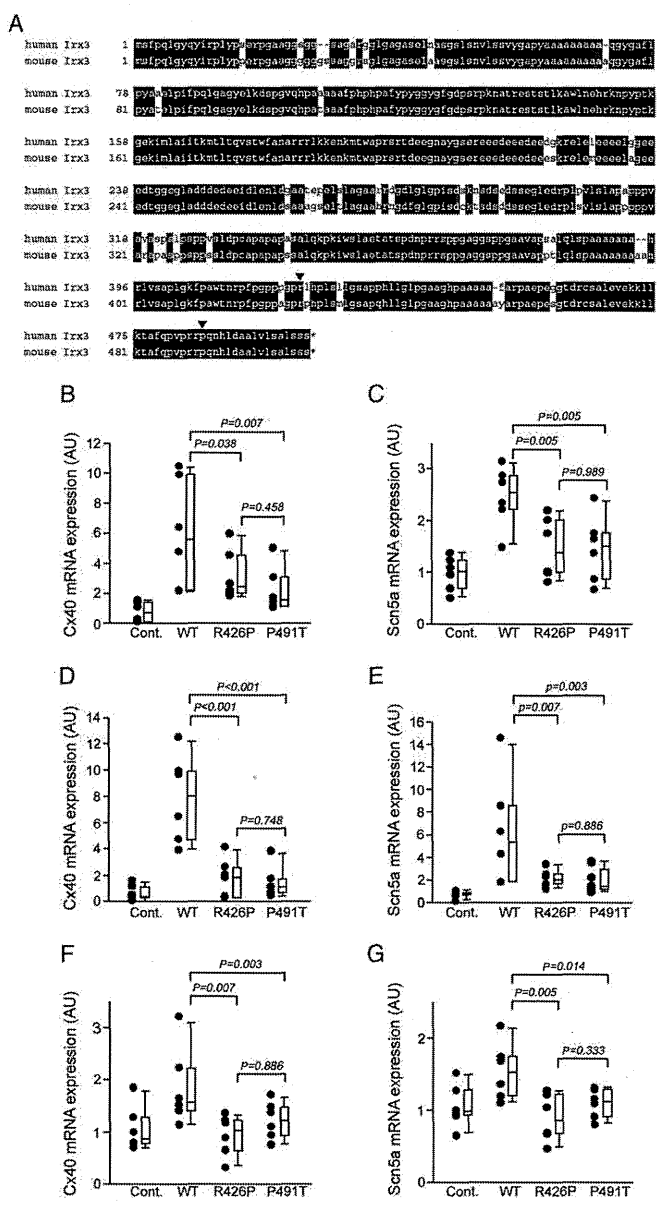

Figure 4 Irx3 mutations were less effective in up-regulation of Cx40 and Scn5a. (*A*) Homology of human IRX3 and murine Irx3. Amino acids conserved between human IRX3 and mouse Irx3 are shown by white letters in black box. Two missense mutation sites found in ventricular fibrillation patients in this study are shown by reverse triangles. (*B* and *C*) Effects of adenoviral infection with Irx3 into HL-1 cells on the expression of Cx40 (*B*) and Scn5a (*C*). The expression of Cx40 and Scn5a was normalized to that of Irx3. Adenoviral infection with wild-type Irx3 increased the expression of Cx40 and Scn5a. Up-regulation of Cx40 and Scn5a was significantly less in R426P ($n = 6$) and P491T ($n = 6$) infection than in wild-type Irx3 infection ($n = 6$). The data are presented actual plots beside the box whisker plot in these and following figures. (*D* and *E*) Effects of adenoviral infection with Irx3 into neonatal murine ventricular myocytes on the expression of Cx40 (*D*) and Scn5a (*E*). The expression of Cx40 and Scn5a was normalized to that of Irx3. Adenoviral infection with wild-type Irx3 into neonatal murine ventricular myocytes increased the expression of Cx40 and Scn5a. Up-regulation of Cx40 and Scn5a was significantly less in R426P ($n = 6$) and P491T ($n = 6$) infection than in wild-type Irx3 infection ($n = 6$). (*F* and *G*) Effects of transfection of HL-1 cells with Irx3 in pcDNA3 vector on the expression of Cx40 (*F*) and Scn5a (*G*). The expression of Cx40 and Scn5a was normalized to that of Irx3. Transfection of HL-1 cells with wild-type Irx3 increased the expression of Cx40 and Scn5a. Up-regulation of Cx40 and Scn5a was significantly less in R426P ($n = 6$) and P491T ($n = 6$) infection than in wild-type Irx3 transfection ($n = 6$).

exclusively affects the electrophysiological properties without morphological insults. Thus, the IRX3 mutation could be a genetic risk for VF in healthy hearts, or idiopathic VF.

Irx3$^{-/-}$ mice had previously shown to exhibit reduced expression of Scn5a and Cx40, and disturbed ventricular conduction represented by prolonged QRS duration.[5] In this report, arrhythmogenicity in *Irx3*$^{-/-}$ mice had not been reported.[5] We believe that their finding does not contradict with our finding, because in *Irx3*$^{-/-}$ mice in our study arrhythmias were also hardly detected during daytime, and they were detected mostly at night, during application of a sympathetic nerve β-receptor agonist, isoproterenol, during exercise, or in the acute phase of myocardial infarction. Thus, *Irx3*$^{-/-}$ mice are arrhythmogenic only when the sympathetic nervous system is activated.

The finding that the proband in the Family #1 exhibited type 1 Brugada-type ECG should be discussed with care. In the Family #1, R421P mutation was segregated with conduction disturbance (AV block, complete RBBB), but not with Brugada-type ECG. In general, arrhythmic events in Brugada syndrome occur when parasympathetic nerve activity is elevated,[12] whereas in our study the proband with Brugada-type ECG exhibited VF related to physical activities. We found no genetic defects in 13 proposed Brugada syndrome-related genes in human (*SCN5A*, *GPD1-L*, *CACNA1C*, *CACNB2*, *KCNE3*, *SCN1B*, *SCN3B*, *KCNJ8*, *MOG1*, *HCN4*, *KCND3*, *KCNE5*, and *SLMAP*). Augmented transient outward currents or diminished voltage-dependent Na^+ or Ca^{2+} currents are implicated in the ionic mechanism of Brugada-type ECG.[13,14] *Irx3*-null mouse exhibited no alterations in the expression of mRNAs encoding transient outward potassium channels (Kv4.2, Kv4.3 and KChIP2), or voltage-dependent Na^+ or Ca^{2+} channels (Sna5a and Cacna1c), in the ventricle (Supplementary material online, *Figures S8* and *S9*). Thus, the relation of VF with Brugada-type ECG is not currently identified.

Irx3 up-regulates the expression of Cx40 and Scn5a. The Irx family commonly acts as a negative regulator. Thus, it is reasonable to assume that the direct target of Irx3 could be some un-identified negative regulator, and that down-regulation of an un-known negative regulator by Irx3 could result in up-regulation of Cx40 and Scn5a. Overexpression of Irx3 with each of two mutations resulted in less up-regulation of Cx40 and Scn5a mRNA compared with overexpression of WT Irx3, suggesting that two IRX3/Irx3 mutations act in a loss-of-function manner.

In conclusion, IRX3 genetic defects and resultant functional perturbation in the His-Purkinje system are novel genetic risk factors of idiopathic VF, and would improve risk stratification and preventive therapy for SCD in otherwise healthy hearts, especially under the condition with elevated sympathetic nerve activity.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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REVIEW

Genetics of long-QT syndrome

Yukiko Nakano[1] and Wataru Shimizu[2]

Congenital long QT syndrome (LQTS) is an inherited arrhythmia syndrome characterized by a prolonged QT interval in the 12-lead ECG, torsades de pointes and not negligible prevalence of sudden cardiac death. The genetic testing plays an important role in the diagnosis of LQTS. A total of 15 genes have been reported for autosomal-dominant forms of Romano–Ward-type congenital LQTS and 2 genes for autosomal-recessive forms of the Jervell and Lange–Nielsen syndrome. In this review, we summarize the recent advances in genetics of LQTS and briefly describe forward perspectives of LQTS investigation.
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INTRODUCTION

Long-QT syndrome (LQTS) is characterized by a prolonged QT interval on 12-lead electrocardiograms (ECGs) that can progress to a polymorphic ventricular tachycardia (VT) known as torsades de pointes. Clinically, torsades de pointes can produce syncope, ventricular fibrillation or even sudden cardiac death. The prevalence of congenital LQTS is reportedly 1 in 2000.[1] In this review, we focus on the advances in our understanding of the genetics of LQTS.

CRITERIA FOR DIAGNOSIS

The Schwartz score[2] is used to diagnose congenital LQTS (Table 1). Patients with a Schwartz score $\geqslant 3.5$ points in the absence of a secondary cause for QT prolongation are diagnosed with LQTS. Recently, in 2013, an expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes was published by the Heart Rhythm Society, the European Heart Rhythm Association and the Asia Pacific Heart Rhythm Society.[3] They reported that congenital LQTS should be diagnosed when the following criteria are fulfilled:

(1) An LQTS risk score $\geqslant 3.5$ without a secondary cause for QT prolongation.
(2) An unequivocally pathogenic mutation in one of the LQTS genes.
(3) In the presence of a corrected QT interval (QTc) $\geqslant 500$ ms on repeated 12-lead ECGs using Bazett's formula in the absence of a secondary cause for QT prolongation.

They adjunctively mentioned that LQTS can be diagnosed when the QTc is between 480 and 499 ms on repeated 12-lead ECGs in patients with unexplained syncope, without a secondary cause for QT prolongation, and in the absence of a pathogenic mutation.

As just described, the genetic testing is included in the diagnosis criteria and has an important role in the diagnosis of LQTS.

GENETICS

The three main types of LQTS and genetic testing

A total of 15 genes have been reported in autosomal dominant forms of Romano–Ward-type congenital LQTS (Table 2). Moreover, most of the genetic abnormalities identified thus far appear to prolong the duration of action potentials by decreasing the potassium current (loss-of-function mutation) or increasing the sodium or calcium current (gain-of-function mutation), resulting in clinical QT prolongation on the ECG.

