Long QT syndrome and associated gene mutation carriers in Japanese children: results from ECG screening examinations

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ABSTRACT

LQTS (long QT syndrome) is caused by mutations in cardiac ion channel genes; however, the prevalence of LQTS in the general population is not well known. In the present study, we prospectively estimated the prevalence of LQTS and analysed the associated mutation carriers in Japanese children. ECGs were recorded from 7961 Japanese school children (4044 males; mean age, 9.9 ± 3.0 years). ECGs were examined again for children who had prolonged QTc (corrected QT) intervals in the initial ECGs, and their QT intervals were measured manually. An LQTS score was determined according to Schwartz’s criteria, and ion channel genes were analysed. In vitro characterization of the identified mutants was performed by heterologous expression experiments. Three subjects were assigned to a high probability of LQTS (3.5 ≤ LQTS score), and eight subjects to an intermediate probability (1.0 < LQTS score ≤ 3.0). Genetic analysis of these 11 subjects identified three KCNH2 mutations (M124T, 547–553 del GGCGGCG and 2311–2332 del/ins TC). In contrast, no mutations were identified in the 15 subjects with a low probability of LQTS. Electrophysiological studies showed that both the M124T and the 547–553 del GGCGGCG KCNH2 did not suppress the wild-type KCNH2 channel in a dominant-negative manner. These results demonstrate that, in a random sample of healthy Japanese children, the prevalence of a high probability of LQTS is 0.038% (three in 7961), and that LQTS mutation carriers can be identified in at least 0.038% (one in 2653). Furthermore, large-scale genetic studies will be needed to clarify the real prevalence of LQTS by gene-carrier status, as it may have been underestimated in the present study.

INTRODUCTION

LQTS (long QT syndrome) is an inherited disorder characterized by delayed cardiac repolarization and a possibility of lethal ventricular tachyarrhythmias, resulting in fainting spells and sudden death [1]. Mutations in various genes encoding cardiac ion channels and membrane adaptor proteins are known to cause this syndrome [2], and many LQTS mutations have been identified [3–6]. A previous study [7] has shown that approx. 36% of patients...

Key words: cardiovascular screening, ion channel, long QT syndrome, molecular genetics.

Abbreviations: CHO, Chinese-hamster ovary; CNBD, cyclic nucleotide-binding domain; CRBBB, complete right bundle branch block; HR, heart rate; LQTS, long QT syndrome; QTc, corrected QT; SSCP, single-strand conformation polymorphism; TEV, two-electrode voltage-clamp; WT, wild-type.

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with untreated genetically defined LQTS had cardiac events, such as syncope, cardiac arrest and sudden death, before the age of 40 years. That study also established that the association between genetic locus, gender and QTc (corrected QT) interval could be used to define three levels of risk of sudden death: high (> 50 %); intermediate (30–49 %) and low (< 30 %), and proposed that prophylactic treatment was warranted in patients at high and intermediate risk. In addition, a recent study [8] has demonstrated age-specific risk factors for aborted cardiac arrest or sudden cardiac death in children with LQTS. It is, therefore, important to make an early diagnosis of patients with LQTS, both clinically and genetically, and to perform a risk stratification for their management.

The prevalence of LQTS in the general population is not well known. Most investigators have reported that one in every 10 000 people are affected by Romano–Ward syndrome, an autosomal-dominant form of LQTS [9–12]; however, these estimates were not based on actual data. To date, only two studies have determined the prevalence of LQTS or the frequency of cardiac channel mutations [13,14]. One of these studies has shown the prevalence of LQTS to be one in 1164 [13]; the other showed that the frequency of LQTS mutation carriers was at least one in 2500 [14]. In the present study, we took advantage of a cardiovascular screening programme for Japanese school children. A total of 7961 Japanese school children were screened for LQTS and, based on preliminary evidence from ECGs indicating the possible presence of LQTS, a subgroup were evaluated further. For example, we also performed genetic analysis on this subgroup of LQTS subjects for the presence of the major LQTS genes, such as KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2, and estimated the frequency of LQTS mutation carriers.

