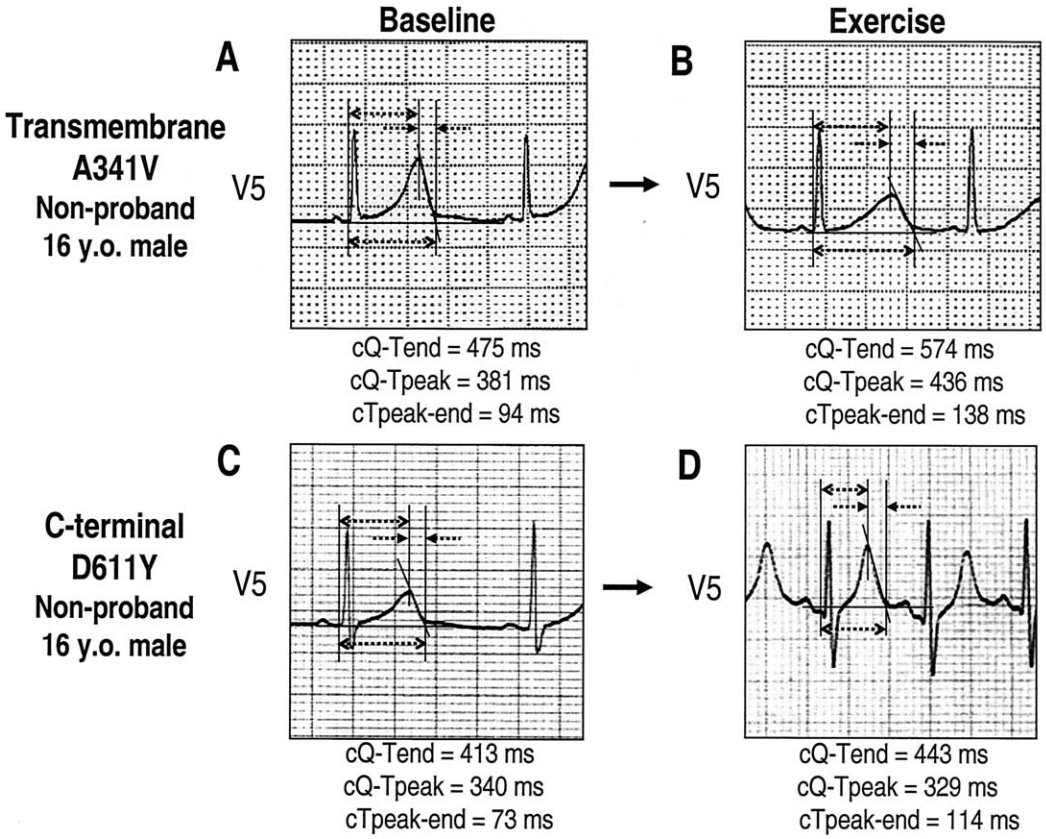

Figure 1. Electrocardiographic parameters in lead V_5 before and after exercise in LQT1 patients with mutations located in the transmembrane region (A341V, non-proband, 16-year-old male) **(A and B)** and in the C-terminal region (D611Y, non-proband, 16-year-old male) **(C and D)**. The baseline corrected Q-Tend (cQ-Tend), Q-Tpeak (cQ-Tpeak), and Tpeak-end (cTpeak-end) intervals were greater in the patient with a transmembrane mutation than in the patient with a C-terminal mutation **(A and C)**. Exercise produced more prominent increases in the cQ-Tend and cTpeak-end in the patient with a transmembrane mutation than in the patient with a C-terminal mutation **(B and D)**.

of whether the patient was a proband. Moreover, hypokalemia, which is known to prolong repolarization, unmasked the LQTS proband in a patient with C-terminal mutation in the present study. These findings suggest that careful follow-up is needed in LQT1 patients with C-terminal mutations, despite their less severe phenotype, by limiting exposure of these patients to QT prolonging conditions.