Between 1995 and 1996,[4–6] three major causative genes were recognized for LQTS and associated with LQTS types 1–3: *KCNQ1*-encoding Kv7.1 (for LQT1), *KCNH2* encoding Kv11.1 (for LQT2) and *SCN5A* encoding Nav1.5. Napolitano *et al.*[7] reported that they identified 235 different mutations in 310 (72%) of 430 probands (49% KCNQ1, 39% KCNH2 and 10% SCN5A). In Japan, Shimizu *et al.*[8] reported the three major genes constituted more than 80% of total genotyped patients with LQTS. According to the Heart Rhythm Society/European Heart Rhythm Association Expert Consensus Statement on the State of Genetic Testing for the Channelopathies and Cardiomyopathies, abnormalities of the three LQTS-associated genes are detected in ~75% of clinically definite LQTS, with rates of 30%–35%, 25%–40% and 5%–10% for LQTS types LQT1, LQT2 and LQT3, respectively.[9] LQTS genetic testing contributes to not only diagnosis but also gene-specific and mutation-specific risk stratification and patient management. They recommended that comprehensive or specific genetic testing for *KCNQ1*, *KCNH2* and *SCN5A* be performed for any patient who fulfills the following criteria:[9]

(1) where a cardiologist has established a strong suspicion for LQTS based on clinical examination,
(2) where a patient has asymptomatic QT prolongation in the absence of other clinical conditions that may prolong the QT interval,

[1]Programs for Biomedical Research Graduate School of Biomedical Science, Division of Frontier Medical Science, Department of Cardiovascular Medicine, Hiroshima University, Hiroshima, Japan and [2]Department of Cardiovascular Medicine, Graduate School of Medicine, Nippon Medical School, Tokyo, Japan
Correspondence: Dr Y Nakano, Programs for Biomedical Research Graduate School of Biomedical Science, Division of Frontier Medical Science, Department of Cardiovascular Medicine, Hiroshima University, 1-2-3,Kasumi, Minami-ku, Hiroshima 7348551, Japan.
E-mail: nakanoy@hiroshima-u.ac.jp
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Table 1 Diagnosis of long QT syndrome

Parameters	*Points*
Electrocardiographic findings[a]	
A) QTc time[b]	
⩾480 ms	3
460–479 ms	2
450–459 ms (male)	1
B) Four-minute recovery QTc after exercise test ⩾480 ms	1
C) Torsade de pointes[c]	2
D) T-wave alternance	1
E) Notched T wave	1
F) Low heart rate for age[d]	0.5
Clinical manifestations	
A) Syncope[c]	
With stress	2
Without stress	1
B) Congenital deafness	0.5
Family history	
A) Family members with definite LQTS[e]	1
B) Unexpected sudden cardiac death at age <30 years in family members	0.5

Abbreviation: LQTS, long QT syndrome.
⩾3.5 points, diagnosed as LQTs; 1.5–3 points, suspect of LQTs; ⩽1 point, unlikely LQTS.
[a]In the absence of medications or disorders known to affect these electrocardiographic features.
[b]QTc calculated by Bazett's formula where $QTc = QT/\sqrt{RR}$.
[c]Mutually exclusive.
[d]Resting heart rate below the second percentile for age.
[e]The same family member cannot be counted in A and B.

(3) where a patient is asymptomatic, with QTc values >460 ms (prepuberty) or >480 ms (adults) on serial 12-lead ECGs and
(4) when an LQTS-causative mutation is identified in an index case, mutation-specific genetic testing is recommended for the family members.

Risk stratification of LQTS using the genetic information

In 600 patients with LQT1, Moss *et al.*[10] demonstrated that those with mutations in the transmembrane region of Kv7.1, those with missense mutations and those with mutations resulting in dominant-negative ion currents had greater risk of arrhythmic events than those with other mutations. In a Japanese multicenter study, Shimizu *et al.*[11] also reported that patients with LQT1 and transmembrane mutations are at a higher risk of cardiac events and had a greater sensitivity to sympathetic stimulation than those with C-terminal mutations. Subsequently, Barsheshet *et al.*[12] demonstrated that patients with C-loop missense mutations in the *KCNQ1* gene exhibited a high risk for life-threatening events, and that β-blocker therapy was effective for them.

Regarding LQT2, Shimizu *et al.*[13] demonstrated in 858 patients that missense mutations in the transmembrane pore region are associated with significantly higher rates of cardiac events than are other missense mutations. Recently, Liu *et al.*[14] also reported that a trafficking-deficient mutation in the transmembrane non-pore region of Kv11.1 causes a dominant-negative effect and a severe clinical course.

Patients with LQTS having both the pathogenic variants and a QTc>500 ms are also at high risk, in particular when they are symptomatic. In contrast, the asymptomatic genetically diagnosed LQTS patients are evaluated at lower risk. An important risk factor for these patients comes from drugs that block the I_{Kr} current and conditions that lower their plasma potassium level.[3]

Table 2 Genes associated with the long QT syndrome

LQTS type	*Gene*	*Protain*	*Current*
Romano–Ward syndrome			
LQT1	*KCNQ1*	Kv7.1	I_{Ks} ↓
LQT2	*KCNH2*	KV11.1	I_{Kr} ↓
LQT3	*SCN5A*	Nav1.5	I_{Na} ↑
LQT4	*Ankyrin-B*	Ankyrin	Na+/K+ ATPase and so on
LQT5	*KCNE1*	MinK	I_{Ks} ↓
LQT6	*KCNE2*	MiRP1	I_{Kr} ↓
LQT7	*KCNJ2*	Kir2.1	I_{K1} ↓
LQT8	*CACNA1C*	CaV1.2	$I_{Ca\text{-}L}$ ↑
LQT9	*CAV3*	Caveolin3	I_{Na} ↑
LQT10	*SCN4B*	SCNβ4subunit	I_{Na} ↑
LQT11	*AKAP-9*	Yotiao	I_{ks} ↓
LQT12	*SNTA1*	Syntrophin-α1	I_{Na} ↓
LQT13	*KCNJ5*	Kir3.4	I_{KACh} ↓
LQT14	*CALM1*	Calmodulin1	Disorder of calcium signaling
LQT15	*CALM2*	Calmodulin2	
Jervell and Lange–Nielsen syndrome			
JLN1	*KCNQ1*	Kv7.1	I_{Ks} ↓
JLN2	*KCNE1*	MinK	I_{Ks} ↓

Genome-wide association study about QT times

In 2009, two genome-wide association studies of QT intervals, the QTGEN and QTSCD, have been reported in *Nature Genetics*.[15,16] Common single-nucleotide polymorphisms in *NOS1AP*, *KCNQ1*, *KCNH2*, *SCN5A*, *KCNJ2* and *RNF207* were detected and the genes were reported as candidates for ventricular arrhythmias and sudden cardiac death. Earle *et al.*[17] demonstrated that the single-nucleotide polymorphisms reported in *NOS1AP* and *KCNQ1*, which affect the QT interval, were also associated with an increased risk of cardiac events in patients with LQTS. Recently, they reported that single-nucleotide polymorphisms of *NOS1AP* increased the risk of cardiac events in patients with LQT2 and they also reported regarding the link between the *KCNQ1* single-nucleotide polymorphism and the *KCNH2* mutations.[18] Other researchers have tried to clarify the drug–gene interactions that influence the QT interval, but numerous problems remain unresolved.[19]

The other genes beyond the three common LQTs

Beyond the three common LQTS gene variants, several mutations encoding ion-channel subunits, except for those associated with LQT4, LQT9, LQT11, LQT12, LQT14 and LQT15, have also been found. Notably, mutations in *KCNQ1* (that is, JLN1, also associated with LQT1) and *KCNE1* (that is, JLN2, also associated with LQT5) have also been found to be causal in autosomal-recessive forms of the Jervell and Lange–Nielsen syndrome attributable to a decrease in the *Iks*. They are accompanied by neurosensorial deafness and a markedly prolonged QT interval[19] (Table 2).

Mutations in the potassium channel genes

The LQTS types associated with slowly activating delayed rectifier potassium current (I_{Ks}) dysfunction include LQT1, LQT5, LQT11, JLN1 and JLN2 (Table 2), although most are associated with LQT1. Mutations in *KCNE1*, which are associated with LQT5, cause defective trafficking of the I_{Ks} channel, reduce amplitude of the I_{Ks} current and influence disease pathogenesis.[20] A mutation in *AKAP9*, which is associated with LQT11, has been shown to reduce the interaction

between Kv7.1 and A-kinase anchor protein 9, reduce the cyclic AMP-induced phosphorylation of the channel, eliminate the functional response of the I_{Ks} channel to cyclic AMP and prolong the action potential in a computational model of the ventricular cardiomyocyte.[21]

The LQTS types associated with rapidly activating delayed rectifier potassium current (I_{Kr}) dysfunction include LQT2 and LQT6, with the former accounting for the majority of cases. Most of the mutations in *KCNH2* disrupt the maturation and trafficking process before reducing the number of functional ion channels at the cell surface membrane.[22] However, mutations of *KCNE2* (the β-subunit of Kv11.1), which are associated with LQT6, have been reported to modulate Kv11.1 channel gating and currents, and to be proarrhythmic.[23]

Mutations in the sodium channel genes

The LQTS types with dysfunctional late-activating sodium channels (I_{Na}) include LQT3, LQT9, LQT10 and LQT12, although most are LQT3. Notably, numerous mutations have been characterized as leading to or predisposing to LQT3. In addition, mutations of Nav1.5 have been linked to a variety of cardiac diseases such as LQTS, Brugada syndrome, cardiac conduction defects, atrial fibrillation and dilated cardiomyopathy.[24,25] Phenotypic overlap of LQT3 with Brugada syndrome is also observed in some carriers of *SCN5A* mutations.[26] For example, the α-subunit of Nav1.5 interacts with several regulatory proteins and the mutations of these genes cause disease related to sodium-channel dysfunction.[27] *SCN4B* encodes the β-subunit of the sodium channel that is critical to the regulation of sarcolemmal expression and the gating of Nav1.5. An *SCN4B* mutation associated with LQT10 has been shown to increase the persistence of the I_{Na} with a positive shift toward inactivation.[28] Caveolin-3 acts in conjunction to increase peak I_{Na} through a cyclic AMP-independent pathway; a *CAV3* mutation associated with LQT9 subsequently results in QT prolongation only during β-blocker therapy.[29] *SNTA1* is associated with LQT12, which encodes α-1-syntrophin, and is associated with the Nav1.5 channel as part of the neuronal nitric oxide synthesis complex. A mutation in *SNTA1* is associated with an increase in both the peak and the persistence of the I_{Na} due to a change in the binding of the PDZ domain.[30]

Mutations in other genes

A type of LQTS that is associated with a mutation in *KCNJ2* is LQT7, which creates a condition known as Andersen–Tawil syndrome. This is characterized by a triad of periodic paralysis, a long QU interval associated with ventricular arrhythmias and skeletal development anomalies. In this condition, a reduction in Kir2.1 due to the *KCNJ2* mutation prolongs the terminal phase of the cardiac action potential. Because of the reduced extracellular potassium levels, sodium/calcium exchanger-dependent delays after depolarization are induced, resulting in spontaneous arrhythmias.[31] The common clinical characteristics of LQT7 and cathecolaminergic polymorphic VT (CPVT), such as biphasic premature ventricular contractions, make diagnosis difficult. Several factors can assist in the differential diagnosis: T-U patterns (i.e. prolonged terminal T downslope, wide T-U junction, and biphasic and enlarged U waves), relatively slow polymorphic or biphasic VT and frequent ventricular ectopic beats at rest may be useful in distinguishing LQT7 from CPVT. After KCNJ2 mutations were identified in patients with CPVT phenotype, differential diagnosis between LQT7 and CPVT by genotyping became more challenging.[32]

The most severe phenotypic form of LQTS is Timothy syndrome (LQT8). This is associated with point mutations in *CACNA1C*, which cause slowed inactivation of CaV1.2 that increase the influx of calcium, prolong the cardiac action potential and promote lethal arrhythmias. Timothy syndrome is quite rare because of the fatal phenotype and multisystem manifestations, including congenital heart disease, syndactyly, immunodeficiency, cognitive abnormalities and autism.[33] Recently, mild LQT8 cases with *CACNA1C* mutation without phenotype of Timothy syndrome were reported.[34,35]

Crotti *et al.*[36] performed exome sequencing in infants with recurrent cardiac arrest and dramatically prolonged QTc intervals, discovering heterozygous *de novo* mutations in *CALM1* and *CALM2* encoding calmodulin (that is, LQT14 and LQT15, respectively). In addition, Makita *et al.*[37] reported the presence of *CALM1* and *CALM2* mutations in LQTS probands by next-generation sequencing approaches. They revealed that these calmodulin mutations disrupted calcium-ion binding to the protein and were associated with LQTS and with overlapping features of LQTS and CPVT.