MATERIALS AND METHODS

Cardiovascular screening

In Kanazawa city, which is representative of a major urban city in Japan, there is a cardiovascular ECG screening programme aimed at all school children in first, seventh and tenth grades to identify congenital heart problems. In accordance with this programme, ECGs were recorded in 7961 school children (4161 and 3800 in first and seventh grade respectively), and the QTc interval was automatically determined by Bazett’s formula (QTc interval = QT/RR\(^{1/2}\)) as an initial cardiovascular screening. School children were also checked, during this initial screening, for clinical and familial findings associated with LQTS (i.e. syncope, congenital deafness and unexplained sudden cardiac death among family members). ECGs were recorded mainly using an ECP-7641 machine (Fukuda Denshi) at a paper speed of 25 mm/s, which automatically determined the average of the QT intervals measured from all cardiac beats during 8 s on four leads. Because the QTc interval of males is generally shorter than that of females, males were screened against different criteria. Children having a prolonged QTc interval (> 450 ms for males and > 460 ms for females) and/or having abnormal clinical and familial findings were referred to the Cardiovascular Checkup Committee for School Pupils of the Kanazawa Medical Association for a re-check of their ECGs and their clinical and familial findings. This committee participated in the design of the present study and conducted secondary cardiovascular screening (see Acknowledgements section).

All of these referred subjects had standard 12-lead ECGs recorded at Kanazawa University Hospital at a paper speed of 25 mm/s and at an amplification of 0.1 mV/mm. They were also asked about clinical and family history again in order to assign an LQTS score [14–16]. The ECG tracings were enlarged to twice the normal size and their measurements were carried out by two investigators, who were blinded to the subjects’ genetic status, clinical history and automated QTc interval values prior to making the manual measurements. QT interval was measured manually, and was taken as the time interval between QRS onset and the point at which the isoelectric line intersected a tangential line drawn at the maximal downslope of the positive T wave. QT interval measurements were the means of three consecutive beats at leads II, aVF and V3–V5. We took the longest QT interval from one of these leads. The LQTS score was determined according to the 1993–2006 LQTS Diagnostic Criteria [14–16]. We divided the subjects into three categories: (i) those with a score of 1 (defined as a low probability of LQTS), (ii) those with a score of > 1 to 3.0 (an intermediate probability), and (iii) those with a score of >= 3.5 (a high probability).

DNA isolation and mutation analysis

To determine the frequency of the gene mutation carriers in the children, we performed genetic analysis, after obtaining written informed consent from their guardians in accordance with the guidelines of the Bioethical Committee on Medical Research. We screened for mutations by PCR–SSCP (single-strand conformation polymorphism) analysis, as described previously [4], in all exons of KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2. Normal and aberrant SSCP products were isolated and sequenced by ABI PRISM 310 (PerkinElmer). With respect to these mutations, we also determined whether they were present in 200 healthy controls. For the detection of deletion or insertion mutations, we performed TA cloning using the TOPO TA cloning kit (Invitrogen), and sequenced normal and mutant alleles separately.

Plasmid constructs and electrophysiology

KCNH2 cDNA in the mammalian expression vector pSI (Promega) was kindly provided by Dr Sabina Kupershmidt (Vanderbilt University School of Medicine,
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Nashville, TN, U.S.A.). Both the M124T and the 547–553 del GCCGCGC KCNH2 cDNAs were constructed using an overlap extension strategy.

CHO-K1 cells (where CHO is Chinese-hamster ovary; Riken BRC Cell Bank) were cultured and transiently transfected with WT (wild-type) KCNH2 (1 μg) alone, WT KCNH2 (1 μg) plus mutant KCNH2 (1 μg) and mutant KCNH2 (1 μg) alone, using FuGENE 6™ transfection reagent (Roche). We did not use Xenopus oocytes with the TEV (two-electrode voltage-clamp) technique because the oocyte provides a non-ideal space clamp, and fast kinetic parameters that are derived from TEV measurements are not always comparable with the same parameters measured with the patch-clamp technique [17]. Membrane currents were studied essentially as described previously [18]. Data acquisition and analysis were performed using a DIGIDATA 1321 A/D converter and pCLAMP8.2 software (Axon Instruments). Data analysis was carried out using Clampfit (version 8.2; Axon Instruments).

