defined as occurrence of 2 or more premature atrial complexes after atrial stimulation; and 5) induced AF defined as AF that was induced by extrastimulus and persisted for >30 s (6,17–19). If RAF or AF was induced during the paired pacing, S2 was no longer decreased and ERP was defined as the minimum S2 interval that induced RAF or AF.

Programmed electrical stimulation was also performed at the ventricle to induce VF. As described previously (15), programmed electrical stimulation was performed at an intensity twice threshold and 2-ms in duration through the distal electrodes in the right ventricular apex, free-wall region, septal region of the right ventricular outflow tract, and posterolateral wall of the left ventricle using pulse generator as described before. The protocol of ventricular stimuli included up to 3 extrastimuli at the basic cycle length of 600 ms and 400 ms and the minimum coupled extrastimuli of 180 ms.

Statistical analysis. Data are expressed as mean values ± standard deviation. Student *t* test was performed to test for statistical differences between 2 unpaired mean values, and categorical data and percentage frequencies were analyzed by the chi-square test (SPSS II for Windows, SPSS Inc., Chicago, Illinois). A value of $p < 0.05$ was considered to be statistically significant.

Results

Patients' characteristics. The population consisted of a total of 73 probands. None of the patients in this study were members of the same family. Patients' characteristics are summarized in Table 1. Spontaneous AF was documented in 10 (13.7%) of the patients and VF was documented in 13 (17.8%) of the patients. Nineteen (26.0%) of the patients had an FH, and syncopal episodes occurred in 20 (27.4%) of the patients. Gene analysis revealed that *SCN5A* mutation was present in 15 (20.5%) of the patients. Spontaneous type 1 ECG was observed in 23 (31.5%) of the patients. In EP study, VF was induced in 34 (47%) of the patients and 33 (45.2%) of the patients had received ICD implantation.

Table 1 Patients' Characteristics (n = 73)

Men/women	72/1
Age (yrs)	49.5 ± 12.0
Syncopal episode (%)	20 (27.4%)
Documented VF (%)	13 (17.8%)
Spontaneous AF (%)	10 (13.7%)
Family history of sudden death (%)	19 (26.0%)
SCN5A mutation (%)	15 (20.5%)
Spontaneous type 1 ECG	23 (31.5%)
VF induction during EP study (%)	34 (46.6%)
ICD implantation (%)	33 (45.2%)

Values are mean ± SD or number of patients.
AF = spontaneous documented atrial fibrillation; ECG = electrocardiogram; EP = electrophysiological; ICD = implantable cardioverter defibrillator; SCN5A = pore-forming region of the human cardiac sodium channel; VF = ventricular fibrillation.

Circadian variation of spontaneous AF and VF. Spontaneous AF episodes were detected at night (12:00 AM to 6:00 AM) in 7 (70%) of the 10 patients with documented AF and 3 of 10 patients in the daytime (6:00 AM to 6:00 PM). Documented VF episodes were observed in 13 patients (46 episodes). Among them, 7 patients (55%) (22 episodes [48%]) were detected at night (12:00 AM to 6:00 AM), and 2 patients (15%) (7 episodes [15%]) in the daytime (6:00 AM to 6:00 PM).

Clinical and genetic differences in BrS patients with AF. Clinical and genetic parameters were compared in BrS patients with spontaneous AF and those without spontaneous AF (Table 2). None of the patients in this study showed chronic AF. Age was not different between the groups. In the clinical parameters, syncopal episode, documented VF, and spontaneous type 1 ECG were observed in larger percentage of patients with spontaneous AF (syncope: 60.0% vs. 22.2%, $p < 0.03$; documented VF: 40.0% vs. 14.3%, $p < 0.05$; and spontaneous type 1 ECG: 60.0% vs. 27.0%, $p < 0.04$). However, FH, *SCN5A* mutation, and VF induction during EP study were not related to spontaneous AF episodes (Table 2).

EP parameters in BrS patients with AF. In EP study, there was no significant difference between the ERP of the RAA in the AF (+) group (254.3 ± 44.7 ms) and that in the AF (−) group (243.9 ± 25.5 ms). However, CT was more prolonged in the AF group at S1 (CT at S1: 138.4 ± 23.8 ms vs.122.3 ± 20.1 ms, $p < 0.03$) and at S2 (172.4 ± 33.3 ms vs. 154.2 ± 18.0 ms, $p < 0.03$). Sinus node recovery time was significantly prolonged in the AF (+) group (1,971 ± 1,007 ms vs. 1,288 ± 488 ms, $p < 0.01$). Other parameters, including RAF, induction of AF, and local atrial electrograms (A1: A at S1 and A2: A at S2) were not different between the groups (Table 2).

Clinical and EP parameters in BrS patients with *SCN5A* mutation. Next we examined the relationships of genetic mutation with clinical and EP parameters in patients with BrS. None of the clinical parameters (age, syncopal episode, documented VF, spontaneous AF, FH, spontaneous type 1 ECG, and ICD implantation) were different in patients with *SCN5A* mutation and patients without *SCN5A* mutation. However, AF induction (in 46.7% of the patients with *SCN5A* mutation and in 20.7% of the patients without *SCN5A* mutation, $p < 0.05$), CT at S1 (138.1 ± 18.1 ms with *SCN5A* mutation and 121.5 ± 20.9 ms without *SCN5A* mutation, $p < 0.03$), CT at S2 (167.9 ± 14.2 ms with *SCN5A* mutation and 153.4 ± 21.3 ms without *SCN5A* mutation, $p < 0.03$), local A2 (103.9 ± 17.4 ms with *SCN5A* mutation and 89.8 ± 18.7 ms without *SCN5A* mutation, $p < 0.03$), and sinus node recovery time (1,682 ± 1,036 ms with *SCN5A* mutation and 1,300 ± 433 ms without *SCN5A* mutation, $p < 0.04$) during EP study were significantly different between the groups (Table 3).

Table 2 Characteristics of Patients With and Without AF

	Without AF	With AF	p Value
Clinical/genetic parameters			
Number of patients (men/women)	63 (62/1)	10 (10/0)	
Age (yrs)	48.4 ± 11.5	53.7 ± 14.2	NS
Syncopal episode (%)	14 (22.2%)	6 (60.0%)	<0.03
Documented VF (%)	9 (14.3%)	4 (40.0%)	<0.05
Family history of sudden death (%)	17 (27.0%)	2 (20.0%)	NS
SCN5A mutation (%)	13 (20.6%)	2 (20.0%)	NS
Spontaneous type 1 ECG (%)	17 (27.0%)	6 (60.0%)	<0.04
VF induction during EP study (%)	29 (46.0%)	5 (50.0%)	NS
ICD implantation (%)	27 (42.9%)	6 (60.0%)	NS
EP parameters of the atrium			
RAF	31 (49.2%)	6 (60.0%)	NS
AF induction	14 (22.2%)	5 (50.0%)	NS
ERP (ms)	243.9 ± 25.5	254.3 ± 44.7	NS
CT at S1 (ms)	122.3 ± 20.1	138.4 ± 23.8	<0.03
CT at S2 (ms)	154.2 ± 18.0	172.4 ± 33.3	<0.03
A1 (ms)	65.7 ± 12.9	72.5 ± 20.4	NS
A2 (ms)	92.4 ± 18.9	99.2 ± 21.8	NS
A2/A1	1.42 ± 0.25	1.39 ± 0.24	NS
Sinus node recovery time (ms)	1,288 ± 488	1,971 ± 1,007	<0.01

Values are mean ± SD or number of patients.
A1 = local atrial potential at S1; A2 = local atrial potential at S2; CT = interatrial conduction time; ERP = effective refractory period; RAF = repetitive atrial firing; other abbreviations as in Table 1.