FUTURE PERSPECTIVES

Numerous ion-channel mutations have been reported and various approaches have been used to confirm the associated functional change, including expression models (using human embryonic kidney cells, Chinese hamster ovary cells or *Xenopus* oocytes), experimental mouse models, computational approaches, neonatal mouse cardiomyocytes and induced pluripotent stem cells.[38,39] The assessment of gene expression with induced pluripotent stem cells is useful when confirming drug efficacy, in particular in cases with complex genotypes.[40,41]

Furthermore, gene analysis techniques have advanced remarkably. As previously mentioned, we can obtain significant amount of data using next-generation sequencing approaches, exome analysis[42] and genome-wide association studies. Next-generation sequencing technology allows a comprehensive genetic analysis of LQTS such as a copy number variation.[43] Whole-exome sequencing is efficient to elucidate the underlying genetic mechanism of diseases. However, an investigator often faces difficulties to identify the possible disease causative variant among hundreds of variants per patient DNA sample and it takes up a lot of energy. We have to identify potential candidate genes by considering genotype–phenotype association, the relationship between genotype and disease development of each family member, publicly available internet-based gene-prioritization tools, *in silico* variant annotation prediction and functional studies.[44,45]

In addition, we can use the large bioinformatics data registries of genome browsers, such as HapMap (http://hapmap.ncbi.nlm.nih.gov/), NCBI (http://www.ncbi.nlm.nih.gov/), UCSC (https://genome.ucsc.edu/), 1000 genome data (http://www.1000genomes.org/) and recently publicly available database, the ExAC browser, which contains exome sequencing data on up to 60 000 individuals (http://exac.broadinstitute.org/).

A recent epochal report demonstrated that rapid whole-genome sequencing could be performed with a speed-optimized bioinformatics platform in LQTS patients that could provide comprehensive diagnostic information at 10 days of life. Their approach was certified by the Clinical Laboratory Improvement Amendments.[46] The goal of identifying the genetic basis of the disease is to individualize and optimize treatment strategies. Finally, it is important to identify and profile the relatives of LQTS probands to identify their risk. In a review article reported by Semsarian *et al.*[47], in sudden cardiac death cases where no cause of death is identified at post mortem, genetic testing of post-mortem blood in a specialized multidisciplinary clinic setting may identify a cause of death in up to 30%. The ultimate goal is

to prevent future adverse clinical outcomes and sudden cardiac death (SCD) events in surviving relatives.

Porta *et al.*[48] reported that patients with higher sympathetic control of the QT interval and reduced vagal control of the heart rate were at lower risk of complications than other patients with LQT1 carrying the same mutation. Myerburg[49] has also stated that gene expression was modulated by physiological fluctuations, drug and electrolytes, and environmental factors, and that we must consider the role of epistasis with other modifying gene variants.

Thus, both genomic and non-genomic factors are important when diagnosing congenital ion-channel diseases and the need to advance genetic analysis is therefore apparent.

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Genotype-dependent differences in age of manifestation and arrhythmia complications in short QT syndrome☆

Daniel Toshio Harrell [a], Takashi Ashihara [b], Taisuke Ishikawa [a], Ichiko Tominaga [a,1], Andrea Mazzanti [c], Kazuhiro Takahashi [d], Yasushi Oginosawa [e], Haruhiko Abe [f], Koji Maemura [g], Naokata Sumitomo [h], Kikuya Uno [i], Makoto Takano [j], Silvia G. Priori [c,k], Naomasa Makita [a,*]

[a] Department of Molecular Physiology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[b] Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Heart Rhythm Center, Shiga, Japan
[c] Molecular Cardiology, IRCCS Salvatore Maugeri Foundation, Pavia, Italy
[d] Department of Pediatric Cardiology, Okinawa Children's Medical Center, Okinawa, Japan
[e] Second Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan
[f] Department of Heart Rhythm Management, University of Occupational and Environment Health, Kitakyushu, Japan
[g] Department of Cardiovascular Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[h] Department of Pediatric Cardiology, Saitama International Medical Center, Hidaka, Japan
[i] Sapporo Heart Center, Sapporo, Japan
[j] Department of Physiology, Kurume University School of Medicine, Kurume, Japan
[k] Department of Molecular Medicine, University of Pavia, Pavia, Italy

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ABSTRACT

Background: Short QT syndrome (SQTS) is a rare inheritable arrhythmia, associated with atrial and ventricular fibrillations, caused by mutations in six cardiac ion channel genes with high penetrance. However, genotype-specific clinical differences between SQTS patients remain to be elucidated.
Methods and results: We screened five unrelated Japanese SQTS families, and identified three mutations in *KCNH2* and *KCNQ1*. A novel mutation *KCNH2*-I560T, when expressed in COS-7 cells, showed a 2.5-fold increase in peak current density, and a positive shift (+14 mV) of the inactivation curve compared with wild type. Computer simulations recapitulated the action potential shortening and created an arrhythmogenic substrate for ventricular fibrillation. In another family carrying the mutation *KCNQ1*-V141M, affected members showed earlier onset of manifestation and frequent complications of bradyarrhythmia. To determine genotype-specific phenotypes in SQT1 (*KCNH2*), SQT2 (*KCNQ1*), and other subtypes SQT3–6, we analyzed clinical variables in 65 mutation-positive patients among all the 132 SQTS cases previously reported. The age of manifestation was significantly later in SQT1 (SQT1: 35 ± 19 years, n = 30; SQT2: 17 ± 25 years, n = 8, SQT3–6: 19 ± 15 years, n = 15; $p = 0.011$). SQT2 exhibited a higher prevalence of bradyarrhythmia (SQT2: 6/8, 75%; non-SQT2: 5/57, 9%; $p < 0.001$) and atrial fibrillation (SQT2: 5/8, 63%; non-SQT2: 12/57, 21%; $p = 0.012$). Of 51 mutation-positive individuals from 16 SQTS families, nine did not manifest short QT, but exhibited other ECG abnormalities such as atrial fibrillation. The resulting penetrance, 82%, was lower than previously recognized.
Conclusion: We propose that SQTS patients may exhibit different clinical manifestations depending upon their genotype.

1. Introduction

Short QT syndrome (SQTS)[2] is a rare, inheritable cardiac electrical disease characterized by a shortened corrected QT interval (QTc)[3] and associated with a risk of sudden cardiac death (SCD),[4] ventricular fibrillation (VF), and atrial fibrillation (AF), but without structural abnormalities [1,2]. SQTS is characterized by accelerated cellular repolarization, owing to either an enhanced outward repolarizing potassium current or a reduced inward depolarizing calcium current. To date, mutations responsible for SQTS have been identified in six ion channel

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* Corresponding author at: Department of Molecular Physiology, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan.
E-mail address: makitan@nagasaki-u.ac.jp (N. Makita).

[1] Current address: NTT Medical Center Tokyo, Tokyo, Japan.
[2] SQTS: short QT syndrome.
[3] QTc: corrected QT interval.
[4] SCD: sudden cardiac death.

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genes: *KCNH2* (SQT1) [3], *KCNQ1* (SQT2) [4], *KCNJ2* (SQT3) [5], *CACNA1C* (SQT4) [6], *CACNB2* (SQT5) [6], and *CACNA2D1* (SQT6) [7]. Previous reports suggest that the penetrance of these mutations is extremely high in families with SQT1, SQT2, and SQT3 [8,9].

Congenital long QT syndrome (LQTS)[5] is another repolarization disorder, for which mutations in 13 genes have been reported. Extensive genetic studies over two decades have improved the risk stratification and management of patients with LQTS [10,11]. In contrast, much less information is available about the genotype–phenotype correlations and natural history of SQTS. A cohort study of the European SQTS registry has shown that *KCNH2* mutation carriers (SQT1) show significantly shorter QT intervals and better response to hydroquinidine therapy as compared with non-SQT1 patients [12]. Conversely, a unique clinical phenotype characterized by neonatal AF and bradycardia was reported in SQT2 with the *KCNQ1* mutation V141M [13]. These studies suggest that SQTS may have some genotype-specific characteristics; however, because of the small number of cases studied to date, a robust genotype–phenotype correlation has not been identified.

By genetic screening of five unrelated Japanese SQTS probands, we found three mutations including a novel *KCNH2* mutation I560T, and previously reported mutations *KCNH2*-T618I [14] and *KCNQ1*-V141M [15]. SQT2 patients carrying the *KCNQ1*-V141M mutation exhibited earlier onset and frequent complications of bradyarrhythmia, a clinical scenario similar to that previously reported [13]. To explore the potential genotype-specific phenotypes underlying SQTS, we included 32 previously reported genotyped SQTS families [6–9,12–19], and further analyzed their clinical variables. We found that SQT1 patients exhibit later onset of manifestation and SQT2 patients have a higher prevalence of bradyarrhythmias and AF, implying that clinical characteristics of SQTS can differ by genotype as seen in LQTS.

2. Methods

2.1. Study cohorts

We studied five unrelated Japanese families consisting of 10 affected family members. The QT interval was corrected for heart rate using Bazett's equation ($QTc = QT/(\sqrt{RR})$). The diagnosis of SQTS was made based on $QTc \leq 330$ ms or $QTc < 360$ ms with presence of VF episode, pathogenic mutations, or family history of SQTS or SCD before age of 40 years [20]. All individuals who participated in the study gave written informed consent prior to genetic and clinical investigations in accordance with the standards of the Declaration of Helsinki and the local ethics committees at each participating institution. To further characterize the genotype-specific characteristics, we created an additional cohort, which consisted of 132 SQTS patients including five families (10 cases) from the current study, 33 families (61 cases) from a review by Gollob et al. [8], and an additional 36 SQTS families (61 cases) that have been previously reported and available on PubMed as of May 2014 [6–9,12–19] (Supplemental Table S1). A full description of the methods is available as supplementary information.

2.2. Genetic analysis

Genomic DNA was extracted from the blood by a standard protocol. All the exons of *KCNH2*, *KCNQ1*, and *KCNJ2* were PCR amplified as previously described [21–23] (Supplemental Table S2). Direct sequencing was performed using the ABI Genetic Analyzer 3130 (Life Technologies, Carlsbad, CA).

[5] LQTS: long QT syndrome.

2.3. Biophysical analysis of KCNH2-I560T

Site-directed mutagenesis was performed using the QuikChange site-directed mutagenesis kit (Agilent Technologies, Santa Clara CA) on human *KCNH2* cDNA cloned in an expression plasmid pcDNA3.1 (Life Technologies). Oligonucleotide sequences are available in Supplemental Table S2. The COS-7 cell line was transiently transfected with wild-type (WT) or I560T-*KCNH2* plasmid, and the potassium current was recorded by whole-cell patch-clamp techniques as previously described [24] with modifications.

2.4. Western blotting

To test the cell surface expression and the glycosylation of the I560T mutant channel protein, we carried out Western blotting analysis using WT channel and a neighboring LQTS mutation A561V as a control which is characterized by the membrane trafficking defect [25]. HeLa cells were transfected with *KCNH2* plasmid of either WT, I560T, or A561V and the proteins extracted from the cell lysate were subjected to Western blotting as described previously [26]. Anti-*KCNH2* polyclonal antibodies (1:400, Life Technologies) and anti-β actin polyclonal antibodies (1:200, Cell Signaling Technology, Danvers, MA) were used as primary antibodies, and the signals were visualized by ECL Prime Detection Reagent (GE Healthcare).

