hearts transfected with a dominant negative *KCNJ2* mutant, Miake et al. demonstrated that suppression of I_{K1} decelerated the action potential repolarization, prolonged the action potential duration, and depolarized the resting membrane potential. They also observed that the suppression of I_{K1} caused QTc prolongation on surface ECGs.

In order to clarify the genotype-phenotype correlations in Japanese ATS patients, we carried out a complete screening of *KCNJ2* in 23 ATS patients and investigated their clinical manifestations.

METHODS

Study subjects

Twenty-three clinically-diagnosed Japanese ATS patients (from 13 unrelated families) were enrolled in this study from 12 institutes in Japan. Individuals were diagnosed as being affected with ATS if 2 or 3 of the following criteria were present: (1) episodes of muscle weakness, (2) cardiac involvement, and/or (3) dysmorphology as previously described (Donaldson, et al., 2003; Tristani-Firouzi, et al., 2002). The presence of periodic paralysis was based on standard criteria (McManis, et al., 1986). Cardiac involvement was determined by the presence of ventricular arrhythmias (frequent premature ventricular contractions (PVCs), bigeminy, ventricular tachycardia (VT)), prolongation of the corrected QT interval (QTc) and/or a prominent U wave. Subjects were classified as having QT prolongation if the QTc exceeded 440 milliseconds (ms) for males and 460 ms for females, in accordance with standard criteria (Keating, et al., 1991). The QT interval was defined from the onset of QRS to the end of the T wave. The U wave was defined as an early diastolic deflection after the end of the T wave. The QU interval was defined from the onset of QRS to the end of the U wave. QT and QU intervals were corrected according to Bazett's formula (Bazzett, 1920; Zhang, et al., 2005). The end of the T or U wave was the point at which a tangent drawn to the steepest portion of the terminal part of the T or U wave crossed the isoelectric line (Yan and Antzelevitch, 1998). Because a prominent U wave is fused to the next PQ segment in some cases, we defined the isoelectric line as a segment connecting two points preceding consecutive QRS complexes. Abnormal U waves were judged by the following criteria: (a) wave amplitude $\geq$ 0.2 mV (Lepeschkin, 1972) or (b) amplitude larger than preceding T wave (Lepeschkin, 1969).

Dysmorphology was defined by the presence of 2 or more of the following: (a) low-set ears, (b) hypertelorism (wide-set eyes), (c) small mandible, (d) clinodactyly (permanent lateral or medial curve of a finger or toe), and (e) syndactyly (persistent webbing between fingers or toes) (Tristani-Firouzi, et al., 2002).

In order to elucidate the genetic differences between ATS and LQTS, we also screened *KCNJ2* mutations in 74 LQTS probands from 74 unrelated families. They displayed no clinical signs compatible with ATS except for cardiac manifestations.

DNA isolation and mutation analysis

The protocol for genetic analysis was approved by the Institutional Ethics Committee and was performed under its guidelines. All patients provided an informed consent before the genetic analysis was carried out. Genomic DNA was isolated from leukocyte nuclei using a

DNA extraction kit, QIAamp DNA Blood midi kit, (QIAGEN GmbH, Hilden, Germany). Genetic screening for *KCNJ2* was performed by polymerase chain reaction/single-strand conformation polymorphism (PCR-SSCP) analysis (Yoshida, et al., 1999) or denaturing high-performance liquid chromatography (DHPLC) using WAVE System Model 3500 (Transgenomic, Omaha, NE, USA) (Ning, et al., 2003). Abnormal conformers were amplified via PCR, and sequencing was performed on an ABI PRISM3100 DNA sequencer (Applied Biosystems, Wellesley, MA, USA).

In vitro mutagenesis

With regard to the novel *KCNJ2* mutations, site-directed mutagenesis was employed to construct mutants as described previously (Hosaka, et al., 2003). Briefly, human *KCNJ2* cDNA was subcloned into the pCMS-EGFP plasmid (Clontech, Palo Alto, CA, USA). We engineered *KCNJ2* mutants using a site-directed mutagenesis kit, QuickChange II XL (Stratagene, La Jolla, CA, USA). The presence of mutations was confirmed by sequencing.

Electrophysiological experiments and data analysis

To assess the functional modulation of *KCNJ2* channels, we employed a heterologous expression system with COS7 cells (Kubota, et al., 2000). Briefly, the cells were transiently transfected by the LipofectAMINE method as directed in the manufacture's instructions (Invitrogen, Carlsbad, CA, USA), using a 1.0 μg/35 mm dish of pCMS-EGFP/*KCNJ2* (wild type (WT) and/or mutant). For electrophysiological experiments, GFP-positive cells were selected 24 to 72 hours after transfection. Current measurement was conducted by the conventional whole-cell configuration of patch-clamp techniques at 37°C, using an Axopatch 200A patch clamp amplifier and a Digidata 1322A digitizer (Axon Instruments, Foster City, CA, USA). pClamp software (version 9.0, Axon Instruments) was used to generate voltage pulse protocols and for data acquisition. Currents were evoked by 150 ms square pulses applied in 10 mV increments to potentials ranging from –140 mV to +30 mV from a holding potential of –80 mV. Pipettes were filled with a solution containing (in mM): K-aspartate 60, KCl 65, KH_2PO_4 1, $MgCl_2$ 2, EDTA 3, K_2ATP 3 and HEPES 5 adjusted to pH 7.4 using KOH, and had a resistance of 3.0 to 5.0 MΩ. The bath solution contained (in mM): NaCl 140, KCl 5.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8, NaH_2PO_4 0.33, glucose 5.5, and HEPES 5 (pH 7.4 with NaOH) (Yoshida, et al., 1999). All the data are shown as mean ± SEM. Where appropriate, Student's unpaired *t*-test was used; a value of $P < 0.05$ was considered statistically significant.

Immunocytochemistry

The hemaggulutinin (HA) epitope (YPYDVPDVA) was introduced into the pCMS-EGFP/*KCNJ2* (WT and mutants) between Ala-115 and Ser-116 (extracellular lesion between TM1 and TM2) as previously described (Ballester, et al., 2006). COS7 cells were transfected with 1.0 μg of plasmid DNA in 35 mm glass-bottom dishes. Forty-eight hours later, the cells were washed twice with phosphate buffered saline (PBS), followed by incubation with a mouse anti-HA primary antibody (1:500,) for 30 minutes at 37°C. The cells were then washed twice with PBS and incubated with an anti-mouse antibody conjugated to the Alexa 594 fluorophor (1:500)(Molecular Probes, Eugene, OR, USA) as a secondary antibody for 30 minutes at 37°C. Finally, cells were washed with and immersed in PBS, and confocal imagings were obtained with a Zeiss LSM 510 (Carl Zeiss GmbH, Jena, Germany).

RESULTS

***KCNJ2* mutation analysis**

Figure 1 and Table 1 show mutations and clinical findings of 23 ATS patients (9 males/14 females; mean 23.0 ± 3.1 years old). There were 13 probands from 13 unrelated families. We found 10 *KCNJ2* heterozygous mutations (two were novel) in ATS patients: c.199C>T/p.R67W (Andelfinger, et al., 2002), c.200G>A/p.R67Q, c.430G>A/p.G144S (Kobori, et al., 2004), c.436G>A/p.G146S, c.574A>G/p.T192A(Ai, et al., 2002), c.644G>A/p.G215D (Hosaka, et al., 2003), c.652C>T/p.R218W (Plaster, et al., 2001), c.653G>A/p.R218Q (Tristani-Firouzi, et al., 2002), c.899G>T/p.G300V (Tristani-Firouzi, et al., 2002), and c.926C>T/p.T309I (Bendahhou, et al., 2005). R67Q and G146S were novel mutations. Arginine at codon 67 is implicated in binding membrane-associated phoshatidylinositol 4,5-bisphosphate (PIP_2), and has been reported to be a hot spot for ATS mutations (Donaldson, et al., 2003; Zhang, et al., 2005), but the substitution of arginine with glutamine (R67Q) is a novel mutation. G146S is also a novel mutation located at an essential region known as the K^+ channel signature sequence that serves as a principal ion selective filter (Doyle, et al., 1998). Therefore, both R67Q and G146S are supposed to cause substantial effects to *KCNJ2* channels.