Clinical, genetic, and EP parameters in BrS patients with spontaneous type 1 ECG. Next we examined the relationship of the basal ECG pattern to the clinical, genetic, and EP parameters in patients with BrS. Spontaneous type 1 ECG was observed in 23 of the patients (31.5%) and drug (pilsicainide)-induced type 1 ECG (type 2 or 3 ECG before the drug administration) in the remaining 50 patients (68.5%) in this study. Spontaneous AF was significantly more observed in patients with spontaneous type 1 ECG (26.1% vs. 8.0%, $p < 0.04$). Documented VF tended to be more observed but not statistically significant (30.4% vs. 12.0%, $p = 0.06$). Other parameters including age, syncopal episodes, FH, frequency of *SCN5A* mutation, VF induction, ICD implantation, and

Table 3 Clinical and EP Parameters in Patients With and Without *SCN5A* Mutation

	SCN5A Mutaion (−)	*SCN5A* Mutaion (+)	p Value
Clinical parameters			
Number of patients (men/women)	58 (57/1)	15 (15/0)	
Age (yrs)	49.6 ± 11.3	47.5 ± 14.5	NS
Syncopal episode (%)	15 (25.9%)	5 (33.3%)	NS
Documented VF (%)	9 (15.5%)	4 (26.7%)	NS
Spontaneous AF (%)	8 (13.8%)	2 (13.3%)	NS
Family history of sudden death (%)	13 (22.9%)	6 (40.0%)	NS
Spontaneous type 1 ECG (%)	16 (27.6%)	7 (46.7%)	NS
VF induction during EP study (%)	30 (51.7%)	4 (26.7%)	NS
ICD implantation (%)	26 (44.8%)	7 (46.7%)	NS
EP parameters of the atrium			
RAF	29 (50.0%)	8 (53.3%)	NS
AF induction	12 (20.7%)	7 (46.7%)	<0.05
ERP (ms)	240.2 ± 24.2	264.5 ± 35.6	NS
CT at S1 (ms)	121.5 ± 20.9	138.1 ± 18.1	<0.03
CT at S2 (ms)	153.4 ± 21.3	167.9 ± 14.2	<0.03
A1 (ms)	64.5 ± 13.2	73.0 ± 11.4	NS
A2 (ms)	89.8 ± 18.7	103.9 ± 17.4	<0.03
A2/A1	1.41 ± 0.26	1.45 ± 0.20	NS
Sinus node recovery time	1,300 ± 433	1,682 ± 1,036	<0.04

Values are mean ± SD or number of patients.
Abbreviations as in Tables 1 and 2.

Table 4 Clinical, Genetic, and EP Parameters in Patients With and Without Spontaneous Type 1 ECG

	Type 2 or 3 ECG	Type 1 ECG	p Value
Clinical/genetic parameters			
Number of patients (men/women)	50 (49/1)	23 (23/0)	
Age (yrs)	49.7 ± 12.0	47.8 ± 12.0	NS
Syncopal episode (%)	12 (24.0%)	8 (34.8%)	NS
Documented VF (%)	6 (12.0%)	7 (30.4%)	NS ($p = 0.06$)
Spontaneous AF (%)	4 (8.0%)	6 (26.1%)	<0.04
Family history of sudden death (%)	13 (28.0%)	6 (26.1%)	NS
SCN5A mutation (%)	8 (16.0%)	7 (30.4%)	NS
VF induction during EP study (%)	20 (40.0%)	14 (60.9%)	NS
ICD implantation (%)	19 (38.0%)	14 (60.9%)	NS
EP parameters of the atrium			
RAF	26 (52.0%)	11 (47.8%)	NS
AF induction	11 (22.0%)	8 (34.8%)	NS
ERP (ms)	246.2 ± 27.4	242.9 ± 32.0	NS
CT at S1 (ms)	122.9 ± 22.8	128.6 ± 17.4	NS
CT at S2 (ms)	155.8 ± 22.3	157.6 ± 16.9	NS
A1 (ms)	65.3 ± 12.1	69.9 ± 15.8	NS
A2 (ms)	91.1 ± 18.4	99.2 ± 20.8	NS
A2/A1	1.4 ± 0.3	1.4 ± 0.2	NS
Sinus node recovery time (ms)	1,310 ± 460	1,523 ± 855	NS

Values are mean ± SD or number of patients.
Abbreviations as in Tables 1 and 2.

all EP parameters were not different between the groups (Table 4).

Clinical, genetic, and EP parameters in BrS patients with and without VF episodes. Finally, we examined the relationships of disease severity (documented VF) with other clinical, genetic, and EP parameters in BrS patients. Spontaneous AF was observed in a large percentage of patients with VF episodes (30.8%) in comparison with that seen in patients without VF episodes (10.0%) ($p < 0.05$), but the frequency of *SCN5A* mutation was not different between the groups (Table 5). Spontaneous type 1 ECG tended to be more observed in patients with VF episodes but not statistically significant ($p = 0.06$). As for the EP parameters, ERP at RAA was not different, but the rate of AF induction

Table 5 Clinical, Genetic, and EP Parameters in Patients With and Without Documented VF Episode

	Documented VF (−)	Documented VF (+)	p Value
Clinical/genetic parameters			
Number of patients (men/women)	60 (59/1)	13 (13/0)	
Age (yrs)	48.3 ± 12.0	52.8 ± 11.1	NS
Spontaneous AF (%)	6 (10.0%)	4 (30.8%)	<0.05
Family history of sudden death (%)	17 (28.3%)	2 (15.4%)	NS
SCN5A mutation (%)	11 (18.3%)	4 (30.8%)	NS
Spontaneous type 1 ECG (%)	16 (26.7%)	7 (53.8%)	NS ($p = 0.06$)
VF induction during EP study (%)	28 (46.7%)	6 (46.2%)	NS
ICD implantation (%)	20 (33.3%)	13 (100%)	<0.01
EP parameters of the atrium			
RAF	29 (48.3%)	8 (61.5%)	NS
AF induction	12 (20.0%)	7 (53.8%)	<0.03
ERP (ms)	242.0 ± 26.2	261.1 ± 34.8	NS
CT at S1 (ms)	121.9 ± 19.6	137.6 ± 24.6	<0.02
CT at S2 (ms)	153.7 ± 16.8	171.3 ± 33.9	<0.02
A1 (ms)	66.1 ± 14.1	68.6 ± 8.5	NS
A2 (ms)	91.6 ± 19.8	100.4 ± 15.0	NS
A2/A1	1.4 ± 0.3	1.5 ± 0.2	NS
Sinus node recovery time	1,313 ± 505	1,658 ± 937	NS

Values are mean ± SD or number of patients.
Abbreviations as in Tables 1 and 2.

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was significantly higher (53.8% vs. 20.0%, $p < 0.03$) and CT was prolonged in patients with VF episodes (CT at S1: 137.6 ± 24.6 ms vs. 121.9 ± 19.6 ms, $p < 0.02$; CT at S2: 171.3 ± 33.9 ms vs. 153.7 ± 16.8 ms, $p < 0.02$) (Table 5). Sinus node recovery time was not different between the groups ($p = 0.07$).

Discussion

The present study demonstrated that BrS patients with spontaneous AF have more severe clinical and EP backgrounds but not associated with family history or mutations of the gene encoding the cardiac sodium channel, *SCN5A*. Electrical vulnerability across the heart may be closely associated with spontaneous AF and VF occurrence in BrS patients.

