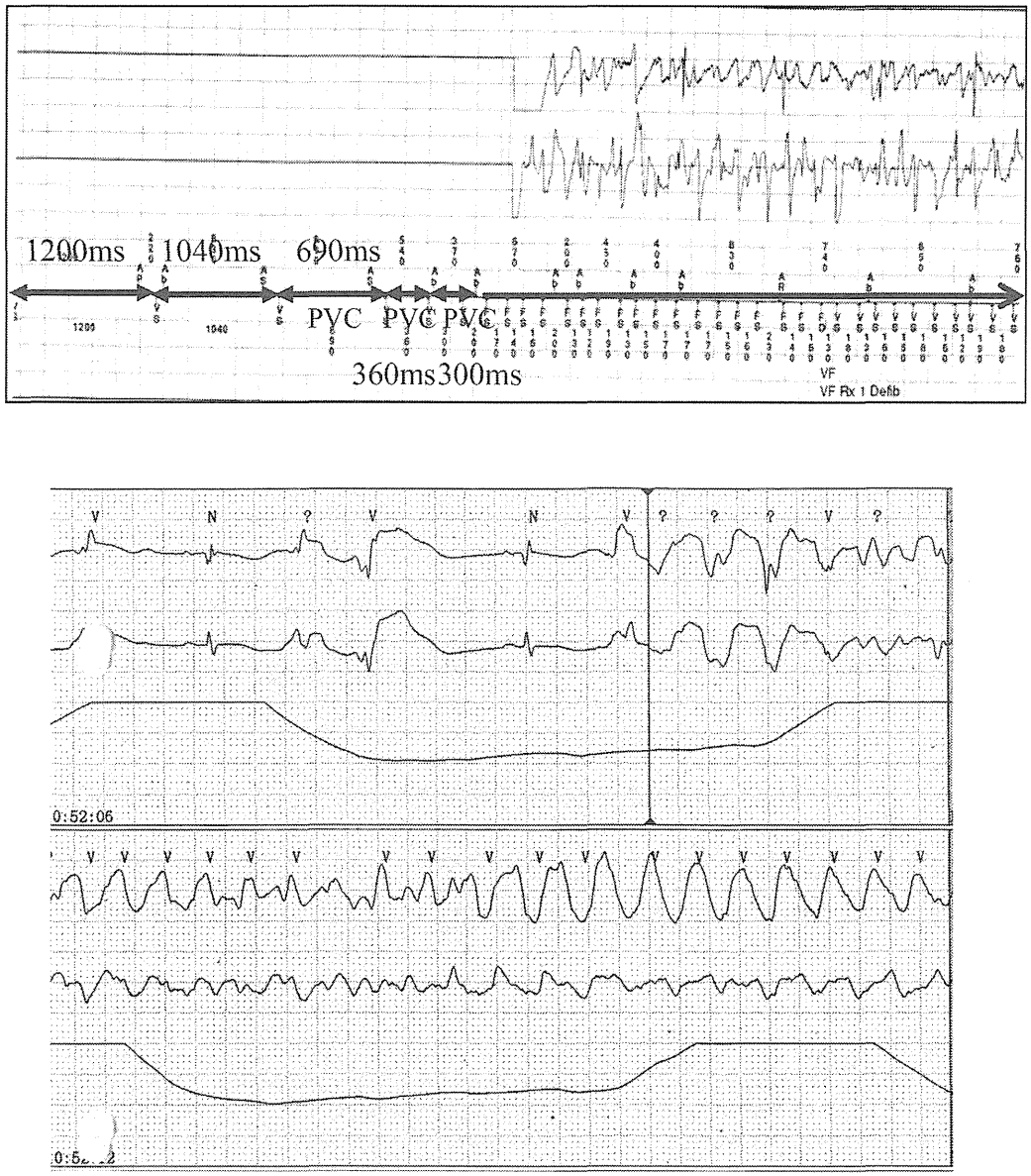

Figure 2. Ventricular fibrillation in patients with *SEMA3A*^{I334V}. After discharge, VF recurred twice and was terminated by ICD shocks in one male patient (patient 1). According to the ICD records, a preceding transient bradycardia was followed by short coupling ectopic ventricular beats and finally VF occurred (upper). The day after admission to the emergency unit, another female patient (patient 2) went into an electrical storm. VF occurred suddenly during sinus bradycardia (lower).
doi:10.1371/journal.pgen.1003364.g002

with UCA and strongly associated with UCA pathophysiology. To our knowledge, this is the first report that investigates the relevance of functional mutations or polymorphisms in *SEMA3A* with respect to human diseases.

We divided the case and control subjects into two geographical groups based on their birthplace in Japan. Significant results observed in Western Japan were replicated in the Eastern Japan group, and the combined P value and odds ratio calculated by the Mantel-Haenszel test were 0.0004 and 3.08, respectively.

According to publicly available data from the 1000 Genomes Project, the frequency of this risk allele of *SEMA3A* is similar among populations other than Europeans, suggesting that this variant may be relevant to the etiology of UCA across these populations. In our study, the G allele frequency was 2.8% in the controls, which was consistent with that reported in Japanese (3.9%) and East Asian populations (2.1%) in the 1000 Genomes Project.

Haïssaguerre et al. reported an increased prevalence of ER characterized by J-point elevation among patients with a history of UCA [18]. Antzelevitch et al. classified ER patterns for risk stratification of VF [19]. The genetic basis for ER is slowly coming into better focus. Burashnikow et al. identified loss of function mutations in the $\alpha 1$, $\beta 2$, and $\alpha 2\delta$ subunits of the cardiac L-type calcium channels (CACNA1C, CACNB2, and CACNA2D1) in patients with ER syndrome [20]. Abe et al. reported that ER may be closely associated with depolarization abnormalities and autonomic modulation [21]. In this study, only two UCA cases with *SEMA3A*^{I334V} demonstrated ER. Instead, the characteristics of the cases with *SEMA3A*^{I334V} suffered VF attacks in a relaxed state and presented with sinus bradycardia/sinus node dysfunc-

Table 4. Comparison of the clinical and electrocardiographic findings in UCA patients with and without *SEMA3A*^{I334V}.

	SEMA3A^{I334V}: rs138694505 (+)	*SEMA3A*^{I334V}: rs138694505 (−)	
	N = 13	**N = 70**	
Clinical Data			
Age of VF occurrence (y)	48±17	42±16	P = 0.273
Gender (Male%)	9 (69.2%)	55 (78.5%)	P = 0.462
History of syncope	3 (23.1%)	15 (21.5%)	P = 0.894
History of atrial fibrillation	3 (23.1%)	15 (21.5%)	P = 0.895
Family History of SCD	4 (30.1%)	13 (18.5%)	P = 0.317
VF occurred during night time	9 (69.2%)	26 (37.1%)	P = 0.032*
VF occurred at rest	9 (69.2%)	24 (34.3%)	P = 0.015*
Twelve Lead ECG Findings			
RR (ms)	1031±111	932±182	P = 0.039*
PQ (ms)	180±36	171±30	P = 0.312
QRS (ms)	110±43	97±17	P = 0.498
QTc (ms)	421±35	413±42	P = 0.238
Presence of J wave	2 (15.4%)	34 (48.6%)	P = 0.020*
Signal Averaged ECG Findings			
fQRSd (ms)	128±31	121±24	P = 0.705
RMS 40 (uV)	39±28	30±26	P = 0.511
LAS 40 (ms)	36±7	37±10	P = 0.760
Echocardiographic Findings			
LVDd (mm)	46.5±5.2	48.1±5.7	P = 0.411
IVSTd (mm)	9.0±1.1	9.2±2.0	P = 0.881
EF (%)	66.2±6.9	63.4±7.6	P = 0.242

UCA: unexplained cardiac arrest, VF:ventricular fibrillation, SCD:sudden cardiac death, SEMA3A: Semaphorin 3A, LVDd: left ventricular end diastolic volume, IVSTd: interventricular septum thickness, EF:ejection fraction, fQRSd: filtered QRS duration, RMS 40: root mean square 40 ms, LAS 40: under 40 uV duration, Data are presented as mean ± SD,
*p<0.05 between *SEMA3A*$^{I334V\ (+)}$ vs *SEMA3A*$^{I334V(-)}$.
doi:10.1371/journal.pgen.1003364.t004

tion. These findings are consistent with the report by Ieda et al. [9,10] that *SEMA3A*$^{-/-}$ mice lacked a cardiac sympathetic innervation gradient and exhibited satellite ganglia malformations, which led to marked sinus bradycardia due to sympathetic dysfunction. Some of the UCA cases in our study may have a mild degree of depolarization or repolarization abnormalities, although we could not detect any obvious organic diseases such as cardiomiopathy by diagnostic imaging or manifest conduction disturbances. The other patients did not have any depolarization or repolarization abnormalities. The patients with *SEMA3A*^{I334V} do not have a homogeneous phenotype and we have to follow up the clinical course of the UCA patients with *SEMA3A*^{I334V} for a long period.

The frequency of AF was 21.6% and rather high in the UCA subjects of our study for unknown reasons and the frequency was similar in the patients with and without *SEMA3A*^{I334V}. One possible reason was that the episodes of AF after resuscitation were included in the past history of AF.

In our study, immunofluorescence staining of the RV revealed that sympathetic nerves were distributed in the subendocardial layer only in patients with *SEMA3A*^{I334V}. If *SEMA3A* exists in adequate quantities in the endocardial layer and functions normally, sympathetic nerves extending to the endocardial layer are suppressed. We assumed that in UCA patients with *SEMA3A*^{I334V}, the epicardial-to-endocardial transmural sympathetic innervation patterning had deteriorated.

An *SEMA3A*^{WT}- and *SEMA3A*^{I334V}-concentrated media did not grossly affect the expression, stability, or secretion of the ligand. As for the molecular weight of *SEMA3A*, when it was expressed in HEK293, the full semaphorin domain (65 kDa) was cleaved and detected in a conditioned media [22]. The sizes of the secreted proteins in both *SEMA3A*^{WT} and *SEMA3A*^{I334V} were equal and coincident with the semaphorin domain including a dimerization interface and Neurolipin-1 (Nrp-1)-binding residue, and the biological activity was sufficient for the acquisition of a high repulsive activity [22].

The function of repelling the DRG axons was weaker and growth cone collapse was less frequent in *SEMA3A*^{I334V} than in *SEMA3A*^{WT}. Therefore, one allele of *SEMA3A* leads to a disruption of the sympathetic innervation of the heart under relevant conditions. These findings were consistent with immunofluorescence observations strongly suggesting that *SEMA3A*^{I334V} can disrupt the ability of *SEMA3A* to repel or collapse DRG axons and sensory neuron growth cones under equal conditions of the neural attractant NGF.

Merte et al. reported that a forward genetic screen in mice identified a novel loss of function *SEMA3A*^{K108N} mutation, which bound to Nrp-1 but failed to repel or collapse DRG axons in vitro [23]. *SEMA3A*^{I334V} exists in blade 5 of the 7-bladed propeller structure of the semaphorin domain and performs a crucial function in *SEMA3A*. Residues 333–335 in 5S of *SEMA3A* constitute the dimerization interface. The *SEMA3A*-65K dimerization interface overlaps with sites responsible for the initial high-

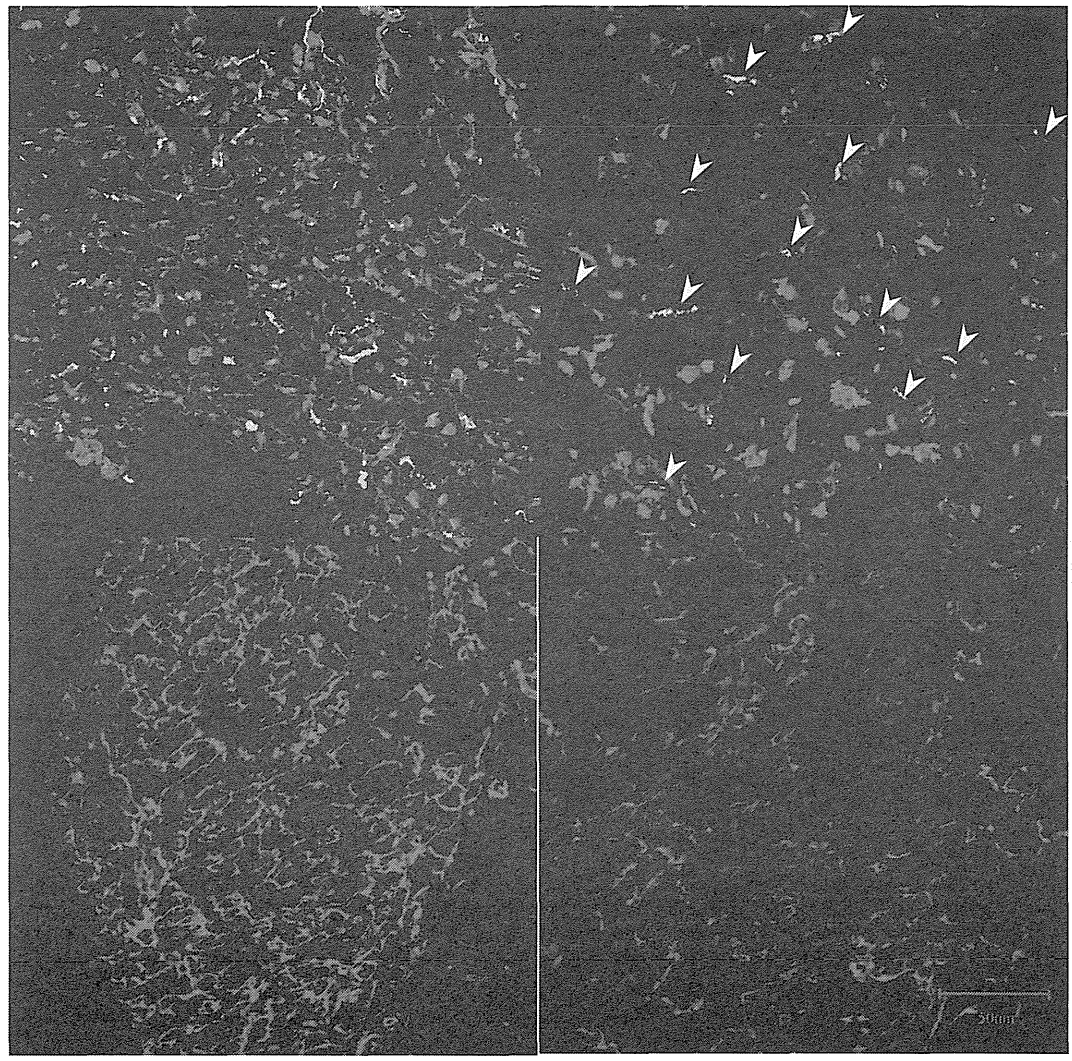

Figure 3. Immunofluorescence staining for Vinculin and anti-TH in the subendocardial layer of patients with and without *SEMA3A*^{I334V}. Immunofluorescence staining of cardiac biopsy specimens revealed that TH positive nerves, as sympathetic nerves, which are absent in the subendocardial layer in normal hearts, extended to the subendocardial layer only in patients with *SEMA3A*^{I334V} (red; anti-Vinculin, green; anti-TH). The samples were examined using a confocal microscope and captured with a 20×objective lens in the figures on the left and with a 40× objective lens in the figures on the right. The arrowheads show the TH positive nerves (Upper panels: *SEMA3A*^{I334V}+, Lower panels: *SEMA3A*^{I334V}−).
doi:10.1371/journal.pgen.1003364.g003

affinity binding to the domain of Nrp-1. Binding of *SEMA3A* to Nrp-1 leads to a conformational change in Plexin-A1, which is transmitted to the cytosolic domain [17].

