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

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

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

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

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

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

Moss et al¹¹ extensively examined the relationships between the site of mutation and clinical phenotype in approxi-

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

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

In Japanese LQT2 patients, mutation site dependency is unclear, although this has been reported in Caucasian patients. Therefore, in the present study we aimed to compare the genotype and phenotype relationship, according to the classification adopted by Moss et al¹¹ in 118 Japanese LQT2 patients who were genetically identified in the 3 genetic centers in Japan and had no other mutations in LQTS-related genes (except LQT4 and 8).

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

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

*Novel mutation.

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

Table 2 Clinical Characteristics of Pore and Non-Pore Mutations

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

Data are mean value ± SD or number (%) of subjects.

ECG, electrocardiography; SCD, sudden cardiac death.

Methods

Study Population

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

Genetic Analysis and Characterization

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

analysis were subsequently sequenced by the dideoxynucleotide chain termination method with fluorescent dideoxynucleotides in an ABI 3130 genetic analyzer (PE Applied Biosystems).

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

Clinical Characterization

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

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

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

Statistical Analyses

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

Results

Genetic Characteristics

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

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

Clinical Characteristics

Table 2 is a comparison of the clinical characteristics of the 56 patients with pore mutations and the 62 patients with non-pore mutations. There were no significant differences between the 2 groups regarding gender, the percentage of probands and the age at baseline ECG recording. Diagnostic LQTS scores of Schwartz et al³⁰ were noticeably greater in the pore group. RR and QT_{peak} intervals were comparable; however, corrected QT_{end} and T_{peak–end} intervals were much longer in the pore than in the non-pore group. Although the incidence of TdP and T-wave alternans did not differ between groups, notched T waves were more frequently seen in the pore group ($p=0.007$ vs non-pore group). The incidence of cardiac events and the introduction of β -blocker therapy were not statistically different between the 2 groups.

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

Clinical Course by Mutation Location

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

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

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

Data are mean value ± SD or number (%) of subjects.

Abbreviations see in Table 2.

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

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

Discussion

This study demonstrates that the clinical features of 118 Japanese LQT2 patients who had 62 different *KCNH2* mutations correlated with the mutation sites, but only in non-probands. In probands, there was no significant relationship between mutation site and prognosis. Moss et al¹¹ reported approximately 179 LQT2 patients based on 44 different *KCNH2* mutations and those patients with pore mutations had significantly ($p<0.0001$) higher frequency of LQTS-related cardiac events and longer QTc intervals than those with non-pore mutations. In contrast to their results, in the present study the mutation-dependent difference in prognosis was relatively small, though significant ($p=0.049$), when analyzed in the total patient cohort (Fig 1A). Indeed, the beneficial outcome of the non-pore patients stemmed from their family members (Fig 1C), and probands showed virtually similar prognosis to that of pore mutation carriers. Although Moss et al did not report separate sub-analysis of

probands and family members, the percentage of family members in their non-pore group was significantly larger than that of the pore group (84% vs 57%, $p<0.001$). The very good prognosis of the non-pore mutation group in their study may have reflected that large number of family members.

The character of the mutation per se may be important as another reason for the variance between these 2 studies, as both had a similar number of LQT2 patients. Compared with the study by Moss et al¹¹ the type of mutation in the present study was quite different: in our non-pore group, there were significantly more complex mutations, such as nonsense or frameshift, that caused the truncation of channel proteins (15/35, 43%) than in the report of Moss et al (4/30, 13%). For example, nonsense-mediated mRNA decay (NMD) has recently been reported to play an important role in reducing dominant negative suppression effects.³¹ Premature termination codon caused by either a deletion or insertion mutation would also cause NMD and thereby attenuate the severity of cardiac phenotypes. This different nature of the mutations may cause the apparently different prognosis of the non-pore mutation groups in each study.

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

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

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

Study Limitations

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

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

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