AF in BrS. It has been reported that spontaneous AF is often observed in patients with BrS. The incidence of AF in this syndrome has been reported to be 10% to 53% (1,4,6). In this study, the incidence of spontaneous AF was 13.7% and most cases (70%) were documented at night. Matsuo et al. (20) reported that VF in patients with BrS was most frequently detected in the midnight to early morning period during sleep. Our finding of a circadian pattern in spontaneous AF and VF episodes is in agreement with their findings, and these findings suggested that nocturnal vagal activity and withdrawal of sympathetic activity may play an important role in arrhythmogenesis in both AF and VF occurrence in this syndrome.

The treatment for AF in BrS is an important issue. It has been reported that quinidine sulfate, isoproterenol, cilostazole (1), and bepridil chloride (21,22) are recommended in Brugada patients with repeated VF by a mechanism of augmenting the calcium current or reducing the *Ito* current. In this study, none of the patients received antiarrhythmic drugs for AF because their episodes were paroxysmal with few symptoms. However, 2 AF patients that experienced recurrent VF episodes had received antiarrhythmic drugs to prevent recurrent VF (1 patient received quinidine sulfate 0.3 g and the other received bepridil hydrochloride 100 mg). While these patients never experienced AF episodes with taking these drugs, indicating antiarrhythmic drugs that were effective to prevent VF might be also effective in AF.

EP parameters in patients with BrS. It has also been reported that atrial vulnerability was increased in patients with BrS, compared with that in a normal control group (6). Among the various indexes of EP parameters, we found the interatrial conduction delay (CT) was significantly increased in BrS patients with AF, indicating that global conduction of the atrial myocardium was impaired. Interestingly, atrial vulnerability (induced AF) was more impaired in BrS patients with VF episodes, indicating that electrical vulnerability may be across the whole heart including the atrium and ventricle. The fact that patients with AF have more episodes of VF or syncopal episodes supports this possibility.

There was no difference in VF inducibility between the patients with and without documented VF. In this study, all patients who had documented VF experienced at least 1 VF episode before ICD implantation; therefore, asymptomatic patients never experienced VF attacks during the follow-up period after ICD implantation. These results indicate that VF inducibility during EP study has a low specificity to identify high-risk BrS patients as reported before (23).

***SCN5A* mutation is not associated with AF in BrS.** The gene encoding the cardiac sodium channel, *SCN5A*, has been reported to be linked causally to BrS. We speculated AF is more common in patients with *SCN5A* mutation, but we found no difference between patients with *SCN5A* mutation and those without *SCN5A* mutation in spontaneous AF episodes or in other clinical parameters (spontaneous VF, syncopal episode, FH, and spontaneous type 1 ECG). The reason is still unclear, but this finding is perhaps of most interest. These results indicate that a defect in the *SCN5A* gene is not associated with AF events or with VF events as was previously reported (1), suggesting that genetic analysis is not useful for risk stratification.

Clinical implications. This study showed that spontaneous AF and atrial vulnerability are important predictors of VF events that cause sudden cardiac death. The fifth-generation ICD is preferable for patients with BrS, even for BrS patients who have never experienced an attack of AF, because atrial vulnerability is common and AF could occur during the follow-up period.

Study limitations. The number of patients in this study was small, and further study is needed to reach definitive conclusion regarding the impact of AF episodes for BrS. Moreover, we analyzed only the coding regions of *SCN5A* for mutations in this study, and the possibility of mutations occurring in regions of the gene other than coding regions cannot be excluded. The functional impact has not been studied for all identified *SCN5A* mutations; therefore, a causal relationship in individual patients has not been proved yet.

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REFERENCES

1. 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;111:659–70.
2. Brugada J, Brugada R, Brugada P. Right bundle-branch block and ST-segment elevation in leads V1 through V3: a marker for sudden

death in patients without demonstrable structural heart disease. Circulation 1998;97:457–60.
3. 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;20:1391–6.
4. Babai MA, Aslani A, Shahrzad S. Clinical predictors of atrial fibrillation in Brugada syndrome. Europace 2007;9:947–50.
5. Eckardt L, Kirchhof P, Loh P, et al. Brugada syndrome and supraventricular tachyarrhythmias: a novel association? J Cardiovasc Electrophysiol 2001;12:680–5.
6. Morita H, Kusano-Fukushima K, Nagase S, et al. Atrial fibrillation and atrial vulnerability in patients with Brugada syndrome. J Am Coll Cardiol 2002;40:1437–44.
7. Chen Q, Kirsch GE, Zhang D, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature 1998;392:293–6.
8. Wang Q, Shen J, Splawski I, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell 1995;80:805–11.
9. Benson DW, Wang DW, Dyment M, et al. Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (SCN5A). J Clin Invest 2003;112:1019–28.
10. Morita H, Fukushima-Kusano K, Nagase S, et al. Sinus node function in patients with Brugada-type ECG. Circ J 2004;68:473–6.
11. Laitinen-Forsblom PJ, Makynen P, Makynen H, et al. SCN5A mutation associated with cardiac conduction defect and atrial arrhythmias. J Cardiovasc Electrophysiol 2006;17:480–5.
12. Chen LY, Ballew JD, Herron KJ, Rodeheffer RJ, Olson TM. A common polymorphism in SCN5A is associated with lone atrial fibrillation. Clin Pharmacol Ther 2007;81:35–41.
13. Bezzina CR, Rook MB, Wilde AA. Cardiac sodium channel and inherited arrhythmia syndromes. Cardiovasc Res 2001;49:257–71.
14. Wang Q, Li Z, Shen J, Keating MT. Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. Genomics 1996;34:9–16.
15. Morita H, Fukushima-Kusano K, Nagase S, et al. Site-specific arrhythmogenesis in patients with Brugada syndrome. J Cardiovasc Electrophysiol 2003;14:373–9.
16. Morita H, Morita ST, Nagase S, et al. Ventricular arrhythmia induced by sodium channel blocker in patients with Brugada syndrome. J Am Coll Cardiol 2003;42:1624–31.
17. Hashiba K, Tanigawa M, Fukatani M, et al. Electrophysiologic properties of atrial muscle in paroxysmal atrial fibrillation. Am J Cardiol 1989;64:20J–3J.
18. Ohe T, Matsuhisa M, Kamakura S, et al. Relation between the widening of the fragmented atrial activity zone and atrial fibrillation. Am J Cardiol 1983;52:1219–22.
19. Shimizu A, Fukatani M, Tanigawa M, Mori M, Hashiba K. Intraatrial conduction delay and fragmented atrial activity in patients with paroxysmal atrial fibrillation. Jpn Circ J 1989;53:1023–30.
20. Matsuo K, Kurita T, Inagaki M, et al. The circadian pattern of the development of ventricular fibrillation in patients with Brugada syndrome. Eur Heart J 1999;20:465–70.
21. Ohgo T, Okamura H, Noda T, et al. Acute and chronic management in patients with Brugada syndrome associated with electrical storm of ventricular fibrillation. Heart Rhythm 2007;4:695–700.
22. Sugao M, Fujiki A, Nishida K, et al. Repolarization dynamics in patients with idiopathic ventricular fibrillation: pharmacological therapy with bepridil and disopyramide. J Cardiovasc Pharmacol 2005;45:545–9.
23. Priori SG, Napolitano C, Gasparini M, et al. Natural history of Brugada syndrome: insights for risk stratification and management. Circulation 2002;105:1342–7.

Fragmented QRS as a Marker of Conduction Abnormality and a Predictor of Prognosis of Brugada Syndrome

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Background—Conduction abnormalities serve as a substrate for ventricular fibrillation (VF) in patients with Brugada syndrome (BS). Signal-averaged electrograms can detect late potentials, but the significance of conduction abnormalities within the QRS complex is still unknown. The latter can present as multiple spikes within the QRS complex (fragmented QRS [f-QRS]). We hypothesized that f-QRS could indicate a substrate for VF and might predict a high risk of VF for patients with BS.

