Genetic Characteristics of Children and Adolescents with Long QT Syndrome Diagnosed by School-Based Electrocardiographic Screening Programs

Yoshinaga; Genetic characteristics of screened LQTS patients

Masao Yoshinaga, MD, PhD; Yu Kucho, MD; Jav Sarantuya, MD, PhD; Yumiko Ninomiya, MD; Hitoshi Horigome, MD, PhD; Hiroya Ushinohama, MD, PhD; Wataru Shimizu, MD, PhD, Minoru Horie, MD, PhD.

Department of Pediatrics (M.Y., Y.K., Y.N.), National Hospital Organization Kagoshima Medical Center, Kagoshima, Japan; Department of Molecular Biology and Genetics (J.S.), School of Bio-medicine, Health Sciences University of Mongolia, Ulaanbaatar, Mongolia Department of Child Health (H.H.), Faculty of Medicine, University of Tsukuba, Tsukuba, Japan; Department of Cardiology (H.U), Fukuoka Children's Hospital and Medical Center for Infectious Diseases, Fukuoka, Japan; Division of Arrhythmia and Electrophysiology (W.S.), Department of Cardiovascular Medicine (MH), National Cerebral and Cardiovascular Center, Suita, Japan; Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu, Japan.

Address for correspondence: Masao Yoshinaga, MD, PhD, HAHA, Department of Pediatrics, National Hospital Organization Kagoshima Medical Center, 8-1 Shiroyama-cho, Kagoshima 892-0853, Japan.

Tel: +81-99-223-1151, Fax: +81-99-223-7918, E-mail: m-yoshi@biscuit.ocn.ne.jp

Total word count: A total of 3937 words

Subject Code: [171] Electrocardiology

Abstract

Background—A school-based electrocardiographic screening program has been developed in Japan. However, few data are available regarding the genetic characteristics of pediatric patients with long QT syndrome (LQTS) who were diagnosed by this program.

Methods and Results—A total of 117 unrelated probands aged ≤18 years referred to our centers for genetic testing were the subjects. Of these, 69 subjects diagnosed by the program formed the screened group. A total of 48 subjects were included as the disease control group and were diagnosed with LQTS-related symptoms, familial study, or by chance (hospital-based group). Mutations were classified as radical, of high probability of pathogenicity, or of uncertain significance. Two subjects in the hospital-based group died. Genotypes were identified in 50 (72%) and 23 (50%) subjects in the screened and hospital-based groups, respectively. Of mutations yielded in *KCNQ1* or *KCNH2*, 31 of 33 (94%) mutations in the screened group and 15 of 16 (94%) mutations in the hospital-based groups were radical and/or of high probability of pathogenicity. Prevalence of symptoms before (9/69 vs 31/48, $p<0.0001$) and after (12/69 vs 17/48, $p=0.03$) diagnosis was significantly lower in the screened group compared with the hospital-based group, although the QTc values, family history of LQTS, sudden death, and follow-up periods were not different between the groups.

Conclusion—These data suggest that the screening program may be effective for early diagnosis of LQTS and prevention of symptoms. In addition, screened patients should be followed equivalently to hospital-based patients.

Key Words: long QT syndrome, screening, genetic, pathogenic

Congenital long QT syndrome (LQTS) is a genetic disorder characterized by delayed repolarization and by a long QT interval on 12-lead electrocardiograms (ECGs). Although many patients are symptomless, the hallmark of the condition is syncope or sudden death due to torsade de pointes.[1,2] Thirteen genes have so far been identified.[3,4] There have been many reports concerning the clinical and genetic backgrounds of patients with LQTS. However, these were mainly based on data collected from patients who suffered from LQTS-related symptoms or familial studies and from combined adult and pediatric populations.[2,5-9]

A nationwide school-based ECG screening program for heart diseases in first, seventh, and 10th graders in Japan has revealed children and adolescents with prolonged QT intervals. The prevalence of subjects with prolonged QT intervals was around 1:1200 in the seventh grade.[10] Differences in clinical characteristics between patients who were screened by the program and those who visited hospitals because of symptoms has already been reported.[11] However, prior to this study, few data have been reported concerning the genetic characteristics of pediatric patients who were diagnosed by ECG screening programs and whose genetic testing was performed.[12-14] Additionally, because of a lack of reports containing large numbers of patients who were screened alongside genetic testing, it is unclear whether screened subjects have similar mutations of a high possibility of pathogenesis to those who suffered LQTS-related symptoms.