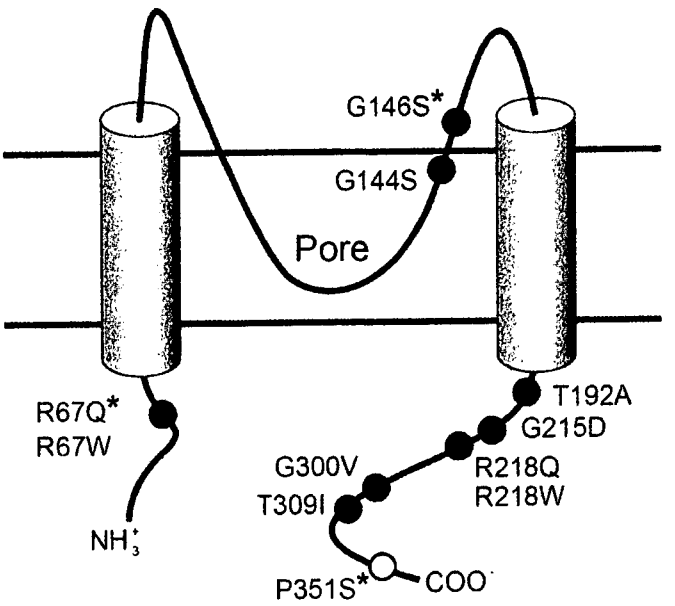

Figure 1. Topology of the Kir 2.1 channel. Scheme showing the topology of the Kir 2.1 channel and the location of 10 *KCNJ2* mutations (filled circles) found in 23 ATS and 1 (open circle) in a LQTS patient. Asterisks indicate novel mutations.

Table 1 **Characteristics of *KCNJ2* Mutation Carriers**

No. of kindred	Mutation in *KCNJ2*		Age	Sex	Cardiac Manifestations								Paralysis	Dysmorphology	Symptom	Seizure	Therapy
	cDNA	Protein			HR (bpm)	QTc (ms)	QUc (ms)	T amplitude (mV)	U amplitude (mV)	Tp-Up (ms)	U/T[††]	Ventricular Arrhythmia					
KJ-01	199C>T	R67W*	30	F	66	383	766	0.60	0.15	240	0.25	a, b, d	+	+	-	-	-
	199C>T	R67W	55	F	71	479	761	0.30	0.15	200	0.50	-	-	+	-	-	-
	199C>T	R67W	20	F	93	430	647	0.15	0.30	200	2.00	b, c	-	+	-	-	-
K-323	200G>A	R67Q*	13	F	54	360	626	1.10	0.30	232	0.27	a, c, d	-	+	Syncope	-	Carvediloi, Mexiletine
K-024	430G>A	G144S[†]*	36	F	67	480	803	0.40	0.30	280	0.75	a, d, f	+	+	Syncope	-	Propranorol, Verapamil, K
	430G>A	G144S[†]	11	M	70	460	756	NA	NA	250	NA	b, d	-	+	Aborted SD	+	Propranorol, Verapamil
K-179	436G>A	G146S*	28	F	126[†††]	N	NA	NA	NA	NA	NA	a, b, d, f	+	+	-	-	Atenolol
K-037	574A>G	T192A*	13	M	89	467	731	0.30	0.32	208	1.07	a, b	-	-	-	+	Acetazolamide
	574A>G	T192A	11	F	86	431	790	0.66	0.22	280	0.33	a, f	+	-	-	-	Acetazolamide
N-01	644G>A	G215D*	34	F	73	408	794	0.78	0.33	280	0.42	a, b, f, g	+	+	Aborted SD	-	ICD
K-180	652C>T	R218W*	6	F	80	483	716	0.05	0.15	200	3.00	a, b, c, d, e	+	-	Syncope	+	Flecainide
	652C>T	R218W	38	M	54	384	693	0.80	0.20	250	0.25	a	+	-	-	+	-
	652C>T	R218W	73	M	52	410	670	0.50	0.25	220	0.50	-	+	-	-	-	-
K-240	652C>T	R218W*	11	F	78	365	753	0.30	0.35	240	1.17	b, c, d, e	-	+	-	-	Mexiletine
	652C>T	R218W	47	M	91	394	640	0.45	0.20	240	0.44	-	-	+	-	-	-
	652C>T	R218W	5	M	78	342	684	0.30	0.15	220	0.50	b, c	-	+	Myalgia	-	-
K-324	652C>T	R218W*	6	M	82	339	701	0.14	0.20	255	1.43	a, c, d	+	+	-	-	Verapamil
K-178	653G>A	R218Q*	13	F	60	420	700	0.40	0.20	220	0.50	b, c, d	+	+	-	-	-
	653G>A	R218Q	42	M	55	402	651	0.80	0.28	230	0.35	b	+	-	-	-	-
K-201	653G>A	R218Q*	12	F	105	423	741	0.65	0.40	200	0.62	b, c, d	+	+	Palpitation	-	-
KJ-02	899G>T	G300V*	16	M	103	393	786	0.90	0.30	240	0.33	b, d	-	+	Syncope	-	Propranorol, Verapamil
	899G>T	G300V	36	F	67	444	740	0.45	0.30	240	0.67	a, b, c, d	-	+	Syncope	-	Propranorol, K
KJ-03	926C>T	T309I*	17	F	NA	NA	NA	NA	NA	NA	NA	a, b, c, d	-	+	Malaise	-	Metoprolol, Verapamil
			23 ± 3.1		77 ± 4.1	406 ± 10	715 ± 12	0.51 ± 0.07	0.25 ± 0.02	231 ± 5.6	0.76 ± 0.16						

Table 1. Characteristics of *KCNJ2* mutation carriers. *: proband. †: compound heterozygous *KCNQ1*(c.1550T>A/p.A341V) mutation carrier. ††: U/T means the ratio of U amplitude compared to T amplitude. †††: Because this patient had frequent extra ventricular systoles, heart rate was recorded as 126 bpm. NA: non available information. As for ventricular arrhythmias, a: PVC, b: bigeminy, c: couplet, d: bidirectional VT, e: monomorphic VT, f: polymorphic VT, g: VF. SD: sudden cardiac death. As for treatments, K indicates potassium supplement, and ICD: implantable cardioverter defibrillator.

In 8 kindred (K-024, K-037, K-178, K-180, K240, K-323, KJ-01, and KJ-02, Table 1) the mutations were inherited from parents. In contrast, they were *de novo* in 3 probands of 3 kindred (K-179, K-201, and K-324) and were undetermined in 2 kindred (N-01 and KJ-03) because detailed family information was not available. These *KCNJ2* variants were not present in 100 normal controls (200 alleles). Analysis for other known LQTS-responsible genes (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2*) revealed that K-024 proband and her son were also heterozygous for a *KCNQ1* mutation (c.1550T>C/p.A341V)(Kobori, et al., 2004).

In 74 LQTS patients, we found one novel *KCNJ2* variant (c.1051C>T /p.P351S) in a 74-year-old female who had suffered from syncope and received a pacemaker implantation. Also, genetic analysis had revealed that she was a carrier of c.2089G>A/p.G643S-*KCNQ1*. This variant (P351S-*KCNJ2*) could not be identified in 100 healthy controls.

General clinical findings

Table 1 summarizes ECG findings and two other major phenotypes in 23 ATS patients. Seven of the 23 mutation carriers (30 %) showed the full clinical triad, and 16 (70 %) had two of the three phenotypes.

(1) Cardiac manifestations (Table 1 and Figure 2)

In 2 cases (G146S: a proband, and T309I: a proband), it was difficult to measure the RR interval and recognize the U wave because of very frequent premature contractions and sustained ventricular bigeminy. Two G144S patients had A341V-*KCNQ1* mutation. We therefore excluded these 4 cases from ECG parameter analyses. In the remaining 19 cases, the mean QTc interval was 406 ± 10 ms, and only 3 (16%) had a QTc ≥ 460 ms. Based on the criteria described in Methods, 16 patients (84%) showed abnormal U waves (Figure 2A-C and Table 1). Their serum K^+ concentrations were within normal range. There were no other factors influencing the U wave formation such as bradycardia or drugs.

