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EDITORIAL COMMENT

Importance of Clinical Analysis in the Era of New Technology in Molecular Genetic Screening*

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For the past 2 decades, a number of inherited cardiac arrhythmia syndromes have been shown to be linked to mutations in genes encoding cardiac ion channels or other membrane components. These include congenital and acquired long-QT syndrome (LQTS), Brugada syndrome (BrS), progressive cardiac conduction defect, Lenegre disease, catecholaminergic polymorphic ventricular tachycardia (CPVT), short-QT syndrome, early repolarization syndrome, and familial atrial fibrillation (AF) (1). In congenital LQTS, 13 genotypes have been identified in approximately 75% of subjects with clinically diagnosed congenital LQTS (1,2), and genotype-phenotype correlations have been investigated in detail. Thus, genetic testing is now a gold standard for diagnosing congenital LQTS, enabling risk stratification of cardiac events and better patient management (1). Mutations in the *RyR2* gene or *calsequestrin* gene can be identified in approximately 60% of typical patients with CPVT associated with bidirectional and/or multifocal ventricular tachycardia (1,2). However, the yield associated with disease-specific genetic testing is far short of 100%, even in congenital LQTS or CPVT. Moreover, causative mutations have been identified in a small number of patients with other inherited arrhythmia syndromes (1). The yield of disease-specific genetic testing is only 20% to 30% in BrS and is still unknown in progressive cardiac conduction defect, short-QT syndrome, early repolarization syndrome, and familial AF (1,2).

In BrS, the first mutation was identified in an alpha subunit of a sodium channel gene, *SCN5A*, in 1998 (2). Subsequently, genetic studies have identified 13 responsible genes on chromosomes 1, 3, 7, 10, 11, 12, 17, and 19 (1). Among 13 genotypes, more than 300 mutations have been identified in the major player, *SCN5A* (>75% of genotyped cases); however, a worldwide cohort reported that *SCN5A* accounts only for 11% to 28% of clinically diagnosed patients with BrS (4). Moreover, the majority of mutations were found in a single family or a small number of families. Therefore, a genotype-phenotype correlation is not available in most cases (1,5).

The relatively lower yield of disease-specific genetic testing except for congenital LQTS or CPVT is due mainly to the technology of genetic testing. Candidate gene analysis has long been used to identify a causative mutation in a gene, which is expected to relate to the pathophysiology of each inherited arrhythmia syndrome, such as cardiac ion channel genes. However, causative mutations do not always involve genes of ion channels or membrane components. Innovative advances in molecular genetic testing are overcoming this issue with the advent of more powerful molecular genetic screening tools, including genome-wide association study (GWAS) using gene array, as well as targeted, whole-exome and whole-genome next-generation sequencing techniques.

Several recent GWASs have disclosed significant association of numerous loci in some genes with electrocardiographic markers or arrhythmia syndromes. Arking et al. (6) first identified *NOS1AP (CAPON)*, a regulator of neuronal nitric oxide synthase, as a gene that is significantly associated with QT-interval variation in a general population derived from 3 cohorts (6). Subsequently, 2 groups conducted a meta-analysis of the GWAS and observed associations of single-nucleotide polymorphisms (SNPs) in

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several genes in addition to *NOS1AP* with QT interval, suggesting that these genes are candidate genes for LQTS or sudden cardiac death (7,8). Several GWASs also identified associations of SNPs in several genes, including *SCN10A*, with cardiac conduction parameters, such as QRS duration and PR interval (9-11). Regarding associations with cardiac arrhythmias, some SNPs in several genes, including *ZFHX3* and *KCNN3*, have been reported to be associated with AF (12-14). The association of a SNP in *CXADR* with ventricular fibrillation in acute myocardial infarction also has been reported (15). However, no responsible mutations have thus far been reported in these candidate genes in patients with clinically diagnosed inherited arrhythmia syndromes, such as congenital LQTS, familial AF, and familial conduction abnormalities.

Bezzina et al. (16) recently conducted a GWAS in 312 patients with BrS with type 1 electrocardiographic pattern and 1,115 controls. They detected 2 significant association signals at the *SCN10A* intronic locus (rs10428132) in chromosome 3p22 and near the *HEY2* gene (rs9388451) in chromosome 6q22 with BrS. *SCN10A*, which encodes the sodium channel isoform Nav1.8, was originally reported as highly expressed in cardiac neurons. Recent evidence indicates that *SCN10A* also is expressed in the working myocardium and the specialized conduction system, indicating a possible role for Nav1.8 in cardiac electrical function. *HEY2* is involved in patterning Nav1.5 (*SCN5A*) expression across the ventricular wall. In an experiment using *HEY2* knockout mouse, Bezzina et al. (16) suggested that loss of *HEY2* might affect the transmural expression gradient of sodium channel implicated in BrS.