From the genetic testing viewpoint, the few percent background rate of the rare *KCNQ1* and *KCNH2* nonsynonymous single nucleotide variants among healthy individuals has lessened the ability to distinguish rare pathogenic mutations from similarly rare, yet presumably innocuous, variants.[15,16] Novel mutations have been found in every study,[12,17,18] but it is difficult to perform electrophysiological studies for each novel mutation except in large laboratories. Recently, an algorithm designed to guide the interpretation of genetic testing results for *KCNQ1* and *KCNH2* has been developed.[16]

Thus, the aim of the present study was to determine the genetic characteristics of pediatric patients with LQTS who were diagnosed by a school-based screening program and whose genetic testing was performed, and to compare results with subjects who visited hospitals because of the presence of LQTS-related symptoms, familial history, or who were diagnosed by chance.

Methods

Study Population

The study population included 117 unrelated probands ≤18 years of age who were referred to the Department of Pediatrics, Kagoshima University Hospital, Japan, between November 1993 and March 2005, or to the National Hospital Organization Kagoshima Medical Center from April 2005 to December 2012 for genetic evaluation. The population included 69 subjects who were screened by a school-based ECG screening program (Table 1). Genetic testing was performed when pediatric cardiologists thought that the subjects might have clinical LQTS or when a family had a history of LQTS-related symptoms, and genetic testing was conducted. In the present study, LQTS-related symptoms were defined as syncope, aborted cardiac arrest, or sudden cardiac death at less than 30 years old. Subjects were divided into two groups on the basis of index events; subjects who were diagnosed by the school-based ECG screening program (screened group), and those who visited hospitals because of the presence of symptoms and/or family history, or who were diagnosed by chance (hospital-based group) (Table 1).

Genetic Testing

Genomic DNA was isolated from blood after obtaining written informed consent. Genetic screening for all exons of *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, *KCNE2*, *KCNJ2*, and *CAV3* was re-performed for the present study using PCR and direct DNA sequencing. When a

patient was suspected to have Timothy syndrome, which is a multisystem disorder characterized by cardiac (QT prolongation and sometimes congenital heart diseases), hand/foot, facial, and neurodevelopmental features, the exons of *CACNA1C* were amplified. When a patient had a prolonged QT interval and hyperaldosteronism, the exon of *KCNJ5* was amplified. The exons of *ANKB*, *SCN4B*, *AKAP9*, and *SNTA1* were not analyzed because of a lack of reported cases of these mutations in the Japanese population. Genomic DNA was isolated using a QIAmp DNA Blood Midi Kit (QIAGEN, Maryland, USA). PCR products were purified using AMPure (Beckman Coulter, California, USA). After treating with BigDye Terminator v1.1 Cycle Sequence Kit (ABI, Warrington, UK) and BigDye X Terminator, direct sequencing was performed by a genetic analyzer, ABI3130x1 Genetic Analyzer (ABI). The study was approved by the Ethics Committee of the Kagoshima University Hospital between November 1993 and March 2005 and the National Hospital Organization Kagoshima Medical Center from April 2005.

Nucleotide changes reported as single nucleotide polymorphisms[18,19] were excluded from mutation analysis in the present study. However, amino acid changes of G643S in *KCNQ1*[20] and D85N in *KCNE1*[21] were included in the present study, because previous reports have shown that these mutations are associated with an approximately 30% reduction in potassium channel currents.[20,21] When multiple mutations were present, each mutation was counted in each genotype.