Ventricular tachyarrhythmias were observed in 15 patients (65%; 12 of 13 probands and 3 of 10 family members): monomorphic VT in 2 (9%), polymorphic VT in 4 (17%), bidirectional VT in 13 (57%) (Figure 2D), and ventricular fibrillation (VF) in 1 (4%).

β blockers (propranolol, atenolol, metoprolol, or carvedilol) were prescribed in 7 individuals, calcium channel blocker (verapamil) in 5 and sodium channel blockers (one is flecainide, the others are mexiletine) in 3. These drugs appeared to prevent cardiac events effectively in each case.

(2) Periodic paralysis

Periodic paralysis was observed in 13 patients (57%). In some of them, muscle weakness was triggered by elevated fever, exercise, and menstruation. Biopsy of skeletal muscle demonstrated tubular aggregates in 2 probands (Ai, et al., 2002; Hosaka, et al., 2003). Carbonic anhydrase inhibitors prevented or reduced the attack in 4, however, they were not effective in 2 cases.

(3) Dysmorphology

Dysmorphology was observed in 17 patients (74%). Mandibular micrognathia (small mandible) was most frequently observed (11; 48%). Short stature was found in 8 (35%);

clinodactyly in 6 (26%); hypertelorism in 6 (26%); low-set ears in 5 (22%); broad forehead in 4 (17%); and scoliosis in 1 (4%).

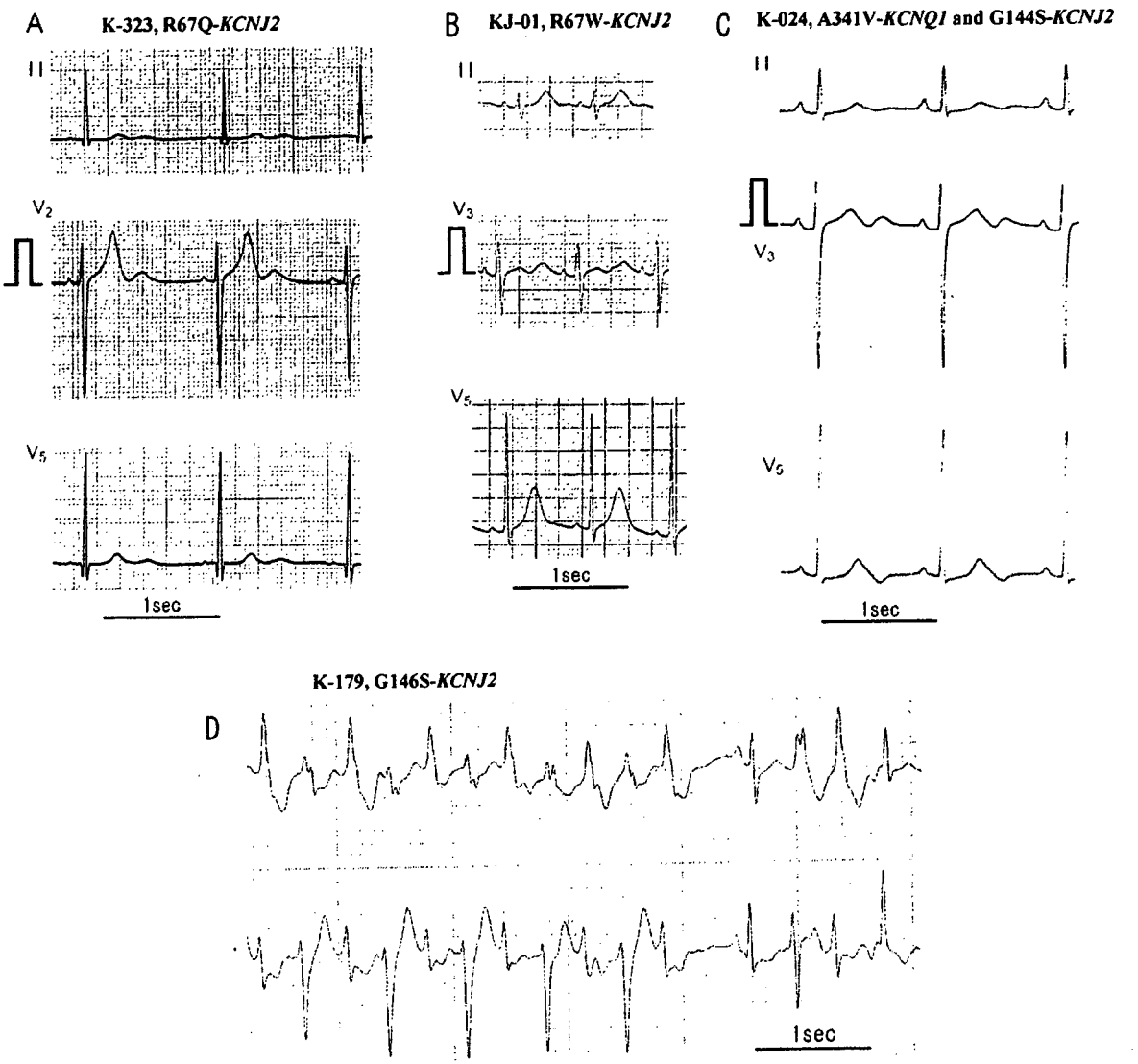

Figure 2. Representative ECG traces of *KCNJ2* mutation carriers. **A:** Normal QTc (360 ms) and abnormal U wave (U amplitude 0.30 mV) (K-323, R67Q). **B:** Normal QTc (373 ms) and abnormal U wave (U amplitude 0.30 mV and U amplitude > T amplitude) (KJ-01, R67W). **C:** QTc prolongation (460 ms) and abnormal U wave (U amplitude 0.30 mV) (K-024, Carrier of compound mutation A341V-*KCNQ1* and G144S-*KCNJ2*). **D:** bidirectional ventricular tachycardia in a Holter recording (K-179, G146S).

(4) Other manifestations in ATS patients

Interestingly, 4 patients (17%) had episodes of afebrile seizures during infancy. Because Kir 2.1 channels are known to be distributed in various types of cells—including major parts of the brain (Raab-Graham, et al., 1994)—seizures as an episodic electrical phenotype of the central nervous system could be a clinical sign of ATS. Mild thyroid dysfunction was observed in one case.