In this issue of the *Journal*, Hu et al. (17) report on a clinical analysis and direct sequencing of *SCN10A* and all known BrS genes in 150 unrelated patients with BrS and 17 family members, as well as more than 200 ethnically matched healthy controls. They identified 17 *SCN10A* mutations in 25 of 150 patients with BrS (a yield of 16.7%). Twenty-three of the 25 (92.0%) displayed overlapping phenotypes, such as early repolarization syndrome and cardiac conduction defect. Patients with BrS with *SCN10A* mutations were more symptomatic and displayed significantly longer PR and QRS intervals than *SCN10A*-negative patients with BrS. Heterologous coexpression of *SCN10A* mutants (R14L and R1268Q) with wild-type *SCN5A* caused 79.4% and 84.4% reductions in sodium channel current, strongly implicating *SCN10A* as a major susceptibility gene for BrS. This study provides the first major step forward in more than 16 years in the identification of new BrS susceptibility genes, advancing the yield for detection of a genotype to more than 50%.

New molecular genetic screening technologies, such as GWAS and whole-exome and whole-genome next-generation sequencing, are promising tools for identifying new candidate genes responsible for inherited arrhythmia syndromes. However, no responsible mutations have been reported in the candidate genes identified by GWAS in patients with clinically diagnosed inherited arrhythmia syndromes. To the best of my knowledge, the *SCN10A* is the first gene to be suggested as a BrS susceptibility gene by both GWAS and direct sequencing techniques. Direct sequencing using the Sanger technique combined with a detailed clinical analysis, including genotype-phenotype correlation and functional expression studies, continue to play an important role in molecular genetic testing, even in the new era in which gene arrays and next-generation sequencing are available. The importance of a detailed clinical analysis including genotype-phenotype correlation as well as functional expression studies cannot be overemphasized. Even in GWAS and whole-genome or whole-exome studies, clinical misdiagnosis can contribute to confounding genetic noise. A detailed, precise clinical diagnosis is therefore a prerequisite for the identification of new potential candidate genes.

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KEY WORDS Brugada syndrome, direct sequencing, genetic study, GWAS, sudden death

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

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

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

Conclusions—These data suggest that the screening program may be effective for early diagnosis of long-QT syndrome that may allow intervention before symptoms. In addition, screened patients should have follow-up equivalent to clinically identified patients. (***Circ Arrhythm Electrophysiol.* 2014;7:107-112.**)

Key Words: diagnosis ■ genetic testing ■ QT interval electrocardiography ■ screening

Congenital long-QT syndrome (LQTS) is a genetic disorder characterized by delayed repolarization and by a long-QT interval on 12-lead ECGs. Although many patients do not have symptoms, the hallmark of the condition is syncope or sudden death because of torsade de pointes.[1,2] To date, 13 genes have been identified.[3,4] There have been many reports on the clinical and genetic backgrounds of patients with LQTS. However, these were mainly based on data collected from patients who had LQTS-related symptoms or familial studies and from combined adult and pediatric populations.[2,5–9]

Clinical Perspective on p 112

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 ≈1:1200 in the seventh grade.[10] Differences in clinical characteristics between patients who were screened by the program and those who visited hospitals with symptoms have been previously reported.[11] However, before this study, few data have been reported about the genetic characteristics of pediatric patients who were diagnosed by ECG screening programs and whose genetic testing was performed.[12–14] In addition, 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 have LQTS-related symptoms.

From the genetic testing viewpoint, the few percentage 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,[17,18] but it is difficult to perform electrophysiological studies for

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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). In the present study, LQTS-related symptoms were defined as syncope, aborted cardiac arrest, or sudden cardiac death at <30 years old. Subjects were divided into 2 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 family history or who were diagnosed by chance (clinical group; Table 1).