Data analysis

Values are means ± S.E.M. Differences among these values were evaluated using ANOVA and unpaired Student’s t tests, where appropriate. A Fisher’s exact test was used to assess the hypothesis of independence between categorical variables in 2 × 2 tables. P < 0.05 was considered to be significant.

RESULTS

Cardiovascular screening

From April 2004 to March 2005, a total of 7961 junior high-school students and elementary school children (4044 males; mean age, 9.9 ± 3.0 years) underwent ECG testing and were checked for clinical and familial findings associated with LQTS. Eventually, 73 of these subjects were selected for further study, due to abnormal QTc intervals and/or abnormal clinical and familial findings. None had a history of syncope, but two had a family history of unexplained sudden cardiac death below 30 years of age. At the initial cardiovascular screening, ECGs of these 73 subjects were categorized based on the LQTS Diagnostic Criteria (Schwartz score).

Table 1 Characteristics of the 73 school children referred to Kanazawa University Hospital following initial screening

<table>
<thead>
<tr>
<th></th>
<th>First grade (n = 41)</th>
<th>Seventh grade (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Cases (n)</td>
<td>26</td>
<td>15</td>
</tr>
<tr>
<td>Initial screening</td>
<td></td>
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<tr>
<td>HR (beats/min)</td>
<td>98 ± 2</td>
<td>103 ± 3</td>
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<tr>
<td>QTc interval (ms)</td>
<td>458 ± 1</td>
<td>467 ± 2</td>
</tr>
<tr>
<td>University Hospital</td>
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<tr>
<td>HR (beats/min)</td>
<td>78 ± 2*</td>
<td>85 ± 2*</td>
</tr>
<tr>
<td>QTc interval (ms)</td>
<td>425 ± 5*</td>
<td>431 ± 6*</td>
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</tbody>
</table>

Figure 1 Cardiovascular screening for male (A) and female (B) school children

N, numbers of children who were categorized based on the LQTS Diagnostic Criteria (Schwartz score).

average QTc interval determined manually under the appropriate HR was significantly shorter than that determined under rapid HR during the initial cardiovascular screening (P < 0.05; Table 1).

For males, 45 out of 4044 children were identified for further investigation based on the initial cardiovascular screening at school (Figure 1A). Of these, two were excluded because of CRBBB (complete right bundle branch block), whereas the ECGs of 43 others were re-examined. Eventually, one subject with a high probability...
Table 2  Clinical characteristics of 11 children with high and intermediate probabilities of LQTS

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Gender</th>
<th>PR (ms)</th>
<th>QRS (ms)</th>
<th>QT (ms)</th>
<th>RR (ms)</th>
<th>QTc (ms)</th>
<th>Tpeak to end (ms)</th>
<th>Tpe</th>
<th>LQTS score</th>
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<td>389</td>
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<tr>
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<td>84</td>
<td>500</td>
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<td>490</td>
<td>147</td>
<td>0.29</td>
<td>3</td>
<td>Intermediate</td>
</tr>
</tbody>
</table>