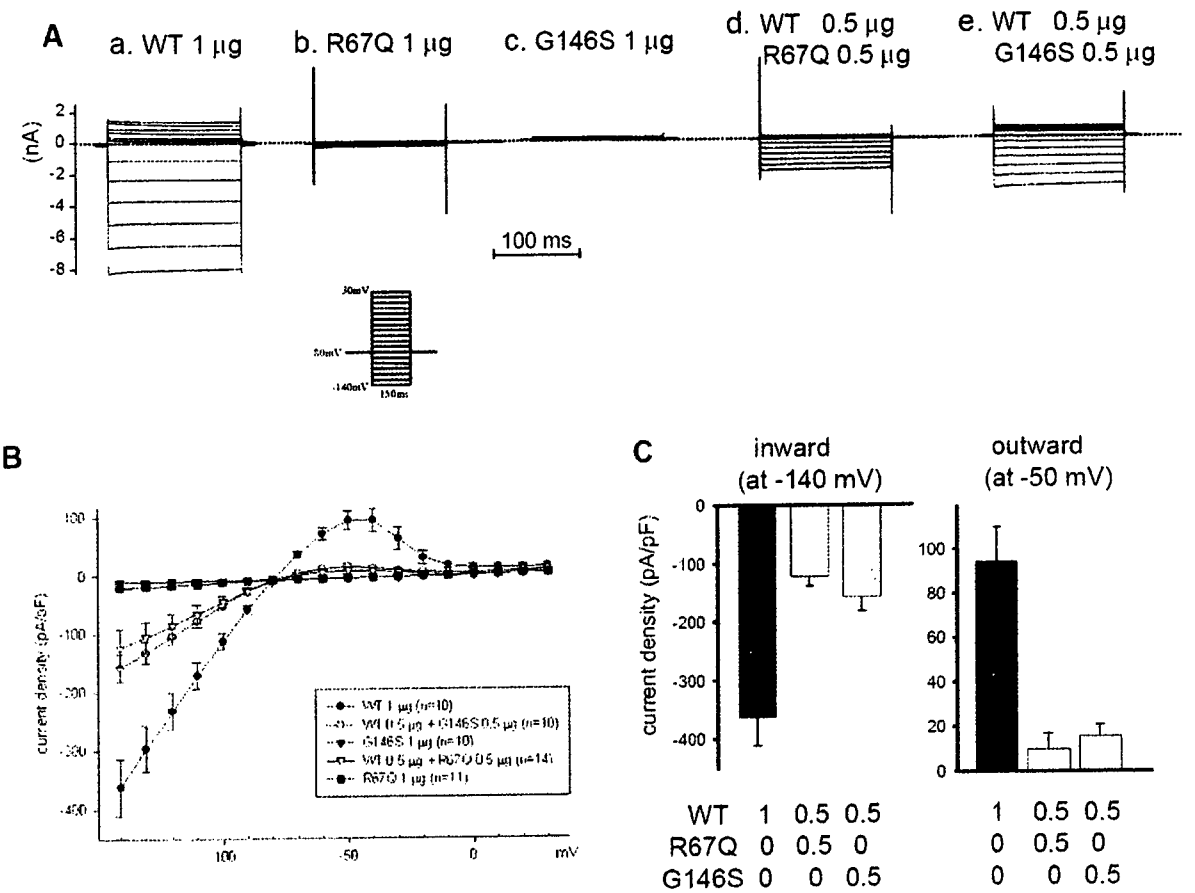

Figure 3. Both R67Q-*KCNJ2* and G146S-*KCNJ2* exert dominant negative effects on wild type function. **A:** Representative Kir 2.1 currents expressed in COS7 cells: **(a)** wild type (WT) cDNA 1 μg, **(b)** R67Q 1 μg, **(c)** G146S 1 μg, **(d)** co-transfection with WT 0.5 μg and R67Q 0.5 μg and **(e)** co-transfection with WT 0.5 μg and G146S 0.5 μg. Holding potential was set at –80 mV. Square pulses of 150 ms duration were applied to the potentials between –140 mV and +30 mV with a 10 mV increment. Time scale is given in the middle of graph. **B:** Plots for current-voltage relationships obtained by multiple experiments of the same protocol as shown in A. Current densities were calculated by dividing with cell capacitance. **C:** Bar graphs showing mean current densities in WT (black bars), co-transfection with WT and R67Q (white bars) and co-transfection with WT and G146S (gray bars); left panel: those at –140 mV and right panel: those at –50 mV. Vertical bars indicate SEM.

Electrophysiological functional assays

(1) R67Q and G146S-*KCNJ2* channels

We performed biophysical assays for the two novel *KCNJ2* mutations using the whole-cell patch clamp method in COS7 cells.

COS7 cells transfected with WT-*KCNJ2* cDNA (1 μg/dish: Fig. 3A-a) displayed K^+ currents with a strong inward rectification, which are typical of I_{K1} as previously described (Kubo, et al., 1993; Raab-Graham, et al., 1994). However, neither of the mutants—R67Q nor G146S—displayed measurable currents when transfected alone (Fig. 3A-b,c).

To simulate the allelic heterozygosity, WT and each mutant-*KCNJ2* were co-transfected at an equimolar ratio (0.5 μg/dish, respectively, Fig. 3A-d,e). Panel B of Figure 3 shows current-voltage relations. The currents co-expressed of WT with either mutant-*KCNJ2* showed the inward rectification. As summarized in bar graphs of Figure 3C, current densities for co-expression of WT-*KCNJ2* with R67Q (white bar) or G146S (gray bar) were –123 ± 32 pA/pF and –157 ± 23 pA/pF at –140 mV, and –9 ± 6 pA/pF and –15 ± 5 pA/pF at –50 mV, respectively. They were significantly smaller than those displayed by WT (–362 ± 48 pA/pF at –140 mV and 94 ± 16 pA/pF at –50 mV, indicated by closed bars). Both mutants showed a larger suppression at –50 mV, which is more physiological membrane potential.

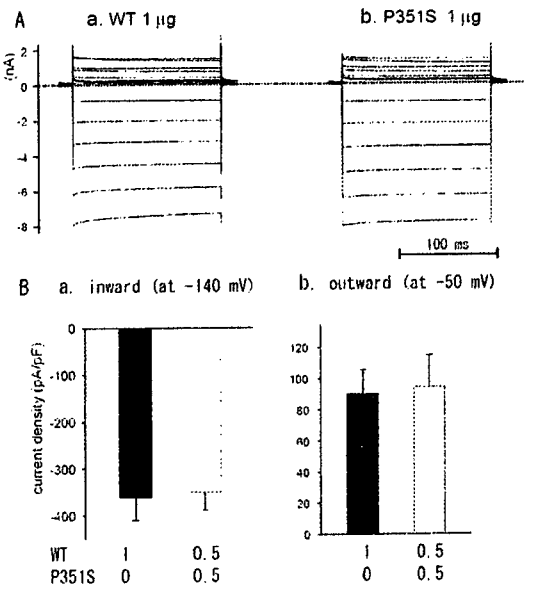

Figure 4. The Kir 2.1 variant in LQTS displays inwardly-rectifying currents, which are indistinguishable from that of the WT. **A:** Representative Kir 2.1 currents expressed in COS7 cells: **(a)** WT cDNA 1 μg, **(b)** P351S 1 μg. Pulse protocols were the same in Fig. 3. **B:** Bar graphs showing averaged current densities induced by WT (black) and co-transfection of WT/P 351S (white) at test of **(a)** –140 mV and **(b)** –50 mV (n=8 respectively).

(2) P351S-*KCNJ2* channel

We found one novel *KCNJ2*-variant (P351S) among 74 LQTS probands (1.4%). Functional assays revealed that the mutant channel displayed inwardly-rectifying currents of similar size (Figure 4A). In a heterozygous condition (Figure 4B), the current densities of WT and WT/P351S were –362 ± 48 pA/pF and –350 ± 38 pA/pF at –140 mV, and 90 ± 15 pA/pF and 94 ± 20 pA/pF at –50 mV, respectively. Student *t*-tests revealed these changes in current were not significant. These findings suggested that the P351S-*KCNJ2* was a non-pathological variant associated with the LQT1 patient who lacked extra-cardiac signs of ATS.

Immunocytochemistry of mutant channels (R67Q and G146S)

In order to investigate whether the R67Q and G146S mutations affect *KCNJ2* trafficking, immunocytochemistry and confocal imaging of mutant channels was performed. An HA tag was introduced into an extracellular loop lesion of *KCNJ2* in the pCMS-EGFP vector which carried GFP as a reporter gene. The HA-tagging procedure itself did not affect the functional expression of inwardly-rectifying I_{K1} currents (data not shown).

Figure 5 illustrates typical results of confocal imaging. COS7 cells were transfected with HA-*KCNJ2*, HA-R67Q, HA-G146S and *KCNJ2* (without HA tag)(Fig 5, upper panel). All of HA-tagged *KCNJ2* (HA-*KCNJ2*, HA-R67Q and HA-G146S) exhibited red fluorescence at the plasma membrane (Fig 5, middle panel), indicating that both R67Q and G146S were trafficking-competent mutants.