Diagnosis of LQTS and Screening of QT Intervals in the School-based ECG Screening Program

The present study was a retrospective study, and diagnosis of LQTS and screening for prolonged QT intervals was based on the judgment of the chief medical doctors in each hospital or doctors who participated in the program in each area. Many Japanese cardiologists use a scoring system published in 1993[19] and recently[1] for the final diagnosis of LQTS. To screen subjects with prolonged QT intervals in the program, the Japanese Society of Pediatric Cardiology and Cardiac Surgery recommended that children and adolescents be screened when they have a QTc value, using Bazett formula, of ≥450 ms at a heart rate of <75 beats per minute or a QTc≥500 ms at a hours of ≥75 beats per minute.[20] Bazett formula overcorrects the QT interval at high heart rates. Pediatric cardiologists who participated in the program used age- and sex-specific criteria using an exponential formula ($QT/RR^{0.31}$)[21] or Fridericia formula.[22] In the screening program, cardiologists use computer-based QTc values as a reference because all ECG machines used in Japan are generally equipped with a function for automated measurement of QT intervals. However, manual measurement using the tangent method is usually applied to obtain QT intervals in Japan.[22,23]

Table 1. Characteristics of Probands

Subjects	Screened Group	Clinical Group	*P* Value
No. of subjects	69	48	...
Age at diagnosis*	10.4±3.4	7.4±6.0	0.04
Age at diagnosis (median and range)	12.2 (6.2–18.8)	8.9 (0–17.2)	...
Sex (men/women)	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±52	0.84
History of symptoms†	9 (13%)	31 (65%)	<0.0001
Syncope	9	28	...
Aborted cardiac arrest	0	7‡	...
Family history of long-QT syndrome†	27 (39%)	18 (38%)	>0.99
Family history of sudden death†	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
Syncope	12	17	...
Aborted cardiac arrest	0	2§	...
Sudden cardiac death	0	2#	...

*The mean value±SD.

†Number of subjects and percentage in parenthesis.

‡Of 7 subjects with aborted cardiac arrest (ACA), 4 experienced both syncope and ACA.

#Of each 2 subjects with ACA or sudden cardiac death (SCD), all 4 subjects experienced syncope and ACA or SCD.

Genetic Testing

Referral for genetic testing was based on the opinion of the chief medical doctors in the present study. Pediatric cardiologists in the present study recommended genetic testing based on the following criteria: (1) for a patient in whom they had established a strong clinical index of suspicion for LQTS based on examination of the patient's clinical history, family history, and expressed ECG phenotype or; (2) for an asymptomatic patient with QT prolongation in the absence of other clinical conditions that might prolong the QT interval, as detailed in the recent consensus recommendation report.[24]

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 reperformed for the present study using polymerase chain reaction 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 QIAamp DNA Blood Midi Kit (Qiagen, Gaithersburg, MD). Polymerase chain reaction products were purified using AMPure (Beckman Coulter, Brea, CA). After treating with BigDye Terminator version 1.1 Cycle Sequence Kit (ABI, Warrington, United Kingdom) 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[18,25] were excluded from mutation analysis in the present study. However, amino acid changes of G643S in *KCNQ1*[26] and D85N in *KCNE1*[27] were included in the present study because previous reports have shown that these mutations are associated with an ≈30% reduction in potassium channel currents.[26,27] 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 of the C-terminal of *KCNQ1*, the Per-Arnt-Sim domain, Per-Arnt-Sim–associated C-terminal domain, and the cyclic nucleotide–binding domain 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 those of uncertain significance.

Statistical Analysis

Differences in the mean values and prevalence values were examined using the Mann–Whitney *U* test and Fisher exact probability test, respectively. Tukey multiple comparison test was used to assess differences in the mean QTc values among first, seventh, and 10th graders. Statistical analysis was performed using IBM SPSS Statistics version 21.0 (IBM Japan, Ltd, Tokyo, Japan). A 2-tailed *P* value of <0.05 was considered statistically significant.

Results

Population

Characteristics of the 117 subjects, including 69 screened and 48 clinical patients, are shown in Table 1. Of the 48 subjects included in the clinical group, 36 were diagnosed with LQTS-related symptoms, 6 were diagnosed by familial study, and 6 were diagnosed by chance. Subjects who were diagnosed by chance included those who visited hospitals for medical checks and for examination of heart murmurs at 1 month (4 patients), those who had been followed with Kawasaki disease (1 patient), and as Ehlers–Donlos syndrome (1 patient). 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 clinical groups. The mean age was lower in the clinical group when compared with the screened group (P=0.04). Prevalence of subjects having LQTS-related symptoms before and after diagnosis was significantly lower in the screened group when compared with that in the clinical group (P<0.001 and P=0.03, respectively). Symptoms before and after diagnosis in the screened group were all syncope. Of 117 subjects, 2 subjects in the clinical group 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. The treatment of subjects with symptoms during follow-up period is shown in Table I in the online-only Data Supplement.