In the association analysis, *SEMA3A*^{I334V} was highly prevalent in patients with UCA and associated with the UCA pathophysiology. On the other hand, none of the control subjects with *SEMA3A*^{I334V} had any signs of disease at the time of the study, indicating incomplete penetrance or additional environmental or genetic factors.

Our study had several limitations. First, it was very difficult to congregate many UCA cases and therefore the size of our study population was too small to obtain any robust findings. Secondly, we were not able to study the segregation data in the UCA patients with SEMA3A^{I334} because their families refused screening. A future prospective study with a larger cohort will be required to obtain these data. A further functional study would also be desirable to determine whether any abnormal innervation can be observed in healthy carriers by using autopsy specimens

In conclusion, a polymorphism of *SEMA3A*^{I334V} diminishes the cardiac sympathetic innervation gradient and partially contributes to the etiology of UCA. This finding is important in elucidating the pathogenesis of UCA.

Materials and Methods

Subjects

We recruited a total of 83 UCA patients (64 male and 19 female, mean age 43±16 years) from Hiroshima University Hospital, Nagasaki University Hospital, Shiga University of Medical Science, and the National Cerebral and Cardiovascular Center. We recruited 2958 controls (1540 male and 1452 female, mean age 54±18 years) from Hiroshima University Hospital, Osaka-Midosuji Rotary Club (Osaka, Japan), Shiga University of Medical Science, and Niigata University Graduate School of Medical and Dental Sciences. All patients and controls in this paper were unrelated Japanese individuals.

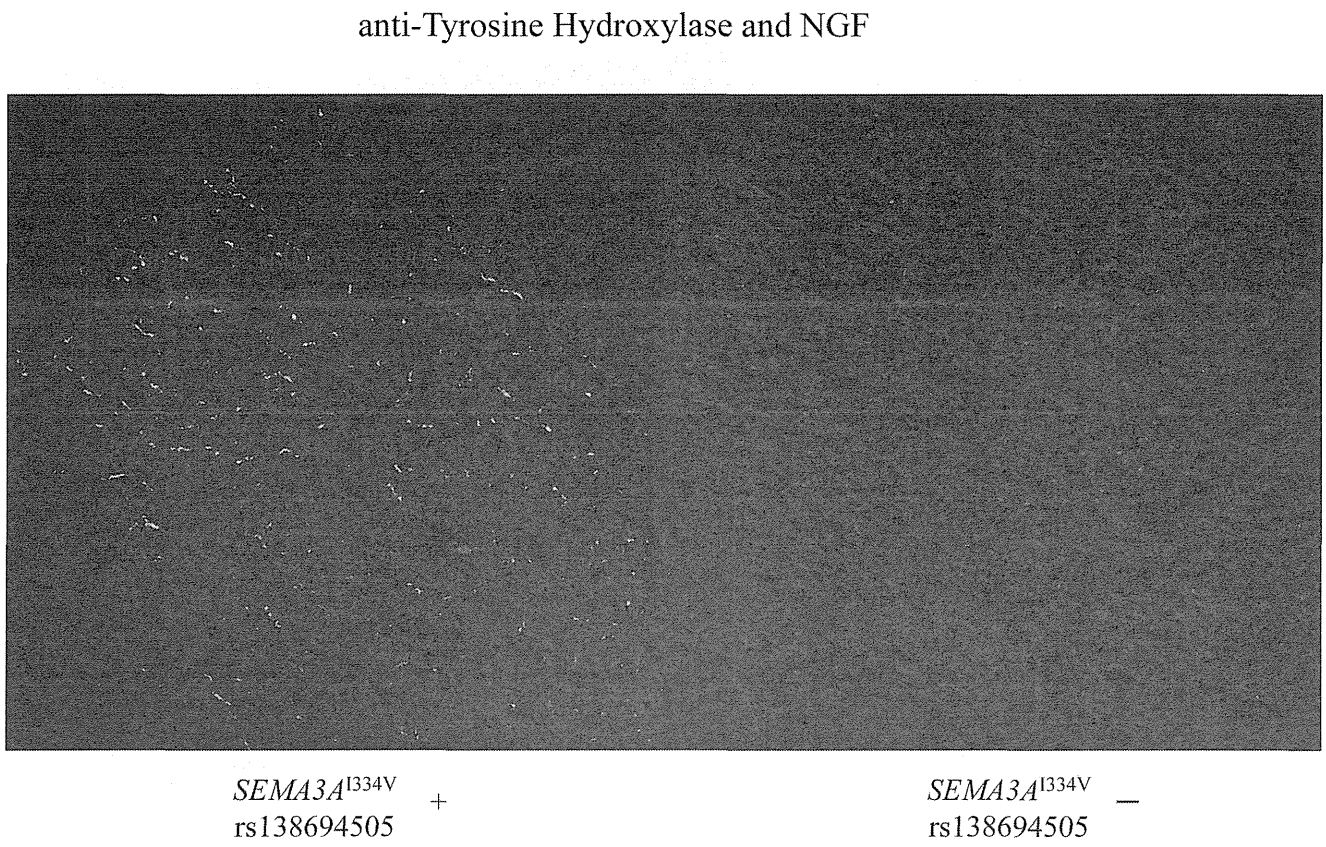

Figure 4. Immunofluorescence staining for Vinculin and NGF in the subendocardial layer of patients with and without *SEMA3A*^{I334V}. On the other hand, the levels of the NGF, a neural attractant factor, were expressed in the subendocardial layer and are comparable between patients with (left panel) and without (right panel) *SEMA3A*^{I334V} (red; anti-NGF; green: anti-TH).
doi:10.1371/journal.pgen.1003364.g004

Case and control subjects were collected from various regions of Japan. Although the Japanese population has rather low genetic diversity, it has been shown that population structures may lead to spurious associations [24]. Therefore, to eliminate the possibility of a population stratification, we divided case and control subjects into two groups geographically based on their birthplace information (i.e., Western Japan and Eastern Japan) (Figure S1).

The Institutional Ethics Committee of the Graduate School of Biomedical Science at Hiroshima University approved all procedures involving human tissue usage. Written informed consent was obtained from all subjects prior to participation.

Twelve subjects enrolled in the study were diagnosed and treated at the Hiroshima University Hospital; the other subjects were diagnosed and treated at other affiliated hospitals and their information was provided to us.

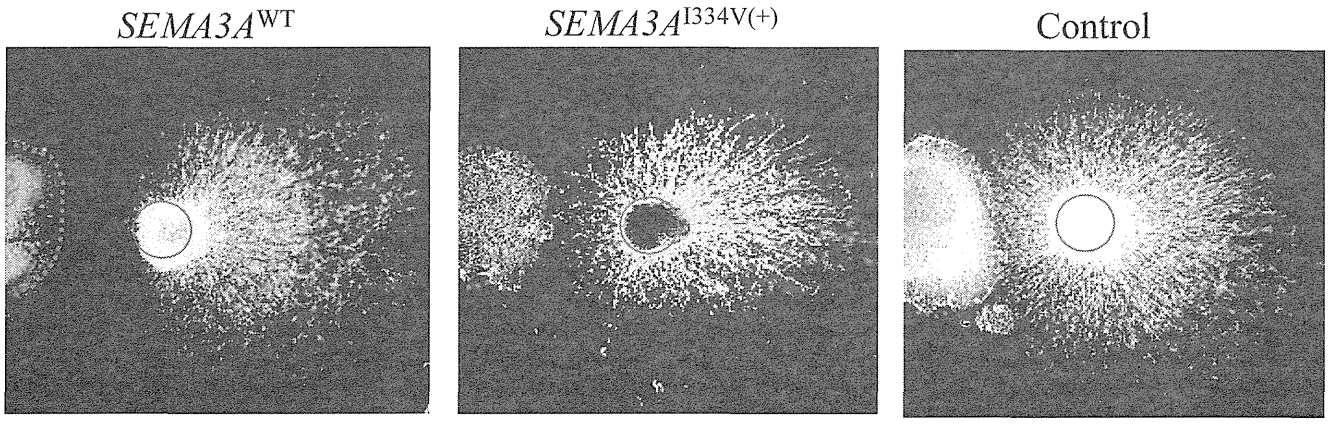

Figure 5. DRG repulsion assay of the *SEMA3A*^{WT}, *SEMA3A*^{I334V}, or control. *SEMA3A*^{WT} expressing cells repelled DRG axons on the proximal side of the ganglia (left). In contrast, DRG explants were less responsive to *SEMA3A*^{I334V} (middle).
doi:10.1371/journal.pgen.1003364.g005

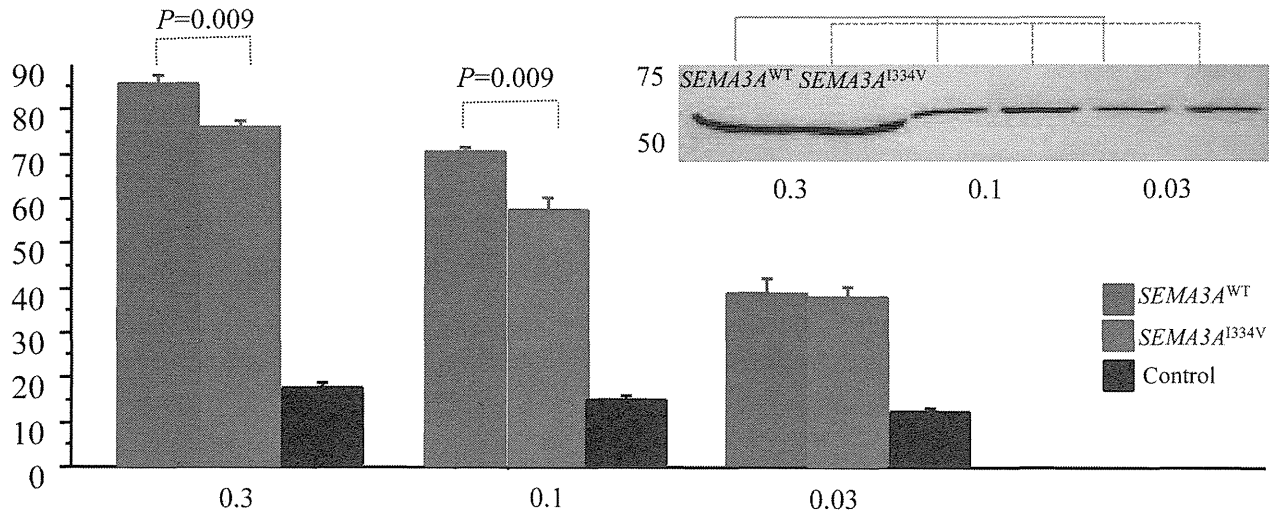

Figure 6. Growth cone collapse assay of the *SEMA3A*^{WT}, *SEMA3A*^{I334V}, or control. The percent of collapsed growth cones of the E8 chick embryos incubated with medium containing vector only, *SEMA3A*^{WT}, or *SEMA3A*^{I334V} at dilutions (0.03, 0.1, and 0.3) of a concentrated media. All dilutions of the concentrated media of the *SEMA3A* or *SEMA3A*^{I334V} expressed in HEK293T cells were similarly secreted. *SEMA3A*^{WT} and *SEMA3A*^{I334V} led to a collapse of the DRG neuron growth cones in all concentrations, but growth cone collapses by *SEMA3A*^{I334V} (red bar) were significantly less than those by *SEMA3A*^{WT} (blue bar) at the dilutions (0.3, 0.1) of the concentrated media ($P = 0.009$).
doi:10.1371/journal.pgen.1003364.g006

Diagnosis of UCA

We defined UCA as that without structural heart disease and in the absence of signs of an arrhythmia syndrome such as Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia and long QT syndrome. All patients with cardiac arrest underwent a physical examination, 12 lead ECG [25], echocardiography and coronary angiography to rule out any underlying heart disease. Those who met the inclusion criteria were enrolled and underwent additional testing (signal averaged ECG, T wave alternance, cardiac magnetic resonance imaging, computer tomography, provocation tests, cardiac biopsy or an electrophysiological study), if possible. The numbers of further noninvasive or invasive tests against UCA patients varied from institute to institute. Patients with exonic mutations in SCN5A and a positive pilsicainide challenge test were excluded from the sample. Early repolarization (ER) was defined as a QRS slurring or notching of $\geq$0.1 mV in more than two consecutive leads of the 12-lead ECG.

Sequence analysis of *SEMA3A* genomic DNA and genotyping

Peripheral blood was obtained from all the subjects. Genomic DNA was extracted from leukocytes using a QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the standard protocol. Using Go Taq (Promega, Madison, WI, USA), all coding regions of the *SEMA3A* located at chromosome 5 were amplified by PCR from 2.5-ng genomic DNA using our original primers in 17 UCA patients and 15 healthy controls entered from Hiroshima University. These amplified coding regions were then resequenced using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) to identify mutations and polymorphisms.

Subsequently, SNP genotypes were genotyped in All of the UCA subjects and healthy control subjects using the Invader assay or the TaqMan assay, as described previously [26,27].

Tag SNP selection

The 47 tag SNPs were genotyped only in the UCA patients and the healthy controls entered from Hiroshima University and Nagasaki University. Using the HapMap database (public release #27, hapmap.ncbi.nlm.nih.gov) and the Haploview program (www.broad.mit.edu/mpg/haploview) and based on selection criteria of $r^2>0.8$ and a minor allele frequency of >0.01 for the Japanese population, tagging-SNPs were selected from the *SEMA3A* region spanning approximately 247 kb, from approximately 5 kb upstream of the transcription start site to 5 kb downstream of the 3′ untranslated region.