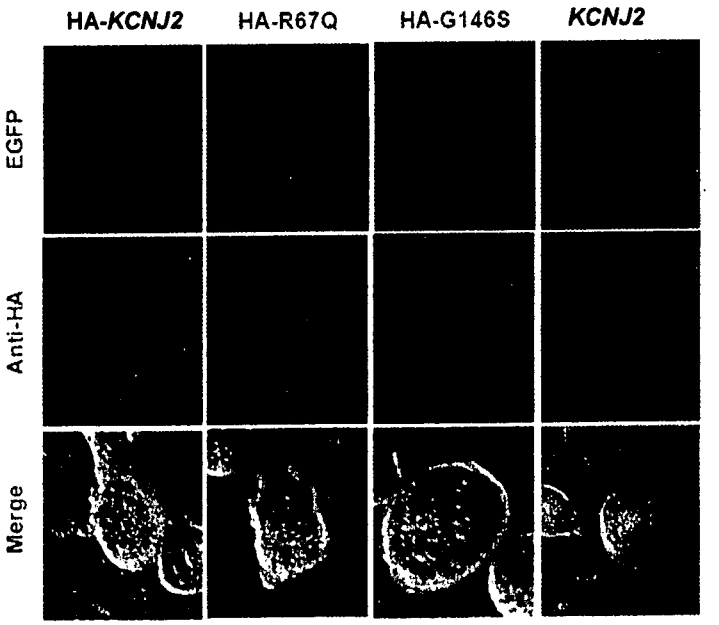

Figure 5. Cellular localization of WT and mutant Kir 2.1 channels In figure, HA-*KCNJ2* indicates HA-tagged WT-*KCNJ2* (positive control), *KCNJ2*; WT-*KCNJ2* without HA tagging (negative control), and HA-R67Q and HA-G146S; HA-tagged mutant *KCNJ2*. Upper panel shows green fluorescence of GFP, middle panel; red fluorescence of secondary anti-HA antibody, bottom panel; merge of green fluorescence, red fluorescence and transmission.

DISCUSSION

ATS is a rare inherited disorder characterized by periodic paralysis, mild dysmorphic features, and QT or QU prolongation with ventricular arrhythmias in ECGs. Mutations of *KCNJ2* have been identified in patients with ATS (ATS1). However, about 20-30% of clinically diagnosed ATS patients do not have the *KCNJ2* mutation (Plaster, et al., 2001; Tristani-Firouzi, et al., 2002; Zhang, et al., 2005), suggesting genetic heterogeneity. In the present study, we identified 10 variations of *KCNJ2* mutations (including 2 novels, R67Q and G146S) in all of our 23 clinically-diagnosed ATS patients. Therefore, all were ATS1 patients. As in our study, a recent genetic survey conducted in the UK (Davies, et al., 2005) demonstrated that all 11 probands from 11 unrelated ATS families were *KCNJ2*-positive (100% ATS1). Our findings, along with that of the UK, seem to contradict the prevalence rate mentioned above, however, ATS is a rare disorder and we should await future studies.

With regard to the cardiac manifestation of ATS, the mean QTc interval was within normal range (406 ± 10 ms), and only 3 of 19 cases (16%) showed prolonged QT intervals. In contrast, abnormal U waves were present in the majority (80%), thereby causing a markedly prolonged QUc interval (715 ± 12 ms). Although ATS had been suggested as a subtype of LQTS (LQT7), Zhang et al. showed the median QTc interval was within the normal range in 96 *KCNJ2*-positive ATS patients and suggested that the QTc prolongation reported in earlier studies was due to the inclusion of U waves in the QT measurement. Our study supported their conclusion, and *KCNJ2*-positive ATS should be classified as ATS1 but not LQT7. In most patients, QTc intervals were within normal range, and the QTc prolongation was observed in only 3 pure ATS patients and 2 ATS patients with compound *KCNQ1* mutation (K-024) (Data of these two compound mutation patients were not included in the analysis of ECG parameters as described above). In our study, if cardiac manifestations were defined as abnormal U waves and/or ventricular arrhythmias, the penetration of cardiac manifestations was up to 96% (22 of 23).

The clinical severity of ventricular arrhythmias was reported to be milder in ATS than other subtypes of LQTS (Plaster, et al., 2001); however, 2 unrelated patients in our cohort experienced aborted sudden death (Hosaka, et al., 2003; Kobori, et al., 2004). Aborted sudden deaths have also been reported in the past (Junker, et al., 2002). It is much too early to conclude that arrhythmias in ATS are, for the most part, benign. Further studies are required to determine long-term prognosis, risk stratification for life threatening cardiac phenotypes, and for effective treatment.

Afebrile seizures were noted during infancy in 4 of 23 ATS patients (17%). This episodic electrical disorder related to the central nervous system was previously reported in one ATS patient having tonic clonic seizures associated with vomiting (Canun, et al., 1999). In our patients with a history of seizures, there were no identified fundamental conditions, including elevated fever, electrolyte abnormality, and/or organic central nervous system disorders. As for a possible mechanism underlying the seizures, Neusch and coworkers (Neusch, et al., 2003) demonstrated that reduced K^+ conductance induced by Kir mutants would disturb the clearance of external K^+ by glial cells during neuronal activities. Therefore, the impaired spatial K^+ buffering induced stronger and prolonged depolarization of glial cells and neurons in response to activity-dependent K^+ release, which may generate the seizures. Furthermore, the Kir current density has been noted to be reduced in temporal lobe epilepsy (Bordey and Sontheimer, 1998;

Schroder, et al., 2000). It remains, however, unknown why these patients experienced the seizures only during infancy.

Subsequent functional assays for novel *KCNJ2* mutations (R67Q and G146S) revealed that they were non-functional and trafficking-competent mutations. In a heterozygous condition, they both caused strong dominant negative suppression effects (91% and 84% reduction at –50 mV respectively)(Figure 3). These biophysical properties may be compatible with pathological roles in expression of ATS phenotypes as well as other mutations previously reported (Lange, et al., 2003).

In conclusion, Japanese ATS patients were exclusively associated with *KCNJ2* mutations (100% ATS1) and presented a high penetrance of cardiac manifestations (96%). ATS1 is a disorder distinct from LQTS. The disease entity is more characterized by normal QTc interval, abnormal U waves and ventricular arrhythmias, typically bidirectional VT.

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Acute and chronic management in patients with Brugada syndrome associated with electrical storm of ventricular fibrillation

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BACKGROUND Some patients with Brugada syndrome experience an electrical storm of ventricular fibrillation (VF).

OBJECTIVE The purpose of this study was to investigate the clinical, laboratory, electrocardiographic, and electrophysiologic characteristics, acute and subsequent chronic treatment, and follow-up data of patients with Brugada syndrome associated with electrical storm of VF.

METHODS Sixty-seven patients with Brugada syndrome (65 men and 2 women, age 46 ± 14 years) were divided into three groups: 7 patients with a history of electrical storm of VF (group I), 39 symptomatic patients with documented VF and/or syncope (group II), and 21 asymptomatic patients (group III). Electrical storm was defined as three or more episodes of VF per day recorded by the memory of an implantable cardioverter-defibrillator.

RESULTS No significant differences were observed among the three groups with regard to clinical (age at diagnosis, familial history of sudden cardiac death), laboratory (SCN5A mutation and serum potassium level), electrocardiographic and electrophysiologic characteristics, and follow-up duration after diagnosis. However, arrhythmic events during follow-up after diagnosis and number of arrhythmic events per patient were significantly higher in group I compared with groups II and III. Isoproterenol infusion (0.003 ± 0.003 μg/kg/min for 24 ± 13 days) completely suppressed electrical storm of VF in all five patients treated and was successfully replaced with oral medications, including denopamine, quinidine, isoproterenol, cilostazol, and bepridil alone or in combination.

CONCLUSION No specifically clinical, laboratory, electrocardiographic, and electrophysiologic characteristics were recognized in patients with Brugada syndrome associated with electrical storm of VF. Isoproterenol infusion was effective as an acute treatment in suppressing electrical storm of VF and was successfully replaced with chronic oral medications.

KEYWORDS Brugada syndrome; Ventricular fibrillation; Electrical storm; Sudden cardiac death; Isoproterenol; Quinidine

(Heart Rhythm 2007;4:695–700)

Introduction

In 1992, Brugada and Brugada[1] described eight patients with a history of aborted sudden cardiac death (SCD) due to ventricular fibrillation (VF) and a distinct ECG pattern consisting of right bundle branch block and ST-segment elevation in the right precordial leads (V_1–V_3) in the absence of any structural heart diseases.[1–7] At present, there is no specific pharmacologic treatment to prevent sudden death in patients with Brugada syndrome. Some patients with Brugada syndrome experience an electrical storm of VF. Isoproterenol, a β-adrenergic agonist, is reported to decrease ST elevation and suppress repetitive episodes of VF in patients with Brugada syndrome probably because of its effect to augment L-type calcium current ($I_{Ca\text{-}L}$).[8–12] However, clinical characteristics and subsequent chronic management following acute therapy with isoproterenol infusion in patients with Brugada syndrome associated with electrical storm of VF is still unclear. In the present study, we investigated the clinical, electrocardiographic, and electrophysiologic characteristics and acute and subsequent chronic treatment in patients with Brugada syndrome associated with electrical storm of VF.