Mutations Determined in the Present Study

The yield of genetic testing in the present study by QTc values using Bazett formula is shown in Table II in the Data Supplement. The data show that there was no difference in yield between subjects with a QTc<500 ms and those with a QTc≥500 ms in both screened and clinical groups in the present study. Of 50 subjects who were screened and whose mutations were identified, 29, 18, and 3 subjects were screened in the first, seventh, and 10th grade, respectively. Their QTc values using Bazett formula were 491±35, 503±43, and 500±49 ms, respectively. There were no differences in QTc values among the screened periods. Of 117 subjects, mutations were found in 50 of 69 (72%) screened and 23 of 48 (48%) clinical subjects (Table III in the Data Supplement). The prevalence of LQT1, LQT2, and LQT3 between the 2 groups was not different. LQTS-related mutations in the present study are summarized in Table IV in the Data Supplement.

Genetic Characteristics of Subjects

All mutations found in *KCNQ1* from 18 mutations in the screened and 9 mutations in the clinical groups were located in regions of a high probability of pathogenicity (Table 2; Figure 1A and 1B). In the screened group, 8 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 1 mutation was present in the subunit assembly domain. About 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 family members, including 4 (22%) with family history of sudden death in the screened group. Eight of 9 mutations in the clinical 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 6 (86%) of 7 mutations in the clinical group were located in regions of a high probability of pathogenicity (Table 2; Figure 2A and 2B). In the screened group, 1 mutation was both radical and present in the cyclic nucleotide–binding domain. Another 5 mutations were radical, and 1 each was present in Per-Arnt-Sim and Per-Arnt-Sim–associated C-terminal regions. However, only 4 (31%) of 13 mutations were associated with the presence of LQTS-related symptoms in probands or family members in the screened group. In the clinical group, 6 (86%) of 7 mutations were associated with the presence of symptoms in probands and family members, and the remaining mutation was found in a subject by ECG screening during a medical check-up at 1 month.

Discussion

Mutations in subjects with LQTS who were diagnosed by school-based ECG screening programs were mostly of high possibility of pathogenicity, similar to clinical subjects. Clinical background, such as QTc values, family history of LQTS, or sudden death, and follow-up periods, was not different between the 2 groups. However, prevalence of symptoms before and after diagnosis in the screened group was significantly lower when compared with the clinical group.

Table 2. Number of Patients With Mutations at High Risk in Each Group

Genes	Mutations	Screened Group	Clinical Group	*P* Value
*KCNQ1**	Radical mutation†	4	1	>0.99
	High probability‡	14	8	0.59
	Variants of uncertain significance	0	0	>0.99
KCNH2†	Radical mutation† and high probability‡	1	1	0.53
	Radical mutation†	5	1	0.66
	High probability‡	7	4	0.73
	Variants of uncertain significance	2	1	>0.99

*Variants of uncertain significance include mutations other than radical or of high probability of pathogenicity.

†Radical mutations include splice-site, nonsense, frame-shift, and insertion/deletions.

‡Mutations of high probability include subunit assembly domain, transmembrane/linker/pore, and C-terminal regions of *KCNQ1*, and the Per-Arnt-Sim (PAS) domain, PAS-associated C-terminal domain, the cyclic nucleotide–binding domain, transmembrane/linker/pore region of *KCNH2*.