2.5. Computer simulation of action potential shortening and inducibility of lethal ventricular arrhythmia in KCNH2-I560T

Computer simulations were carried out to determine whether the gating abnormalities and the increased current density of the *KCNH2*-I560T are sufficient to cause shortening of the action potential duration (APD)[6] and QT interval. Membrane kinetics were represented by the O'Hara-Rudy dynamic human ventricular model with modified Markovian I_{Kr} equations [27–29], and the pseudo-ECG was calculated as described previously [30]. To demonstrate the relative arrhythmogenicity of *KCNH2*-I560T, we conducted simulations of VF in the bidomain endocardial sheet, and an S1–S2 cross-field protocol was applied to induce a spiral wave reentry.

2.6. Clinical characteristics of SQTS with different genotypes

In the study cohort of 132 SQTS patients, 65 patients were mutation-positive (6 from the current study and 59 from previous reports [6–9, 12–19]) of whom 30 (46%) were male and had a mean age of manifestation of 28 ± 20 years (ranging from 0 to 72). Clinical variables for each reported patient were extracted for sex, age of manifestation, QT, QTc, heart rate (HR), causative gene and mutation, and clinical history: AF, SCD/cardiac arrest, palpitations/syncope, and sick sinus syndrome/bradycardia. Bradycardia for adult patients was evaluated based on $HR < 50$ bpm. For children, bradycardia was determined based on $HR < 100$ bpm for ages 0 to 3 years and on $HR < 60$ bpm for ages 3–9 years [31]. A patient was considered symptomatic in the presence of the aforementioned clinical episodes.

2.7. Statistical analysis

Data are reported as mean ± SD and analyzed by one-way ANOVA with Bonferroni correction. The univariate clinical variables are presented as percentages, and analyzed by χ^2-test or Fisher exact test. Statistical significance was set at $p < 0.05$.

[6] APD: action potential duration.

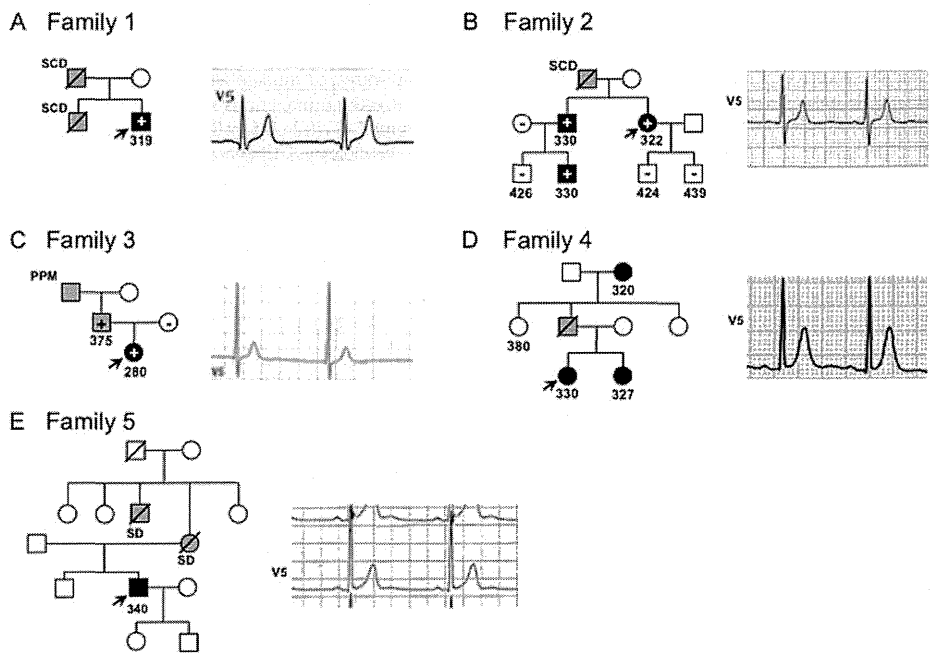

Fig. 1. SQTS family pedigrees and proband's ECG. The arrow indicates the proband of each family (A)–(E). Numbers under the symbols are QTc (ms). Closed boxes (male) and circles (female) indicate phenotype-positive SQTS patients. Gray and open symbols mean suspected and unaffected, respectively. Plus and minus indicate mutation carrier (heterozygous) and non-carrier, respectively. SCD: sudden cardiac death, SD: sudden death, PPM: permanent pacemaker. V5 lead ECG of each proband is shown.

3. Results

3.1. Case presentation and genetic analysis of SQTS families (Figs. 1 and 2 and Table 1)

3.1.1. Family 1

A 64 year-old man experienced palpitations and near syncope due to paroxysmal AF and atrial flutter, for which he underwent catheter ablation (Fig. 1A). After the catheter ablation, short QT (QTc = 319 ms, HR = 68 bpm) with peaked T waves on the precordial leads became manifested, and he was diagnosed with SQTS (Supplemental Fig. S1). His father and brother died suddenly from unknown causes. Genetic screening revealed a novel heterozygous missense mutation in exon 7 of *KCNH2* (c.1679T>C), resulting in the amino acid substitution I560T, located in transmembrane segment S5 (Fig. 2A). *KCNH2*-I560T was absent in the genomic DNA of 200 healthy Japanese individuals, and in the public databases: dbSNP; 1000 Genomes project; Exome Variant Server; and Human Genetic Variation Database. The amino acid residue I560 is 100% conserved among eight different species (Fig. 2B). The proband has no offspring, and declined insertion of an implantable cardioverter defibrillator (ICD).

3.1.2. Family 2

A 39-year-old woman with aborted VF was diagnosed with SQTS (QTc = 322 ms, HR = 74 bpm) and had an ICD implanted (Fig. 1B, Supplemental Fig. S1, B). Combined prescription of bepridil with bisoprolol successfully prolonged her QTc to 341 ms, and she has been free from VF attack for 2 years. Her father died suddenly from unknown causes at the age of 40 years and her brother and nephew exhibited short QTc (330 ms). Genetic screening identified a heterozygous missense mutation T618I (c.1853C>T) located in the pore region. This mutation was detected in all affected members (Fig. 2C). This mutation was previously reported [14].

3.1.3. Family 3

The index patient, a 10-year-old girl, was diagnosed with congenital sick sinus syndrome (SSS)[7] due to fetal bradycardia (HR = 72 bpm) at the gestational age of 22 weeks (Fig. 1C). She was born by cesarean section at the age of 37 weeks, and exhibited sinus bradycardia (HR = 50 bpm) with bradycardia-induced heart failure, although echocardiography showed no organic heart diseases. As a result of a complete atrioventricular block at the age of 12 days, a permanent pacemaker was implanted. At 4 years of age, the patient demonstrated severe QT shortening (QTc = 280 ms) with bradycardia (HR = 64 bpm) (Supplemental Fig. S1, C). Genetic screening revealed a heterozygous missense mutation V141M (c.421G>A) of *KCNQ1* located in transmembrane segment S1 (Fig. 2D). This mutation was previously reported [15]. Her father had experienced chronic AF with bradycardia since the age of 3 years, but recorded a QTc value of 375 ms (HR = 37 bpm) by ECG, which is outside the diagnostic criteria of SQTS (Supplemental Fig. S1, D) [20]. Interestingly, despite this, he carried the V141M mutation. Her paternal grandfather had a pacemaker implanted at the age of 50 years, but declined genetic testing.

3.1.4. Family 4

A 17-year-old woman who survived an episode of VF was diagnosed with SQTS (QTc = 330 ms, HR = 83 bpm), and an ICD was implanted (Fig. 1D, Supplemental Fig. S1, E). Her sister and grandmother also displayed short QT. Genetic screening was negative.

3.1.5. Family 5

A 42-year-old man exhibited short QT (QTc = 340 ms, HR = 53 bpm) without presence of organic heart disease (Fig. 1E). Coved-type ST elevation on V1 and V2 was observed after intravenous

[7] SSS: sick sinus syndrome.

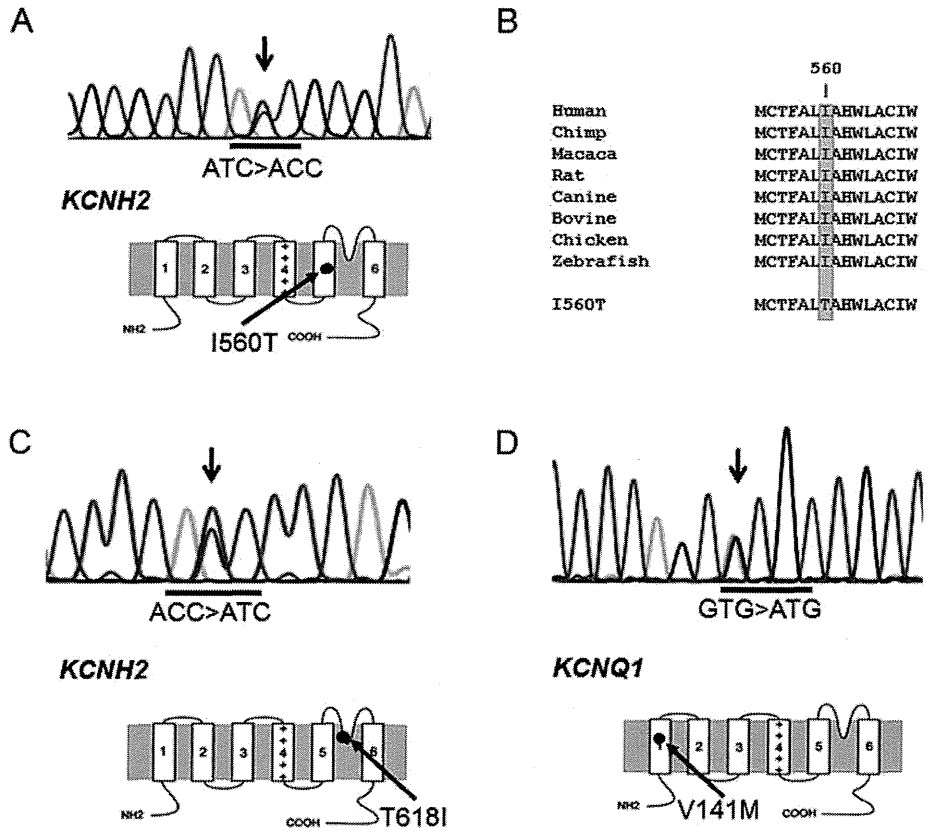

Fig. 2. Sequence analysis of the SQTS probands. (A) An electropherogram within exon 7 of *KCNH2* of the Family 1 proband. I560T is located in the S5 transmembrane segment. (B) Amino acid sequence between 554 and 568 of *KCNH2* is identical among eight species. (C) An electropherogram within exon 7 of *KCNH2* of the Family 2 proband. T618I is located in the pore region of *KCNH2*. (C) An electropherogram within exon 3 of *KCNQ1* of the Family 3 proband. V141M is located in the S1 transmembrane segment.

administration of pilsicainide, indicating a complication of Brugada syndrome (Supplemental Fig. S1, F). A programmed electrical stimulation failed to induce VF. His mother died at 43 years and an uncle died suddenly from unknown causes. Genetic testing was negative for all six SQTS genes, as well as Brugada syndrome candidate genes including *SCN5A*, *HCN4*, *KCND3*, *KCNE3*, *SCN1B*, *SCN3B*, *SCN10A*, and *TRPM4*. An ICD was implanted as a primary preventative measure.