Dr. W Shimizu was supported by the Uehara Memorial Foundation, the Hoansha Research Foundation, Japan Research Foundation for Clinical Pharmacology, Ministry of Education, Culture, Sports, Science and Technology Leading Project for Biosimulation, and Health Sciences Research Grants (H18-Research on Human Genome-002) from the Ministry of Health, Labour and Welfare, Japan. **Address reprint requests and correspondence:** Dr. Wataru Shimizu, Division of Cardiology, Department of Internal Medicine, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka, 565-8565 Japan. E-mail address: wshimizu@hsp.ncvc.go.jp. (Received December 14, 2006; accepted February 10, 2007.)

Methods

Study population

The study population consisted of 67 consecutive patients (65 men and 2 women, age 19–67 years, mean 46 ± 14 years) with Brugada syndrome who were admitted to the

1547-5271/$ -see front matter doi:10.1016/j.hrthm.2007.02.014

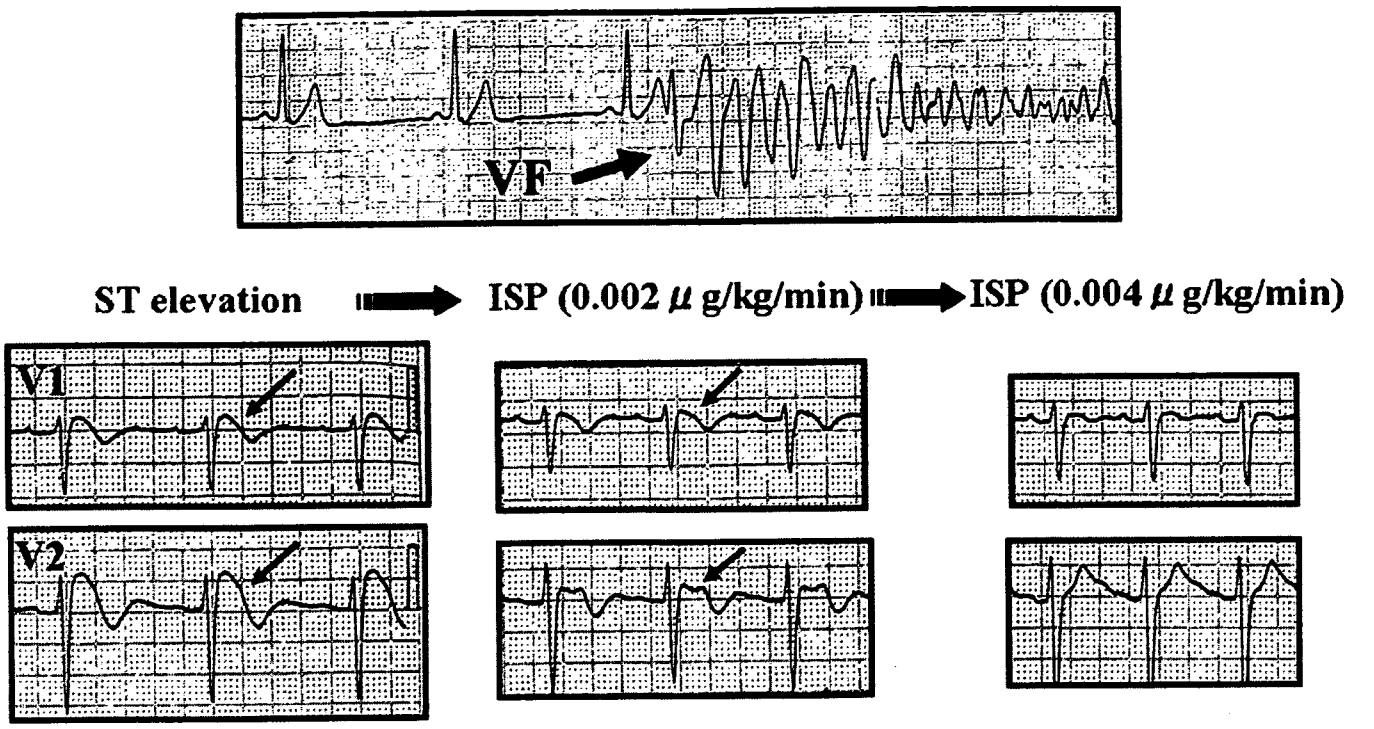

Figure 1 Effects of isoproterenol (ISP) infusion on ST-segment elevation and ventricular fibrillation (VF) in a patient with electrical storm of VF. Isoproterenol (0.002 μg/kg/min) decreased J-point amplitude and changed coved-type to saddleback-type ST-segment elevation in lead V_2. Increasing dose of isoproterenol (0.004 μg/kg/min) normalized ST-segment elevation in lead V_2 and completely suppressed repetitive episodes of VF.

National Cardiovascular Center, Osaka, Japan, between 1994 and 2004. Brugada syndrome was diagnosed when type 1 coved-type ST-segment elevation (≥0.2 mV at J point) was observed in more than one of the right precordial leads (V_1–V_3) in the presence or absence of a sodium channel blocker and in conjunction with one of the following: documented VF, polymorphic ventricular tachycardia (VT), family history of SCD at age younger than 45 years, coved-type electrocardiogram (ECG) in family members, inducibility of VF with programmed electrical stimulation, syncope, or nocturnal agonal respiration.[13] Physical examination showed no abnormal findings, and no evidence of structural heart diseases was demonstrated by echocardiogram in any patients. Informed consent was obtained from all patients. The 67 patients with Brugada syndrome were divided into three groups; 7 patients (6 men) with a history of electrical storm of VF (group I), 39 symptomatic patients (38 men) with documented VF and/or syncope (group II), and 21 asymptomatic patients (21 men, group III).

The 21 patients in group III were diagnosed as having Brugada syndrome according to the following combination of diagnostic criteria in addition to the type 1 Brugada ECG: 11 patients with VF induction during electrophysiologic study, 4 patients with a family history of SCD, 2 patients with documented nonsustained polymorphic VT, 2 patients with nocturnal agonal respiration, and 2 patients with augmentation of ST elevation at early recovery phase after exercise. Electrical storm was defined as three or more episodes of VF per day recorded by the memory of an implantable cardioverter-defibrillator (ICD) for at least 1 day. We retrospectively compared clinical, laboratory, electrocardiographic and electrophysiologic characteristics and follow-up data among the three groups. In the present study, patients were entered into the study upon diagnosis of Brugada syndrome. Study procedures, including 12-lead ECG, signal-averaged ECG, and electrophysiologic study, were performed during first symptomatic in-hospital admission (groups I and II) or in-hospital admission for evaluation of Brugada ECG (group III).

Twelve-lead ECG

Twelve-lead ECG data were recorded at a paper speed of 25 mm/s during sinus rhythm in the supine resting state.

Signal-averaged ECG

The late potential was analyzed using a signal-averaged ECG system (Arrhythmia Research Technology 1200EPX, Milwaukee, WI, USA). Three parameters were assessed using a computer algorithm: (1) total filtered QRS duration, (2) root mean square voltage of the terminal 40 ms of the filtered QRS complexes (V_{40}), and (3) duration of low-amplitude signals <40 μV of the filtered QRS complex (T_{40}). A late potential was considered present when the two criteria (V_{40} 38 ms) were fulfilled.