Plasmid construction

The complete coding region of human *SEMA3A* was amplified from cDNA with forward (tgttagtgttgccatgaggtct) and reverse (gcattcacctgtgttctctgttag) primers. To generate Flag- *SEMA3A*, the coding sequence DYKDDDD was introduced between the codons for G25 and K26 (NM_006080.2). The I334V mutation was introduced by site-directed mutagenesis using the QuickChange (Stratagene, La Jolla, CA, USA). Full-length human wild-type (*SEMA3A*^{WT}) or mutant *SEMA3A* (*SEMA3A*^{I334V}) cDNA was cloned into pcDNA3.1(+) (Invitrogen, Carlsbad, CA, USA).

Immunofluorescence staining of anti-tyrosine hydroxylase (TH), nerve growth factor (NGF), and vinculin

Transverse sections of a septal site of the RV outflow tract were obtained by biopsy from 12 UCA subjects (4 patients with *SEMA3A*^{I334V} and 8 patients without *SEMA3A*^{I334V}). These sections were embedded in an OCT compound (Sakura, Torrance, CA, USA) and frozen with liquid nitrogen. Immunofluorescence staining was performed using the frozen sections with rabbit anti-TH (AB152, Millipore, Billerica, MA, USA) antibodies and mouse anti-vinculin (Sigma-Aldrich, St. Louis, MO, USA) antibodies diluted at concentrations of 1:100 and 1:200, respectively, in 1% BSA/PBS. Alexa 488-conjugated goat anti-rabbit and Alexa 568-conjugated goat anti-mouse antibodies (Invitrogen) were used as secondary antibodies. As for NGF, sheep polyclonal to NGF (ab49205, Abcam, Cambridge, MA, USA) and rabbit anti-TH (ab152, Millipore) were used as primary antibodies at concentrations of 1:100 in 1% BSA/PBS. Alexa568 donkey anti-sheep (A21099) and Alexa488 donkey anti-rabbit (A21206) antibodies were used as secondary antibodies. Nuclei were stained

with 10 μM of Hoechst 33342 (Molecular probes). Samples were examined using a confocal microscope and captured with a 20× and 40× objective lens on a Zeiss LSM 510 laser scanning microscopy system (Carl Zeiss, Thornwood, NY, USA).

DRG repulsion assay and growth cone collapse assay of *SEMA3A*^{WT} and *SEMA3A*^{I334V}

The DRG were dissected from E8 chick embryos. HEK293T cells were transfected with Flag- *SEMA3A*^{WT} or *SEMA3A*^{I334V} expression vector or equal amounts of empty vector (control) using Gene Juice Transfection Reagent (Novagen, Madison, WI, USA). The DRG and *SEMA3A* -expressing HEK293T cell aggregates were embedded as described previously [28]. Samples were incubated at 37°C in a 5% CO_2 humidified incubator for 48 h and examined using an inverted microscope. For DRG repulsion assays, 10–15 DRG cells were examined, each with Sema3A^{WT}, Sema3A^{I334V}, or a control.

For the purpose of a growth cone collapse assay, the conditioned medium of the *SEMA3A* -expressing HEK293T cells was concentrated [22]. A Western blot analysis was performed using both dilutions of the *SEMA3A*^{WT} and *SEMA3A*^{I334V} concentrated media with anti-FlagM2 (Sigma). Growth cone collapse assays were performed as previously described using chick E8 DRG explants grown on laminin (Invitrogen)- and poly-L-lysine (Sigma)-coated 48-well plates (BD Falcon/353078). The dilution series of the *SEMA3A*^{WT}, *SEMA3A*^{I334V} and vector only concentrates were added to each well and incubated at 37°C in a 5% CO_2 humidified incubator for 30 min. The explants were fixed with 4% paraformaldehyde in 10% sucrose PBS (pH 7.4), and the samples were examined using an inverted microscope [29]. In each dilution series, 5 or 6 growth cone collapse assays were investigated. Each in vitro assay was performed in triplicate.

For quantification, we counted at least 50 growth cones to score on each explant. We assigned each growth cone as either collapsed or not collapsed, and the results were expressed as the percentage of collapsed to all counted growth cones. We compared the percentage of those collapsed between the *SEMA3A*^{WT} and *SEMA3A*^{I334V}.

Statistical analysis

Normally distributed continuous variables are presented as the mean ± SD. Continuous data between the two groups were analyzed using the nonparametric Mann–Whitney U test. For testing the genetic associations in the case–control studies, the chi-square test and Cochran–Armitage trend test were used. Tests for the Hardy–Weinberg equilibrium among the cases and controls were conducted for observed and expected genotype frequencies using an ordinary chi-square test, where a *P*-value of <0.05 was considered statistically significant. For a meta-analysis of 3 individual cases and controls, we used the Mantel-Haenszel test.

Supporting Information

Figure S1 The case and control subjects were divided into two groups geographically based on their birthplace information (i.e., Western Japan and Eastern Japan).
(PDF)

Table S1 Forty-seven tag SNPs of *SEMA3A* were additionally genotyped in the UCA patients and the healthy controls from Hiroshima University. The I334V variant had a moderate linkage disequilibrium only with rs740948 ($r^2 = 0.43$). None of the other SNPs were significantly associated with the UCA after a Bonferroni correction. a: Tagging-SNPs other than I334V were selected based on the selection criteria of an r 2 of >0.8 and minor allele frequency of >0.01in the HapMap-JPT population. b: chi-square test P value in the allele frequency model (uncorrected). c: Hardy-Weinberg equilibrium tests in the control subjects.
(DOCX)

Table S2 The number of tests that we performed for the UCA in VF patients.
(DOCX)

Table S3 Phenotype characterizations in each UCA patient with *SEMA3A*^{I334V}. Patient 3 had persistent AF and patient 13 had chronic AF. Patients 1,2,5,7 and 9 had 1st degree atrioventricular block. Patient 1 had positive late potentials and the fQRSd was increased in a number of patients.
(DOCX)

Acknowledgments

We thank Osaka-Midosuji Rotary Club (Osaka, Japan) for participating in this study. We thank Yasuhiko Kitadai and Kei Shinagawa (Department of Medicine and Molecular Science, Hiroshima University) for suggesting the use of the confocal microscope. We thank Yoshihiro Nakata (Department of Pharmacology, Hiroshima University) for teaching us the technique of extracting the DRG. We thank Yuji Shimatani (Hiroshima City Hospital) and Yoshioka Koichiro (Department of Cardiology, Tokai University School of Medicine) for their advice regarding tissue sampling. We appreciate the efforts of Kaori Ishi (Department of Dermatology, Hiroshima University), Keiei Son (Department of Cellular Biology, Hiroshima University), Yukiji Tonouchi and Shinya Matsuura (Department of Genetics and Cell Biology, Hiroshima University) for their assistance and advice on the experiments. Finally, we thank the members of the clerical and medical staff at Hiroshima University Hospital for their assistance.

Author Contributions

Conceived and designed the experiments: YN KC HO ST YK. Performed the experiments: MT HS DM CNH TT KA YH KH SO. Analyzed the data: NO KS YU-M KK CM MF YW HW TA WS MH. Contributed reagents/materials/analysis tools: AS YY KO IW NM AN NE. Wrote the paper: YN HO.

References

1. No author listed. (1997). Consensus Statement of the Joint Steering Committees of the Unexplained Cardiac Arrest Registry of Europe and of the Idiopathic Ventricular Fibrillation Registry of the United States. Survivors of out-of-hospital cardiac arrest with apparently normal heart. Need for definition and standardized clinical evaluation. Circulation 95: 265–272.
2. Smith ML, Hamdan MH, Wasmund SL, Kneip CF, Joglar JA, et al. (2010). High-frequency ventricular ectopy can increase sympathetic neural activity in humans. Heart Rhythm 7: 497–503.
3. Nishisato K, Hashimoto A, Nakata T, Doi T, Yamamoto H, et al. (2010). Impaired cardiac sympathetic innervation and myocardial perfusion are related to lethal arrhythmia quantification of cardiac tracers in patients with ICDs. J Nucl Med 51: 1241–1249.
4. Paul M, Schäfers M, Kies P, Acil T, Schäfers K, et al. (2006). Impact of sympathetic innervation on recurrent life-threatening arrhythmias in the follow-up of patients with idiopathic ventricular fibrillation. Eur J Nucl Med Mol Imaging 33: 866–870.
5. Bifli M, Fallani F, Boriani G, Fanti S, Kowoll L, et al. (2003). Abnormal cardiac innervation in patients with idiopathic ventricular fibrillation. Pacing Clin Electrophysiol 26: 357–360.
6. André NG, Brack KE, Patel VH, Coote JH. (2007). Autonomic modulation of electrical restitution, alternans and ventricular fibrillation initiation in the isolated heart. Cardiovascular Research 73: 750–760.
7. Tanelian DL, Barry MA, Johnston SA, Le T, Smith GM. (1997). Semaphorin III can repulse and inhibit adult sensory afferents in vivo. Nat Med 3: 1398–1401.
8. Kawasaaki T, Barry MA, Johnston SA, Le T, Smith GM. (2002). Requirement of neuropilin 1-mediated Sema3A signals in patterning of the sympathetic nervous system.. Development 129: 671–680.

9. Ieda M, Kanazawa H, Kimura K, Hattori F, Ieda Y, et al. (2007). *Sema3A* maintains normal heart rhythm through sympathetic innervation patterning. Nature Med 13: 604–612.
10. Kimura K, Ieda M, Fukuda K. (2012). Development, maturation, and transdifferentiation of cardiac sympathetic nerves. Circ Res 110(2): 325–36.
11. Chirumamilla A, Travin MI. (2011). Cardiac applications of 123I-MIBG imaging. Semin Nucl Med 41: 374–387.
12. Haïssaguerre M, Chatel S, Sacher F, Weerasooriya R, Probst V, et al. (2009). Ventricular fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/KATP channel. Cardiovasc Electrophysiol 20: 93–98.
13. Alders M, Koopmann TT, Christiaans I, Postema PG, Beekman L, et al. (2009). Haplotype-sharing analysis implicates chromosome 7q36 harboring DPP6 in familial idiopathic ventricular fibrillation. Am J Hum Genet 84: 468–476.
14. Postema PG, Christiaans I, Hofman N, Alders M, Koopmann TT, et al. (2011). Founder mutations in the Netherlands familial idiopathic ventricular fibrillation and DPP6. Neth Heart J 19: 290–296.
15. Lorentz CU, Alston EN, Belcik T, Lindne JR, Giraud GD, et al. (2010). Heterogeneous ventricular sympathetic innervation, altered beta-adrenergic receptor expression, and rhythm instability in mice lacking the p75 neurotrophin receptor. Am J Physiol Heart Circ Physiol 298: H1652–1660.
16. Ieda M, Fukuda K. (2009). Cardiac innervation and sudden cardiac death. Curr Cardiol Rev 5: 289–295.
17. Antipenko A, Himanen JP, van Leyen K, Nardi-Dei V, Lesniak J, et al. (2003). Structure of the semaphorin-3A receptor binding module. Neuron 39: 589–598.
18. Haïssaguerre M, Derval N, Sacher F, Jesel L, Deisenhofer I, et al. (2008). Sudden cardiac arrest associated with early repolarization. N Engl J Med 358: 2016–2023.
19. Antzelevitch C, Yan GX. (2010). J wave syndromes. Heart Rhythm 7: 549–558.
20. Burashnikov E, Pfeiffer R, Barajas-Martinez H, Delpón E, Hu D, et al. (2010). Mutations in the cardiac L-type calcium channel associated J wave syndrome and sudden cardiac death. Heart Rhythm 7: 1872–1882.
21. Abe A, Ikeda T, Tsukada T, Ishiguro H, Miwa Y, et al. (2010). Circadian variation of late potentials in idiopathic ventricular fibrillation associated with J waves insights into alternative pathophysiology and risk stratification. Heart Rhythm 7: 675–682.
22. Adams RH, Lohrum M, Klostermann A, Betz H, Püschel AW. (1997). The chemorepulsive activity of secreted semaphorins is regulated by furin-dependent proteolytic processing. EMBO J 16: 6077–6086.
23. Merte J, Wang Q, Vander Kooi CW, Sarsfield S, Leahy DJ, et al. (2010). A forward genetic screen in mice identifies *Sema3A* (K108N), which binds to neuropilin-1 but cannot signal. J Neurosci 30: 5767–5775.
24. Yamaguchi-Kabata Y et al. Japanese Population Structure, Based on SNP Genotypes from 7003 Individuals Compared to Other Ethnic Groups:Effects on Population-Based Association Studies(2008). Am J Hum Genetics 83, 445–456
25. Krahn AD, Healey JS, Chauhan V, Birnie DH, Simpson CS, et al.(2009) Systematic assessment of patients with unexplained cardiac arrest: Cardiac Arrest Survivors With Preserved Ejection Fraction Registry (CASPER). Circulation.120(4):278–85.
26. Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, et al. (2001). A high-throughput SNP typing system for genome-wide association studies. J Hum Genet 46: 471–477.
27. Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, et al. (2003). Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 34: 395–402.
28. Moore SW, Kennedy TE. (2008). Dissection and coculture of embryonic spinal commissural neurons. Curr Protoc Neurosci 4: 3.20.1–3.20-17.
29. Kapfhammer JP, Xu H, Raper JA. (2007). The detection and quantification of growth cone collapsing activities. Nature Protocols 2: 2005–2011.

Prognostic significance of early repolarization in inferolateral leads in Brugada patients with documented ventricular fibrillation: A novel risk factor for Brugada syndrome with ventricular fibrillation

Hiro Kawata, MD,[*†] Hiroshi Morita, MD, PhD,[*‡] Yuko Yamada, MD,[*] Takashi Noda, MD, PhD,[*] Kazuhiro Satomi, MD, PhD,[*] Takeshi Aiba, MD,[*] Mitsuaki Isobe, MD,[†] Satoshi Nagase, MD, PhD,[‡] Kazufumi Nakamura, MD, PhD,[‡] Kengo Fukushima Kusano, MD, PhD,[‡] Hiroshi Ito, MD, PhD,[‡] Shiro Kamakura, MD, PhD,[*] Wataru Shimizu, MD, PhD[*]

From the [*]Division of Arrhythmia and Electrophysiology, Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Suita, Japan, [†]Department of Cardiology, Tokyo Medical and Dental University, Tokyo, Japan, and [‡]Department of Cardiovascular Medicine/Cardiovascular Therapeutics, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan.