3.2. Electrophysiological properties of KCNH2-I560T

KCNH2-I560T heterologously expressed in COS-7 cells resulted in a significant 2.5-fold increase in the peak I_{Kr} current density versus WT (I560T: 99.7 ± 10.2 pA/pF; WT: 40.6 ± 10.4 pA/pF; $p < 0.005$) (Fig. 3A,B), whereas the voltage dependence of activation was comparable (I560T: −19.7 ± 3.2 mV; WT: −18.5 ± 1.6 mV; NS) (Fig. 3C).

Table 1
Summary of clinical characteristics of SQTS families.

Family	Proband/family	Gender	Age of manifestation (year)	QTc (ms)	Mutations	Arrhythmias	Symptoms	Family history
1	Proband	M	64	319	*KCNH2*-I560T	Paroxysmal AF, AFL	Palpitation, syncope	SCD
2	Proband	F	39	322	*KCNH2*-T618I	Aborted VF	Palpitation	SCD
	Brother	M	42	330		–	–	
	Nephew	M	14	330		–	–	
3	Proband	F	4*	280	*KCNQ1*-V141M	SSS, fetal bradycardia	–	PPM
	Father	M	37	375		Chronic AF, bradycardia	–	
4	Proband	F	17	330	Negative	Aborted VF	–	–
	Sister	F	19	327		–	–	
	Grandmother	F	77	321		–	–	
5	Proband	M	42	340	Negative	BrS	–	SD

AF: atrial fibrillation, AFL: atrial flutter, VF: ventricular fibrillation, SCD: sudden cardiac death, BrS: Brugada syndrome, SD: sudden death, SSS: sick sinus syndrome, PPM: permanent pacemaker.
*: Diagnosed at 10 years. Severe short QT (QTc = 280 ms) demonstrated at 4 years.

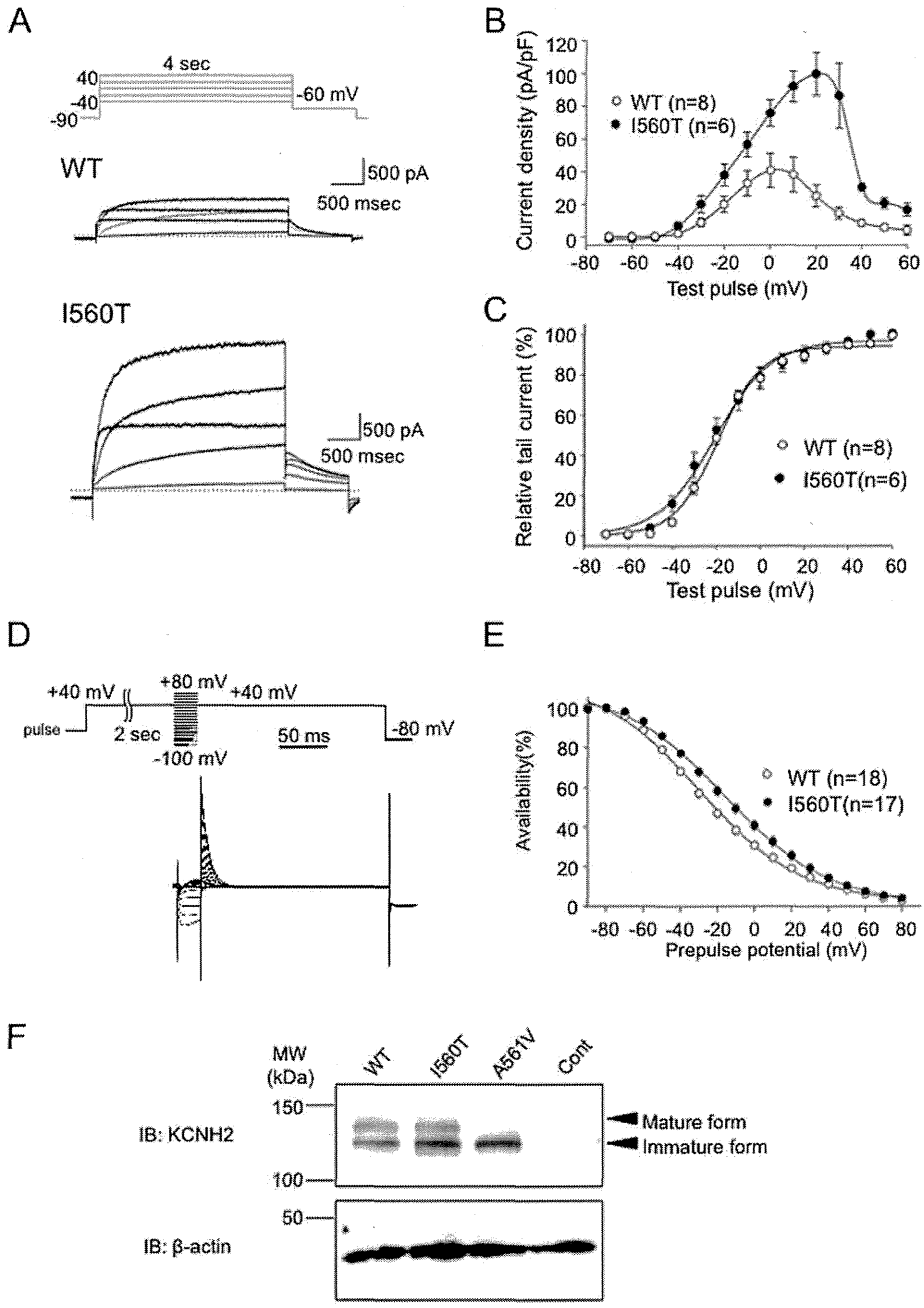

Fig. 3. Electrophysiological properties and protein expression of *KCNH2*-I560T. (A) I_{Kr} current traces of WT and *KCNH2*-I560T obtained from COS-7 cells. (B) Current–voltage relationship showing that I560T showed a 2.5-fold increase of the current density. (C) Voltage dependence of activation of WT and I560T were nearly identical. (D) Representative traces of I560T obtained with a multi-pulse protocol to determine channel availability. (E) Steady-state inactivation of I560T showed a significant 14 mV positive shift of $V_{1/2}$, but the slope factor was nearly identical. (F) I560T expressed glycosylated mature protein at similar levels to WT. The trafficking-defective LQTS mutation A561V expresses unglycosylated immature proteins.

Steady-state inactivation showed a 14 mV positive shift in the mutant channel (I560T: -13.2 ± 4.1 mV; WT: -27.3 ± 2.4 mV; $p < 0.005$). The slope factor was nearly identical (I560T: -26.5 ± 1.2 mV; WT: -25.4 ± 1.1 mV; NS) (Fig. 3E). These results suggest that the mutant channel may cause a gain of function in I_{Kr} current, which is a known trait of SQTS caused by *KCNH2* mutations.

3.3. Protein expression of KCNH2-I560T

To test if the I_{Kr} gain of function observed in the *KCNH2*-I560T channel may be attributed to increased membrane expression levels or hyperglycosylation of the mutant channel protein, we performed Western blotting using a trafficking-defective neighboring *KCNH2* mutation

A561V as a control [25]. While the A561V mutant showed only minimal expression of mature glycosylated high-molecular-weight protein, the I560T mutant showed nearly identical expression levels and pattern to the WT protein (Fig. 3F). This confirms that the gain of function in I_{Kr} observed in the I560T mutant is not due to the altered membrane expression but most likely due to the changes in channel gating properties.

3.4. In silico simulation of KCNH2-I560T

To explore whether the relatively modest gating modulation caused by the *KCNH2*-I560T mutation is sufficient to cause shortenings of APD and QT interval, we performed simulations of human ventricular action potentials with and without the *KCNH2*-I560T mutation in the 1-D myofiber model, representing the electrical behaviors of the left

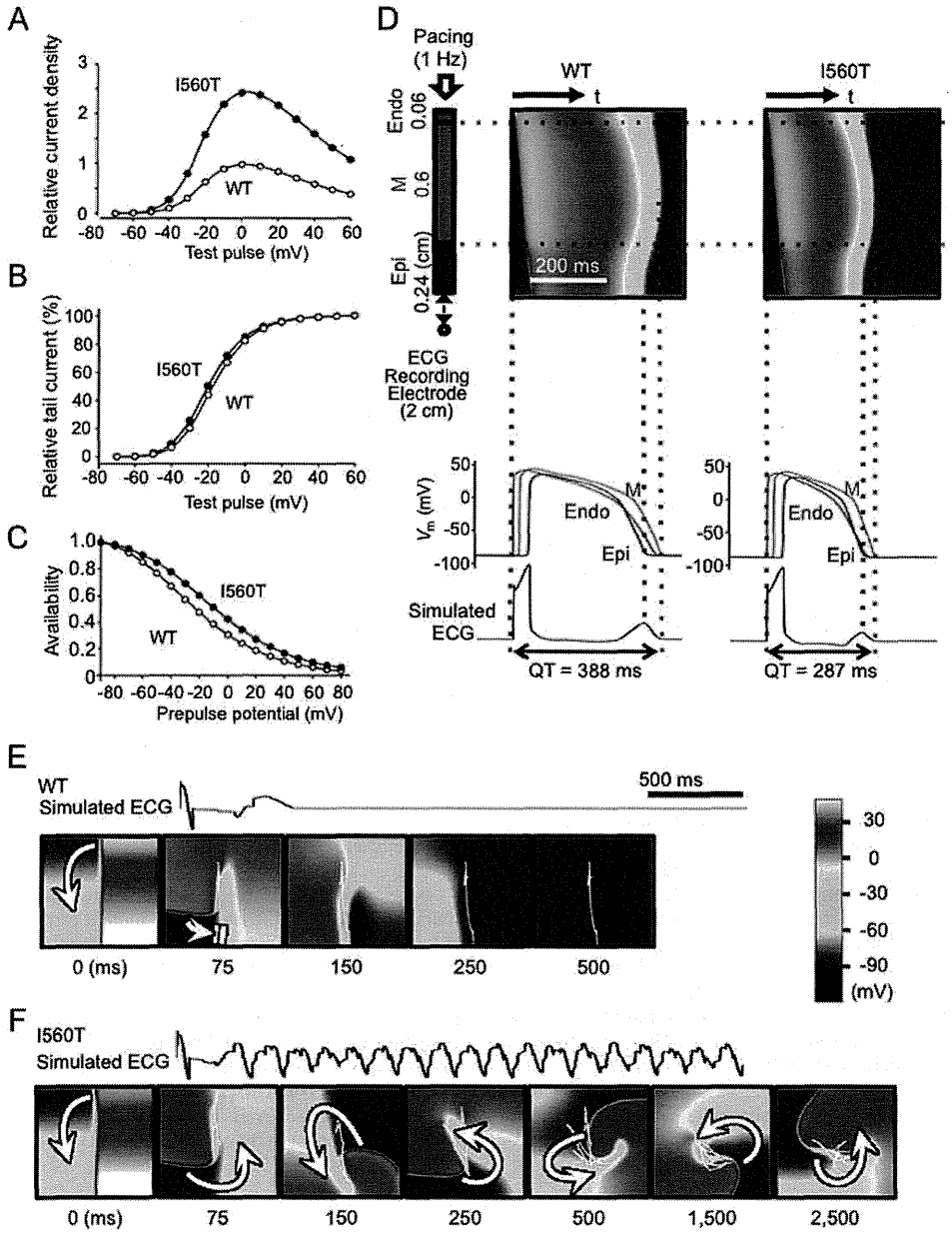

Fig. 4. Simulations of Markovian I_{Kr}, action potentials, ECGs, and effect on ventricular arrhythmogenicity of *KCNH2*-I560T. (A–C) Current–voltage relationship, voltage dependence of activation, and steady-state inactivation curves generated from the Markovian I_{Kr} model. (D) Using the myofiber model incorporating the above Markovian I_{Kr} models, transmural dispersions of action potentials with WT and I560T mutation were made. The spatial distributions of membrane potentials were color coded according to the color bar. Endo, M, and Epi denote endocardial, mid-myocardial, and epicardial layers. (E and F) Simulated ECGs and consecutive snapshots of the myocardial sheet model, incorporating the Markovian I_{Kr} models, after S1–S2 cross-field stimulation are shown. White open arrows and double short lines indicate the direction of wave front propagation and collision between wave fronts, respectively. Curved thin white lines inside the snapshots represent trajectories of the spiral wave phase singularities during the last 500 ms of activity.