Electrophysiologic study

Electrophysiologic study was conducted without any antiarrhythmic drugs after informed consent was obtained. Pro-

Table 1 Clinical, laboratory, electrocardiographic, and electrophysiologic characteristics and follow-up

	Group I (n = 7)	Group II (n = 39)	Group III (n = 21)	*P* value
Clinical characteristics				
Age at diagnosis (years)	49.5 ± 15.9	45.5 ± 12.5	47.5 ± 11.4	NS
Previous VF or aborted cardiac arrest before diagnosis (%)	4/7 (57%)	24/39 (62%)	0/21 (0%)	<.05
Previous syncope alone before diagnosis (%)	3/7 (43%)	15/39 (38%)	0/21 (0%)	<.05
Family history of sudden cardiac death (%)	1/7 (14%)	3/39 (8%)	4/21 (19%)	NS
ICD placement (%)	7/7 (100%)	32/39 (82%)	12/21 (57%)	<.05
Duration after ICD placement (years)	8.2 ± 7.4	8.2 ± 2.3	5.3 ± 0.9	NS
Laboratory characteristics				
SCN5A mutation (%)	1/7 (14%)	3/39 (8%)	2/21 (10%)	NS
Serum potassium (mEq/L)	4.0 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	NS
Electrocardiographic characteristics				
Spontaneous coved-type ST elevation (%)	4/7 (57%)	12/39 (31%)	10/21 (48%)	NS
J-point amplitude (mV)	0.35 ± 0.1	0.29 ± 0.2	0.37 ± 0.2	NS
QRS duration (ms)	103 ± 15	106 ± 17	103 ± 20	NS
PQ interval (ms)	159 ± 45	176 ± 37	167 ± 18	NS
Late potential (%)	4/6 (67%)	22/36 (62%)	9/19 (47%)	NS
Augmentation of ST elevation at early recovery phase after exercise (%)	5/6 (83%)	15/31 (48%)	8/17 (47%)	NS
Electrophysiologic characteristics				
Induction of VF (%)	4/7 (57%)	21/30 (70%)	11/15 (65%)	NS
Mode				
Triple	2	9	7	
Double	2	10	4	
Single	0	0	0	
HV interval (ms)	45 ± 10	44 ± 13	46 ± 12	NS
Follow-up				
Follow-up duration after diagnosis (years)	9.5 ± 4.8	8.7 ± 4.5	5.4 ± 1.2	NS
Arrhythmic events during follow-up (%)	7/7 (100%)	11/39 (28%)	2/21 (9%)	<.05
No. of arrhythmic events per patient	14.7	1.1	0.1	<.01
Electrical storm during follow-up (%)	7/7 (100%)	0/39 (0%)	0/7 (0%)	<.01
Duration between diagnosis and first electrical storm (years)	4.6 ± 4.7	NA	NA	
Follow-up duration after electrical storm (years)	5.0 ± 1.5	NA	NA	
Arrhythmic events after electrical storm (%)	5/7 (71%)	NA	NA	

ICD = implantable cardioverter-defibrillator; VF = ventricular fibrillation.

grammed electrical stimulation was performed from the right ventricular apex and right ventricular outflow tract with up to triple extrastimuli. The last extrastimulus was given up to 180 ms in older cases and up to 200 ms in recent cases. Induction of VF requiring direct cardioversion and/or polymorphic VT lasting >30 seconds was considered positive.

Acute treatment

If a patient had at least one episode of VF due to electrical storm of VF after admission, isoproterenol infusion was started until heart rate increased by 20% (Figure 1). If VF did not occur after admission, isoproterenol infusion was not used as an acute treatment, but oral medication (denopamine, an $\alpha+\beta$–adrenergic agonist, or quinidine) was prescribed.

Chronic treatment

After repetitive episodes of VF were completely suppressed by isoproterenol infusion for more than 3 days, isoproterenol infusion was replaced with oral medications. Oral denopamine was prescribed initially. If VF recurred, other oral medications also were prescribed (quinidine, isoproterenol, cilostazol, bepridil).

Follow-up

All patients were followed up at the outpatient clinic of the National Cardiovascular Center.

Statistical analysis

Quantitative values are expressed as mean ± SD. Statistical significance of differences was analyzed by Chi-square test or one-way analysis of valiance among the three groups (group I vs group II vs group III). $P < .05$ was considered significant.

Results

Clinical, laboratory, electrocardiographic, and electrophysiologic characteristics of the three groups

The average number of VF episodes at electrical storm was 9.1 ± 6.8 (3–20) in the 7 group I patients. No specific triggers (e.g., fever, stress, drugs or concomitant illness) for the electrical storm have been noted.

Comparison of the clinical, laboratory, electrocardiographic, and electrophysiologic characteristics among the three groups is given in Table 1. There were no significant differences with regard to age at diagnosis, familial history

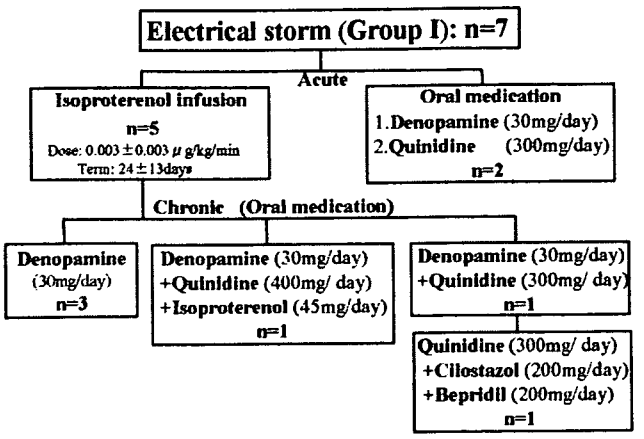

Figure 2 Summary of acute and subsequent chronic treatment in the seven patients associated with electrical storm of ventricular fibrillation.

of SCD, duration after ICD placement, *SCN5A* mutation, and serum potassium level among the three groups. There were no significant differences in previous VF or aborted cardiac arrest before diagnosis and previous syncope alone before diagnosis between groups I and II. Four patients had a history of VF or aborted cardiac arrest before diagnosis, and 3 patients in group I had previous syncope alone before diagnosis. An ICD was implanted before electrical storm of VF in all 7 patients.

No significant differences were observed among the three groups in the electrocardiographic characteristics at diagnosis with regard to J-point amplitude, QRS duration, PQ interval, and incidence of spontaneous coved-type ST elevation, late potentials, and augmentation of ST elevation at early recovery phase after exercise testing.

No significant differences in the frequency and mode of VF induction and HV interval during electrophysiologic study were observed among the three groups.

No significant differences were observed among the three groups during follow-up after diagnosis. However, arrhythmic events during follow-up after diagnosis and number of arrhythmic events/patients were significantly higher in group I vs groups II and III. Average duration between diagnosis and first electrical storm in group I was 4.6 ± 4.7 years. Subsequent arrhythmic events after electrical storm were observed in 5 of the 7 group I patients.

Acute and chronic treatment for electrical storm of VF

At the electrical storm in the 7 group I patients, no intravenous antiarrhythmic agents (e.g., lidocaine or amiodarone) or sedation had been used before starting isoproterenol infusion. ST-segment elevation was augmented at the electrical storm compared with that at baseline (V_1; 0.14 ± 0.07 vs 0.09 ± 0.04 mV; V_2: 0.38 ± 0.09 vs 0.29 ± 0.07 mV). However, this difference did not reach statistical significance.

Figure 1 shows the acute effect of isoproterenol infusion on the electrical storm of VF in a representative patient with Brugada syndrome. Continuous infusion of isoproterenol (0.002 μg/kg/min) decreased the J-point amplitude and changed coved-type to saddleback-type ST-segment elevation in lead V_2. Increasing dose of isoproterenol (0.004 μg/kg/min) normalized ST-segment elevation in lead V_2 and completely suppressed repetitive episodes of VF.

Figure 2 summarizes the acute and subsequent chronic treatment of the 7 patients with electrical storm of VF. Isoproterenol infusion was used as acute treatment in 5 of the 7 patients with electrical storm. Average dose of isoproterenol infusion was 0.003 ± 0.003 μg/kg/min. Average term of isoproterenol infusion (24 ± 13 days) was required because of difficulty in discontinuing or decreasing isoproterenol infusion because of VF recurrence. Isoproterenol completely suppressed electrical storm of VF in all 5 patients. The remaining 2 patients were prescribed oral medication (denopamine 30 mg/day and quinidine 300 mg/day, respectively) because no additional VF episodes occurred after admission.