BACKGROUND Little is known about the clinical and prognostic impact of early repolarization (ER) on patients with Brugada syndrome (BrS), especially those with documented ventricular fibrillation (VF).

OBJECTIVE To investigate the prevalence and prognostic significance of ER in inferolateral leads in patients with BrS and documented VF.

METHODS We investigated 10 different 12-lead electrocardiograms (ECGs) recorded on different days to identify the presence of ER, which was defined as J-point elevation ≥0.1 mV in inferior (II, III, aVF) or lateral leads (I, aVL, V_4–V_6), in 49 individuals (46 men; age 46 ± 13 years) with a type 1 ECG of BrS and previous history of VF.

RESULTS ER was observed persistently (in all ECGs) in 15 patients (31%; P group), intermittently (in at least one but not in all ECGs) in 16 patients (33%; I group), and not observed in 18 patients (37%; N group), yielding an overall ER incidence of 63% (31/49). During the follow-up period (7.7 years), recurrence of VF was documented in all 15 patients (100%) in the P group, and less in 12 patients (75%) in the I group and in 8 patients (44%) in the N group. The P group showed a worse prognosis than N group ($P = .0001$) by Kaplan-Meier analysis. Either persistent or intermittent ER in an inferolateral lead was an independent predictor of fatal arrhythmic events (hazard ratio 4.88, 95% confidence interval 2.02–12.7, $P = .0004$; and hazard ratio 2.50, 95% confidence interval 1.03–6.43, $P = .043$, respectively).

CONCLUSION The prevalence of ER in inferolateral leads was high and an especially persistent form of ER was associated with a worse outcome in BrS patients with documented VF.

KEYWORDS Early repolarization; J wave; Idiopathic ventricular fibrillation; Brugada syndrome; Sudden death

ABBREVIATIONS **BrS** = Brugada syndrome; **ECG** = electrocardiogram; **ER** = early repolarization; **ICD** = implantable cardioverter-defibrillator; **IVF** = idiopathic ventricular fibrillation; **PES** = programmed electrical stimulation; **RV** = right ventricle; **SCD** = sudden cardiac death; **VF** = ventricular fibrillation

(Heart Rhythm 2013;10:1161–1168)

Introduction

Early repolarization (ER) or J wave is often found in the general population and had been considered a benign electrocardiographic (ECG) finding. Its prevalence has been estimated in 1% to 5% of healthy adults.[1–3] Recently, several reports have suggested the association of idiopathic ventricular fibrillation (IVF) with ER in the inferior and/or lateral leads of the ECG.[3–11] ER is more common among patients with IVF than among healthy control subjects, and IVF patients with ER had a worse prognosis than patients without ER.[4]

Brugada syndrome (BrS)[12] is characterized by ST-segment and J-point elevation in the right precordial leads V_1–V_3 and is considered to have a high propensity for sudden cardiac death (SCD).[13,14] Experimental studies have shown that a similar mechanism of repolarization abnormality initiating VF underlies both IVF with ER/J wave and BrS.[15] Some researchers

The first two authors contributed equally to this work. Presented in part at the American Heart Association Scientific Sessions, November 12–16, 2011, Orlando, Florida. Dr. Shimizu was supported in part by the Research Grant for the Cardiovascular Diseases (21C-8, 22-4-7, H23-114, H24-033) from the Ministry of Health, Labour and Welfare, Japan; and Grant-in-Aid for Scientific Research on Innovative Areas (22136011). **Address reprint requests and correspondence:** Dr. Wataru Shimizu, Department of Cardiovascular Medicine, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603 Japan. E-mail address: wshimizu@nms.ac.jp.

1547-5271/$-see front matter http://dx.doi.org/10.1016/j.hrthm.2013.04.009

consider IVF presenting prominent ER in the inferior or lateral leads as a variant of BrS,[6,9] and inferolateral ER also can be present in patients with BrS.[16–19] However, little is known about the clinical and prognostic impact of ER on patients with BrS, especially those with documented ventricular fibrillation (VF). The aim of this study was to investigate the prevalence and prognostic significance of ER in the inferolateral leads among BrS patients with documented VF and implantable cardioverter-defibrillator (ICD) implantation for secondary prevention.

Methods

Patient characteristics

Forty-nine BrS patients with documented VF and ICD implantation (46 men, age range 22–73 years, mean age 46 ± 13 years) from two Japanese institutes (National Cerebral and Cardiovascular Center and Okayama Univ Graduate School of Medicine) were studied. All subjects had a history of VF or aborted SCD and were diagnosed as having BrS if they met the following inclusion criteria: (1) proband, (2) amplitude of QRS-ST junction ≥0.2 mV (2 mm) with coved-type ST-segment elevation in at least 2 of the 3 right precordial leads (V_1–V_3) at baseline or after provocation with a Class I antiarrhythmic drug, (3) normal findings on physical examination, and (4) no abnormality in either right or left ventricular morphology and/or function demonstrated by chest radiography and echocardiography. Laboratory tests were performed to exclude metabolic or electrolyte abnormalities at the time of ECG diagnosis of BrS. Screening test for SCN5A mutations was performed in 37 individuals, and electrophysiologic study was performed in 43 subjects. Programmed electrical stimulation (PES), with a maximum of 3 ventricular extrastimuli, was delivered from 2 right ventricular (RV) sites (RV apex and RV outflow tract). Premature beats were started in late diastole; the shortest coupling interval of the extrastimulus was limited to 180 ms for safety. Stimulation was performed at twice diastolic threshold. Induced VF was defined as any ventricular tachyarrhythmias lasting >15 seconds, causing syncope, circulatory collapse, or requiring defibrillation. Among the 49 patients, 37 showed spontaneous Brugada-type ECG. In the remaining 12 patients, sodium channel blocker infusion test unmasked Brugada-type ECG.

Drug challenge test

Drug provocation test of sodium channel blocker was performed in patients without spontaneous Brugada-type ECG. Intravenous sodium channel blocker pilsicainide (1 mg/kg, maximum 50 mg, 5 mg/min), flecainide (2 mg/kg, maximum 100 mg, 10 mg/min), or disopyramide (2 mg/kg maximum 100 mg, 10 mg/min) was used.[20–24] The test result was considered positive if a type 1 Brugada ECG appeared in more than 1 right precordial lead (V_1–V_3).[16]

ECG

Twelve-lead ECG were recorded at a paper speed of 25 mm/s with amplification of 10 mm/mV in all patients. According to previous study, ER or J-point elevation was defined as an elevation of the QRS-ST junction (J point) in at least two inferior (II, III, aVF) and/or lateral (I, aVL, V_4–V_6) leads (Figures 1–3).[4] ER amplitude had to be at least 1 mm (0.1 mV) above the baseline level, either as QRS slurring (smooth transition from QRS to the ST segment) or notching (positive J deflection inscribed on the S wave).[4,6,11] In general, ER amplitude varies day by day, and determining the definite ER amplitude in all patients is difficult. Moreover, in some patients ER appears clearly in 1 ECG but disappears in another ECG. To solve this problem, we investigated ten 12-lead ECGs recorded on different days for all patients. All ECGs were interpreted by 2 independent cardiologists who were blinded to the clinical result.

ST-segment patterns after the J wave also were analyzed using Tikkanen criteria.[25,26] Horizontal/descending type was defined as ≤0.1-mV elevation of the ST segment within 100 ms after the J point. The concave/rapidly ascending ST segment was defined as >0.1-mV elevation of the ST segment within 100 ms after the J point or a persistently elevated ST segment >0.1 mV throughout the ST segment. Patients were counted as having horizontal ST segment when the ST segment was ascending type in some leads and horizontal type in others.

Classification of groups

Forty-nine patients were categorized into 3 groups based on the stability of ER in the inferior and/or lateral leads. Patients

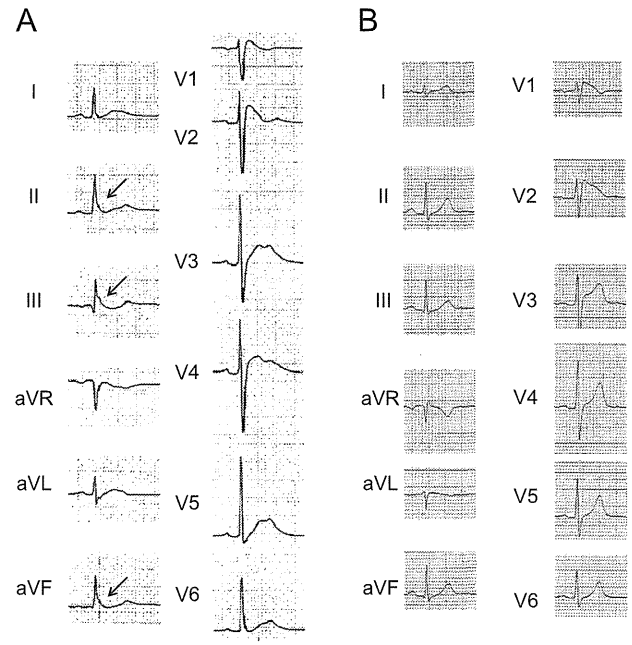

Figure 1 **A:** Twelve-lead ECGs in a patient with Brugada syndrome and early repolarization (ER) in the inferior leads. *Arrows* indicate ER. **B:** Twelve-leads ECG in a patient with Brugada syndrome but without ER repolarization in the inferolateral leads.

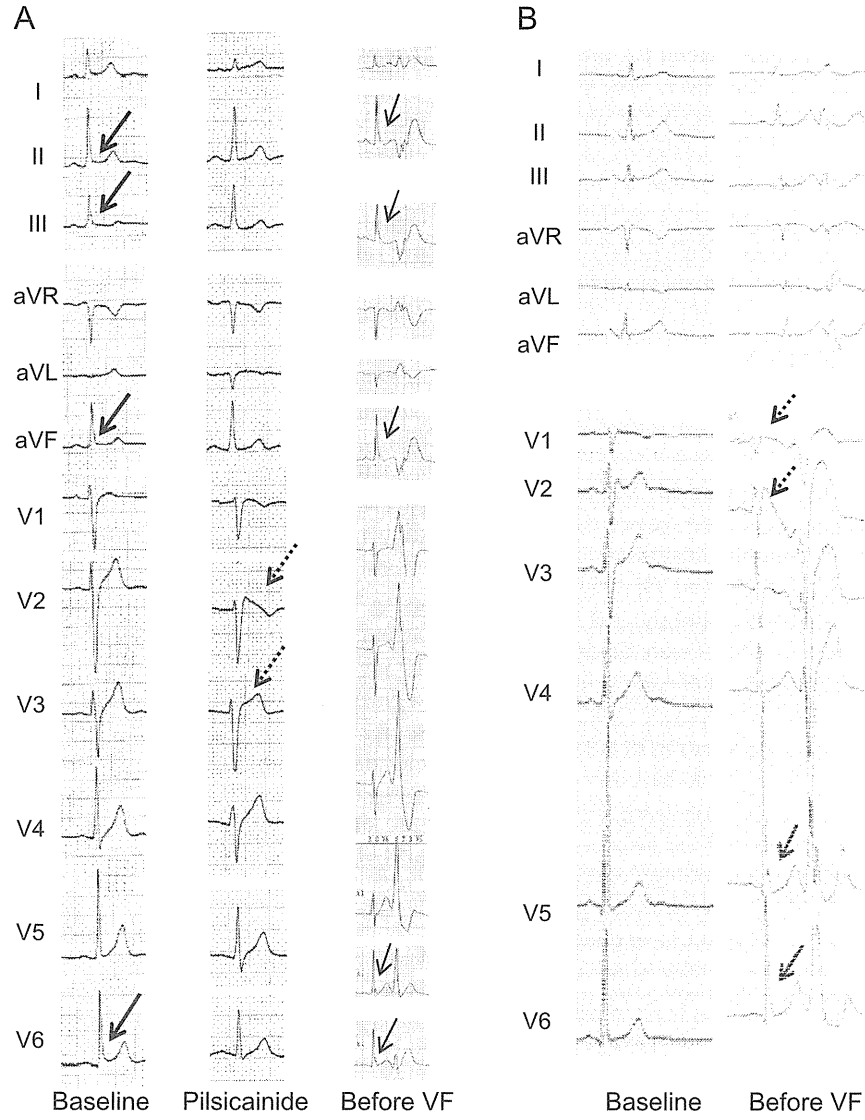

Figure 2 **A:** Twelve-lead ECGs recorded at baseline, during pilsicainide stress test, and recorded just before an episode of ventricular fibrillation (VF) in a Brugada patient with early repolarization (ER) in inferolateral leads. Baseline ECG showed small ER in leads II, III, aVF, and V_6 (*arrows*). During the pilsicainide stress test, despite unmaking of type 1 ST elevation in the right precordial leads (*dotted arrows*), no enhancement or even decrease of ER was observed. On the other hand, ER was clearly accentuated just before an episode of VF compared with baseline (*arrows*), whereas ST elevation in right precordial lead was not observed. **B:** Twelve-lead ECGs recorded at baseline and just before an episode of VF in a Brugada patient with ER in lateral leads. Baseline ECG showed no apparent ER. Just before an episode of VF, accentuation of ER was observed in leads V_5 and V_6 (*arrows*) and was followed by a ventricular premature beat; a similar beat triggered VF a few minutes later. In this patient, ST elevations in right precordial leads (V_2 and V_3, *dotted arrows*) and ER in lateral leads (V_5 and V_6, *arrows*) were observed simultaneously.

with persistent ER (in all ECGs; P group), patients with intermittent ER (in at least one but not all ECGs; I group), and patients without ER (not observed in any ECGs; N group). The prognosis and occurrence of VF were compared among these 3 groups.

Therapy and follow-up

All case subjects received an ICD implantation that provided accurate information on VF recurrence. The subjects visited the hospital routinely every 4 to 6 months for clinical review and device interrogation or whenever patients experienced symptoms relating to ventricular arrhythmia or device discharge at the outpatient clinic. Life-threatening arrhythmic events during follow-up periods include an occurrence of SCD and documented ventricular tachycardia/VF confirmed by intracardiac ECG of their ICD. Electrical storm was defined as 3 or more episodes of VF per day recorded by the memory of the ICD. All patients were followed up at the outpatient clinic of the National Cerebral and Cardiovascular Center (n = 27) or the Okayama University Graduate School of Medicine (n = 22).