Mutations of High Probability of Pathogenicity

Mutations of a high probability of pathogenicity were based on data published by Giudicessi, et al.[16] Radical mutations included splice-site, nonsense, frame-shift, and insertion/deletions.[16] Mutations of a high probability of pathogenicity in the present study were defined as those present in the subunit assembly domain (SAD) of the C-terminal of *KCNQ1*, the Per-Arnt-Sim (PAS) domain and PAS-associated C-terminal (PAC) domain of

the N-terminal of *KCNH2*, and the cyclic nucleotide-binding domain (cNBD) of *KCNH2*. Mutations present in the transmembrane/linker/pore and C-terminal regions of *KCNQ1*, and the transmembrane/linker/pore regions of *KCNH2* were also defined as those of a high probability of pathogenicity.[16] Remaining mutations were defined as of uncertain significance.

Statistical Analysis

Differences in the mean values and prevalence values were examined using the Mann-Whitney *U* test and Fisher's exact probability test, respectively. Statistical analysis was performed using IBM SPSS Statistics Version 21.0 (IBM Japan, Ltd., Tokyo). A two tailed probability value of <0.05 was considered statistically significant.

Results

Population

Characteristics of the 117 subjects, 69 screened and 48 hospital-based patients, are shown in Table 1. There were no differences in sex, mean QTc values, family history of LQTS, family history of sudden death, or follow-up period between the screened and hospital-based groups. The mean age was lower in the hospital-based group compared with the screened group ($p=0.04$). Prevalence of suffering LQTS-related symptoms before and after diagnosis was significantly lower in the screened group compared with the hospital-based group ($p<0.001$ and $p=0.03$, respectively). Of 117 subjects, two subjects died. A girl had a history of aborted cardiac arrest at 2 months, and died suddenly in her sleep at 5 years of age. An 11-year-old boy had frequent symptoms and died suddenly during class. Genetic analysis failed to show the presence of any of the mutations analyzed in this study.

Mutations Determined in the Present Study

Of 117 subjects, mutations were found in 50 of 69 (72%) screened and 23 of 48 (50%)

hospital-based subjects (Table 2). The prevalence of LQT1, LQT2, and LQT3 between the two groups was not different. LQTS-related mutations in the present study are summarized in Table 3.

Genetic Characteristics of Subjects

All mutations found in *KCNQ1* from 18 mutations in the screened and nine mutations in the hospital-based groups were located in regions of a high probability of pathogenicity (Table 4, Figures 1a and b). In the screened group, eight mutations were located in the transmembrane/linker/pore regions and 10 were present in the C-terminal regions (Figure 1a). Three mutations were radical and one mutation was present in the SAD. Regarding the association between locations of mutation and the presence or absence of LQTS-related symptoms, 14 (78%) of 18 mutations were associated with the presence of symptoms in probands and/or family members, including four (22%) with family history of sudden death in the screened group. Eight of nine mutations in the hospital-based group were associated with the presence of symptoms, and the remaining mutation was found in a subject who was diagnosed by a familial study.

Among mutations yielded in *KCNH2*, 13 (87%) of 15 mutations in the screened group and six (86%) of seven mutations in the hospital-based group were located in regions of a high probability of pathogenicity (Table 4, Figures 2a and b). In the screened group, one mutation was both radical and present in the cNBD domain. Another five mutations were radical and one each was present in PAS and PAC regions. However, only four (31%) of 13 mutations were associated with the presence of LQTS-related symptoms in probands or family members in the screened group. In the hospital-based group, six (86%) of seven mutations were associated with the presence of symptoms in probands and/or family members, and the remaining mutation was found in a subject by ECG screening during a medical check-up at 1 month of age.

Discussion

Mutations in subjects with LQTS who were diagnosed by school-based ECG screening programs were mostly of high possibility of pathogenicity, similar to hospital-based subjects. Clinical background such as QTc values, family history of LQTS, or sudden death, and follow-up periods were not different between the two groups. However, prevalence of symptoms before and after diagnosis in the screened group was significantly lower compared with the hospital-based group. These data suggest that screening programs may be effective for early diagnosis of LQTS and prevention of symptoms, and that screened patients should be followed similarly to hospital-based patients.

Clinical and genetic backgrounds of patients with LQTS have been reported widely for infants, children, adolescents, and adults.[5-9] These data were mostly based on symptomatic probands and family members. Few data are available regarding the genetic background of subjects who were diagnosed by ECG screening programs. Schwartz *et al.* reported that LQTS-related mutations were identified in 16 neonates out of 43,080 who underwent neonatal ECG screening;[12] nine *KCNQ1*, six *KCNH2*, and one each of *KCNE1* and *KCNE2*; one infant had digenic mutation of KCNQ1 and KCNH2.