Figure 2  ECGs of three subjects with a high probability of LQTS

(A) Case 1 was a 7-year-old boy with an HR of 81 beats/min and a QTc interval of 487 ms, which are the means of three consecutive beats at lead V3. Notched T waves were present at leads V3 to V4. His aunt (see Figure 3B, II-2) also had prolonged QTc intervals of 529 ms and the presence of notched T waves at leads V3–V5. The boy’s final LQTS score was 4 (QTc interval ≥480 ms and family members with definite LQTS). (B) Case 2 was a 7-year-old girl with an HR of 74 beats/min and a QTc interval of 489 ms, which are the means of three consecutive beats at lead II. The notched T waves were identified at leads II, aVF and V3–V5. Her aunt (see Figure 4B, II-2) also had a prolonged QTc interval of 510 ms and the presence of notched T waves at leads V2–V5. The LQTS score was 5 (QTc interval ≥480 ms and notched T wave in three leads, and family members with definite LQTS). (C) Case 3 was a 13-year-old girl with an HR of 61 beats/min and a QTc interval of 534 ms, which are the means of three consecutive beats at lead II. The notched T waves were observed at leads II, aVF and V4–V6. Her LQTS score was 4 (QTc interval ≥480 ms and notched T wave in three leads).

of LQTS (Case 1 in Table 2 and Figure 2A), four with an intermediate probability of LQTS (Table 2), and 38 with a low probability of LQTS were identified (Figure 1A). For females, 28 out of 3917 subjects were identified for further investigation based on the initial cardiovascular screening at school (Figure 1B), and the ECGs of all 28 female subjects were re-examined. Eventually, two subjects with a high probability of LQTS (Case 2 in Table 2 and Figure 2B; and Case 3 in Table 2 and Figure 2C), four with an intermediate probability of LQTS (Table 2) and 22 with a low probability of LQTS were identified. Thus, out of 7961 school children, there were three subjects (0.38 in 1000) with a high probability and eight (1.0 in 1000) with an intermediate probability (Table 2).

Molecular genetic analyses and clinical characteristics of the genotyped subjects

From these 73 subjects (45 boys and 28 girls) identified by the initial screening, we obtained 26 blood samples (three subjects with a high probability, eight with an intermediate probability and 15 with a low probability...
of LQTS) and screened them for mutations in the five different ion channel genes. The remaining 47 subjects, all with a low probability, did not undergo genetic testing, either because their LQTS scores were zero or because they refused consent. Genetic analyses revealed three mutations in the \( \text{KCNH2} \) gene (Table 3: M124T in the N-terminus (Figure 3A), 2311–2332 del/ins TC in the CNBD (cyclic nucleotide-binding domain) (Figure 4A) and 547–553 del GGGCGCG in the N-terminus (Figure 5B). All three of the identified mutations were absent in the 200 healthy control subjects. Although we have reported previously on the point mutation M124T in 27- and 58-year-old females [19], the other frame-shift mutations are novel findings in the present study.

The M124T mutation was identified from Case 1, who was diagnosed as having a high probability of LQTS (QTc interval \( \geq 480 \) ms and family members with definite LQTS) (Tables 2 and 3, Figures 2A and 3). In this proband, five members of his family also carried this same mutation, of which four had prolonged QTc intervals (Figure 3B). Moreover, two of them experienced TdP (torsade de pointes), accompanied by faintness, after they had started taking probucol [19]. The 2311–2332 del/ins TC mutation was identified in Case 2, who was diagnosed as having a high probability of LQTS (Figure 4B). The 547–553 del GGGCGCG mutation was identified from another 7-year-old boy (Case 4 in Tables 2 and 3, and Figure 5), who was diagnosed as having an intermediate probability of LQTS (QTc interval 460–479 ms and notched T wave in three leads). This mutation was only found in the proband and is thought to be \textit{de novo} (Figure 5B). A further familial study of each detected mutation revealed that there was no other first or seventh grade school children with the same gene mutations identified in each proband. In contrast with these high- and intermediate-probability cases, no mutation was identified in the 15 subjects with a low probability of LQTS. There was a statistically significant difference in the frequency of the LQTS mutation carriers between the subjects with a high or intermediate probability and the those with a low probability (\( P = 0.045 \), as determined by a Fisher’s exact test).

Genetic analysis also revealed two polymorphisms in the \( \text{KCNQ1} \) gene [P448R in one subject with a low probability and G643S in two cases (Cases 4 and 6) with an intermediate probability of LQTS]. We also found a polymorphism in the \( \text{SCN5A} \) gene [H558R in a subject (Case 10) with an intermediate probability of LQTS] and one polymorphism in the \( \text{KCNE1} \) gene [S38G in three cases (Cases 7, 8 and 9) with an intermediate probability and eight with a low probability of LQTS] (Table 3).