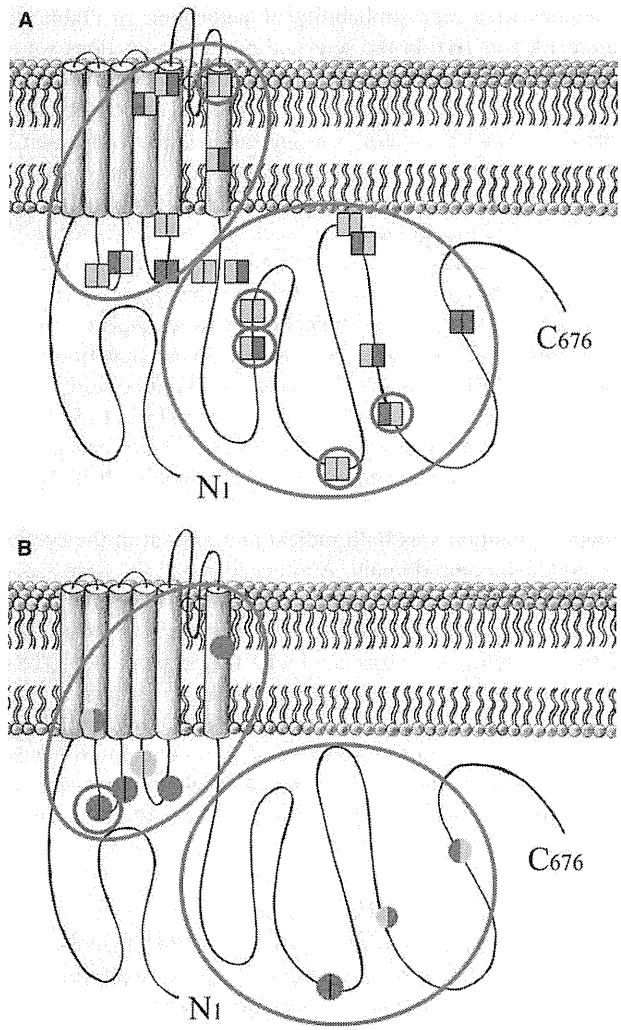

Figure 1. Topological depiction of *KCNQ1* in the present study in the screened (**A**) and clinical (**B**) groups. Mutations found in the screened group are shown as boxes (**A**) and those in the clinical group as circles (**B**). Each box or circle is divided into 2 parts: **left** and **right** sides. Each part represents the presence or absence of long-QT syndrome–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 subunit assembly domain. Two big purple circles symbolize locations of transmembrane/linker/pore and C-terminal regions of *KCNQ1*.

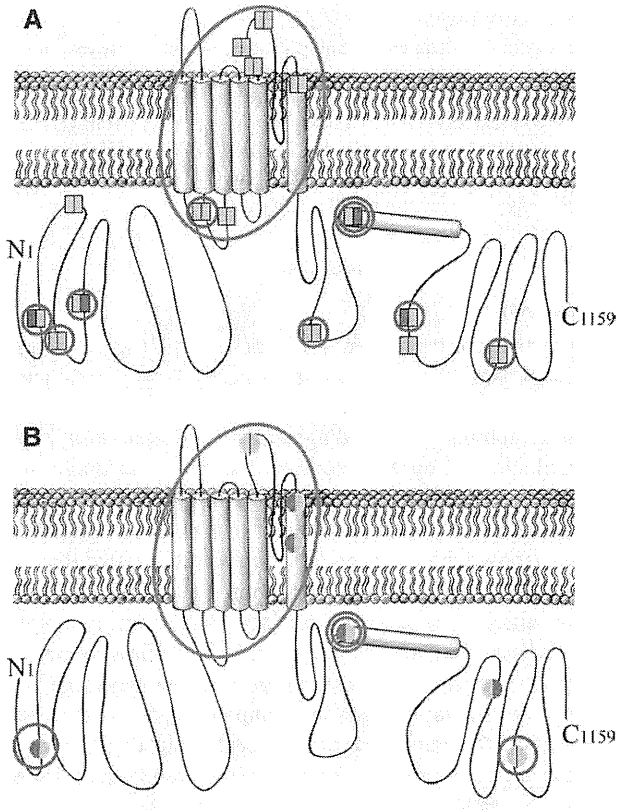

Figure 2. Topological depiction of *KCNH2* in the present study in the screened (**A**) and clinical (**B**) groups. Explanations of symbols and shapes are the same as in Figure 1. Bold blue circles surrounding mutations in this figure represent Per-Arnt-Sim (PAC), PAC-associated C-terminal, and cyclic nucleotide–binding domains, respectively, from the **left** side. A big purple circle symbolizes locations of transmembrane/linker/pore regions.

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 similar to clinical 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 on the genetic background of subjects who were diagnosed by ECG screening programs. Schwartz et al[12] reported that LQTS-related mutations were identified in 16 neonates of 43 080 who underwent neonatal ECG screening; 8 *KCNQ1*, 5 *KCNH2*, and 1 each of *KCNE1* and *KCNE2*. One infant had a digenic mutation of *KCNQ1* and *KCNH2*.

A school-based ECG screening program for heart diseases was initiated in 1994 for 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[13] reported that mutations were identified in 3 subjects with high or intermediate probabilities of LQTS using Schwartz criteria from 7961 school children; all 3 mutations were present in *KCNH2*. Yasuda et al[14] reported that *KCNQ1* mutations were found in 8 of 13 pediatric patients and that 7 of 8 patients were diagnosed by the ECG screening program.

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 clinical subjects. All 16 mutations in the *KCNQ1* gene in the screened group were radical or of high probability of pathogenicity similar to the clinical group. The ratio of mutations of radical and of high probability of pathogenicity in the *KCNH2* gene in the screened group (13/15; 87%) was remarkably similar to that in the clinical 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 when compared with clinical patients when ECG screening was developed in Japan.