A school-based ECG screening program for heart diseases was initiated in 1994 for the screening of first, seventh, and 10th graders in Japan. The program screened subjects with QT prolongation. However, few studies have confirmed the genetic background in these screened subjects.[13,14] Hayashi *et al.* reported that mutations were identified in three subjects with high or intermediate probabilities of LQTS using Schwartz's criteria from 7,961 school children;[13] all three mutations were present in *KCNH2*. Yasuda *et al.* reported that *KCNQ1* mutations were found in eight out of 13 pediatric patients and that seven out of eight patients were diagnosed by the ECG screening program.[14]

In the present study, a relatively large number of subjects, who were diagnosed by ECG screening programs accompanied by genetic testing, were included. The clinical backgrounds of the screened subjects such as QTc values, family history of LQTS, or sudden death were similar to hospital-based subjects. All 16 mutations in the *KCNQ1* gene in the screened group were radical or of high probability of pathogenicity similar to the hospital-based group. The ratio of mutations of radical and/or of high probability of pathogenicity in the *KCNH2* gene in the screened group (13/15, 87%) was remarkably similar to that in the hospital-based group (6/7, 86%). These data suggest that pediatricians, who asked for genetic testing in the present study, chose patients with similar clinical backgrounds in both groups, and that demand for genetic testing was more prevalent in screened patients compared with hospital-based patients when ECG screening was developed.

Conversely, prevalence of symptoms before and after diagnosis was significantly lower in the screened group compared with the hospital-based group. A low prevalence of symptoms before diagnosis suggests that the ECG screening program is effective for early diagnosis of LQTS. The reason for low prevalence of symptoms after diagnosis in the screened group is uncertain. Doctors may recommend pediatric LQTS patients and/or their parents for their lives, for example, not doing vigorous exercise, not swimming a lap, not diving,[22] and other activities in both the screened and hospital-based subjects. The reason should be exactly clarified.

There are some limitations of the current study. Firstly, we did not discuss subjects with the *SCN5A* gene. One fourth of pediatric patients with LQTS had the *SCN5A* gene. We need similar algorithms designed to guide the interpretation of genetic testing results for the *SCN5A* mutation, to determine the possibility of pathogenesis in patients with SCN5A in the future. Secondly, the hospital-based group showed a low rate (50%) of genotypic determination. We could not find mutations in two cases who died in the present study. The

reasons for this were unclear. One potential reason was that we did not screen copy number variations (CNVs) in genes associated with LQTS.[23-25] Eddy *et al.*[24] and Barc *et al.*[25] reported that three of 26 (12%) and three of 93 (3%) unrelated mutation-negative probands showed CNVs, indicating that some mutation-negative patients may have CNVs. Another reason may be that numerous previously undetected mutations exist in symptomatic patients.

In conclusion, mutations in subjects with LQTS who were diagnosed by screening programs were of high possibility of pathogenicity similar to hospital-based subjects. Clinical backgrounds were not different, although the prevalence of symptoms before and after diagnosis in the screened group was significantly lower compared with the hospital-based group. These data suggest that the school-based screening program may be effective for early diagnosis of LQTS and prevention of symptoms, and that screened patients should be followed equivalently to hospital-based patients.

Funding Source

This study was partly supported by a Health and Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare of Japan (Research on Intractable Diseases [H22-032] and [H24-033]).

Disclosure

None

References

1. Schwartz PJ, Crotti L, Insolia R. Long-QT syndrome: from genetics to management. Circ Arrhythm Electrophysiol. 2012;5:868-77.

2. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, Vicentini A, Spazzolini C, Nastoli J, Bottelli G, Folli R, Cappelletti D. Risk stratification in the long-QT syndrome. *N Engl J Med.* 2003; 348:1866-74.

3. Ackerman MJ, Mohler PJ. Defining a new paradigm for human arrhythmia syndromes: phenotypic manifestations of gene mutations in ion channel- and transporter-associated proteins. *Circ Res.* 2010; 107:457-65.