Isoproterenol infusion was successfully replaced with oral medication in the first 5 patients: 3 with denopamine (30 mg/day), 1 with a combination of denopamine (30 mg/day), quinidine (400 mg/day), and isoproterenol (45 mg/day), and 1 with a combination of denopamine (30 mg/day) and quinidine (300 mg/day).

Average follow-up duration after electrical storm in the 7 group I patients was 5.0 ± 1.5 years. In the 3 patients discharged with denopamine alone following isoproterenol infusion, two VF episodes were recorded in ICD memory for 68 months in 1 patient but no VF episodes in the remaining 2 patients (51 months and 77 months, respectively). Four VF episodes were recorded for 76 months in the patient who was discharged with a combination of denopamine, quinidine, and isoproterenol. The last patient, who was discharged with a combination of denopamine and quinidine, experienced another electrical storm of VF 6 months later after discontinuation of denopamine due to palpitation. Isoproterenol infusion was used again after readmission, and a combination of quinidine (300 mg/day), cilostazol (200 mg/day), and bepridil (200 mg/day) could successfully replace the isoproterenol infusion. VF did not recur for 18 months after readmission in this case. In the 2 patients in whom isoproterenol infusion was not used as an acute treatment, 1 patient who was discharged with denopamine experienced 6 VF episodes for 47 months, and the other patient who was discharged with quinidine had 7 VF episodes for 72 months.

Discussion

The major findings of this study were as follows: (1) no specifically clinical, laboratory, electrocardiographic, and electrophysiologic characteristics were recognized in patients with Brugada syndrome associated with electrical storm of VF, (2) continuous infusion of isoproterenol normalized ST-segment elevation and completely suppressed the electrical storm of VF as an acute treatment, and (3) oral medications including denopamine, quinidine, isoprotere-

nol, cilostazol, and bepridil successfully replaced isoproterenol infusion as a chronic treatment.

Characteristics of Brugada patients associated with electrical storm of VF

Identification of high-risk patients with Brugada syndrome associated with electrical storm of VF and elucidation of their clinical characteristics are important issues. Brugada syndrome usually manifests during adulthood, with a mean age at sudden death of 41 ± 15 years.[14] It is reported that a family history of unexplained sudden death is present in approximately 20%–40% of Brugada Probands in Western countries and less (15–20%) in Japan, and that *SCN5A* mutations account for only 18%–30% of clinically diagnosed Brugada patients.[8,14–16] Low serum potassium level is suggested to be a predisposing factor for VF in patients with Brugada syndrome.[14] However, no significant differences in these clinical characteristics were observed between patients with and without a history of electrical storm of VF. Moreover, 12-lead electrocardiographic parameters and HV interval during electrophysiologic study were no different between patients with and those without an electrical storm of VF. Approximately 60%–70% of patients with Brugada syndrome show late potentials detected by signal-averaged ECG.[14,17] During treadmill exercise testing, augmentation of ST-segment elevation in the right precordial leads compared with that at baseline occasionally is recorded at early recovery phase after exercise (1 or 2 minutes) in Brugada patients. VF or sustained polymorphic VT is induced in approximately 50%–70% of Brugada patients during electrophysiologic study.[8,15,16,18] However, in the present study, frequency of late potentials and ST-segment augmentation after exercise, and inducibility of VF were no different between patients with and those without a history of electrical storm of VF. Although triggering or predisposing factors for electrical storm of VF and characteristics of Brugada patients associated with an electrical storm of VF remain unclear, in this study all 7 patients who experienced electrical storm of VF had arrhythmic events during follow-up after diagnosis. Therefore, our data provided further support for the requirement of ICD placement in Brugada patients with previous episodes of arrhythmic events.

Acute management of Brugada patients associated with electrical storm of VF

Experimental studies have suggested that isoproterenol, a β-adrenergic agonist, decreases ST-segment elevation and suppresses VF by strongly augmenting $I_{Ca\text{-}L}$ in an experimental model of Brugada syndrome.[19,20] Several clinical studies reported the protective effect of isoproterenol in normalizing ST-segment elevation and suppressing episodes of VF.[8–12] Watanabe et al[21] systematically reported that isoproterenol suppressed repetitive ventricular arrhythmia in patients with Brugada syndrome. In the present study, continuous infusion of isoproterenol attenuated ST-segment elevation and completely prevented repetitive episodes of VF in all 5 patients treated and therefore is considered to be first-line acute treatment of electrical storm of VF in Brugada syndrome.

Adjunctive chronic oral treatment

Although isoproterenol infusion is effective in preventing repetitive episodes of VF at electrical storm, discontinuation or decrease of isoproterenol infusion often is difficult because of VF recurrence. In such cases, chronic oral medication usually is required to decrease and discontinue isoproterenol infusion. Several oral agents can be candidates as adjunctive chronic treatment to replace isoproterenol infusion and reduce the incidence of VF episodes subsequently in patients with Brugada syndrome associated with electrical storm of VF.

In the present study, several oral agents, including denopamine, quinidine, isoproterenol, cilostazol, and bepridil alone or in combination, were effective in replacing isoproterenol infusion. Especially, oral denopamine, an α+β–adrenergic stimulant, was effective as a chronic treatment, probably by increasing $I_{Ca\text{-}L}$. Quinidine, a class IA sodium channel blocker, has a relatively strong effect in blocking I_{to} and has been proved effective in suppressing a spontaneous episode of VF in patients with Brugada syndrome.[2–26] Cilostazol, a phosphodiesterase III inhibitor that increases $I_{Ca\text{-}L}$, is reported to be effective in suppressing VF in Brugada syndrome.[2–7] More recently, bepridil is reported to suppress the incidence of VF episodes, probably by blocking I_{to}.[2–8] Although there was small number of Brugada patients associated with electrical storm of VF in whom adjunctive chronic effect of these agents could be examined, each agent alone or in combination was effective as an oral chronic treatment. Further systematic evaluation of the usefulness of these oral agents in larger numbers of Brugada patients is required to make a definitive conclusion.

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differentiate symptomatic Brugada patients from the asymptomatic ones. The sensitivity of MCG could be enhanced by evaluating the effect of Flecainide [or Pilsicainide, more used in Japan[7]] on the WHEBEM pattern during phase 2 of ventricular repolarization.

Therefore, given the considerable number of Brugada patients investigated with MCG, we would be really greatful to Kandori et al. if, in responding to this letter, they could provide also the imaging and a table with the quantitative estimate of current density during the ST interval. Finally, we suggest them to study the effects of Flecainide (or Pilsicainide) on the WHEBEM patterns of ventricular depolarization and repolarization of asymtomatic patients, to evaluate if the pharmacological test might enhance the predictive accuracy of MCG. In fact, such informations would be of major interest as reference landmarks for MCG evaluation of Brugada patients in other institutions.

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To The Editor:

We greatly appreciate the comments from Dr. Riccardo Fenici and Dr. Donatella Brisinda on our article.[1] Their suggestion that magnetocardiography (MCG) could be a sensitive method of risk-stratification of asymptomatic individuals with Brugada syndrome coincide with our future goals.

It is generally agreed upon that any method, either noninvasive or invasive, is not able to stratify future risks among asymptomatic individuals with Brugada syndrome.[2,3] Therefore, new parameters remain to be tested. As suggested, MCG analysis, such as "whole heart electrical activation diagram" (W-HEAD)[4] or "whole heart electrical bull's eye map" (WHEBEM),[1] in combination with pharmacological challenge is a candidate approach. We first reported MCG current-density abnormalities at the right ventricular outflow tract in Brugada syndrome in 2004,[5] followed by W-HEAD and WHEBEM analysis in 2006. These parameters could detect the current abnormalities in Brugada syndrome with type 1, 2, or 3 Brugada electrocardiogram. However, as Brugada syndrome has a complicated pathogenesis, i.e. various electrocardiographic changes within an individual, genetic heterogeneity, and sex and ethnic differences,[3] prospective large population trials are required to conclusively link the relationship between risk of cardiac events and abnormal MCG currents in a quantitative fashion.

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