Conversely, prevalence of symptoms before and after diagnosis was significantly lower in the screened group when compared with that in the clinical 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 patients with LQTS and their parents adopt changes to their lifestyles, for example, not doing vigorous exercise, not swimming a lap, and not diving,[28] in both the screened and clinical subjects. The precise reason remains to be clarified.

The reason for no difference in the prevalence of family history between the screened and clinical groups is unclear. The authors posit that even now in Japan the general population may not be familiar with LQTS, and that the parents in the present study did not think that syncope in their children was a serious condition. In addition, they may have been unaware that LQTS is an inherited disease. The reason of the high prevalence of family history of LQTS in the screened group is also unclear. The authors speculate that doctors did not ask the parents (grandparents of the probands in the present study) to perform familial studies 2 or 3 decades ago, when parents of the probands of the present study and their family members experienced symptoms at younger ages; however, no data were obtained addressing this hypothesis from the families.

There are some limitations of the current study. First, 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 and to determine the possibility of pathogenesis in patients with SCN5A in the future. Second, the clinical group showed a low rate (48%) of genotypic determination. We could not find mutations in 2 cases of death in the present study. The reasons for this are unclear. One potential reason was that we did not screen copy number variations in genes associated with LQTS.[29,30] Eddy et al[29] and Barc et al[30] reported that 3 of 26 (12%) and 3 of 93 (3%) unrelated mutation-negative probands showed copy number variations, indicating that some mutation-negative patients may have copy number variations. 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 had a high probability of pathogenicity similar to clinical subjects. Clinical backgrounds were not different although the prevalence of symptoms before and after diagnosis in the screened group was significantly lower when compared with that in the clinical 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 have follow-up equivalent to clinical patients.

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Disclosures

None.

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CLINICAL PERSPECTIVE

This study aimed to determine the genetic characteristics of 69 pediatric patients with long-QT syndrome who were diagnosed by a school-based screening program (screened group) and in whom genetic testing was performed. The screened group was compared with 48 subjects who visited hospitals because of the presence of long-QT syndrome–related symptoms, familial history, or who were diagnosed by chance (clinical group). A recently developed algorithm, designed to guide the interpretation of genetic testing results for *KCNQ1* and *KCNH2*, enabled us to classify the mutations as probably pathogenic or variant of uncertain significance. Using the algorithm, the authors found that of mutations yielded in *KCNQ1* or *KCNH2*, 31 of 33 (94%) mutations in the screened group and 15 of 16 (94%) mutations in the clinical group were radical and of high probability of pathogenicity. They also found that prevalence of symptoms before ($P<0.0001$) and after ($P=0.03$) diagnosis was significantly lower in the screened group when compared with that in the clinical group although the QTc values, family history of long-QT syndrome, sudden death, and follow-up periods were not different between the groups. Demand for genetic testing is now more prevalent in screened patients when compared with clinical patients because ECG screening was developed in Japan. This study may help to clarify the benefits of ECG screening. In addition, this study provides valuable genetic information and confirms that patients identified by ECG screening have the condition and are similar in many ways to those identified via a clinical setting.

A Kir3.4 mutation causes Andersen–Tawil syndrome by an inhibitory effect on Kir2.1

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Supplemental data at Neurology.org

ABSTRACT

Objective: To identify other causative genes for Andersen-Tawil syndrome, which is characterized by a triad of periodic paralysis, cardiac arrhythmia, and dysmorphic features. Andersen-Tawil syndrome is caused in a majority of cases by mutations in *KCNJ2*, which encodes the Kir2.1 subunit of the inwardly rectifying potassium channel.

Methods: The proband exhibited episodic flaccid weakness and a characteristic TU-wave pattern, both suggestive of Andersen-Tawil syndrome, but did not harbor *KCNJ2* mutations. We performed exome capture resequencing by restricting the analysis to genes that encode ion channels/associated proteins. The expression of gene products in heart and skeletal muscle tissues was examined by immunoblotting. The functional consequences of the mutation were investigated using a heterologous expression system in *Xenopus* oocytes, focusing on the interaction with the Kir2.1 subunit.

Results: We identified a mutation in the *KCNJ5* gene, which encodes the G-protein-activated inwardly rectifying potassium channel 4 (Kir3.4). Immunoblotting demonstrated significant expression of the Kir3.4 protein in human heart and skeletal muscles. The coexpression of Kir2.1 and mutant Kir3.4 in *Xenopus* oocytes reduced the inwardly rectifying current significantly compared with that observed in the presence of wild-type Kir3.4.