Statistical analysis

Continuous variables are expressed as mean ± SD and were compared using the Student *t* test or analysis of variance for normally distributed variables. Fisher exact tests, when

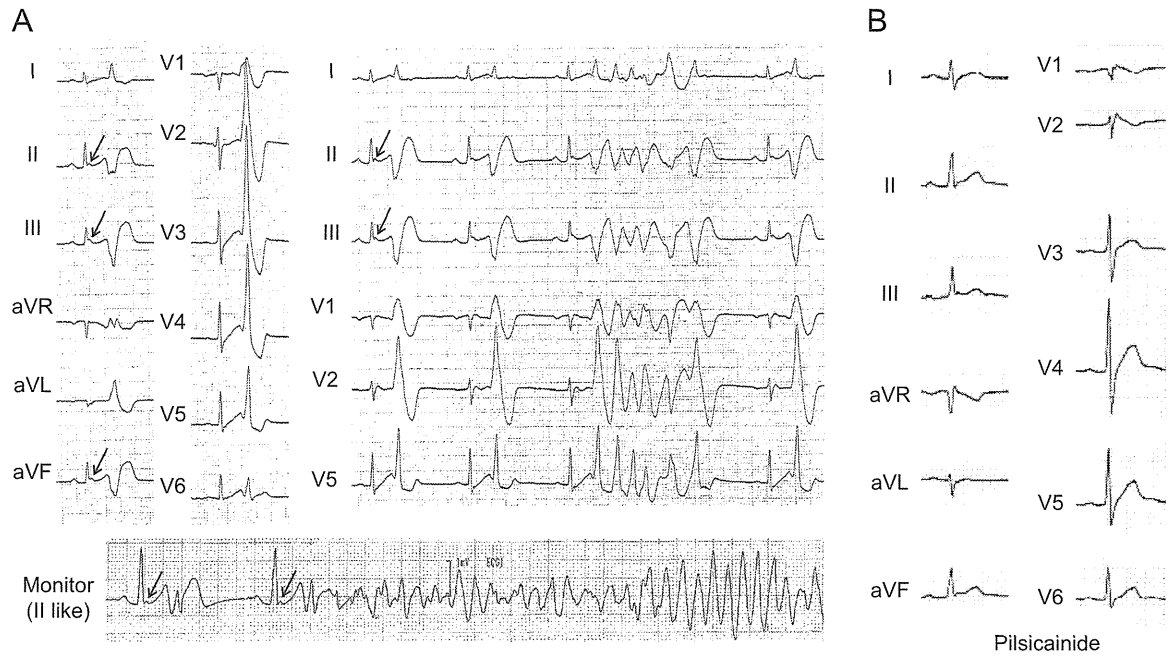

Figure 3 **A, left:** Twelve-lead ECGs in a Brugada patient with early repolarization (ER) in inferior leads II, III, and aVF. A premature ventricular contraction (PVC) with a right bundle branch block morphology and superior axis was observed, indicating that the PVC originated from the left ventricular inferior wall. **Right:** Similar morphology of PVC triggered nonsustained polymorphic ventricular tachycardia. A few seconds later, ventricular fibrillation was recorded on monitor ECG (**bottom panel**). **B:** In this patient, ST elevation in right precordial lead was induced by pilsicainide infusion.

appropriate, were used to compare differences between categorical variables. Survival curves were plotted using Kaplan-Meier methods and analyzed by log-rank test. Event analysis during the follow-up period was performed using the Cox proportional hazards regression model. In the Cox proportional hazards regression model, 95% confidence (CI) limits for the hazard ratio are profile likelihood limits. $P < .05$ was considered significant.

The study was approved by the medical ethical review committees of National Cerebral and Cardiovascular Center. Written informed consent was obtained from all individuals.

Some patients were included in our previous studies: 14 of 49 patients in one study[27] and 19 of 49 patients in another.[18]

Results

Prevalence of inferolateral ER

The inferolateral ER was observed persistently (in all ECGs) in 15 patients (P group), intermittently in 16 patients (I group), and not observed in 18 patients (N group). The prevalence of ER in BrS with a history of VF in the present study was 63% (31/49 patients). Among the 31 patients with the inferolateral ER, 11 showed ER in inferior leads, 11 patients in lateral leads, and 9 patients in both inferior and lateral leads, respectively. As shown in Figures 1, 2, and 3, manifestation of inferolateral ER did not always relate to ST elevation in the right precordial leads.

Clinical, ECG, electrophysiologic, and genetic characteristics

Table 1 compares the clinical, ECG, electrophysiologic, and genetic characteristics and recurrent events during the follow-up period across the P, I, and N groups. Mean age of the 49 patients was 46 years (median 48 years, range 22–73 years), and 46 patients (94%) were male. Thirty-seven of the 49 patients (76%) showed spontaneous ST elevation in the right precordial leads. Family history of SCD occurring before the age of 50 years and SCN5A mutation were observed in 16% and 19%, respectively. PES induced VF in 72% of the patients. There were no significant differences with respect to age, gender, spontaneous ST elevation in right precordial leads, family history (SCD <50 years old), maximum altitude of ER in inferior or lateral leads, inducibility of VF by PES during electrophysiologic study, and SCN5A mutation among the 3 groups.

Prognosis and univariate analysis of clinical variables and inferolateral ER

Mean follow-up period for the entire study population was 93.8 ± 45.6 months (Table 1). Follow-up periods were similar among the 3 groups (P group: 88.7 ± 55.2; I group: 96.5 ± 41.9; N group: 95.6 ± 46.3 months). Thirty-five patients (71%) had recurrence of VF, and 18 patients (37%) experienced electrical storm of VF during the follow-up period. Kaplan-Meier analyses of recurrent VF was performed during follow-up, depending on the presence of spontaneous type 1 ST elevation in right precordial lead, family history of SCD, and existence of inferolateral ER. In Kaplan-Meier survival analysis, time to the first event was shorter in the ER group patients (including both P and I groups) than in the N group patients ($P = .02$). There was no significant difference in prognosis by spontaneous type 1 ST elevation or family history of SCD. Figure 4 shows the

Table 1 Comparison of clinical, electrocardiographic, electrophysiologic, and genetic characteristics and recurrence events during follow-up period across the 3 groups

	All	Persistent	Intermittent	No ER	*P* value
No.	49	15	16	18	Three groups
Age (years)	46.0 ± 12.7	42.6 ± 12.9	43.8 ± 14.8	50.7 ± 9.6	.10
Male gender	94% (46/49)	87% (13/15)	94% (15/16)	100% (18/18)	.19
Spontaneous ST elevation	76% (37/49)	73% (11/15)	75% (12/16)	78% (14/18)	1
ER with horizontal/descending ST	51% (16/31)	60% (9/15)	44% (7/16)	NA	1*
Family history of SCD	16% (8/49)	20% (3/15)	13% (2/16)	17% (3/18)	.89
Maximum amplitude of ER in inferolateral leads (mV)	0.228 ± 0.122	0.265 ± 0.150	0.193 ± 0.080	NA	.25*
Inducibility of VF during electrophysiologic study	72% (31/43)	62% (8/13)	85% (11/13)	71% (12/17)	.40
SCN5A	19% (7/37)	33% (3/9)	18% (2/11)	12% (2/17)	.35
Follow-up period (months)	93.8 ± 45.6	88.7 ± 52.2	96.5 ± 41.9	95.6 ± 46.3	.80
Recurrence of VF	71% (35/49)	100% (15/15)	75% (12/16)	44% (8/18)	.0019
Recurrence of electrical storm	37% (18/49)	47% (7/15)	50% (8/16)	17% (3/18)	.087

Analysis of variance for continuous variables between multiple groups. Fisher exact test for categorical variables. T test for continuous variables between two groups.

ER = early repolarization; SCD = sudden cardiac death; VF = ventricular fibrillation.

*Persistent vs intermittent.

Kaplan-Meier analysis of VF recurrence in each ER subgroup. Patients in the P group had a significantly worse prognosis than did those in the N group ($P = .0001$). The I group also had a tendency for worse prognosis than the N group, but this did not reach statistical significance. Figure 5 shows the Kaplan-Meier analysis of the time to first electrical storm after ICD implantation. The P group showed significantly worse results than the N group ($P = .045$). Table 2 lists the results of univariate analysis of several variables. Univariate analysis revealed that the existence of inferolateral ER (either persistent or intermittent) and ER with horizontal/descending ST segment were correlates for worse prognosis. Multivariate analysis was not performed because those 2 parameters are not independent variables to each other. No other factors were associated with patient prognosis. We then compared prognosis according to the localization of ER: inferior, lateral, and both inferior and lateral. There was no significant difference in the frequency of VF recurrence among the 3 groups according to localization of ER.

Discussion

BrS is characterized by a high incidence of life-threatening ventricular tachyarrhythmias and is responsible for a number of SCD in young healthy people. Patients with a history of syncope, especially those with a previous history of cardiac arrest or VF, are at increased risk for subsequent arrhythmic events compared with asymptomatic individuals.[27–33] Prognosis of BrS varies widely from benign to malignant among

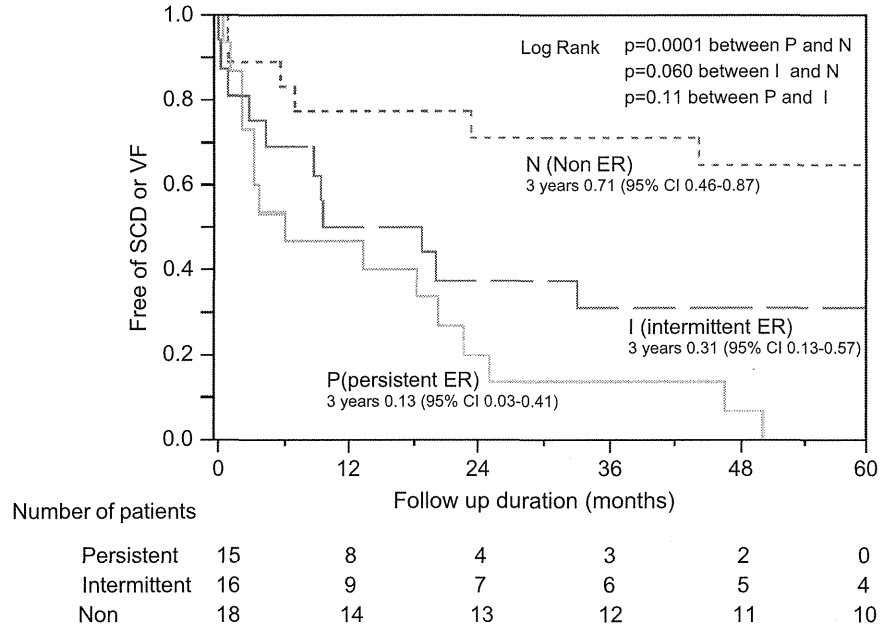

Figure 4 Kaplan-Meier analysis of recurrence of ventricular fibrillation (VF) or sudden cardiac death (SCD) during follow-up depending on frequency of early repolarization (ER) in inferolateral leads (persistent ER vs intermittent ER vs Non ER).

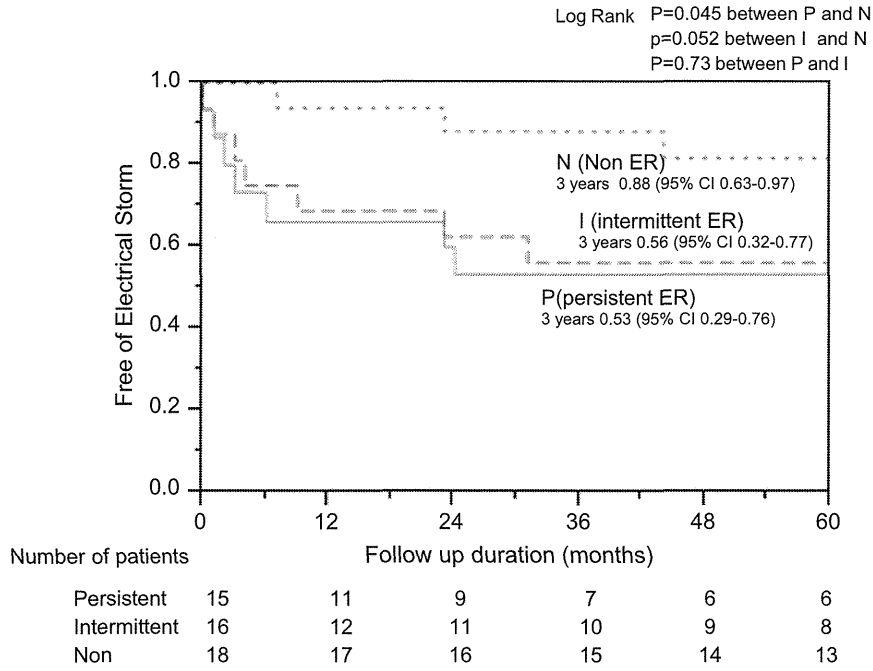

Figure 5 Kaplan-Meier analysis of time to first episode of electrical storm after implantable cardioverter-defibrillator implantation during follow-up depending on frequency of early repolarization (ER) in inferolateral leads (persistent ER vs intermittent ER vs Non ER).

patients and depends on the patient's risk factors.[16,27–36] Accurate prediction of future occurrence of a fatal arrhythmic event is difficult, but all previous studies agreed that previous history of VF was a significant predictor of subsequent VF.[28–34] In this study, we focused on Brugada patients with a previous history of VF episodes in order to investigate the prognostic impact of inferolateral ER on high-risk BrS patients. Eventually, we collected 49 BrS patients with previous VF episode and/or aborted cardiac arrest from 2 large centers in Japan.