Methods and Results—In study 1, we analyzed the incidence of f-QRS in 115 patients with BS (13 resuscitated from VF, 28 with syncope, and 74 asymptomatic). f-QRS was observed in 43% of patients, more often in the VF group (incidence of f-QRS: VF 85%, syncope 50%, and asymptomatic 34%, $P<0.01$). *SCN5A* mutations occurred more often in patients with f-QRS (33%) than in patients without f-QRS (5%). In patients with syncope or VF, only 6% without f-QRS experienced VF during follow-up (43±25 months), but 58% of patients with f-QRS had recurrent syncope due to VF ($P<0.01$). In study 2, to investigate the mechanism of f-QRS, we studied in vitro models of BS in canine right ventricular tissues (n=4) and optically mapped multisite action potentials. In the experimental model of BS, ST elevation resulted from a large phase 1 notch of the action potential in the epicardium, and local epicardial activation delay reproduced f-QRS in the transmural ECG.

Conclusions—f-QRS appears to be a marker for the substrate for spontaneous VF in BS and predicts patients at high risk of syncope. (***Circulation*. 2008;118:1697-1704.**)

Key Words: death, sudden ■ arrhythmia ■ electrocardiography ■ genes ■ tachyarrhythmias

Clinical observations have shown that conduction abnormalities contribute to the occurrence of ventricular fibrillation (VF) in Brugada syndrome,[1–3] possibly by providing a proarrhythmic substrate.[4–7] The existence of late potentials (LPs) in the signal-averaged ECG suggests the presence of regions undergoing depolarization later than most of the ventricle and thus is a useful marker of conduction abnormalities.[8] LPs have been reported to occur frequently in patients with Brugada-type ECG,[2,3,6,7] although VF can also occur in patients without LPs.[2] In addition to LPs, conduction abnormalities can exist within the QRS complex, manifested as a fragmented QRS (f-QRS). In patients with coronary artery disease, f-QRS can be caused by zigzag conduction in the infarcted myocardium, which results in multiple spikes within the QRS complex, and has been used as an indicator of non–Q-wave myocardial infarction and as a predictor of ventricular arrhythmia.[9–11] We hypothesized that f-QRS can detect conduction abnormalities within the QRS complex in Brugada syndrome and can be used to identify high-risk patients. To evaluate the effects of the local epicardial conduction delay on the generation of f-QRS, we studied both patients with Brugada syndrome and in vitro canine right ventricular (RV) tissue preparations with drug-induced Brugada syndrome.

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Methods

Clinical Studies

The subjects of the present study were 113 male and 2 female patients with Brugada-type ECGs (mean age 48±12 years). A spontaneous type 1 ECG as detailed in the consensus report on Brugada syndrome[12] was detected in all patients without drug provocation. We classified these patients into 3 groups: (1) VF group, for patients with VF at admission or within 24 hours after syncopal episodes; (2) syncope group, for patients who had syncope without detected VF; and (3) asymptomatic group, for patients who

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The clinical part of this study was performed at Okayama University, and the experimental study was performed at Indiana University.

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had only mild symptoms (such as palpitations or chest pain) or who were symptomless. No patients were from the same family. Echocardiography and chest roentgenograms were performed in all patients, and no abnormalities were found.

Standard 12-lead ECGs (with 0- to 150-Hz filters) and additional V_1 through V_3 leads at the 3rd intercostal space were recorded simultaneously. ECGs acquired before initiation of drug therapy were used for analysis. We evaluated the RR interval, PQ interval, QRS width, QT interval, ST level at the J point, and number of spikes within the QRS complex in leads V_1 through V_6. ECGs were reviewed blindly by 3 authors (HM, SN, KN).

The presence of LPs was evaluated with a signal-averaged ECG (ART 1200EPX, noise level <0.3 μV, and high-pass filtering of 40 Hz with a bidirectional 4-pole Butterworth). The filtered QRS duration, root-mean-square voltage of the terminal 40 ms in the filtered QRS complex (RMS40), and duration of low-amplitude signals <40 μV in the terminal filtered QRS complex (LAS40) were measured by signal-averaged ECG. LPs were considered to be positive when 2 criteria were met: RMS40 <20 μV and LAS40 >38 ms.[3]

The risks of the electrophysiological study were explained to each patient, and written informed consent was obtained from all patients. The electrophysiological study was performed in 85 patients as reported previously.[13,14] Coronary angiography was performed in all 85 patients and showed no sign of coronary artery disease. Induction of ventricular arrhythmia was initially attempted without the use of any antiarrhythmic drugs. The criterion for the induction of ventricular arrhythmia was induction of sustained polymorphic ventricular tachycardia or VF by programmed electrical stimulation from the RV apex, RV outflow tract (RVOT), or left ventricle (LV) with a maximum of 2 extrastimuli at 2 cycle lengths.

The gene analysis of *SCN5A* was performed in compliance with guidelines for human genome studies of the Ethics Committee of Okayama University. Informed consent was obtained from all patients. Analysis of *SCN5A* mutation was performed as reported previously.[15] Twenty-seven exons and a portion of the introns 20 base pairs before and after exons of the *SCN5A* gene were amplified with previously reported intronic primers.[15] *SCN5A* gene exon 1 is a noncoding region, and this region was not analyzed in the present study. Mutations were analyzed at least 3 times by independent polymerase chain reaction amplification and sequencing. Polymerase chain reaction products were subjected to single-strand conformation polymorphism analysis followed by direct sequence analysis.

Control Subjects

To exclude right bundle-branch block (RBBB) from the definition of f-QRS, we evaluated the ECGs of 80 control subjects who had RBBB but did not have Brugada-type ST elevation (64 males and 16 females, age 63±15 years). The control group included 53 subjects with complete RBBB and 27 with incomplete RBBB without obvious heart disease. The control group consisted of patients who were admitted to Okayama University for noncardiac disease and referred to our department because of the presence of RBBB noted in their ECGs. A physical examination, chest roentgenogram, and echocardiogram were performed in control patients and excluded the presence of heart disease. Standard 12-lead ECGs were recorded in the same way as in the Brugada patients and were evaluated for RR interval, PQ interval, QRS width, QT interval, ST level at J point, and number of positive spikes within QRS complex in leads V_1 through V_6.

In Vitro Studies

The investigation conforms to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). We prepared tissues with procedures similar to those used previously.[16,17]

Our previous clinical finding[6] suggested the presence of a disturbance in local activation within the epicardium of the RVOT in Brugada syndrome. We isolated 4 neighboring pairs of transmural tissue preparations from the RV free wall of 4 male canine hearts and investigated the mechanism of f-QRS in drug-induced Brugada syndrome. The close anatomic relationship of the tissues in each pair minimized their differences in action potential (AP). Each tissue included a branch of the right coronary artery for perfusion. In each pair of tissues from the same heart, 1 tissue (the epicardial tissue) had only a 2- to 3-mm-thick epicardial layer (with the midwall and endocardium removed), and the other tissue (the transmural tissue) had an intact transmural wall. The prepared tissues were mounted parallel to one another in a warmed chamber with the epicardial tissue placed at the epicardial side of the transmural tissue and their cut-exposed transmural surfaces facing a mapping camera (Figure 1). Both tissues were perfused simultaneously with the same Tyrode's solution. We paced the endocardium of the transmural tissue and the epicardium of the epicardial tissue at a cycle length of 2000 ms. Two silver electrodes were placed in the bath, 5 mm away from the epicardium of the epicardial tissue (anode) and the endocardium of the transmural tissue (cathode), to register the compound transmural ECG of the paired tissue. To mimic local epicardial conduction delay, we delayed the timing of activation in the thin epicardial tissue to 1 ms near the end of the QRS complex of the paired transmural tissue and evaluated the effect of the local activation delay on the transmural ECG.