Conclusions: We propose that *KCNJ5* is a second gene causing Andersen-Tawil syndrome. The inhibitory effects of mutant Kir3.4 on inwardly rectifying potassium channels may account for the clinical presentation in both skeletal and heart muscles. **Neurology® 2014;82:1-7**

GLOSSARY

cRNA = complementary RNA; **LQT** = long QT; **SNP** = single nucleotide polymorphism; **SNV** = single nucleotide variant.

Periodic paralysis is a heterogeneous disorder caused by mutations in several ion channel genes, including sodium, calcium, and potassium channels.[1–3] Andersen–Tawil syndrome is a form of periodic paralysis that is characterized by a triad of periodic muscle weakness, cardiac arrhythmia, and dysmorphic features.[4,5] Although dominantly inherited, its phenotypes are highly variable and its penetrance is low.[6,7] The syndrome has been proposed as LQT7; however, the ECG features are distinct from those of classic forms of long QT (LQT) syndrome, i.e., characteristic TU patterns, including enlarged U waves, a wide TU junction, and a prolonged terminal T-wave downslope.[6,8]

KCNJ2 mutation, which encodes the Kir2.1 subunit, causes Andersen–Tawil syndrome.[9] Kir2.1 is predominantly expressed in the brain, heart, and skeletal muscles and forms an inwardly rectifying potassium channel via the homo- or heteromeric assembly of 4 Kir2.x subunits.[10] Most *KCNJ2* mutations cause loss of function or dominant-negative suppression of the inwardly rectifying

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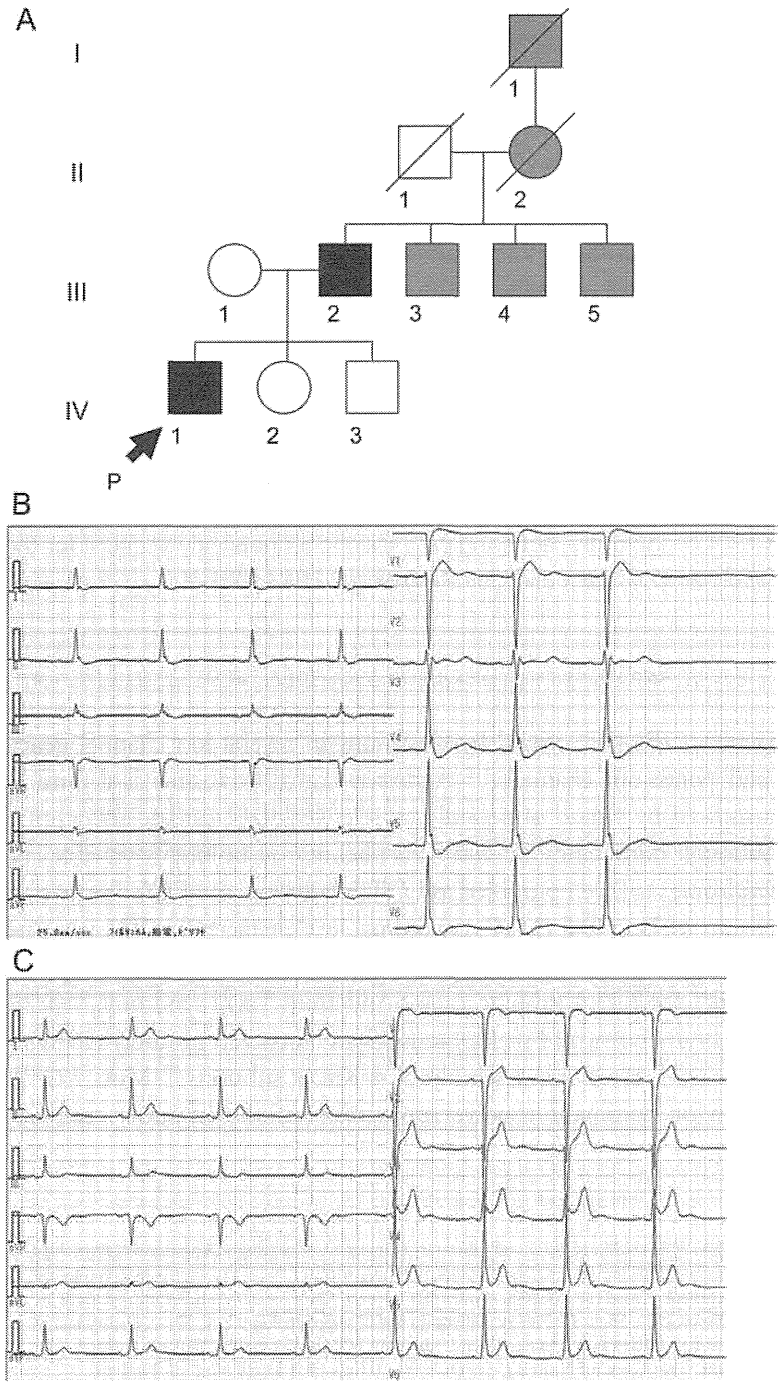
potassium current that stabilizes excitability during the terminal phase of repolarization in the heart and the resting membrane potential in skeletal muscle.[11]