For Case 3, with a high probability of LQTS, we could not detect any genetic anomaly by PCR–SSCP analysis. All exons and exon–intron boundaries of \( \text{KCNQ1} \), \( \text{KCNH2} \), \( \text{SCN5A} \), \( \text{KCNE1} \) and \( \text{KCNE2} \) were analysed.
Electrophysiological findings of the detected mutations

We analysed the two KCNH2 mutations in cultured mammalian cells [18,20,21]. CHO-K1 cells transfected with 1.0 μg of WT KCNH2 cDNA expressed a slowly activating outward current by step depolarizations (Figures 6A and 7A). In contrast, the amplitude of the outward tail current through the M124T channels, was less than half that through the WT KCNH2 channels (Figure 6A). CHO-K1 cells transfected with 1.0 μg of 547–553 del GGCGGCG KCNH2 cDNA did not express functional channels at all (Figure 7A). When equal amounts of 1.0 μg of WT KCNH2 cDNA and 1.0 μg of M124T or 547–553 del GGCGGCG KCNH2 cDNA (i.e. 2.0 μg of cDNA in total) were transfected into CHO-K1 cells, the evoked currents were approximately the same as those in CHO-K1 cells transfected with 1.0 μg of WT KCNH2 cDNA alone (Figures 6A and 7A).

The current–voltage relationships for activating peak currents and tail currents were recorded during depolarizing pulses (Figures 6B and 6C, and Figures 7B and 7C). The mean amplitude of the tail currents measured at −50 mV, after a depolarizing test pulse of +40 mV, was 23.8 ± 2.1 pA/pF (n = 13) for the M124T KCNH2 channels, which was significantly smaller than 47.3 ± 6.5 pA/pF (n = 13) for the WT KCNH2 channels (Figure 6C). The mean amplitude for the M124T + WT KCNH2 channels were 46.4 ± 7.1 pA/pF (n = 13), which were comparable with that of the WT KCNH2 channels. The mean amplitude of the tail currents was 1.0 ± 0.1 pA/pF (n = 5) for the 547–553 del GGCGGCG KCNH2 channels, 21.4 ± 3.7 pA/pF (n = 11) for the WT KCNH2 channels, and 21.5 ± 4.7 pA/pF (n = 13) for the 547–553 del GGCGGCG + WT KCNH2 channels (Figure 7C). These results suggest that both mutant KCNH2 channels did not have dominant-negative effects.
Figure 6  Functional characterization of M124T KCNH2 in CHO-K1 cells
(A) Representative expressed currents in CHO-K1 cells transfected with 1 μg of WT KCNH2 alone (upper trace), 1 μg of M124T KCNH2 alone (middle trace) and 1 μg of WT KCNH2 + 1 μg of M124T KCNH2 (lower trace). Depolarizing pulses were applied from a holding potential of −80 mV to various potentials between −60 mV and +60 mV in 10 mV increments for 2 s, followed by a hyperpolarizing pulse to −50 mV for 2 s. (B) and (C) Current–voltage relationships for peak (B) and tail (C) currents in CHO-K1 cells transfected with WT KCNH2 alone, M124T KCNH2 alone and WT KCNH2 + M124T KCNH2. ∗P < 0.05 compared with WT KCNH2. The n values indicate the number of CHO-K1 cells examined.

Figure 7  Functional characterization of 547–553 del GGCGGCG KCNH2 in CHO-K1 cells
(A) Representative expressed currents in CHO-K1 cells transfected with 1 μg of WT KCNH2 alone (upper trace), 1 μg of 547–553 del KCNH2 alone (middle trace) and 1 μg of WT KCNH2 + 1 μg of 547–553 del GGCGGCG KCNH2 (lower trace). (B) and (C) Current–voltage relationships for peak (B) and tail (C) currents in CHO-K1 cells transfected with WT KCNH2 alone, 547–553 del GGCGGCG KCNH2 alone and WT KCNH2 + 547–553 del GGCGGCG KCNH2. ∗P < 0.05 and †P < 0.01 compared with WT KCNH2.