4. Yang Y, Yang Y, Liang B, Liu J, Li J, Grunnet M, Olesen SP, Rasmussen HB, Ellinor PT, Gao L, Lin X, Li L, Wang L, Xiao J, Liu Y, Liu Y, Zhang S, Liang D, Peng L, Jespersen T, Chen YH. Identification of a Kir3.4 mutation in congenital long QT syndrome. *Am J Hum Genet.* 2010; 86:872-80.

5. Spazzolini C, Mullally J, Moss AJ, Schwartz PJ, McNitt S, Ouellet G, Fugate T, Goldenberg I, Jons C, Zareba W, Robinson JL, Ackerman MJ, Benhorin J, Crotti L, Kaufman ES, Locati EH, Qi M, Napolitano C, Priori SG, Towbin JA, Vincent GM. Clinical implications for patients with long QT syndrome who experience a cardiac event during infancy. J Am Coll Cardiol. 2009 Aug 25;54(9):832-7.

6. Goldenberg I, Moss AJ, Peterson DR, McNitt S, Zareba W, Andrews ML, Robinson JL, Locati EH, Ackerman MJ, Benhorin J, Kaufman ES, Napolitano C, Priori SG, Qi M, Schwartz PJ, Towbin JA, Vincent GM, Zhang L.Risk factors for aborted cardiac arrest and sudden cardiac death in children with the congenital long-QT syndrome. Circulation. 2008 Apr 29;117(17):2184-91.

7. Liu JF, Jons C, Moss AJ, McNitt S, Peterson DR, Qi M, Zareba W, Robinson JL, Barsheshet A, Ackerman MJ, Benhorin J, Kaufman ES, Locati EH, Napolitano C, Priori

SG, Schwartz PJ, Towbin J, Vincent M, Zhang L, Goldenberg I; International Long QT Syndrome Registry. Risk Factors for Recurrent Syncope and Subsequent Fatal or Near-Fatal Events in Children and Adolescents With Long QT Syndrome. J Am Coll Cardiol. 2011 Feb 22;57(8):941-50

8. Hobbs JB, Peterson DR, Moss AJ, McNitt S, Zareba W, Goldenberg I, Qi M, Robinson JL, Sauer AJ, Ackerman MJ, Benhorin J, Kaufman ES, Locati EH, Napolitano C, Priori SG, Towbin JA, Vincent GM, Zhang L. Risk of aborted cardiac arrest or sudden cardiac death during adolescence in the long-QT syndrome. JAMA. 2006 Sep 13;296(10):1249-54.
9. Sauer AJ, Moss AJ, McNitt S, Peterson DR, Zareba W, Robinson JL, Qi M, Goldenberg I, Hobbs JB, Ackerman MJ, Benhorin J, Hall WJ, Kaufman ES, Locati EH, Napolitano C, Priori SG, Schwartz PJ, Towbin JA, Vincent GM, Zhang L. Long QT syndrome in adults. *J Am Coll Cardiol*. 2007; 49:329-37.
10. Fukushige T, Yoshinaga M, Shimago A, Nishi J, Kono Y, Nomura Y, Miyata K, Imamura M, Shibata T, Nagashima M, Niimura I. Effect of age and overweight on the QT interval and the prevalence of long QT syndrome in children. *Am J Cardiol*. 2002; 89:395-398.
11. Yoshinaga M, Nagashima M, Shibata T, Niimura I, Kitada M, Yasuda T, Iwamoto M, Kamimura J, Iino M, Horigome H, Seguchi M, Aiba S, Izumida N, Kimura K, Ushinohama H, Nishi J, Kono Y, Nomura Y, Miyata K. Who is at risk for cardiac events in young patients with long QT syndrome? *Circ J*. 2003; 67:1007-1012.
12. Schwartz PJ, Stramba-Badiale M, Crotti L, Pedrazzini M, Besana A, Bosi G, Gabbarini F, Goulene K, Insolia R, Mannarino S, Mosca F, Nespoli L, Rimini A, Rosati E, Salice P, Spazzolini C. Prevalence of the congenital long-QT syndrome. *Circulation*. 2009;120:1761-7
13. Hayashi K, Fujino N, Uchiyama K, Ino H, Sakata K, Konno T, Masuta E, Funada A, Sakamoto Y, Tsubokawa T, Nakashima K, Liu L, Higashida H, Hiramaru Y, Shimizu M,

Yamagishi M.Long QT syndrome and associated gene mutation carriers in Japanese children: results from ECG screening examinations. *Clin Sci (Lond)*. 2009;117:415-24.