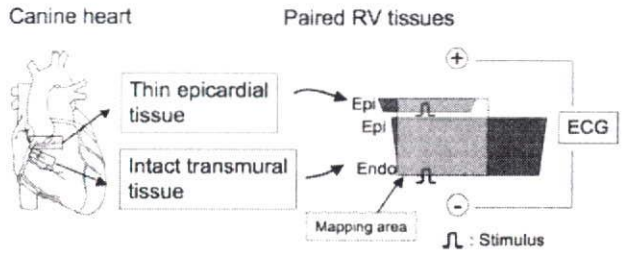

Figure 1. Schematic of paired tissue preparations from canine heart. We isolated a neighboring pair of transmural tissue preparations from each RV free wall. In each pair of tissues, 1 (the epicardial tissue) had only a 2- to 3-mm-thick epicardial layer, and the other (the transmural tissue) had an intact transmural wall. The prepared tissues were mounted parallel to one another in a chamber with the epicardial tissue placed at the epicardial side of the transmural tissue and their cut-exposed transmural surfaces facing a mapping camera. We paced the endocardium of the transmural tissue and the epicardium of the epicardial tissue. Two silver electrodes were placed in the bath, 5 mm away from the epicardium of the epicardial tissue (anode) and from the endocardium of the transmural tissue (cathode), to register the compound transmural ECG of the tissue pair. Epi indicates epicardial; Endo, endocardial.

The tissue preparations were stained with di-4-ANEPPS (Biotium, Inc, Hayward, Calif; ≈4 mmol/L) and immobilized with cytochalasin D (Fermentek Ltd, Jerusalem, Israel; 20 to 30 μmol/L).[18] We evaluated the physiological conditions of the preparations as we have done before.[18,19] Well-perfused preparations had a vivid reddish color, low-noise optical signals with normal AP duration (APD) and strong contractions on stimulation before immobilization. An optical mapping system with a 256-element (16×16) photodiode camera collected the fluorescence from a 19.5×19.5 mm^2 observation area on the tissue surface and converted it into 256 channels of electrical signals (APs).[18,19]

As we have done before,[16,17] we induced a Brugada-type transmural ECG at 36.5±0.5°C with pilsicainide (2.5 to 12.5 mmol/L, Dai-Ichi Suntry Pharma, Tokyo, Japan), pinacidil (1.25 to 12.5 mmol/L, Sigma Chemical, St. Louis, Mo), and terfenadine (2.0 mmol/L, Sigma Chemical). The doses of drugs were increased progressively and simultaneously in each pair of tissue preparations until both tissues developed the characteristic epicardial AP of Brugada syndrome, as reported previously.[16–17,20] We also checked tissue healthiness by direct observations of tissue perfusion and of the amplitude, duration, and signal-to-noise ratio of the fluorescence signals during experiments. As noted previously,[16–20] these procedures produced stable tissues during an experimental period of ≈2 hours.

Table 1. ECG Parameters of Control Subjects Who Had RBBB

	V_1	V_2	V_3
QRS width, ms	124±25	125±22	121±27
QT interval, ms	403±35	405±34	407±33
QTc interval, ms	428±35	431±33	432±30
ST level, mV	0.02±0.05	0.03±0.07	0.03±0.07
No. of spikes	2.1±0.4	2.0±0.15	1.8±0.7
Median	2	2	2
Range	1–3	1–4	1–3

We statistically analyzed APDs at the recording sites along the epicardial and endocardial layers in the transmural preparations. Transmural dispersion of APD was calculated as the difference between the endocardial and epicardial APDs. The depth of the phase 1 notch of the AP relative to the height of phase 0 depolarization was used as a surrogate indicator of the effects of I_{to}.[16,17,21,22]

Statistical Analysis

Continuous data were expressed as mean±SD values. Comparisons among means were performed with 2-way ANOVA coupled with Scheffé's test. Comparisons of 2 groups were made with Student's *t* test for unpaired data (patient data) and paired data (longitudinal experiment data), as appropriate. Fisher's exact test was performed for the comparison of proportions among groups. A Mann-Whitney *U* test was performed to compare the number of spikes within the QRS complex. Survival and event rates were determined with the Kaplan-Meier method and compared between groups with a 2-sample log-rank test. Significance was defined as $P<0.05$.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Results

ECG Characteristics of Control Subjects With RBBB

Most (64%) of the 80 control subjects with RBBB but without known heart disease had 2 to 3 spikes within the QRS complex in each of the right precordial leads (leads V_1 through V_3) and a sum of 5.9±1.0 (median 6, range 4 to 8) spikes in all V_1 through V_3 (Table 1). To exclude these control subjects, we defined abnormal fragmentation within the QRS complex as ≥4 spikes in 1 or ≥8 spikes in all of the leads V_1, V_2, and V_3. Two control subjects (2.5%) were regarded as having f-QRS by these criteria.

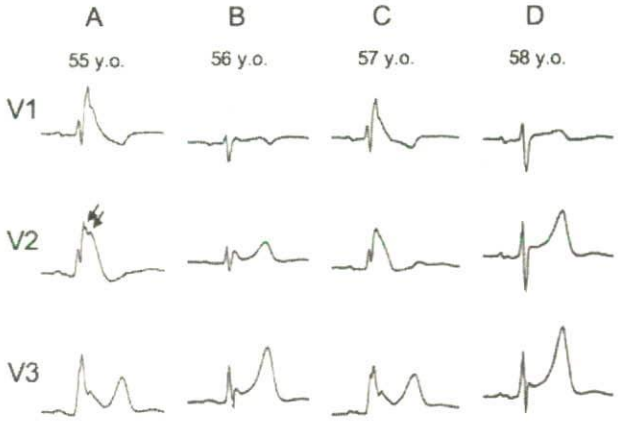

Figure 3. Spontaneous variations of f-QRS and ST elevation. These ECGs were recorded in a patient who experienced cardiac arrest. A, ECG when patient was 55 years old (y.o.). Lead V_2 had f-QRS at the late phase of the QRS complex (arrows). Leads V_1 and V_2 showed coved-type ST elevation. B, ECG at age 56 years. ST elevation decreased and changed to the saddle-back type. f-QRS was not observed. C, ECG at age 57 years. Coved-type ST elevation reappeared, but f-QRS did not appear. D, ECG at age 58 years. ECG converted to normal pattern. This patient died suddenly 6 months after the last ECG recording.

f-QRS in Patients With Brugada Syndrome

With the above criteria, f-QRS was identified in 43% (50 of 115) of patients with Brugada syndrome, more frequently in the VF group than in the other groups ($P=0.0069$; VF group 85% [11/13], syncope group 50% [14/28], and asymptomatic group 34% [25/74]; Figure 2). In 11 patients (22%), f-QRS appeared in leads V_1 through V_3 in the 3rd intercostal space but not in the 4th intercostal space (Figure 3A). Therefore, f-QRS occurred preferably in the right precordial leads within