ventricular free wall. Using a modified computer model of Markovian I_{Kr}, we were able to faithfully reproduce the I_{Kr} current traces and tail currents (Supplemental Fig. S2), 2.5-fold increase in current density (Fig. 4A), no significant shift in activation (Fig. 4B), and a 14 mV positive shift in inactivation (Fig. 4C), consistent with our observations in vitro. When cells were stimulated at 1 Hz, *KCNH2*-I560T showed significantly shorter APD than WT (Fig. 4D). The resulting pseudo-ECG also showed an abbreviated QT interval for the *KCNH2-I560T* mutant (287 ms) compared with WT (388 ms), which meets the diagnostic criteria of SQTS.

To explore the arrhythmogenic potency of VF in *KCNH2*-I560T, an S1–S2 cross-field protocol, with onset of S2 assumed as time zero (0 ms panel), was applied to induce a spiral wave re-entry (as a model of VF) (Fig. 4E,F). In the WT model, a counter-clockwise rotating wavefront terminated immediately (75–150 ms panels) and sustained re-entry was not induced. In contrast, the *KCNH2*-I560T model elicited a sustained meandering spiral wave re-entry with mean cycle length of ~153 ms (Supplemental videos S1 and S2).

3.5. Genotype-dependent differences in clinical characteristics of SQTS

Based on the observations in our SQTS cohort, that *KCNQ1* mutation carriers showed apparently earlier onset and more frequent bradyarrhythmia complications (Fig. 1, Table 1), we explored potential genotype-specific characteristics in the broader SQTS patient population. We combined mutation-positive SQTS patients of our cohort ($n = 6$) and those from previous publications ($n = 59$), and analyzed the clinical variables with respect to different genotypes (Tables 2 and 3). Among the SQT1, SQT2, and SQT3–6 groups, the age of manifestation was significantly later in SQT1 patients (SQT1: 35 ± 19 years, $n = 30$; SQT2: 17 ± 25, $n = 8$; SQT3–6: 19 ± 15, $n = 15$; $p = 0.011$), whereas the QTc values were comparable (Fig. 5, Table 2). Conversely, complications of SSS or bradycardia were significantly more prevalent in SQT2 patients (6/8, 75%) than non-SQT2 patients (5/57, 9%; $p < 0.001$) (Fig. 5C, Table 3), whereas there was no difference between SQT1 and non-SQT1 (Table 3). Furthermore, the prevalence of AF was also present in SQT2 patients (5/8, 63%) than non-SQT2 patients (12/57, 21%, $p = 0.012$) (Fig. 5D, Table 3). Other clinical parameters did not show significant differences between genotypes (Tables 2 and 3).

3.6. Evaluation of penetrance in 16 SQTS families

SQTS has been described as having close to complete penetrance in cohort studies, with only some exceptional cases with normal QTc [8, 9]. Despite carrying the mutation *KCNQ1*-V141M, the father of family 3 exhibited a QTc of 375 ms, which is outside the diagnostic criteria for SQTS [20], but manifested chronic AF and bradycardia, prompting us to reevaluate the genetic penetrance of SQTS (Fig. 1C, Supplemental Fig. S1, D). Among 35 SQTS families, we focused on two families from our study and 14 previously reported families [1,3,5–9,12,14, 32–36] with two or more genetically or phenotypically affected individuals (Table 4). Our family 3 was the only SQTS family in this group carrying a *KCNQ1* mutation. Among a total of 51 mutation-positive individuals, only 42 exhibited short QTc <360 ms. Therefore, the calculated overall genetic penetrance of SQTS was 82%, which was lower than previously recognized [8,9]. Furthermore, we found that the 13 families with K channel mutations (SQT1–3) showed a higher penetrance of 90%, whereas Ca channel mutations (SQT4–6) showed a much lower penetrance of 58%. Interestingly, the low penetrance observed in families with Ca channel mutations is comparable to the well-known incomplete penetrance associated with Brugada syndrome [37]. Of the nine mutation carriers who did not exhibit short QTc, four K channel mutation carriers exhibited syncope, AF, bradycardia, or instances of non-documented arrhythmia [35,36], and the five patients with Ca channel mutations remained asymptomatic [6,7].

4. Discussion

4.1. Common electrophysiological properties in KCNH2 mutations

The novel *KCNH2* mutation I560T was identified in an SQT1 patient with severe QTc shortening, and family history of sudden death. Investigation of the *KCNH2*-I560T channel expressed in COS-7 cells showed a relatively mild gain of function with a +14 mV shift of steady-state inactivation but no activation abnormalities. The computer simulation recapitulated the APD shortening and susceptibility to ventricular reentry. Among four *KCNH2* mutations that have been functionally evaluated, N588K was associated with a severe QTc shortening and exhibited severe gain of function with a 4-fold increase in peak current density, virtually no inactivation over the physiological range, and a large positive shift (+102 mV) of steady-state inactivation [3,38]. Peak current density was increased 6-fold in both T618I and E50D, and the steady-state inactivation was shifted by +20 mV and +11.5 mV in T618I and E50D, respectively [8,14,39,40]. Taken together, these results suggest that augmented peak current density and a positive shift of steady-state inactivation are the functional channel properties commonly affected by *KCNH2* mutations responsible for SQT1. The precise mechanism for the positive shift in inactivation curve in *KCNH2*-I560T is not clear, however, it is speculated that the shift in inactivation may be due to a disruption of the hydrogen bonding between amino acid residues that span the S5 and pore helix as seen in a neighboring residue H562 [41]. These changes in inactivation may be the primary determinants for the clinical manifestations of SQT1 [40]. However, as is observed in the SQT1 case carrying *KCNH2*-I560T reported here, the degree of the gating abnormality and the clinical severity may not always correlate, suggesting the involvement of additional confounding factors. These may include a number of common genetic variations that modulate QTc, as suggested by genome-wide association studies of LQTS [42,43]. Similar mechanisms may underlie the difference between clinical severity of SQTS patients and the electrophysiological properties of the mutant channels.

4.2. Incomplete penetrance of SQTS

Penetrance in LQTS has been recognized to be as low as 25% for some mutations [44], with the latent LQTS mutant carriers still, however, at risk of lethal arrhythmias and SCD [11]. By contrast, nearly complete penetrance has been described in SQTS within cohort studies [8,9]. However, our meta-analysis of 16 SQTS families revealed an incomplete penetrance of 82%, where nine mutation-positive patients from six unrelated SQTS families exhibited longer QTc than 360 ms (Table 4). Responsible mutations for the latent SQTS cases include three K channel mutations: *KCNQ1*-V141M (family 3); *KCNH2*-E50D [8,36]; and *KCNH2*-R1135H [35]. The carriers of *KCNQ1*-V141M and *KCNH2*-R1135H showed arrhythmias in the absence of short QTc. Three other mutations

Table 2
Age of manifestation and QTc of SQTS patients.

Clinical characteristics	SQT1	SQT2	SQT3–6	p*	Non-genotyped
Age of manifestation (year)	35 ± 19 (30)	17 ± 25 (8)	19 ± 15 (15)	0.011	28 ± 18 (57)
QTc (ms)	307 ± 30 (31)	305 ± 33 (8)	329 ± 55 (21)	0.107	311 ± 27 (65)

Mean ± SD (n).
* Comparison between SQT1, SQT2, and SQT3–6.

Table 3
Genotype–phenotype comparison SQTS patients.

Clinical characteristics	SQT1 (n = 34)	non-SQT1* (n = 31)	p†	SQT2 (n = 8)	non-SQT2§ (n = 57)	p‡	Non-genotyped (n = 67)
Male	18 (53%)	12 (39%)	0.25	2 (25%)	28 (49%)	0.19	50
Syncope/palpitation	8 (24%)	3 (10%)	0.123	0 (0%)	11 (20%)	0.17	8
SCD/aborted cardiac arrest	6 (18%)	6 (19%)	0.86	2 (25%)	10 (18%)	0.6	20
AF	8 (24%)	9 (29%)	0.614	5 (63%)	12 (21%)	0.012	11
SSS/bradycardia	4 (12%)	7 (23%)	0.245	6 (75%)	5 (9%)	<0.001	7

n (%). AF: atrial fibrillation, SCD: sudden cardiac death, SSS: sick sinus syndrome.
*Non-SQT1 denotes SQT2–6.
§Non-SQT2 denotes SQT1 and SQT3–6.
†Comparison between SQT1 vs SQT2–6.
‡Comparison between SQT2 vs SQT1, 3–6.

were found in Ca channel genes: *CACNA1C*-G490R, *CACNB2*-S481L and *CACNA2D1*-S755T. The carriers were asymptomatic [6,7]. The mechanisms underlying latent SQTS have not been determined; however, coexisting common polymorphisms that prolong repolarization may be potential candidates that mask the abbreviated QT intervals. In fact, among the mutant carriers of *CACNA1C*-G490R, an individual with a well-known *KCNH2* polymorphism K897T [42] showed normal QTc, whereas the other two family members who only carry G490R manifested SQTS [6].

In SQT2, only three mutations (*KCNQ1*-V307L, -V141M, and -R259H) have to date been reported in de novo or sporadic cases [4,9,13,15,16] and our family 3 carrying V141M is the first familial instance of SQT2. In view of the fact that the proband's father has exhibited chronic AF since 3 years of age without manifesting SQTS, despite carrying the V141M mutation, there may be additional latent carriers who do not show ECG abnormalities or other arrhythmias such as AF in the absence of QT shortening. Lack of familial SQT2 may be because the phenotypic manifestations of *KCNQ1* mutations are milder than other subtypes, or because SQT2 has an extremely low penetrance. Further genetic screening of family members with non-remarkable ECG may help identify more latent *KCNQ1* carriers and better understand the natural history of SQTS.