14. Yasuda K, Hayashi G, Horie A, Taketani T, Yamaguchi S. Clinical and electrophysiological features of Japanese pediatric long QT syndrome patients with KCNQ1 mutations. *Pediatr Int*. 2008;50:611-4.
15. Kapa S, Tester DJ, Salisbury BA, Harris-Kerr C, Pungliya MS, Alders M, Wilde AA, Ackerman MJ. Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants. *Circulation*. 2009; 120: 1752-60.
16. Giudicessi JR, Kapplinger JD, Tester DJ, Alders M, Salisbury BA, Wilde AA, Ackerman MJ. Phylogenetic and physicochemical analyses enhance the classification of rare nonsynonymous single nucleotide variants in type 1 and 2 long-QT syndrome. *Circ Cardiovasc Genet*. 2012;5:519-28.
17. Tester DJ, Will ML, Haglund CM, Ackerman MJ. Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. *Heart Rhythm*. 2005;2:507-17.
18. Kapplinger JD, Tester DJ, Salisbury BA, Carr JL, Harris-Kerr C, Pollevick GD, Wilde AA, Ackerman MJ. Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test. *Heart Rhythm*. 2009;6:1297-303.
19. Genetic mutation and inherited arrhythmias. http://www.fsm.it/cardmoc/ access on 2012/12/14.
20. Kubota T, Horie M, Takano M, Yoshida H, Takenaka K, Watanabe E, Tsuchiya T, Otani H, Sasayama S. Evidence for a single nucleotide polymorphism in the KCNQ1 potassium channel that underlies susceptibility to life-threatening arrhythmias. *J Cardiovasc Electrophysiol*. 2001; 12:1223-9.

21. Nishio Y, Makiyama T, Itoh H, Sakaguchi T, Ohno S, Gong YZ, Yamamoto S, Ozawa T, Ding WG, Toyoda F, Kawamura M, Akao M, Matsuura H, Kimura T, Kita T, Horie M. D85N, a KCNE1 polymorphism, is a disease-causing gene variant in long QT syndrome. *J Am Coll Cardiol*. 2009; 54:812-9.

22. Maron BJ, Chaitman BR, Ackerman MJ, Bayés de Luna A, Corrado D, Crosson JE, Deal BJ, Driscoll DJ, Estes NA 3rd, Araújo CG, Liang DH, Mitten MJ, Myerburg RJ, Pelliccia A, Thompson PD, Towbin JA, Van Camp SP; Working Groups of the American Heart Association Committee on Exercise, Cardiac Rehabilitation, and Prevention; Councils on Clinical Cardiology and Cardiovascular Disease in the Young. Recommendations for physical activity and recreational sports participation for young patients with genetic cardiovascular diseases. Circulation. 2004;109:2807-16.

23. Koopmann TT, Alders M, Jongbloed RJ, Guerrero S, Mannens MM, Wilde AA, Bezzina CR. Long QT syndrome caused by a large duplication in the KCNH2 (HERG) gene undetectable by current polymerase chain reaction-based exon-scanning methodologies. *Heart Rhythm*. 2006; 3:52-5.

24. Eddy CA, MacCormick JM, Chung SK, Crawford JR, Love DR, Rees MI, Skinner JR, Shelling AN. Identification of large gene deletions and duplications in KCNQ1 and KCNH2 in patients with long QT syndrome. *Heart Rhythm*. 2008; 5:1275-81.

25. Barc J, Briec F, Schmitt S, Kyndt F, Le Cunff M, Baron E, Vieyres C, Sacher F, Redon R, Le Caignec C, Le Marec H, Probst V, Schott JJ. Screening for copy number variation in genes associated with the long QT syndrome: clinical relevance. *J Am Coll Cardiol*. 2011; 57:40-7.