ER is a common ECG finding and is considered an innocent sign among healthy young population.[1,3] However, recent data have shown that inferolateral ER is not always benign and is sometimes strongly associated with IVF.[4,35] Inferolateral ER can appear in patients with BrS, but only 4 studies have investigated the significance of ER in Brugada patients.[16,17,19,27] Sarkozy et al[16] showed that inferolateral ER pattern occurs relatively frequently in BrS, which is consistent with our result. Kamakura et al[27] first demonstrated that ER in inferolateral leads was a reliable risk marker in all Brugada patients with and without VF/SCD. A very recent report also showed that patients with BrS who had a J wave in both inferior and lateral leads followed by horizontal ST-segment morphology showed a higher incidence of cardiac events than did those without ER.[19] However, Letsas et al[17] did not find any significant

Table 2 Probability of sudden cardiac death or VF during follow-up depending on clinical, electrocardiographic, and genetic variables in all patients

	Univariate analysis		
	Hazard ratio	95% Confidence interval	*P* value
Age (<40 years)	0.95	0.44–.93	.90
Family history of SCD	1.23	0.46–.79	.65
Spontaneous type 1	1.64	0.76–.10	.22
Inducibility of VF during electrophysiologic study	0.5	0.24–.17	.10
SCN5A positive	1.88	0.68–.44	.21
QRS duration (≥120 ms)	1.19	0.61–.43	.62
Fragmented QRS	1.32	0.67-2.59	.40
Positive signal-averaged ECG	0.63	0.31–.34	.22
Non-ER	1	—	—
Persistent inferolateral ER	4.88	2.02–2.7	.0004
Intermittent inferolateral ER	2.50	1.03–.43	.04
ER with horizontal ST segment	4.48	1.90–1.6	.0006
ER with ascending ST segment	2.53	1.02–.58	.05

ER = early repolarization; SCD = sudden cardiac death; spontaneous type 1 = coved-type ST-elevation on 12-lead ECG at baseline; VF = ventricular fibrillation.

difference in the analysis of all patients. This conflicting result may be due to the unstable existence of ER in inferolateral leads. In order to solve the matter, we first investigated the stability of ER in inferolateral leads in high-risk Brugada patients and the effect on the prognosis. The present study suggested that BrS patients with a history of VF who had inferolateral ER, especially persistent ones, showed a worse prognosis. Electrical heterogeneity in the RV outflow tract is thought to be the main arrhythmogenic substrate for BrS. Antzelevitch and Yan[15] view the J wave in inferolateral leads as a spectrum of disorders that involve action potential heterogeneity in the left ventricular lateral free wall. BrS patients with inferolateral ER may have an electric heterogeneity in extensive regions of ventricles, which can result in life-threatening ventricular arrhythmias. If inferolateral ER represents the substrate of abnormal repolarization leading to initiation of VF, it is reasonable that persistent ER exposes patients to arrhythmic risk more frequently compared with intermittent ER.

Antzelevitch et al[15,36] recently proposed the concept of the J-wave syndrome, and the J-point elevation responsible for the ER pattern is thought to be generated by depression of the epicardial action potential dome, same as with BrS. In the presence of a large I_{to}, commonly seen in the RV epicardium, the same shift of current responsible for an ER pattern in the left ventricle may cause phase 2 reentry and ventricular tachycardia/VF. They hypothesized that the same electrophysiologic mechanism existed in both ST elevation in the right precordial leads and inferolateral ER. However, alterations in ST elevation in the right precordial leads and ER in inferolateral leads do not always occur accordantly. As shown in Figure 3, in some patients from our study, accentuation of ER was observed during the arrhythmic episode, even though ST elevation in the right precordial leads was not observed. In this patient, ST elevation in the right precordial lead was induced by pilsicainide, and diagnosis of BrS was made (Figure 3B). In these patients, not only ST elevation in the right precordial leads but ER in the inferolateral leads may play an important role in VF occurrence. In that sense, it might be better to categorize these patients as ER syndrome with latent Brugada-type ECG rather than BrS with ER. In addition, the effects of sodium channel blockers have been recently reported to be different between ST elevation in the right precordial leads and ER in the inferolateral leads in patients with BrS.[17,37] These facts may indicate that some difference of electrophysiologic mechanism exists between ST elevation in right precordial leads and ER in inferolateral leads in patients with BrS.

Our analysis showed patients with ER and horizontal ST segment had worse prognosis than did those without. Tikkanen et al[25] first showed that a horizontal/descending ST segment is associated with increased risk for arrhythmic death. Takagi et al[19] reported that among Brugada patients, patients with a J wave in both inferior and lateral leads or with horizontal ST-segment morphology after J wave showed a worse prognosis than did those without. Our result reaffirmed that horizontal/descending ST segment morphology is useful for risk stratification of patients with ER among BrS patients.

A spontaneous type 1 ECG and a family history of SCD[27] were reported to be an independent risk factor in patients with BrS.[28,30] However, we could not find any prognostic value of these markers in this study. A reason for the different results from the previous study could be the focus on high-risk BrS patients with previous VF episodes in this study.

The prevalence of ER in BrS was 63% (31/49 patients) in this study and is higher than the incidence reported in previous studies. We checked 10 ECG recordings to evaluate the existence of inferolateral ER. The fact that approximately one third of patients had intermittent appearance of ER in this study indicates that reviewing few ECG recordings could fail to detect ER. Moreover, we focused on high-risk BrS patients who experienced prior VF episodes. The number of evaluated ECGs and the severity of the patients' condition could be influenced on the high incidence of ER in patients with BrS of the present study.

This study would not change the management of BrS patients with documented VF because these patients have the highest risk for recurrent ventricular arrhythmia and should require a defibrillator. Therefore, inferolateral ER is not useful in risk stratification of these patients, but we should pay attention to the possibility of frequent ICD discharge and prophylactic drug therapy (eg, quinidine) to prevent frequent VF episodes in these patients.

Study limitation

Recently, some reports demonstrated that mutations in ion channel genes are responsible for IVF associated with ER.[38–42] In our study, we screened only for SCN5A mutations, and only 19% of patients were positive. Considering the recent reports, screening of other genes might be necessary to elucidate the relationship between ion channel genes and ER in the future.

Conclusion

This study presented the long-term prognosis of high-risk BrS patients (history of previous VF) with ER in inferolateral leads compared to those without ER. The presence of ER is a correlate of VF recurrence. Classification of patients based on the persistence of ER may allow risk stratification over long-term fatal arrhythmic events in patients with BrS and aborted SCD. Repeat ECG recordings and evaluation of the existence of ER are important to identifying the severity of the patients' condition.

Acknowledgment

We gratefully acknowledge the expert statistical assistance of Akiko Kada, MPH, of the National Cerebral and Cardiovascular Center.

References

1. Klatsky AL, Oehm R, Cooper RA, Udaltsova N, Armstrong MA. The early repolarization normal variant electrocardiogram: correlates and consequences. Am J Med 2003;115:171–177.
2. Mehta M, Jain AC, Mehta A. Early repolarization. Clin Cardiol 1999;22:59–65.
3. Gussak I, Antzelevitch C. Early repolarization syndrome: clinical characteristics and possible cellular and ionic mechanisms. J Electrocardiol 2000;33:299–309.
4. Haïssaguerre M, Derval N, Sacher F, et al. Sudden cardiac arrest associated with early repolarization. N Engl J Med 2008;358:2016–2023.
5. Aizawa Y, Tamura M, Chinushi M, et al. Idiopathic ventricular fibrillation and bradycardia-dependent intraventricular block. Am Heart J 1993;126:1473–1474.
6. Kalla H, Yan GX, Marinchak R. Ventricular fibrillation in a patient with prominent J (Osborn) waves and ST segment elevation in the inferior electrocardiographic leads: a Brugada syndrome variant? J Cardiovasc Electrophysiol 2000;11:95–98.
7. Ogawa M, Kumagai K, Yamanouchi Y, Saku K. Spontaneous onset of ventricular fibrillation in Brugada syndrome with J wave and ST-segment elevation in the inferior leads. Heart Rhythm 2005;2:97–99.
8. Potet F, Mabo P, Le Coq G, et al. Novel Brugada SCN5A mutation leading to ST segment elevation in the inferior or the right precordial leads. J Cardiovasc Electrophysiol 2003;14:200–203.
9. Takagi M, Aihara N, Takaki H, et al. Clinical characteristics of patients with spontaneous or inducible ventricular fibrillation without apparent heart disease presenting with J wave and ST segment elevation in inferior leads. J Cardiovasc Electrophysiol 2000;11:844–888.
10. Shinohara T, Takahashi N, Saikawa T, Yoshimatsu H. Characterization of J wave in a patient with idiopathic ventricular fibrillation. Heart Rhythm 2006;3: 1082–1084.
11. Rosso R, Kogan E, Belhassen B, et al. J-point elevation in survivors of primary ventricular fibrillation and matched control subjects: incidence and clinical significance. J Am Coll Cardiol 2008;52:1231–1238.
12. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. J Am Coll Cardiol 1992;20:1391–1396.
13. Shimizu W, Aiba T, Kamakura S. Mechanisms of disease: current understanding and future challenges in Brugada syndrome. Nat Clin Pract Cardiovasc Med 2005;2:408–414.
14. Antzelevitch C, Brugada P, Borggrefe M, et al. Brugada syndrome: report of the second consensus conference: endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. Circulation 2005;111:659–670.
15. Antzelevitch C, Yan G-X. J wave syndromes. Heart Rhythm 2010;7:549–558.
16. Sarkozy A, Chierchia GB, Paparella G, et al. Inferior and lateral electrocardiographic repolarization abnormalities in Brugada syndrome: clinical perspective. Circ Arrhythm Electrophysiol 2009;2:154–161.
17. Letsas KP, Sacher F, Probst V, et al. Prevalence of early repolarization pattern in inferolateral leads in patients with Brugada syndrome. Heart Rhythm 2008;5: 1685–1689.
18. Kawata H, Noda T, Yamada Y, et al. Effect of sodium-channel blockade on early repolarization in inferior/lateral leads in patients with idiopathic ventricular fibrillation and Brugada syndrome. Heart Rhythm 2011;9:77–83.
19. Takagi M, Aonuma K, Sekiguchi Y, Yokoyama Y, Aihara N, Hiraoka M. The prognostic value of early repolarization (J wave) and ST-segment morphology after J wave in Brugada syndrome. Heart Rhythm 2013;10:533–53-29.
20. Brugada R, Brugada J, Antzelevitch C, et al. Channel blockers identify risk for sudden death in patients with ST-segment elevation and right bundle branch block but structurally normal hearts. Circulation 2000;101:510–515.
21. Priori SG, Napolitano C, Schwartz PJ, Bloise R, Crotti L, Ronchetti E. The elusive link between LQT3 and Brugada syndrome: the role of flecainide challenge. Circulation 2000;102:945–947.
22. Miyazaki T, Mitamura H, Miyoshi S, Soejima K, Aizawa Y, Ogawa S. Autonomic and antiarrhythmic drug modulation of ST segment elevation in patients with Brugada syndrome. J Am Coll Cardiol 1996;27:1061–1070.
23. Shimizu W, Antzelevitch C, Suyama K, et al. Effect of sodium channel blockers on ST segment, QRS duration, and corrected QT interval in patients with Brugada syndrome. J Cardiovasc Electrophysiol 2000;11:1320–1329.
24. Roden DM, Wilde AAM. Drug-induced J point elevation. J Cardiovasc Electrophysiol 1999;10:219–223.
25. Tikkanen JT, Junttila MJ, Anttonen O, et al. Early repolarization: electrocardiographic phenotypes associated with favorable long-term outcome. Circulation 2011;123:2666–2673.
26. Rosso R, Glikson E, Belhassen B, et al. Distinguishing "benign" from "malignant early repolarization": the value of the ST-segment morphology. Heart Rhythm 2012;9:225–229.
27. Kamakura S, Ohe T, Nakazawa K, et al. Brugada Syndrome Investigators in Japan. Long-term prognosis of probands with Brugada-pattern ST-elevation in leads V1-V3. Circ Arrhythm Electrophysiol 2009;2:495–503.
28. Brugada J, Brugada R, Antzelevitch C, Towbin J, Nademanee K, Brugada P. Long-term follow-up of individuals with the electrocardiographic pattern of right bundle-branch block and ST-segment elevation in precordial leads V1 to V3. Circulation 2002;105:73–78.
29. Priori SG, Napolitano C, Gasparini M, et al. Natural history of Brugada syndrome: insights for risk stratification and management. Circulation 2002;105: 1342–1347.
30. Brugada J, Brugada R, Brugada P. Determinants of sudden cardiac death in individuals with the electrocardiographic pattern of Brugada syndrome and no previous cardiac arrest. Circulation 2003;108:3092–3096.
31. Eckardt L, Probst V, Smits JP, et al. Long-term prognosis of individuals with right precordial ST-segment-elevation Brugada syndrome. Circulation 2005;111: 257–263.
32. Priori SG, Gasparini M, Napolitano C, et al. Risk stratification in Brugada syndrome: results of the PRELUDE (PRogrammed ELectrical stimUlation preDictive valuE) registry. J Am Coll Cardiol 2012;59:37–45.
33. Probst V, Veltmann C, Eckardt L, et al. Long-term prognosis of patients diagnosed with Brugada syndrome: results from the FINGER Brugada Syndrome Registry. Circulation 2010;121:635–643.
34. Morita H, Kusano KF, Miura D, et al. Fragmented QRS as a marker of conduction abnormality and a predictor of prognosis of Brugada syndrome. Circulation 2008;118:1697–1704.
35. Haïssaguerre M, Sacher F, Nogami A, et al. Characteristics of recurrent ventricular fibrillation associated with inferolateral early repolarization role of drug therapy. J Am Coll Cardiol 2009;53:612–619.
36. Yan GX, Lankipalli RS, Burke JF, Musco S, Kowey PR. Ventricular repolarization components on the electrocardiogram: cellular basis and clinical significance. J Am Coll Cardiol 2003;42:401–409.
37. Roten L, Derval N, Sacher F, et al. Ajmaline attenuates electrocardiogram characteristics of inferolateral early repolarization. Heart Rhythm 2012;9: 232–239.
38. Haïssaguerre M, Chatel S, Sacher F, et al. Ventricular fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/KATP channel. J Cardiovasc Electrophysiol 2009;20:93–98.
39. Burashnikov E, Pfeiffer R, Barajas-Martinez H, et al. Mutations in the cardiac L-type calcium channel associated with inherited J-wave syndromes and sudden cardiac death. Heart Rhythm 2010;7:1872–1882.
40. Valdivia CR, Medeiros-Domingo A, Ye B, et al. Loss-of-function mutation of the SCN3B-encoded sodium channel beta3 subunit associated with a case of idiopathic ventricular fibrillation. Cardiovasc Res 2010;86:392–400.
41. Medeiros-Domingo A, Tan BH, Crotti L, et al. Gain-of-function mutation S422L in the KCNJ8-encoded cardiac KATP channel Kir6.1 as a pathogenic substrate for J-wave syndromes. Heart Rhythm 2010;7:1466–1471.
42. Watanabe H, Nogami A, Ohkubo K, et al. Electrocardiographic characteristics and SCN5A mutations in idiopathic ventricular fibrillation associated with early repolarization. Circ Arrhythm Electrophysiol 2011;4:874–881.