**DISCUSSION**

**Prevalence of LQTS estimated from cardiovascular screening**

The present study provides evidence that three out of 7961 healthy Japanese children have a high probability of LQTS. Previous reviews have estimated the prevalence of Romano–Ward syndrome, the autosomal-dominant form of LQTS, as one in 10000 [9–12]. One review also indicated that 40% of patients with LQTS were asymptomatic at diagnosis [9]. In the present study, we evaluated the ECGs of a large number of school children and diagnosed those patients with LQTS. It should be noted that none of the children had any past history of cardiac events at diagnosis.

A previous study has determined the prevalence of LQTS in 4655 Japanese children by measuring three consecutive QT and RR intervals [13]. That study showed the prevalence of non-familial LQTS to be four in 4655 (i.e. one in 1164), which is slightly higher than the prevalence of LQTS found in the present study. This difference may arise from the different diagnostic criteria for LQTS used in the two studies. We diagnosed LQTS on the basis of the 1993–2006 LQTS Diagnostic Criteria described by Schwartz and co-workers [14–16], whereas the previous study [13] based the final diagnosis of LQTS in accordance with different criteria. A recent prospective study, which examined the ECGs of infants at 3–4 weeks of age, demonstrated that the frequency of LQTS gene mutation carriers was at least one in 2500 [14], which is almost the same as that found in the present study. Consequently, it is possible that the prevalence of LQTS is higher than supposed previously.

In the present study, we identified gene mutations in three out of 11 subjects with a high or intermediate probability of LQTS. Two of the three mutations were identified from subjects with a high probability of LQTS and one from a subject with intermediate probability. In contrast, no mutations were identified in the 15 subjects with a low probability of LQTS, which suggests that this is less frequently associated with gene mutations.

**Clinical characteristics and risk stratification of the children with LQTS in the present study**

We clinically diagnosed three subjects (Cases 1–3) as having a high probability of LQTS (Figure 2 and Table 2), and identified three mutations in the KCNH2 gene (M124T in Case 1 (Figure 3), 2311–2332 del/ins TC in Case 2 (Figure 4), and 547–553 del GGC
text continues...
the clinical findings showing moderate QT prolongation and no incidence of cardiac events in the absence of environmental stressor even with gene mutations [29].

Study limitations
There remain several limitations of the present study. First, the QT interval was determined using an automatic method in the initial cardiovascular screening of 7961 school children. However, it has been reported recently that manual and automated measurements of QT intervals generated similar results in healthy volunteers, and that automated techniques have a clear utility in extensive QT studies in healthy volunteers [30]. Secondly, the PCR–SSCP method is not completely sensitive enough for mutational screening. Therefore we performed a direct DNA sequence analysis on one patient with a high probability and negative for the SSCP method for all exons and exon–intron boundaries of the major LQTS genes. Thirdly, we did not analyse the 2311–2332 del/ins TC KCNQ2 because we expected this del/ins KCNQ2 to have similar electrophysiological properties to the 547–553 del GGCAGCG KCNH2, taking into account a previous study which showed that the protein encoded by the deletions of KCNQ2 did not form functional channels and did not have a dominant-negative effect on WT KCNH2 function [28]. Finally, the true prevalence of LQTS by gene carrier status could be an underestimate, because we did not perform a genetic analysis in all 7961 subjects. LQTS may cause sudden infant death and, therefore, it is quite probable that some candidates had died before they reached the age selected for screening in the present study.

Conclusions
The present study demonstrates that both the prevalence of LQTS and the incidence of LQTS mutation carriers is higher than previous estimates [9–12], although the prevalence of LQTS by gene carrier status has not yet been established. We suggest genetic analysis is performed on all subjects who are diagnosed as having an intermediate risk of LQTS, as well as those with a high probability, even if they are asymptomatic.

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