Figure legends

Figure 1. Topological depiction of *KCNQ1* in the present study in the screened (Figure 1a) and hospital-based (Figure 1b) groups.

Mutations found in the screened group are shown as boxes (a) and those in the hospital-based group as circles (b). Each box or circle is divided into two parts; left and right sides. Each part represents the presence or absence of LQTS-related symptoms in probands (left) and family members (right), respectively. Green, brown, and red colors, symbolize no symptoms, syncope or aborted cardiac arrest, and sudden death, respectively. Bold red circles surrounding mutations represent radical mutations. A bold blue circle represents SAD. Two big purple circles symbolize locations of transmembrane/linker/pore and C-terminal regions of *KCNQ1*.

Figure 2. Topological depiction of *KCNH2* in the present study in the screened (Figure 2a) and hospital-based (Figure 2b) groups.

Explanations of symbols and shapes are the same as for Figure 1. Bold blue circles surrounding mutations in this figure represent PAS, PAC, and cNBD domains, respectively, from the left side. A big purple circle symbolizes locations of transmembrane/linker/pore regions.

Table 1 Characteristics of probands

Subjects	Screened*	Hospital-based*	p value
Number of subjects	69	48	
Age at Diagnosis	10.4 ± 3.4	7.4 ± 6.0	0.04
Age at Diagnosis (median & range)	12.2 (6.2 - 18.8)	8.9 (0 - 17.2)	
Sex (Male/Female)	36/33	27/21	0.66
Mean QT interval (ms)†	466 ± 51	442 ± 83	0.09
Mean RR interval (ms)†	887 ± 170	802 ± 261	0.09
QTc (Bazett) ($ms^{1/2}$)†	496 ± 40	502 ± 543	0.84
QTc (Fridericia) ($ms^{1/3}$)†	486 ± 40	480 ± 55	0.24
Past history of symptoms‡	9 (13 %)	31 (65 %)	<0.0001
Family history of LQTS‡	27 (39 %)	18 (38 %)	>0.99
Family history of SD‡	5 (7 %)	7 (15 %)	0.23
Follow-up periods†	4.6 ± 4.9	5.2 ± 5.7	0.36
Symptoms after Diagnosis†	12 (17 %)	17 (35 %)	0.03

*; Screened group included subjects who were diagnosed by the school-based ECG screening program. Hospital-based group included subjects who visited hospitals because of the presence of symptoms and family history, or who were diagnosed by chance.

†; The mean value ± standard deviation.

‡; Number of subjects and percentage in parenthesis.

Abbreviations: LQTS, long QT syndrome; SD, sudden death less than 30 years old.

Table 2 Mutations determined in the screened and hospital-based patients

	Screened	Hospital-based	Total
No of probands	69	48	117
No of determined	50 (72%)	23 (50%)	73 (62%)
KCNQ1	24 (48 %)*	11 (48 %)*	35 (48 %)
KCNH2	16 (32 %)	6 (30 %)	22 (30 %)
SCN5A	12 (24 %)	5 (26 %)	17 (23 %)
KCNE1	1 (2%)	4 (17 %)	5 (7 %)
KCNJ2	2 (4 %)	0 (0 %)	2 (3 %)
CACNA1c	0 (0 %)	1 (4 %)	1 (1 %)
KCNJ5	1 (2 %)	0 (0 %)	1 (1 %)
Multiple mutations	7 (14 %)	5 (22 %)	12 (16 %)

When multiple mutations were present, each mutation was counted in each genotype.

*; Number of subjects whose mutations were identified and the percentage in parenthesis among subjects in each group