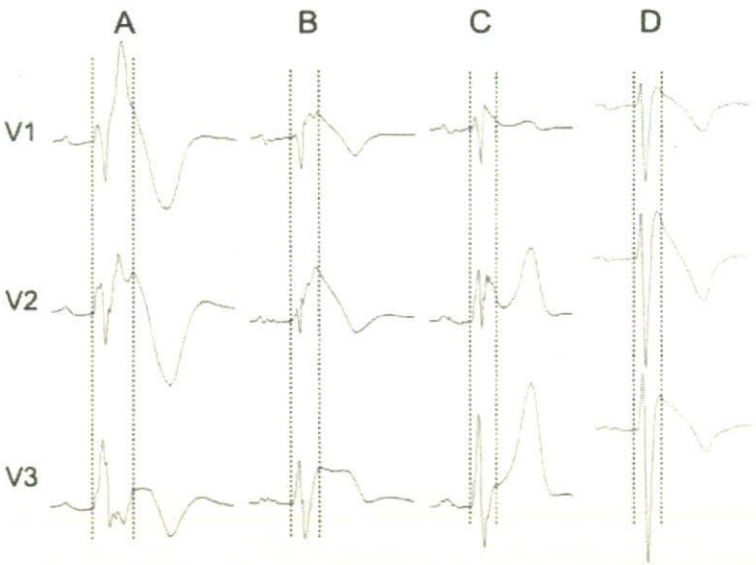

Figure 2. Example of f-QRS in Brugada syndrome. Dotted lines show onset and termination of the QRS complex. A, Multiple spikes between the R wave and the end of the QRS complex in leads V_2 and V_3. B, Multiple spikes were observed at the upstroke of the S wave in leads V_1 and V_2. C, Multiple spikes existed around the late r′ in leads V_1 and V_2. D, No sign of f-QRS. Right precordial lead showed rSr′ pattern without multiple spikes in the QRS complex.

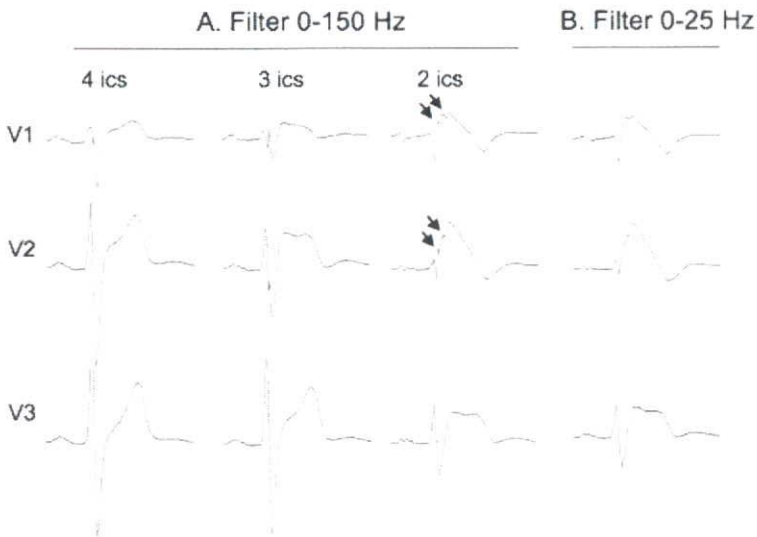

Figure 4. Effects of recording site and filtering on f-QRS. A, ECGs recorded by 0- to 150-Hz filtering. f-QRS appeared in the 3rd intercostal space (ics) and were manifested in the 2nd intercostal space (arrows). f-QRS was not observed at the regular ECG recording (in the 4th intercostal space). B, Reducing the cutoff frequency of the low-pass filter from 150 to 25 Hz diminished f-QRS in the same 2nd intercostal space ECG as in A.

the high (3rd) intercostal space. Multiple spikes were observed in the mid QRS (12%), the upstroke part of the S wave (28%), and the late part of the QRS complex (60%; Figure 2). Spontaneous variations of f-QRS (changing magnitude or altering appearance and disappearance of multiple spikes) were observed in 72% of patients with f-QRS (VF group 91% [10/11], syncope group 57% [8/14], and asymptomatic group 72% [18/25]; Figure 4). f-QRS was detected on the first ECG recording in 43 patients (86%) and was detected for the first time on later recordings of the ECG in 2 patients with VF (0.5 months after the first ECG recording), 2 patients with syncope (0.5 to 1.5 months), and 3 patients in the asymptomatic group (0.2 months to 5 years). Reducing the low-pass filter frequency from 150 to 25 Hz masked the existence of f-QRS in all patients and eliminated f-QRS completely in 37 patients.

Compared with patients without f-QRS, patients with f-QRS had a longer RR interval (899±125 versus 986±226 ms, $P=0.0087$, respectively), a similar PQ interval (174±25 versus 174±23 ms, $P=0.93$), a longer QRS width and QT interval (Figure 5), and a longer HV interval (39±7 versus 44±7 ms, $P=0.0015$). LPs were common in patients with Brugada syndrome (overall group 71%, VF group 83%, syncope group 57%, and asymptomatic group 70%), with no statistical association with f-QRS [incidence of LP: f-QRS(+) 73%, versus f-QRS(−) 69%, $P=0.68$; Figure 6]. Programmed electrical stimulation frequently induced VF in patients with Brugada syndrome (VF group 67%, syncope group 48%, and asymptomatic group 52%). The existence of f-QRS was not associated with the incidence of VF induction [f-QRS(+) 58% versus f-QRS(−) 49%, $P=0.56$]. Among a total of 66 patients (26 with f-QRS and 40 without) screened, an *SCN5A* mutation was identified in 11 (17%; incidence of *SCN5A* mutations 22% in the VF group, 28% in the syncope group, and 10% in the asymptomatic group). An *SCN5A* mutation was strongly associated with f-QRS [incidence of *SCN5A* mutation: f-QRS(+) 34%, f-QRS(−) 5%, $P=0.0027$].

Eleven patients in the VF group, 12 in the syncope group, and 16 in the asymptomatic group received ICD therapy. During follow-up (43±25 months, range 0.5 to 161 months, median 25 months), 8 of the 28 patients in the syncope group and 8 of the 13 patients in the VF group experienced recurrent syncope due to VF, and each case was defibrillated successfully by the ICD or an external defibrillator; however, 1 patient died of brain damage and recurrent VF. Neither LPs, inducibility of VF by programmed electrical stimulation, nor mutation of *SCN5A* predicted recurrent syncope due to VF in the syncope and VF groups. Patients who had f-QRS often experienced recurrence of syncope due to VF within 4 years of the first episode of syncope or VF (Table 2; Figure 7). In contrast, the recurrence of syncope was rare in patients without f-QRS.

In Vitro Model of f-QRS in Brugada Syndrome

In controls, the transmural tissue had a normal APD gradient (epicardial APD 280±42 ms, endocardial APD 297±41 ms,

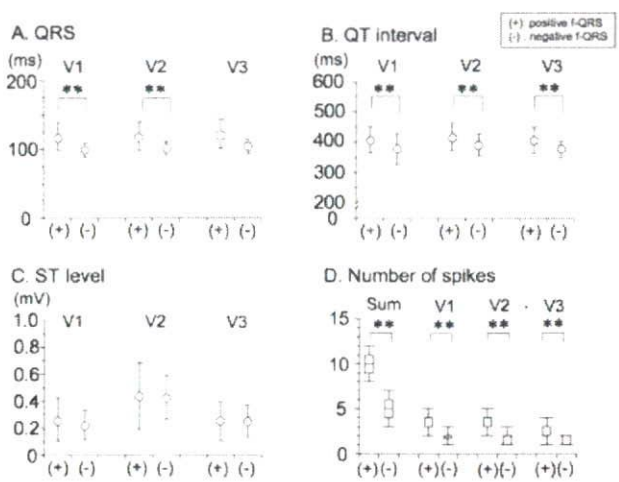

Figure 5. Differences in ECG parameters between patients with and without f-QRS. A, QRS width; B, QTc interval; C, ST level; and D, number of spikes within QRS complex. Patients with f-QRS had longer QRS interval, longer QTc interval, and multiple spikes within QRS compared with patients without f-QRS. There was no difference of ST level between the 2 groups. Sum indicates sum of spikes in all V_1 through V_3. $^{**}P<0.01$.