Although the difference in the clinical course based on

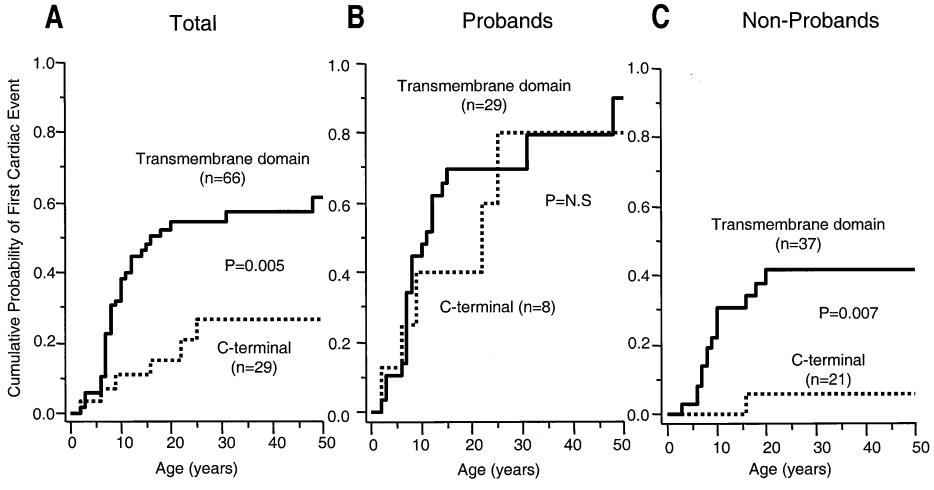

Figure 2. (A) Kaplan-Meier cumulative cardiac event curves from birth through to age 50 years for a total of 95 patients with *KCNQ1* mutations located in the transmembrane regions (n = 66) and C-terminal regions (n = 29). The difference in the clinical course by mutation location was significant (log-rank, p = 0.005), with a greater risk of first cardiac events in patients with transmembrane mutations than in those with C-terminal mutations. Kaplan-Meier cumulative cardiac event curves for 37 probands **(B)** and 58 non-probands **(C)** with transmembrane mutations and C-terminal mutations.

Table 3. Electrocardiographic Measurements Before and After Exercise Testing

	Transmembrane Domain (n = 33)	C-Terminal (n = 16)	p Value
Demographics			
Female gender (%)	18 (55%)	7 (44%)	NS
Proband (%)	22 (67%)	4 (25%)	0.006
Age (yrs) at ECG (range)	26 ± 16 (9–72)	27 ± 13 (6–45)	NS
ECG measurements before exercise			
RR (ms)	862 ± 128	850 ± 106	NS
Corrected Q-T_{end} (ms)	494 ± 40	435 ± 28	<0.0001
Corrected Q-T_{peak} (ms)	403 ± 37	359 ± 26	<0.0001
Corrected $T_{peak-end}$ (ms)	91 ± 12	76 ± 6	NS
ECG measurements after exercise			
RR (ms)	514 ± 87*	503 ± 74*	NS
Corrected Q-T_{end} (ms)	571 ± 45*	470 ± 39*	<0.0001
Corrected Q-T_{peak} (ms)	420 ± 38	365 ± 51	<0.0001
Corrected $T_{peak-end}$ (ms)	151 ± 34*	106 ± 17*	<0.0001
Changes in ECG measurements with exercise			
RR (ms)	349 ± 119	348 ± 137	NS
Corrected Q-T_{end} (ms)	77 ± 32	35 ± 17	<0.0001
Corrected Q-T_{peak} (ms)	17 ± 36	5 ± 30	NS
Corrected $T_{peak-end}$ (ms)	60 ± 34	30 ± 17	0.002

*$p < 0.005$ vs. before exercise. Data are presented as the mean ± SD value or number (%) of subjects. The electrocardiographic (ECG) measurements after exercise were obtained within 2 min after stopping exercise.

mutation location was obvious in the non-probands, no significant difference was observed in the clinical course according to mutation location in the probands. This is not surprising, because probands are usually brought to medical attention by their first cardiac events, especially those with a less prolonged QT interval. There indeed may be no difference in cardiac events based on mutation location for probands. This is because other modifier genes may be contributing to the more severe phenotype, which leads to the individual receiving a label of "proband."