Table 3 Summary of LQTS-related mutations in the present study

	Nucleotide change	Amino acid change	Mutation type	Location	No of Screened patients	No of Hospital-based patients
LQT1	502G>A	G168R	Missense	S2	0	1
	532 G>A	A178T	Missense	S2/S3	1	0
	567 del G (homo)	G189fs+236X*	Frame shift	S2/S3	0	1
	568 C>T	R190W	Missense	S2/S3	1	0
	569 G>A	R190Q	Missense	S2/S3	0	1
	677 C>T	A226V*	Missense	S4	2	0
	724 G>A	D242N	Missense	S4/S5	0	1
	760 G>A	V254M	Missense	S4/S5	1	1
	781 G>T & 782 A>T	E261L*†	Missense	S4/S5	1	0
	830 C>T	S277L	Missense	S5	1	0
	993 C>A	C331X*	Nonsense	S6	1	0
	1022 C>T	A341V	Missense	S6	0	2
	1031 C>A	A344E	Missense	S6	1	0
	1096 C>T	R366W	Missense	C-terminal	1	0
	1097 G>A	R366Q	Missense	C-terminal	1	0
	1252+1 G>A*		Splice site	C-terminal	1	0
	1264 A>T	K431X*	Nonsense	C-terminal	1	0
	1552 C>G	R518G	Missense	C-terminal	0	1
	1552 C>T	R518X	Nonsense	C-terminal	1	0

	1637 C>T	S546L	Missense	C-terminal	1	0
	1663 C>T	R555C	Missense	C-terminal	1	0
	1748 G>A	R583H	Missense	C-terminal	1	0
	1760 C>T	T587N	Missense	C-terminal	0	1
	1772 G>A	R591H	Missense	SAD	2	0
	1927 G>A	G643S	Missense	C-terminal	6	2
LQT2	133 A>G	N45D*	Missense	PAS	0	1
	157 G>A	G53R*	Missense	PAS	1	0
	233 C>T	A78V*	Missense	N-terminal	1	0
	388 G>A	E130K*	Missense	PAC	1	0
	454 ins C	P151fs+179X	Frame shift	N-terminal	1	0
	1401_1402 ins ATGTT	I467_L468ins	In-frame ins	S2/S3	1	0
	CATTGTGGACATC	MFIVDI*				
	1474 C>T	H492Y	Missense	S2/S3	1	0
	1744 C>A	R582S*	Missense	S5/pore	1	0
	1750 G>T	G584C*	Missense	S5/Pore	1	0
	1760 C>T	T587N*	Missense	S5/Pore	0	1
	1801 G>A	G601S	Missense	S5/Pore	1	0
	1882 G>C	G628R*	Missense	Pore	0	1
	1899 C>A	N633K*	Missense	Pore/S6	1	0
	1900 A>G	T634A*	Missense	Pore/S6	0	1
	2146+2 A>G*		Splice site	S6/cNBD	1	0
	2230 del C	T743fs+12X*	Frame shift	cNBD	0	1
	2235-2245 del	G745fs+54X*	Frame shift	cNBD	2	0
	GGCCACCAAGG + ins TTT					

	2637-2638 del TG	G879fs+38X*	Frame shift	C-terminal	1	0
	2690 A>C	K897T*	Missense	C-terminal	1	0
	2730 G>C	C977S*	Missense	C-terminal	0	1
	3019 del C	R1007fs+1056X*	Frame shift	C-terminal	1	0
	3065 del T	L1021fs+34X*	Frame shift	C-terminal	0	1
LQT3	1595 T>G	F532C	Missense	DI/DII	0	1
	2441 G>A	R814G	Missense	DII/S4	0	1
	3578 G>A	R1193Q	Missense	DII/DIII	6	2
	4282 G>T	A1428S	Missense	DIII/S5-S6	0	1
	4937 T>G	L1646R*	Missense	DIV/S4	1	0
	5324 C>G	L1772V*	Missense	C-terminal	0	1
	5349 G>A	E1784K	Missense	C-terminal	1	0
	5963 C>T	L1988R	Missense	C-terminal	1	0
LQT5	47 T>C	L16P*	Missense	C-terminal	0	1
	253 G>A	D85N	Missense	C-terminal	1	3
LQT7	200 G>A	R67Q	Missense	N-terminal	1	0
	652 C>T	R218W	Missense	C-terminal	1	0
LQT8	1216 G>A	G406R	Missense	DI/S6	0	1
LQT13	472A>G	T158A	Missense		1	0

Abbreviations: cNBD, cyclic nucleotide-binding domain; fs, frame shift; ins, insertion; PAC, Per-Arnt-Sim associated C-terminal; PAS, Per-Arnt-Sim; SAD, Subunit assembly domain.

* denotes novel mutations or variants.