Electrocardiographic Screening of 1-Month-Old Infants for Identifying Prolonged QT Intervals

Masao Yoshinaga, MD, PhD, FAHA; Hiroya Ushinohama, MD, PhD; Seiichi Sato, MD, PhD; Nobuo Tauchi, MD, PhD; Hitoshi Horigome, MD, PhD; Hideto Takahashi, PhD; Naokata Sumitomo, MD, PhD; Yuu Kucho, MD; Hirohiko Shiraishi, MD, PhD; Yuichi Nomura, MD, PhD; Wataru Shimizu, MD, PhD, FAHA; Masami Nagashima, MD, PhD

Background—Neonatal electrocardiographic screening is used to screen infants with prolonged QT intervals, as previously shown in whites. However, this procedure needs to be confirmed in other ethnic groups.

Methods and Results—In 8 areas in Japan, an ECG was recorded in 4285 infants at 1-month medical checkup. A prospective study showed that a provisional criterion of QTc ≥470 ms was appropriate for infants. To assess the validity of the criterion, all infants with a QTc between 460 and 470 ms were followed up. Five infants had a QTc ≥470 ms. Four infants were diagnosed with prolonged QT intervals from follow-up ECGs. Four infants showed no symptoms and did not have a family history of long-QT syndrome. Two infants showed progressive prolongation of QT intervals, and medication was started. Genetic testing was performed in 3 of 4 infants with prolonged QT intervals, and it revealed a *KCNH2* mutation (3065 delT, L1021fs+34X) in 1 infant. One infant with a QTc ≥470 ms and 2 infants with a QTc between 460 and 470 ms showed a decline in their QTc values during follow-up. The study screened another infant with Wolff–Parkinson–White syndrome who was diagnosed with noncompaction before symptoms appeared.

Conclusions—Neonatal electrocardiographic screening can identify infants likely to be affected by long-QT syndrome in the Japanese population, as already shown in whites. This screening may also be useful in identifying other important cardiac diseases. **(*Circ Arrhythm Electrophysiol.* 2013;6:932-938.)**

Key Words: arrhythmias, cardiac ■ death, sudden, cardiac ■ diagnosis ■ electrocardiography ■ long-QT syndrome

Long-QT syndrome (LQTS) is characterized by prolonged ventricular repolarization, with a prolonged QT interval on the surface ECG. The clinical presentation of LQTS is the occurrence of syncope or cardiac arrest in children and young adults.[1,2] Patients with LQTS who experience aborted cardiac arrest during infancy are at high risk for subsequent aborted cardiac arrest or death during their next 10 years,[3] indicating that these patients are an extremely high-risk subset.

Clinical Perspective on p 938

Sudden infant death syndrome is one of the major causes of death in infants, with the highest prevalence at ≈2 months of age.[4–6] Sudden infant death syndrome is multifactorial in origin[7]; however, genetic studies have shown that ≈10% of cases diagnosed as sudden infant death syndrome carry functionally significant genetic mutations in LQTS genes.[8,9]

Electrocardiographic screening in infants may permit early detection of a substantial percentage of patients at risk for sudden infant death syndrome.[10] Studies of infants in Italy and a recent study of infants in Japan have shown that QTc intervals were longest at ≈2 months of age.[11,12] A large study conducted in Italy showed that the prevalence of LQTS might be close to 1:2000[13]; however, no studies have been conducted outside Europe. In Japan, medical examinations during infancy are mandatory, and medical examinations at 1 month of age are currently performed on all infants. Therefore, the aim of the present study was to confirm whether electrocardiographic screening of 1-month-old infants identifies Japanese infants with prolonged QT intervals, as previously shown in whites.

Methods

Study Population

The study was conducted in 16 maternity institutes in 8 areas between July 2010 and March 2011 in Japan, including Kagoshima, Fukuoka, Nagoya, Ogaki, Tokyo, Tochigi, Tsukuba, and Niigata. The parents were asked to participate in the study at discharge from the maternity

Received November 28, 2012; accepted August 29, 2013.

From the Department of Pediatrics, National Hospital Organization Kagoshima Medical Center, Kagoshima, Japan (M.Y., Y.K.); Department of the Cardiovascular System, Fukuoka Children's Hospital and Medical Center for Infectious Diseases, Fukuoka, Japan (H.U.); Department of Pediatrics, Niigata City General Hospital, Niigata, Japan (S.S.); Department of Rehabilitation, Aichi Saiseikai Rehabilitation Hospital, Nagoya, Japan (N.T., M.N.); Departments of Child Health (H.H.) and Epidemiology (H.T.), University of Tsukuba, Tsukuba, Japan; Department of Pediatrics and Child Health, Nihon University School of Medicine, Tokyo, Japan (N.S.); Department of Pediatrics, International Pediatric Center Josai Hospital, Ibaraki, Japan (H.S.); Department of Pediatrics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan (Y.N.); and Department of Cardiovascular Medicine, Nippon Medical School, Tokyo, Japan (W.S.).

The online-only Data Supplement is available at http://circep.ahajournals.org/lookup/suppl/doi:10.1161/CIRCEP.113.000619/-/DC1.

Correspondence to Masao Yoshinaga, MD, PhD, Department of Pediatrics, National Hospital Organization Kagoshima Medical Center, 8-1 Shiroyama-cho, Kagoshima 892-0853, Japan. E-mail m-yoshi@biscuit.ocn.ne.jp

Circ Arrhythm Electrophysiol **is available at http://circep.ahajournals.org** **DOI: 10.1161/CIRCEP.113.000619**

institutes. A total of 4319 consecutive infants participated in the study at the time of a 1-month medical checkup after obtaining written informed consent from parents. We obtained permission to use and analyze these data from the Ethics Committee of the National Hospital Organization Kagoshima Medical Center under the condition that the confidentiality of all personal data would be maintained.

Analysis of ECG and Measurement of the QT Interval

Twelve-lead ECGs were recorded at a speed of 25 mm/s with an FCP-4510 recorder (Fukuda Denshi, Tokyo, Japan). The ECGs were initially read in each center, and a written report was sent to the parents of each participant. The ECGs were then transferred to 1 author (M.Y.) of the present study, and all QT/RR data for the present study were remeasured by the same author (M.Y.). The QT intervals of 3 consecutive beats were measured from the onset of the Q wave to the end of the T wave in lead V_5. When the QT interval could not be measured because of instability of isoelectronic levels in lead V_5, the QT intervals in lead II were measured. When a notch was present in >3 leads[14,15] and this notch appeared at the same timing,[16] the T wave was defined as bifid. The QT/RR data of each of 3 consecutive beats were corrected, and the mean values of the 3 consecutive QTc were used.

Screening and Follow-Up of Infants With LQTS in a Preliminary Study

Published diagnostic criteria using the QTc by the Bazett formula recommend additional diagnostic caution when scaling with tachycardic patients.[14] In a preliminary study, a formula to minimize the effect of heart rate in infants was used[12]: $QTc=QT/RR^{0.43}$ and a provisional criterion of QTc ≥440 $ms^{0.43}$ were used.[12] To assess the validity of the criterion, all infants with QTc ≥430 ms and QTc <440 ms were followed up. Infants with QTc ≥420 ms and QTc <430 ms were also followed in the Kagoshima area where the chief investigator (M.Y.) was working and where 56% of the total subjects participated. The screened infants were followed for 2 or 3 weeks.

Screening of Infants Using the Bazett Formula

Because of the current and frequent use of the Bazett formula in the clinical setting, the present study was reconducted retrospectively using the Bazett formula. The QTc values calculated by the formula in the preliminary study ($QT/RR^{0.43}$) were highly associated with those calculated by the Bazett formula (r=0.989; P<0.0001; Figure 1). The QTc values of 440, 430, and 420 $ms^{0.43}$ used in the preliminary study corresponded to the QTc values of 470, 460, and 450 $ms^{0.5}$ calculated by the Bazett formula (Figure 1). Based on this finding, the screening strategy in the reconducted study included a provisional criterion of QTc ≥470 $ms^{0.5}$ to screen infants with a prolonged QT interval. To assess the validity of this criterion, all infants with QTc ≥460 ms and QTc <470 ms were followed up. Infants with QTc ≥450 ms and QTc <460 ms were also followed in the Kagoshima area where the chief investigator (M.Y.) was working and where 56% of the total subjects participated. The screened infants were followed for 2 or 3 weeks. The definition of infants with a prolonged QT interval in the present study was those whose prolonged QTc values were sustained during follow-up at a 2- or 3-week interval.

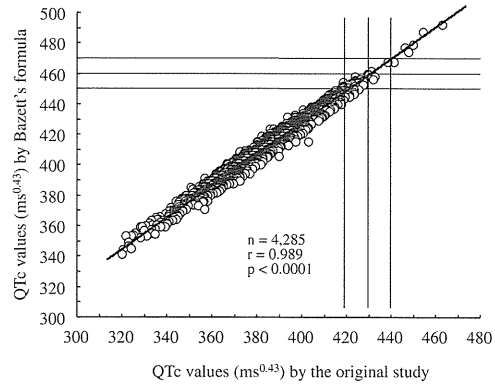

Figure 1. Association of QTc values calculated by the original formula with those calculated by the Bazett formula. QTc values calculated by the formula in a preliminary study ($QT/RR^{0.43}$) were highly associated with those by the Bazett formula ($QT/RR^{0.5}$).

Follow-Up Strategies of Infants With Prolonged QT Intervals

In a nationwide study in Japan, patients with LQTS who showed life-threatening arrhythmias at the perinatal period and whose mutations were determined were mostly those with LQT2 or LQT3.[17] The clinical course of these infants was favorable with administration of β-blockers and mexiletine and with pacemaker implantation or an implantable cardioverter-defibrillator. In this Japanese series, β-blockers and mexiletine were coadministered to 7 of 11 infants with LQT2 and to all 7 LQT3 infants.[17] β-Blockers and mexiletine were coadministered in the present study when the QTc values progressively increased and when the parents accepted medication for their infants.

In the preliminary electrocardiographic screening program, thorough familial electrocardiographic recording and genetic testing were not mandatory. The performance of familial electrocardiographic screening and genetic testing was based on the judgment of the chief physicians.

Genetic Analysis

Genomic DNA was isolated from blood after obtaining written informed consent. Genetic screening for LQT-1 (*KCNQ1*), -2 (*KCNH2*), -3 (*SCN5A*), -5 (*KCNE1*), -6 (*KCNE2*), -7 (*KCNJ2*), -9 (*CAV3*), -10 (*SCN4B*), and -12 (*SNTA1*) was performed by polymerase chain reaction and direct DNA sequencing. When abnormal hand/foot findings were present, screening for LQT-8 (*CACNA1C*) was planned. The exons of LQT-4 (*ANKB*), LQT-10 (*SCN4B*), and LQT-11 (*AKAP9*) were not analyzed because there are no reported cases of these mutations in the Japanese population. Genomic DNA was isolated using a QIAamp DNA Blood Midi Kit (Qiagen, Gaithersburg, MD). Polymerase chain reaction products were purified by AMPure (Beckman Coulter, Brea, CA). After treatment with the BigDye Terminator v1.1 Cycle Sequencing Kit (ABI, Warrington, United Kingdom) and BigDye X Terminator, direct sequencing was performed by the ABI3130x1 Genetic Analyzer (ABI).

Statistical Analysis

The most appropriate cutoff values to screen for QT prolongation in 1-month-old infants in the present study were obtained from the positive predictive value and negative predictive value.

Results

Final Subjects

Of the 4319 infants who participated in the study, a total of 4285 subjects were enrolled in this retrospective study whose QT/RR data of 3 consecutive beats could be measured (2148 male infants, 2038 female infants; sex was not described in 100 infants). Of the 34 infants excluded, 3 consecutive QT/RR data could not be obtained because of the instability of isoelectric lines in 26 infants; however, their QTc values were normal based on 1 or 2 QT/RR data sets. Five infants with complete right bundle branch block and 3 infants with Wolff–Parkinson–White syndrome were also excluded from the QT study.

QTc Intervals of Infants

The mean values of the QT interval, heart rate, and QTc intervals of male infants were 253±17 ms, 160±16 beats per minute, and 410±19 ms, respectively; those of female infants

were 255±17 ms, 158±16 beats per minute, and 413±19 ms, respectively; and those of all infants were 254±17 ms, 159±16 beats per minute, and 412±19 ms, respectively. The mean QTc value of female infants was longer than that of male infants ($P<0.0001$).

Infants With Prolonged QT Intervals

Of the 4285 infants, 5 infants had a QTc of ≥470 ms at the time of the 1-month screening (Table 1). Four infants (3 male infants and 1 female infant) were diagnosed with prolonged QT intervals from the follow-up ECGs (Figure 2). Of these 4 infants, 2 (cases 1 and 2 in Figure 2) showed progressive prolongation of QT intervals (Figures 3 and 4). Propranolol and mexiletine were administered to these 2 infants. Two patients (cases 3 and 4 in Figure 2) were followed without medication. Case 1 was the third child of the parent, and cases 2, 3, and 4 were the first children of their parents. All 4 families had no family history of LQTS-related symptoms, including sudden cardiac death.

One male infant with a QTc of ≥470 and 2 female infants with a QTc between 460 and 470 ms showed a decline in their QTc values during follow-up (Figure 2). One female infant with a QTc between 460 and 470 ms was lost to follow-up. Of the 2420 infants (56% of the final total subjects) who participated in the Kagoshima area, 21 infants (0.87%) had QTc values between 450 and 460 ms, and all infants showed a decrease in QTc values during follow-up.

Genetic Analysis

Genetic analysis was performed in 3 of 4 infants with a prolonged QT interval (cases 1, 2, and 3 in Figure 3), and it demonstrated a frameshift-type mutation in the *KCNH2* gene (3065 delT, L1021fs+34X) in 1 infant (case 2).

Cutoff Values for Screening for QT Prolongation in 1-Month-Old Infants

Assuming that 4 of the 4285 infants had prolonged QT intervals in the present study, the most appropriate cutoff value to screen for QT prolongation in 1-month-old infants was 470 ms, and the next appropriate value was 460 ms (Table 2).

Infants With Miscellaneous Heart Diseases

Of the 4319 infants who participated in the study, including 4285 infants whose QTc values were analyzed, some infants were found to have miscellaneous heart diseases; 1 infant had left ventricular nonimpaction (LVNC), and 1 had situs inversus totalis. A 43-day-old male infant was admitted to our hospital because of the presence of Wolff–Parkinson–White syndrome. He seemed active, but his echocardiography revealed LVNC (Figure 5). His left ventricular ejection fraction and brain natriuretic peptide levels at the first visit were 50% and 89 pg/mL, respectively. He was followed for 2 or 3 weeks, and he showed an ejection fraction of 50% to 60%. His ejection fraction showed a sharp decline to <30% at 81 days of age, but his general status was good. Medication was then started with carvedilol and enalapril. He is currently 28 months old. He experienced supraventricular tachycardia several times since the age of 5 months. However, supraventricular tachycardia was successively treated, and he finally received catheter ablation twice as a treatment for supraventricular tachycardia. His ejection fraction has recovered to 65% with medication (carvedilol, enalapril, and flecainide).