Zareba et al. (32) recently reported on 294 LQT1 patients in the International Long-QT Syndrome Registry and analyzed the QTc interval and cardiac event rates by mutation location. In contrast to the present study, they found no significant differences in QTc or risk of cardiac events when the patients were separated into those with transmembrane mutations and those with C-terminal mutations. However, only six transmembrane mutations were overlapped between the two studies (out of 31 transmembrane mutations in the Registry and 19 transmembrane mutations in this study). Moreover, no overlap was observed in the C-terminal mutations between the two studies (out of 11 C-terminal mutations in the Registry and 8 C-terminal mutations in this study). In transmembrane regions, the S4 to S5 loop (amino acid residues 221 through 300) and the S6 segment (amino acid residues 325 through 354) are known to be important for voltage-dependent I_{Ks} function (22); thus, a more severe phenotype is expected in mutations located in the S4 to S5 loop and the S6 segment than in the S2 to S3 loop (amino acid residues 148 through 220). The transmembrane mutations in the non-pore region in the present study were located in the S4 to S5 loop and the S6 segment, except for two mutations found in the S2 to S3 loop. This may affect the result that cardiac event rates were higher in patients with transmembrane mutations in the present study than those in the Registry. Interestingly, when the patients with transmembrane mutations in the present study were separated into those with pre-pore mutations and those with pore mutations, according to the definition by Zareba et al. (32), the patients with pore mutations had a longer corrected Q-Tend than did those with pre-pore mutations (data not shown). Overall, our data present evidence that mutation site-specific differences in arrhythmic risk exist, in contrast to findings previously reported from the Long-QT Syndrome Registry. Therefore, a larger patient population per mutation and a greater spectrum of *KCNQ1* mutations by corroboration with other investigators are clearly needed to make a definitive conclusion about the mutation site-specific differences in arrhythmic risk in LQT1 syndrome.

Greater sensitivity to sympathetic stimulation in transmembrane mutations of the *KCNQ1* gene. The LQT1 syndrome is reported both clinically and experimentally to be the most sensitive to sympathetic stimulation among the seven forms of LQTS (9–12). Sympathetic stimulation is known to increase the net outward repolarizing current due to a larger increase in outward currents, including Ca^{2+}-activated I_{Ks} and Ca^{2+}-activated chloride current ($I_{Cl(Ca)}$), compared with the inward Na^+/Ca^{2+} exchange current (I_{Na-Ca}), resulting in an abbreviation of action potential duration (APD) and QT interval under normal conditions. A defect in I_{Ks} in LQT1 syndrome could account for the failure of sympathetic stimulation to abbreviate the QT interval and APD, especially in the mid-myocardial regions, resulting in a paradoxical QT prolongation and an increase in transmural dispersion of repolarization reflecting an

increase in the Tpeak-end interval under sympathetic stimulation (12). In fact, recent clinical studies have demonstrated that sympathetic stimulation with epinephrine infusion or exercise produced a more significant increase in Q-Tend and Tpeak-end intervals in LQT1 compared with LQT2 patients (33–35). More recently, the Q-Tend and Tpeak-end intervals both before and after epinephrine and prolongation of Q-Tend with epinephrine were reported to be greater in symptomatic than asymptomatic patients with LQT1 syndrome (9). In the present study, LQT1 patients with transmembrane mutations, who had more frequent LQTS-related cardiac events than those with C-terminal mutations, showed greater baseline Q-Tend and Tpeak-end intervals and a greater increase in both Q-Tend and Tpeak-end intervals with exercise than those with C-terminal mutations. The data in the present study may indicate a stricter exercise limit and a more aggressive use of beta-blockers in LQT1 patients with transmembrane mutations, but once again, we need to evaluate a larger patient population to make a definitive recommendation.

With regard to the sympathetic regulation of I_{Ks}, Marx et al. (36) have suggested that beta-adrenergic modulation of I_{Ks} required targeting of cyclic adenosine monophosphate (cAMP)-dependent protein kinase and protein phosphatase-1 to *KCNQ1* through the targeting protein yotiao. The binding of protein kinase and protein phosphatase-1 to the *KCNQ1* channel through yotiao is mediated by leucine zipper (LZ) motifs located in the C-terminal of the *KCNQ1* gene (amino acid residues 588 to 616). They also reported that the G589D mutant channel located in the LZ motifs of the C-terminus prevented cAMP-dependent regulation of I_{Ks}, and thus may not respond to beta-adrenergic stimulation, resulting in a defect of APD shortening and further prolonging of the APD. The G589D mutation was not included among the C-terminal mutations of the present study. Two C-terminal mutations, R591H and D611Y, located in the LZ motifs were included in the present study. However, the prolongation of the QTc interval was mild in patients with the two mutations (Fig. 1). Further clinical evaluation will be required to conclude the role of LZ motifs in the C-terminus on sympathetic modulation of the I_{Ks} channel.

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