4.3. Genotype-specific clinical characteristics in SQTS

The age of manifestation of mutation-positive SQTS patients spans from in utero to the eighth decade of life [8]. However, the age-distribution of lethal events in SQTS shows two peaks; one at the first year of life and another between 20 and 40 years of age [9]. Because we found that the age of initial clinical manifestation in SQT1 was significantly later than other subtypes, and six *KCNQ1* mutation carriers exhibited apparent early onset of bradyarrhythmia, it is speculated that two distinct peaks of the first arrhythmic events may also be attributed to two genotypes (Fig. 5A, Table 2). A similar genotype-dependent age of manifestation is well known in LQTS; the majority of LQT2 (*KCNH2*) patients manifest their first symptoms after puberty, whereas LQT1

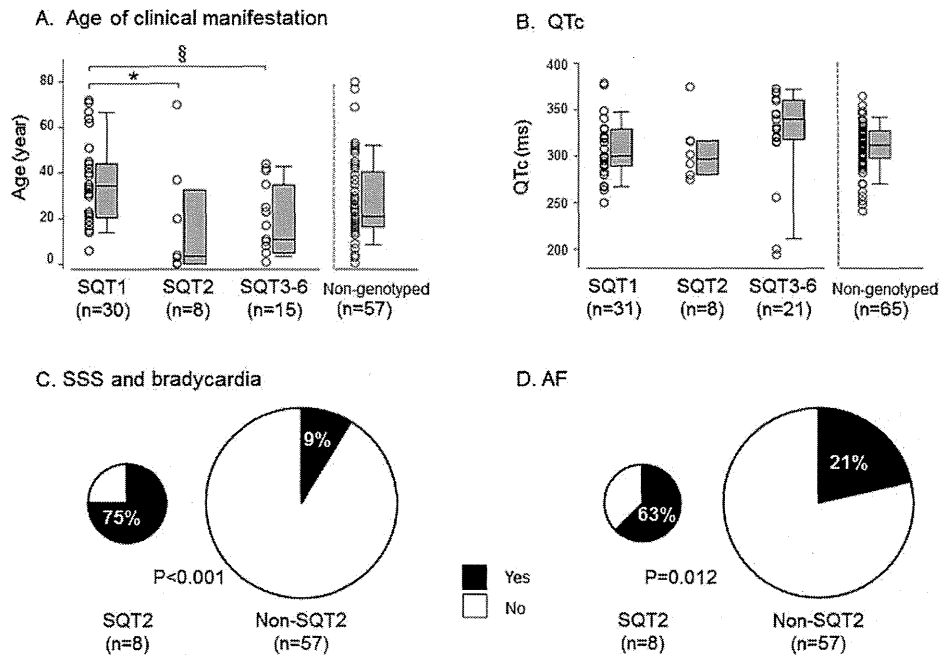

Fig. 5. Genotype-dependent clinical characteristics observed in SQTS patients. (A) Dot represents the age of manifestation of each case. SQT1 patients exhibited significantly later onset than other SQTS subgroups ($p = 0.011$). Pairwise comparison also showed significant later manifestation in SQT1 than SQT2 (*: $p = 0.043$) as well as SQT3–6 (§: $p = 0.019$). Non-genotyped SQTS, shown as a reference, exhibited a wide distribution. Boundaries of the box represent the 25th and 75th percentiles, and a line within a box marks the median. Whiskers of the box indicate the 10th and 90th percentiles. (B) QTc values were similar among SQTS subgroups. (C) Complications of SSS and bradycardia were significantly more prevalent in SQT2 than non-SQT2 subgroup ($p < 0.001$). (D) Complication of AF was significantly more prevalent in SQT2 patients than in non-SQT2 subgroup ($p = 0.012$).

Table 4
Mutations and the penetrance of 16 reported SQTS families.

Family	Genes	Mutations	Affected (n)	Mutation-positive (n)	Penetrance (%)	Other complications	References
1	*KCNH2*	N588K	3	3	100	–	[1, 32]
2	*KCNH2*	N588K	3	3	100	–	[3, 33, 34]
3	*KCNH2*	N588K	4	4	100	–	[3, 33, 34]
4	*KCNH2*	N588K	3	3	100	–	[9]
5	*KCNH2*	R1135H	1	3	33	(a), (b)	[35]
6	*KCNH2*	E50D	1	2	50	Syncope	[8, 36]
7	*KCNH2*	T618I	4	4	100	–	[14]
8	*KCNH2*	T618I	2	2	100	–	[9]
9	*KCNH2*	T618I	3	3	100	–	Current study
10	*KCNH2*	Not reported	6	6	100	–	[12]
11	*KCNQ1*	V141M	1	2	50	AF, (b)	Current study
12	*KCNJ2*	D172N	2	2	100	–	[9]
13	*KCNJ2*	D172N	2	2	100	–	[5]
14	*CACNA1C*	G490R	2	3	67	–	[6]
15	*CACNB2*	S481L	4	6	67	–	[6]
16	*CACNA2D1*	S755T	1	3	33	–	[7]
Total			42	51	82%		

(a): Non-documented arrhythmia, (b): bradycardia, AF: atrial fibrillation.

(*KCNQ1*) patients tend to become symptomatic before the age of 10 years [45].

We found a higher prevalence of bradyarrhythmia and AF in *KCNQ1* mutant carriers than for other genotypes (Fig. 5C,D, Table 3). A gain of function in I_{Ks} has not only been associated with SQTS but also with familial AF [46] and sinus bradycardia [47]. This is thought to be primarily because the APD shortening occurs in the atrium as well as in the ventricle, increasing the susceptibility of atrial tissue to sustained re-entry [48]. Furthermore, computer simulations of the SQTS mutation, *KCNQ1*-V141M, and the familial AF mutation, *KCNQ1*-V241K, demonstrated that a gain of function in I_{Ks} has been found to cause a cessation in spontaneous activity in the sinus node [15,47]. Such mechanisms may explain the observed phenotypic overlap and predominance of bradycardia and AF in SQT2.

5. Study limitations

In eight SQT2 patients we studied, six individuals carried the identical *KCNQ1* mutation V141M. Therefore, the phenotype of the V141M mutation may be over-represented in our SQT2 data. As SQTS is a rare disease, the size of the population studied is small. Further delineation of this rare lethal arrhythmic syndrome warrants more extensive genetic and population studies using larger cohorts.

6. Conclusions

In summary, our study identified two *KCNH2* mutations and one *KCNQ1* mutation in five Japanese families with SQTS. The novel *KCNH2*-I560T mutation causes severe shortening of the QT interval and can trigger VF despite only a modest shift in inactivation. Among SQTS patients, there exist latent mutation carriers with ECG abnormalities such as AF and bradycardia indicating incomplete penetrance. Furthermore, despite the limited number of reported SQTS patients, our study suggests that clinical characteristics of SQTS can differ depending on the patient genotype, as is observed in LQTS.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2015.04.090.

Conflict of Interest

None.

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Genotype-dependent differences in age of manifestation and arrhythmia complications in short QT syndrome☆

Daniel Toshio Harrell [a], Takashi Ashihara [b], Taisuke Ishikawa [a], Ichiko Tominaga [a,1], Andrea Mazzanti [c], Kazuhiro Takahashi [d], Yasushi Oginosawa [e], Haruhiko Abe [f], Koji Maemura [g], Naokata Sumitomo [h], Kikuya Uno [i], Makoto Takano [j], Silvia G. Priori [c,k], Naomasa Makita [a,*]

[a] Department of Molecular Physiology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[b] Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Heart Rhythm Center, Shiga, Japan
[c] Molecular Cardiology, IRCCS Salvatore Maugeri Foundation, Pavia, Italy
[d] Department of Pediatric Cardiology, Okinawa Children's Medical Center, Okinawa, Japan
[e] Second Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan
[f] Department of Heart Rhythm Management, University of Occupational and Environment Health, Kitakyushu, Japan
[g] Department of Cardiovascular Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[h] Department of Pediatric Cardiology, Saitama International Medical Center, Hidaka, Japan
[i] Sapporo Heart Center, Sapporo, Japan
[j] Department of Physiology, Kurume University School of Medicine, Kurume, Japan
[k] Department of Molecular Medicine, University of Pavia, Pavia, Italy

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ABSTRACT

Background: Short QT syndrome (SQTS) is a rare inheritable arrhythmia, associated with atrial and ventricular fibrillations, caused by mutations in six cardiac ion channel genes with high penetrance. However, genotype-specific clinical differences between SQTS patients remain to be elucidated.
Methods and results: We screened five unrelated Japanese SQTS families, and identified three mutations in *KCNH2* and *KCNQ1*. A novel mutation *KCNH2*-I560T, when expressed in COS-7 cells, showed a 2.5-fold increase in peak current density, and a positive shift (+14 mV) of the inactivation curve compared with wild type. Computer simulations recapitulated the action potential shortening and created an arrhythmogenic substrate for ventricular fibrillation. In another family carrying the mutation *KCNQ1*-V141M, affected members showed earlier onset of manifestation and frequent complications of bradyarrhythmia. To determine genotype-specific phenotypes in SQT1 (*KCNH2*), SQT2 (*KCNQ1*), and other subtypes SQT3–6, we analyzed clinical variables in 65 mutation-positive patients among all the 132 SQTS cases previously reported. The age of manifestation was significantly later in SQT1 (SQT1: 35 ± 19 years, n = 30; SQT2: 17 ± 25 years, n = 8, SQT3–6: 19 ± 15 years, n = 15; $p = 0.011$). SQT2 exhibited a higher prevalence of bradyarrhythmia (SQT2: 6/8, 75%; non-SQT2: 5/57, 9%; $p < 0.001$) and atrial fibrillation (SQT2: 5/8, 63%; non-SQT2: 12/57, 21%; $p = 0.012$). Of 51 mutation-positive individuals from 16 SQTS families, nine did not manifest short QT, but exhibited other ECG abnormalities such as atrial fibrillation. The resulting penetrance, 82%, was lower than previously recognized.
Conclusion: We propose that SQTS patients may exhibit different clinical manifestations depending upon their genotype.

1. Introduction

Short QT syndrome (SQTS)[2] is a rare, inheritable cardiac electrical disease characterized by a shortened corrected QT interval (QTc)[3] and associated with a risk of sudden cardiac death (SCD),[4] ventricular fibrillation (VF), and atrial fibrillation (AF), but without structural abnormalities [1,2]. SQTS is characterized by accelerated cellular repolarization, owing to either an enhanced outward repolarizing potassium current or a reduced inward depolarizing calcium current. To date, mutations responsible for SQTS have been identified in six ion channel

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* Corresponding author at: Department of Molecular Physiology, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan.
E-mail address: makitan@nagasaki-u.ac.jp (N. Makita).

[1] Current address: NTT Medical Center Tokyo, Tokyo, Japan.
[2] SQTS: short QT syndrome.
[3] QTc: corrected QT interval.
[4] SCD: sudden cardiac death.

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genes: *KCNH2* (SQT1) [3], *KCNQ1* (SQT2) [4], *KCNJ2* (SQT3) [5], *CACNA1C* (SQT4) [6], *CACNB2* (SQT5) [6], and *CACNA2D1* (SQT6) [7]. Previous reports suggest that the penetrance of these mutations is extremely high in families with SQT1, SQT2, and SQT3 [8,9].

Congenital long QT syndrome (LQTS)[5] is another repolarization disorder, for which mutations in 13 genes have been reported. Extensive genetic studies over two decades have improved the risk stratification and management of patients with LQTS [10,11]. In contrast, much less information is available about the genotype–phenotype correlations and natural history of SQTS. A cohort study of the European SQTS registry has shown that *KCNH2* mutation carriers (SQT1) show significantly shorter QT intervals and better response to hydroquinidine therapy as compared with non-SQT1 patients [12]. Conversely, a unique clinical phenotype characterized by neonatal AF and bradycardia was reported in SQT2 with the *KCNQ1* mutation V141M [13]. These studies suggest that SQTS may have some genotype-specific characteristics; however, because of the small number of cases studied to date, a robust genotype–phenotype correlation has not been identified.