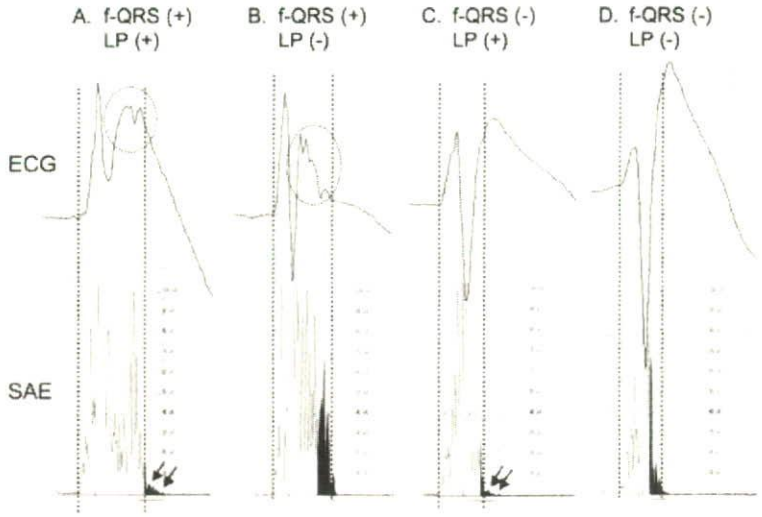

Figure 6. f-QRS and LP. Upper panel shows ECGs in lead V_2, with f-QRS circled. Bottom panel shows signal-averaged ECG (SAE) with the LP indicated by arrows. Patient A had both f-QRS and LPs. Patient B had only f-QRS. Patient C had only LPs. Patient D had neither f-QRS nor LP.

$P=0.0015$) and a small phase 1 notch in the epicardial AP (depth of phase 1 notch 11±3+%) with a small J wave and a positive T wave in the transmural ECG. Delayed pacing in the epicardial tissue resulted in a bundle-branch block type of wide QRS complex in the transmural ECG (epicardial activation time in epicardial tissues: no delay 25±5 ms, delay 56±9 ms, $P<0.01$; QRS width: no delay 35±9 ms, delay 78±12 ms, $P=0.0007$) but had no effect on the J wave (J-wave width: no delay 32±3 ms, delay 32±4 ms, $P=1.00$; Figure 8A). There were no differences in the epicardial AP between the epicardial and transmural tissues.

The combination of pinacidil (10.0±2.0 μmol/L), pilsicainide (10.0±2.0 μmol/L), and terfenadine (2.0 μmol/L) induced Brugada-type ECGs with large J-ST elevation and a negative T wave, deepened the phase 1 notch of the epicardial AP (to 17±5% from 11±3%), and produced longer APDs in the epicardium than in the endocardium, which resulted in reversal of the transmural APD gradient (epicardial APD 292±52 ms, endocardial APD 269±52 ms, $P=0.039$). Delayed pacing of the epicardial tissue widened the QRS and induced multiple spikes during the late phase of the QRS complex in the transmural ECG (epicardial activation time in the epicardial tissues: no delay 37±12 ms, delay 68±19 ms, $P=0.001$; QRS width: no delay 52±13 ms, delay 83±21 ms, $P=$ 0.0073; Figure 8B).

Table 2. Clinical Characteristics and Outcomes of the f-QRS(+) and f-QRS(−) Groups

	f-QRS(+) (n=50)	f-QRS(−) (n=65)	*P*
Male/female, n	50/0	63/2	0.222
Age, y	49±12	47±12	0.370
Family history, n (%)	18 (36)	21 (32)	0.680
Therapy,* n (%)			
ICD	28 (56)	12 (18)	0.006
Ablation	1 (2)	1 (2)	0.852
Drugs	11 (22)	0	<0.001
Disopyramide	2	0	
Quinidine	4	0	
Bepridil	5	0	
Outcome, n (%)			
SD	1 (2)	0	0.254
Recurrent VF	15 (30)	1 (2)	<0.001
New-onset VF	1 (2)	0	0.254
Noncardiac death	1 (2)	0	0.254

ICD indicates implantable cardioverter-defibrillator; SD, sudden death.
*Therapy during follow-up.

Discussion

New Observations

In the present study, we updated the definition of f-QRS to exclude control subjects who had RBBB without obvious heart disease. This definition differed from the previous definition of f-QRS, which was created for the diagnosis of

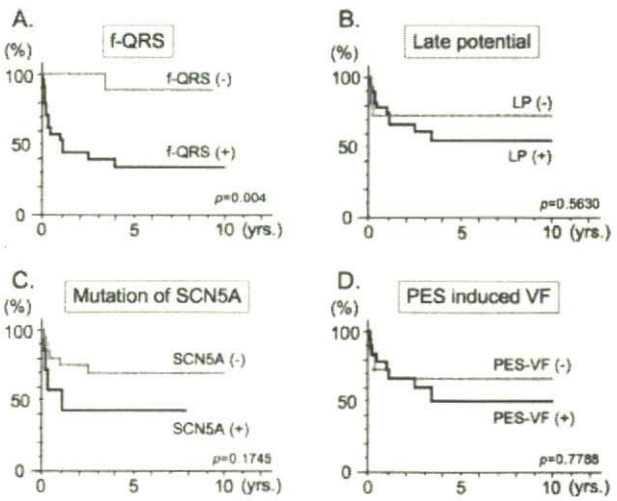

Figure 7. Recurrence of syncope due to ventricular arrhythmia. A, Freedom from events for patients with and without f-QRS. Patients with f-QRS often experienced recurrent syncope due to VF within 4 years from the first episode. The existence of LPs (B), mutation of SCN5A (C), and VF induced by programmed electrical stimulation (PES; D) did not predict the recurrence of syncope.

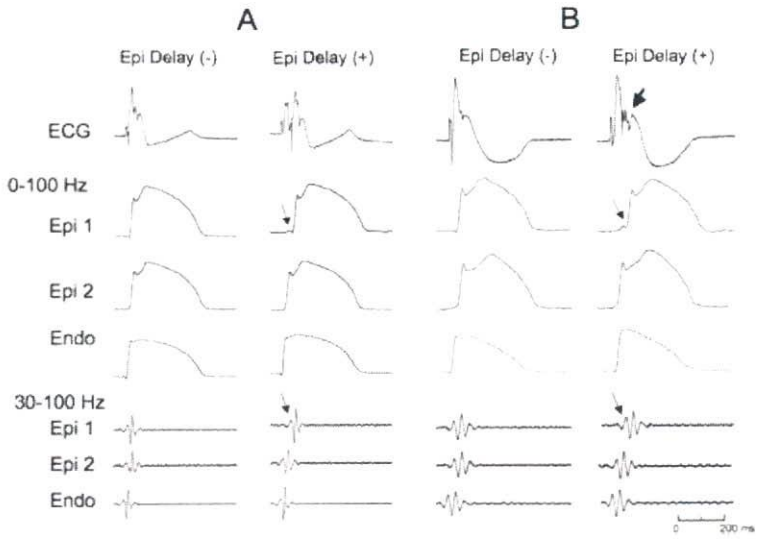

Figure 8. Experimental model of Brugada syndrome. Transmural ECG, APs (filter 0 to 100 Hz), and unipolar potential (filter 30 to 100 Hz) before (A; control) and after (B) administration of drugs that induced the Brugada model. A, Control; left panel shows the transmural ECG and APs when epicardial (Epi) activation was synchronized in both tissues. QRS duration was not prolonged, and a small J wave and positive T wave were observed. Right, Activation of the epicardium was delayed at Epi 1, and the transmural ECG had the RsR′ pattern of the QRS complex, but without Brugada ECG characteristics in the transmural ECG. Arrows show delayed epicardial activation in the epicardial tissue. B, Brugada model. The phase 1 notch of the AP was larger and deeper than the control in A. There was no activation deflection at the upstroke of the phase 2 dome in the unipolar potential (30 to 100 Hz filtering). When epicardial activation was synchronized, the transmural ECG showed ST elevation with a negative T wave but without f-QRS (left). Local epicardial delay caused multiple spikes at the late phase of the QRS complex (thick arrow), followed by ST elevation and a negative T wave (right). Endo indicates endocardium.