Table 1. Distribution of Infants Based on the Duration of QT Intervals in the Present Study and in an Italian Study[13]

QTc, ms	Present Study	Italian Study
≥470	5 (0.12%)	31 (0.07%)
460–470	3 (0.07%)	28 (0.06%)
450–460	34 (0.79%)	177 (0.41%)
440–450	172 (4.01%)	858 (1.99%)
<440	4071 (95.0%)	41 986 (97.5%)
	4285 (100%)	43 080 (100%)

The data are expressed as absolute values and percentages in parentheses. ECGs in the present study were recorded in infants at the 1-month medical checkup, and those in the Italian study were recorded in infants between 15 and 25 days of age.

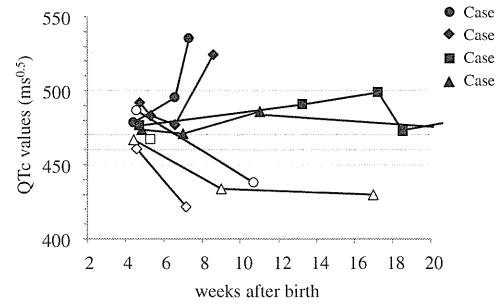

Figure 2. Time course of QTc values of infants whose QTc was >460 ms. Among 5 infants with a QTc >470 ms, cases 1 (·) and 2 (♦) received medication because of progressive prolongation of their QTc values, cases 3 (■) and 4 (▲) were followed without medication, and in 1 infant (○), the QTc value decreased. Among 3 infants with a QTc value between 460 and 470 ms, 1 infant was lost to follow-up.

Discussion

The present study confirmed that electrocardiographic screening of 1-month-old infants is successful in identifying infants with prolonged QT intervals in the Japanese population, which is similar to findings in whites.[13] This screening was also able to identify an infant with life-threatening heart disease during the asymptomatic period.

A large study conducted in Italy showed that 17 infants among a cohort of 44 596 neonates were affected by LQTS and that the prevalence of LQTS was 1:2534 in whites.[13] Of the 17 infants, 16 were diagnosed with LQTS because of the presence of both QT prolongation and disease-causing mutations, and 1 was diagnosed because the father of the infant with a QTc of 482 ms also had an extremely prolonged QTc (581 ms). The authors of this previous study hypothesized that the prevalence of LQTS is close to 1:2000, considering the presence of some infants without genetic analysis in the study.[13] The present study was conducted in the Japanese population. The distribution of infants with a QTc >470 ms was 5 of 4285 (0.12%) in the present study and 31 of 43 080 (0.07%) in a previous study[13] and that of a QTc between 460 and 470 ms was 3 of 4285 (0.07%) in the present study and 28 of 43 080 (0.06%) in a previous study. The distribution was

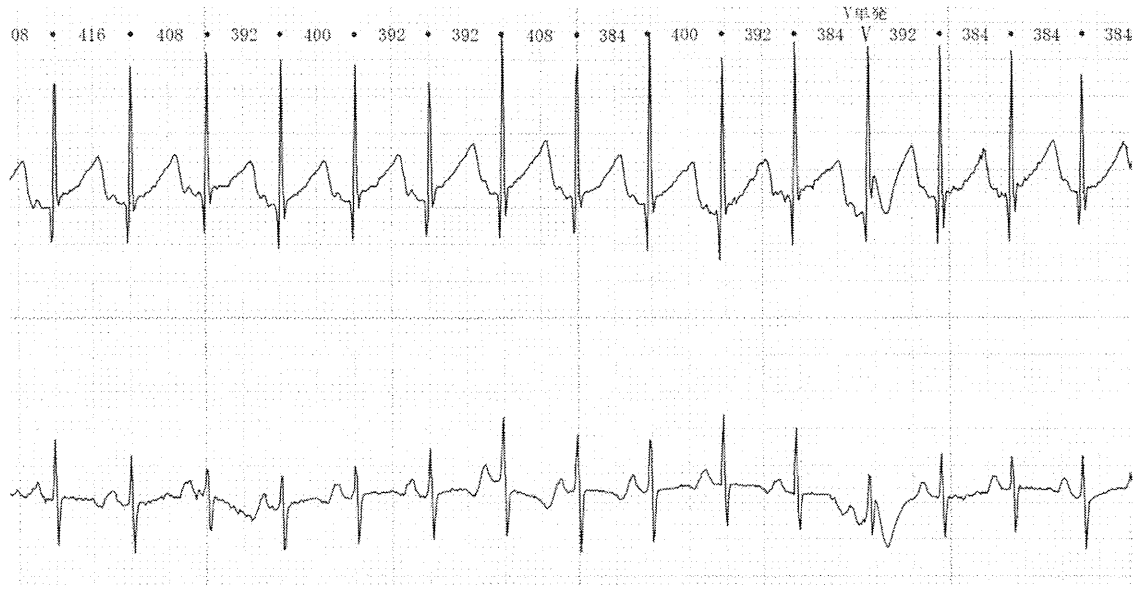

Figure 3. An ECG of a Holter recording at 51 days of age in an infant who received medication. The QTc value was 511 ms.

not different between the present study and this previous study (P=0.38 and P>0.99, respectively).[13]

The mean QTc intervals were similar between the 2 studies (412±19 ms in the present study and 406±20 ms in a previous study).[13] The reason for slightly longer QTc values in the present study than in the previous study[13] might be because of the dates of the electrocardiographic recording. ECGs were recorded in 1-month-old infants in the present study and between the 15th and 25th days of life in the previous study.[13] Mean QTc intervals increase from birth to 2 months of age.[11,12] Finally, 4 infants had prolonged QT intervals in the present study. These data suggest that neonatal electrocardiographic screening is successful for identifying infants with prolonged QT intervals in the Japanese population, as already shown in whites.

QTc values in female children are known to be longer than those in male children, as well as in adolescents and the adult population. Accordingly, LQTS diagnostic criteria recommend using different criteria between male and female infants.[1] A previous study showed that QTc values were not

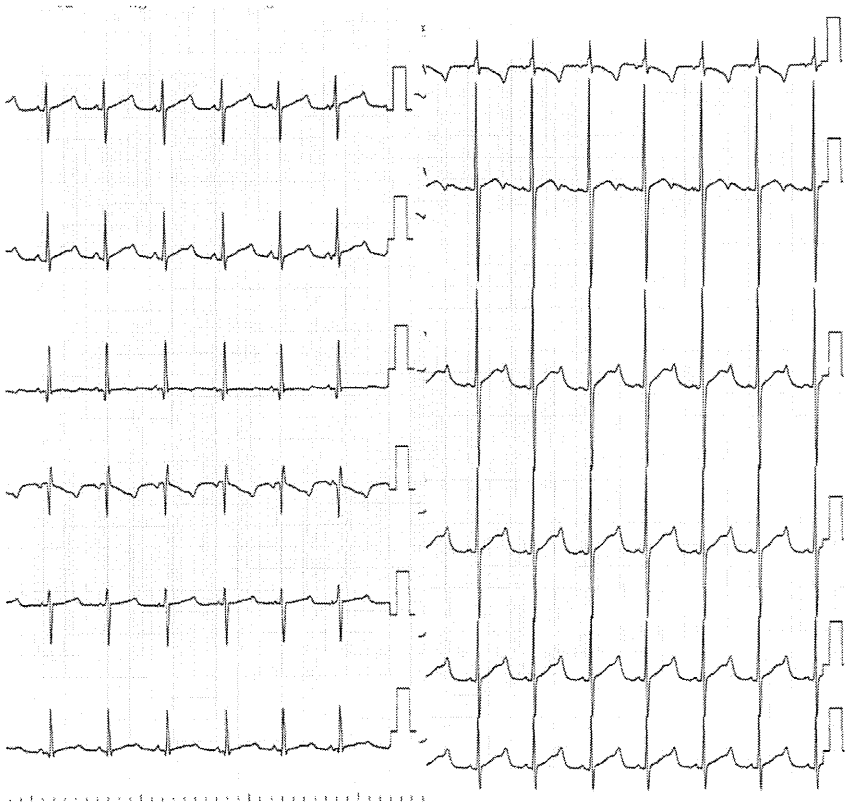

Figure 4. An ECG at 4 months of age in a patient with a *KCNH2* mutation. The QTc value was 533 ms.

Table 2. Positive Predictive Value (PPV) and Negative Predictive Value (NPV)

QTc, ms	PPV	NPV
430	0.0053	1.0000
440	0.0172	1.0000
450	0.0976	1.0000
460	0.5714	1.0000
470	0.8000	1.0000
480	0.7500	0.9998
490	1.0000	0.9991

different among 4867 male and 4858 female infants on the third to fourth day of life (401±19 versus 400±20 ms; *P*=not significant).[18] Another large study showed a sex difference in QTc values among 22 967 male and 21 629 female infants between the 15th and 25th day of life (405±20 versus 407±20 ms; $P<0.001$).[13] In the present study, a sex difference was also present on the 32nd day of life (410±19 versus 413±19 ms; $P<0.001$). However, guidelines of the International Conference on Harmonization reported that concerning the difference in the QT/QTc values in a thorough QT/QTc study, the threshold level of regulatory concern is ≈5 ms, as evidenced by an upper bound of the 95% confidence interval around the mean effect on QTc of 10 ms.[19] This suggests that a difference in QTc of a few milliseconds between male and female infants is clinically irrelevant.

Of the 4319 infants who participated in the study, including 4285 infants whose QTc values were analyzed, some infants were found to have miscellaneous heart diseases that were different from QT prolongation. Of these, echocardiography revealed a 43-day-old male infant with Wolff–Parkinson–White syndrome, LVNC, and heart failure. He showed a sudden decrease in his ejection fraction to <30% at 81 days of age, although his general status still seemed to be good. Clinical manifestations of LVNC are highly variable, ranging from no symptoms to disabling congestive heart failure, even from the neonatal period.[20] Children who are diagnosed with LVNC during infancy are at high risk for severe heart failure and a poor prognosis.[21] Quaglini et al[22] reported that ongoing neonatal electrocardiographic screening in >30 000 infants identified infants with prolonged QT intervals, as well as 4 cases of asymptomatic life-threatening congenital heart disease, 3 cases of coarctation of the aorta, and 1 case of anomalous origin of the left coronary artery from the pulmonary artery, which escaped detection at the initial medical visit. The results from this previous study and the present data indicate that neonatal electrocardiographic screening for QT prolongation, which was the primary objective of both studies, has additive value to screening.

Limitations

There are limitations to the present study. We did not perform genetic analysis of several infants with QTc >460 ms.[13] We are not able to exclude the possibility that some of these

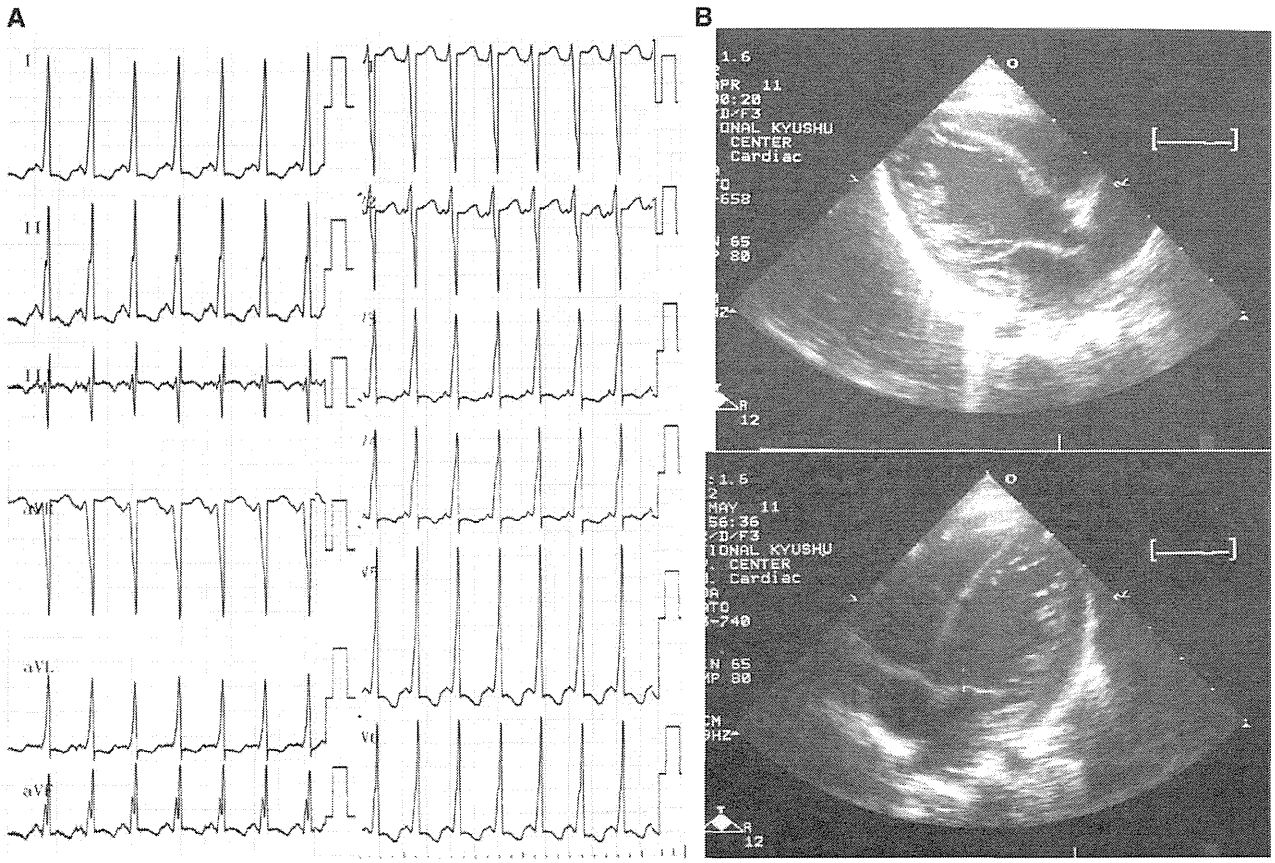

Figure 5. An ECG (**A**) and images of echocardiography (**B**) in an infant. His ECG shows Wolff–Parkinson–White syndrome, and echocardiography shows noncompaction of the left ventricle.