Approximately 40% patients with Andersen–Tawil syndrome do not harbor mutations in *KCNJ2*, suggesting the presence of other causative genes.[12] We identified a patient with periodic paralysis and characteristic ECG features suggestive of Andersen–Tawil syndrome but failed to find mutations in *KCNJ2*. Exome capture resequencing analysis revealed a novel mutation in *KCNJ5*. We investigated the functional consequences of the mutation using a heterologous expression system in *Xenopus* oocytes to understand its pathomechanism.

Figure 1 **Pedigree of the family and ECG recordings of the proband**



(A) Pedigree of the family. Filled symbols represent affected and empty symbols represent unaffected individuals. Gray symbols represent patients with arrhythmia lacking detailed information. DNA was available only from the proband. ECG recordings of the proband during the attack of hypokalemic periodic paralysis (B) and at the interictal state (C). In addition to prominent U waves, atrial standstill was also observed during the paralytic attack (B).

METHODS **Patient.** The proband (IV-1 in figure 1A) was a 35-year-old man who experienced periodic episodes of paralysis with reduced serum potassium concentrations starting at the age of 31 years. The episodes occurred several times in a year and lasted from 1 to 10 days. During a severe attack, the weakness, which initially manifested in the legs, extended to the upper limbs within several days and then gradually improved. The proband showed an abnormal decrement in the amplitude of compound motor action potentials in the prolonged exercise test at the interictal state. He showed no dysmorphic features, but had a family history of arrhythmia (figure 1A). The clinical data of other family members were not available because of lack of consent. His father (III-2) underwent pacemaker implantation because of bradycardia/tachycardia syndrome with atrial fibrillation. Three uncles, a grandmother, and a great-grandfather, all on his father's side, had arrhythmia; however, the details of their conditions were not available. Within the knowledge of the proband, no other family members had experience of paralytic attacks. F1

The proband had no cardiac symptoms, but the ECG recorded during the paralytic attack with a serum potassium concentration of 2.0 mEq/L exhibited prominent U waves and possible sinus arrest (figure 1C, left). The U waves were consistently observed at normal potassium concentration (figure 1C, right). His thyroid function was normal and he did not show hypertension or increased plasma aldosterone levels, which ruled out primary hyperaldosteronism.

Standard protocol approvals, registrations, and patient consents. We obtained informed consent from the patient using protocols approved by the Institutional Ethics Review Board of Osaka University.

Sanger sequencing. Genomic DNA was extracted from blood leukocytes. The regions encompassing known causative mutations for periodic paralysis in *SCN4A* and *CACNA1S* and the entire coding region of *KCNJ2* and *KCNJ18* were amplified using either Platinum Taq DNA Polymerase High Fidelity (Life Technologies, Carlsbad, CA) or the Advantage-GC2 PCR kit (Clontech, Mountain View, CA) (primer sequences are listed in table e-1 on the *Neurology*® Web site at Neurology.org).[13] The purified fragments were sequenced using an automated DNA sequencer (Big Dye Terminator version 3.1 and ABI3100; Life Technologies).

Exome capture resequencing analysis. We enriched exonic regions of genomic DNA using the SureSelect Human All Exon v.2 kit (Agilent Technologies, Santa Clara, CA), which covers 44 mega base pairs of the human genome or 98.2% of the CCDS (Consensus Coding Sequence) database and sequenced 50 base pairs of each tag in a single direction using the SOLiD 4 system (Life Technologies). We obtained 80.0 $\times$ 10^6 tags of 50–base pair reads and mapped 57.7 $\times$ 10^6 tags (72.1%) to the hg19/GRCh37 human genome, which yielded a mean coverage of 53.2 on the targeted exome regions. Next, we removed multiple-aligned reads,