By genetic screening of five unrelated Japanese SQTS probands, we found three mutations including a novel *KCNH2* mutation I560T, and previously reported mutations *KCNH2*-T618I [14] and *KCNQ1*-V141M [15]. SQT2 patients carrying the *KCNQ1*-V141M mutation exhibited earlier onset and frequent complications of bradyarrhythmia, a clinical scenario similar to that previously reported [13]. To explore the potential genotype-specific phenotypes underlying SQTS, we included 32 previously reported genotyped SQTS families [6–9,12–19], and further analyzed their clinical variables. We found that SQT1 patients exhibit later onset of manifestation and SQT2 patients have a higher prevalence of bradyarrhythmias and AF, implying that clinical characteristics of SQTS can differ by genotype as seen in LQTS.

2. Methods

2.1. Study cohorts

We studied five unrelated Japanese families consisting of 10 affected family members. The QT interval was corrected for heart rate using Bazett's equation ($QTc = QT/(\sqrt{RR})$). The diagnosis of SQTS was made based on $QTc \leq 330$ ms or $QTc < 360$ ms with presence of VF episode, pathogenic mutations, or family history of SQTS or SCD before age of 40 years [20]. All individuals who participated in the study gave written informed consent prior to genetic and clinical investigations in accordance with the standards of the Declaration of Helsinki and the local ethics committees at each participating institution. To further characterize the genotype-specific characteristics, we created an additional cohort, which consisted of 132 SQTS patients including five families (10 cases) from the current study, 33 families (61 cases) from a review by Gollob et al. [8], and an additional 36 SQTS families (61 cases) that have been previously reported and available on PubMed as of May 2014 [6–9,12–19] (Supplemental Table S1). A full description of the methods is available as supplementary information.

2.2. Genetic analysis

Genomic DNA was extracted from the blood by a standard protocol. All the exons of *KCNH2*, *KCNQ1*, and *KCNJ2* were PCR amplified as previously described [21–23] (Supplemental Table S2). Direct sequencing was performed using the ABI Genetic Analyzer 3130 (Life Technologies, Carlsbad, CA).

[5] LQTS: long QT syndrome.

2.3. Biophysical analysis of KCNH2-I560T

Site-directed mutagenesis was performed using the QuikChange site-directed mutagenesis kit (Agilent Technologies, Santa Clara CA) on human *KCNH2* cDNA cloned in an expression plasmid pcDNA3.1 (Life Technologies). Oligonucleotide sequences are available in Supplemental Table S2. The COS-7 cell line was transiently transfected with wild-type (WT) or I560T-*KCNH2* plasmid, and the potassium current was recorded by whole-cell patch-clamp techniques as previously described [24] with modifications.

2.4. Western blotting

To test the cell surface expression and the glycosylation of the I560T mutant channel protein, we carried out Western blotting analysis using WT channel and a neighboring LQTS mutation A561V as a control which is characterized by the membrane trafficking defect [25]. HeLa cells were transfected with *KCNH2* plasmid of either WT, I560T, or A561V and the proteins extracted from the cell lysate were subjected to Western blotting as described previously [26]. Anti-*KCNH2* polyclonal antibodies (1:400, Life Technologies) and anti-β actin polyclonal antibodies (1:200, Cell Signaling Technology, Danvers, MA) were used as primary antibodies, and the signals were visualized by ECL Prime Detection Reagent (GE Healthcare).

2.5. Computer simulation of action potential shortening and inducibility of lethal ventricular arrhythmia in KCNH2-I560T

Computer simulations were carried out to determine whether the gating abnormalities and the increased current density of the *KCNH2*-I560T are sufficient to cause shortening of the action potential duration (APD)[6] and QT interval. Membrane kinetics were represented by the O'Hara-Rudy dynamic human ventricular model with modified Markovian I_{Kr} equations [27–29], and the pseudo-ECG was calculated as described previously [30]. To demonstrate the relative arrhythmogenicity of *KCNH2*-I560T, we conducted simulations of VF in the bidomain endocardial sheet, and an S1–S2 cross-field protocol was applied to induce a spiral wave reentry.

2.6. Clinical characteristics of SQTS with different genotypes

In the study cohort of 132 SQTS patients, 65 patients were mutation-positive (6 from the current study and 59 from previous reports [6–9, 12–19]) of whom 30 (46%) were male and had a mean age of manifestation of 28 ± 20 years (ranging from 0 to 72). Clinical variables for each reported patient were extracted for sex, age of manifestation, QT, QTc, heart rate (HR), causative gene and mutation, and clinical history: AF, SCD/cardiac arrest, palpitations/syncope, and sick sinus syndrome/bradycardia. Bradycardia for adult patients was evaluated based on $HR < 50$ bpm. For children, bradycardia was determined based on $HR < 100$ bpm for ages 0 to 3 years and on $HR < 60$ bpm for ages 3–9 years [31]. A patient was considered symptomatic in the presence of the aforementioned clinical episodes.

2.7. Statistical analysis

Data are reported as mean ± SD and analyzed by one-way ANOVA with Bonferroni correction. The univariate clinical variables are presented as percentages, and analyzed by χ^2-test or Fisher exact test. Statistical significance was set at $p < 0.05$.

[6] APD: action potential duration.

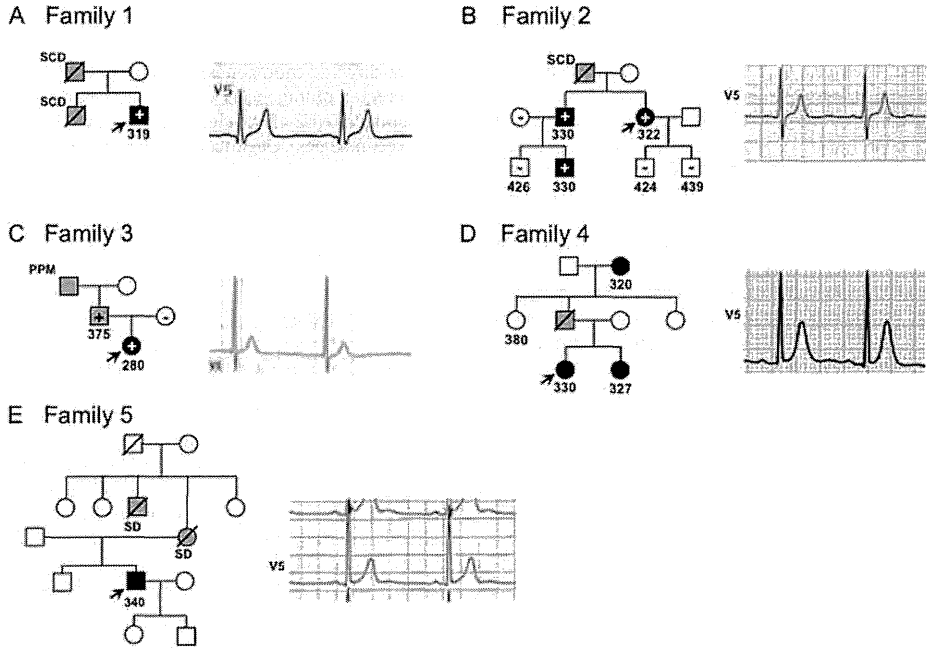

Fig. 1. *SQTS family pedigrees and proband's ECG. The arrow indicates the proband of each family (A)–(E). Numbers under the symbols are QTc (ms). Closed boxes (male) and circles (female) indicate phenotype-positive SQTS patients. Gray and open symbols mean suspected and unaffected, respectively. Plus and minus indicate mutation carrier (heterozygous) and non-carrier, respectively. SCD: sudden cardiac death, SD: sudden death, PPM: permanent pacemaker. V5 lead ECG of each proband is shown.*

3. Results

3.1. Case presentation and genetic analysis of SQTS families (Figs. 1 and 2 and Table 1)

3.1.1. Family 1

A 64 year-old man experienced palpitations and near syncope due to paroxysmal AF and atrial flutter, for which he underwent catheter ablation (Fig. 1A). After the catheter ablation, short QT (QTc = 319 ms, HR = 68 bpm) with peaked T waves on the precordial leads became manifested, and he was diagnosed with SQTS (Supplemental Fig. S1). His father and brother died suddenly from unknown causes. Genetic screening revealed a novel heterozygous missense mutation in exon 7 of *KCNH2* (c.1679T>C), resulting in the amino acid substitution I560T, located in transmembrane segment S5 (Fig. 2A). *KCNH2-I560T* was absent in the genomic DNA of 200 healthy Japanese individuals, and in the public databases: dbSNP; 1000 Genomes project; Exome Variant Server; and Human Genetic Variation Database. The amino acid residue I560 is 100% conserved among eight different species (Fig. 2B). The proband has no offspring, and declined insertion of an implantable cardioverter defibrillator (ICD).

3.1.2. Family 2

A 39-year-old woman with aborted VF was diagnosed with SQTS (QTc = 322 ms, HR = 74 bpm) and had an ICD implanted (Fig. 1B, Supplemental Fig. S1, B). Combined prescription of bepridil with bisoprolol successfully prolonged her QTc to 341 ms, and she has been free from VF attack for 2 years. Her father died suddenly from unknown causes at the age of 40 years and her brother and nephew exhibited short QTc (330 ms). Genetic screening identified a heterozygous missense mutation T618I (c.1853C>T) located in the pore region. This mutation was detected in all affected members (Fig. 2C). This mutation was previously reported [14].

3.1.3. Family 3

The index patient, a 10-year-old girl, was diagnosed with congenital sick sinus syndrome (SSS)[7] due to fetal bradycardia (HR = 72 bpm) at the gestational age of 22 weeks (Fig. 1C). She was born by cesarean section at the age of 37 weeks, and exhibited sinus bradycardia (HR = 50 bpm) with bradycardia-induced heart failure, although echocardiography showed no organic heart diseases. As a result of a complete atrioventricular block at the age of 12 days, a permanent pacemaker was implanted. At 4 years of age, the patient demonstrated severe QT shortening (QTc = 280 ms) with bradycardia (HR = 64 bpm) (Supplemental Fig. S1, C). Genetic screening revealed a heterozygous missense mutation V141M (c.421G>A) of *KCNQ1* located in transmembrane segment S1 (Fig. 2D). This mutation was previously reported [15]. Her father had experienced chronic AF with bradycardia since the age of 3 years, but recorded a QTc value of 375 ms (HR = 37 bpm) by ECG, which is outside the diagnostic criteria of SQTS (Supplemental Fig. S1, D) [20]. Interestingly, despite this, he carried the V141M mutation. Her paternal grandfather had a pacemaker implanted at the age of 50 years, but declined genetic testing.

3.1.4. Family 4

A 17-year-old woman who survived an episode of VF was diagnosed with SQTS (QTc = 330 ms, HR = 83 bpm), and an ICD was implanted (Fig. 1D, Supplemental Fig. S1, E). Her sister and grandmother also displayed short QT. Genetic screening was negative.

3.1.5. Family 5

A 42-year-old man exhibited short QT (QTc = 340 ms, HR = 53 bpm) without presence of organic heart disease (Fig. 1E). Coved-type ST elevation on V1 and V2 was observed after intravenous

[7] *SSS: sick sinus syndrome.*