myocardial infarction without consideration of bundle-branch block.[9–11] We demonstrated that the existence of f-QRS was associated with the prognosis of high-risk patients with Brugada syndrome who had experienced syncope or VF. Although LPs, induced VF, and gene mutation could not predict prognosis, f-QRS was statistically associated with the recurrence of syncope caused by malignant ventricular arrhythmia in patients with Brugada syndrome. Using an isolated canine RV tissue model of Brugada syndrome, we demonstrated that activation delay in the epicardium could reproduce similar f-QRS in the transmural ECG and thus provided a possible mechanism for our clinical observations.

Conduction Delay in Brugada Syndrome

The most important characteristic of Brugada syndrome is repolarization abnormality detected as ST elevation in the right precordial leads,[1,12] which correspond to the RVOT. Repolarization heterogeneity within the epicardium of the RVOT has been identified as the origin of ventricular arrhythmia (by phase 2 reentry) in Brugada syndrome.[16,17,20–22] In addition to the repolarization abnormality, Brugada syndrome is associated with conduction disturbances.[2–7,23] Mutations of the sodium channel gene, *SCN5A*, which are observed in 20% to 30% of patients with Brugada syndrome,[23–25] reduce the cardiac Na^+ current.[24,25] A reduced Na^+ current not only deepens the phase 1 notch of the AP but also slows the conduction velocity and reduces the safety factor of conduction, which results in conduction abnormalities (with regional delay or block of conduction). It has been demonstrated in a model of Brugada syndrome that conduction abnormalities provide a substrate for the degeneration of polymorphic ventricular tachycardia into VF.[5,23] Previously, intraventricular conduction abnormalities were observed and reported as RBBB and a prolonged HV interval in patients with Brugada syndrome.[1,12] Conduction delays and delayed epicardial activation were observed to cause LPs, especially during VF induction by programmed stimulation[2,4] and in the RVOT[6,7] in patients with Brugada syndrome. Although delayed activation within a small mass of ventricular tissue could produce LPs without having significant effects on the QRS complex, delayed activation in a larger ventricular mass can cause multiple spikes within the QRS complex, resulting in f-QRS.[9–11] The presence of f-QRS can be a predictor of prognosis for patients with a typical Brugada-type ECG.

Characteristics and Interpretation of f-QRS in Brugada Syndrome

f-QRS existed in ≈40% of patients with Brugada syndrome and was often observed in patients who had VF episodes. The preferential occurrence of f-QRS in the right precordial leads, especially in the higher intercostal spaces, suggests a localized conduction abnormality within the RVOT area. Because f-QRS consists of multiple small spikes, successful recording of f-QRS requires a low-noise amplifier that has a low-pass filter with a relatively high cutoff frequency (150 Hz). The use of a low-pass filter with a low cutoff frequency (>25 Hz), as has commonly been done to remove the electromyogram signal, can eliminate the f-QRS, underestimate the existence of the f-QRS, and cause pseudo day-by-day variation of the f-QRS.

The present observations of longer QRS width, QT interval, and HV interval in patients with f-QRS than in those without f-QRS suggest that Brugada patients with f-QRS had both prominent depolarization and repolarization abnormalities. Although local conduction slowing can be caused by myocardial fibrosis secondary to myocardial degeneration or myocarditis[4] in addition to Na^+ channel mutation, the present observation of dynamic and spontaneous changes in f-QRS suggests the presence of functional modulation of conduction rather than modulation by a fixed scarred myocardium, eg, by autonomic nerve activity, aging, temperature, or heart rate, in f-QRS.

The occurrence of LPs does not predict the recurrence of syncope, although it suggests the presence of excessive conduction delay (which lasts longer than the QRS) within a small tissue mass that produces the LP but does not affect the QRS complex. In contrast, multiple spikes within the f-QRS complex suggest the presence of an arrhythmogenic substrate that has multiple areas of conduction slowing (within the QRS complex) in a relatively large tissue mass (which affects

the QRS). Thus, f-QRS suggests an increased probability of spontaneous VF and predicts a high risk of sudden cardiac death in patients.

Although *SCN5A* mutation is a clearly identified cause of conduction disease and Brugada syndrome,[23,25] *SCN5A* mutation was identified in only 22% of the VF group and 33% of the patients with f-QRS in the present study. In contrast, f-QRS was identified in 85% of the Brugada patients in the VF group.

Effects of Activation Delay in Experimental Model of Brugada Syndrome

We studied 4 pairs of tissue preparations in the experiment study. To create local epicardial activation delay, we changed the timing of pacing in the thin epicardial tissue and recorded the transmural ECG. Therefore, the experiments represented activation delay, which may affect the ECG differently from the conduction delay in patients with Brugada syndrome.

Previous tissue models of Brugada syndrome (mostly single pieces of isolated RVOT tissues[5,16,17,20–22]) demonstrated the following mechanism of arrhythmogenesis: A reduced membrane Na^+ current in combination with a prominent phase 1 notch of the AP causes the simultaneous presence of APs with and without a phase 2 dome within the epicardium of the RVOT, which leads to phase 2 reentry. In contrast, the present study created a new experimental model of Brugada syndrome with regional epicardial activation delay by the addition of a piece of thin epicardium to an intact transmural tissue, along with delayed stimulation of the thin epicardial tissue. The present model demonstrated that regional epicardial activation delay could cause f-QRS in Brugada syndrome similar to clinical observations. On the basis of the results of the present study, we conclude that f-QRS and the underlying conduction disturbance play an important role in the spontaneous occurrence of VF and that f-QRS is a simple and powerful indicator of prognosis of high-risk patients with Brugada syndrome.

Study Limitation

We defined f-QRS based on data from the control subjects with RBBB but without obvious heart disease. The incidence of f-QRS at the 4th intercostal space was high in patients with Brugada syndrome (39 patients). We only recorded the standard 12-lead ECG with V_1 through V_3 in the 4th intercostal space, not in the 3rd intercostal space, in control subjects. Therefore, the presence of f-QRS in the V_1 through V_3 in the 3rd intercostal space in control patients is unknown.

Sources of Funding

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Disclosures

None.

References

1. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. *J Am Coll Cardiol*. 1992;20:1391–1396.
2. Kanda M, Shimizu W, Matsuo K, Nagaya N, Taguchi A, Suyama K, Kurita T, Aihara N, Kamakura S. Electrophysiologic characteristics and implication of induced ventricular fibrillation in symptomatic patients with Brugada syndrome. *J Am Coll Cardiol*. 2002;39:1799–1805.
3. Morita H, Takenaka-Morita S, Fukushima-Kusano K, Kobayashi M, Nagase S, Kakishita M, Nakamura K, Emori T, Matsubara H, Ohe T. Risk stratification for asymptomatic patients with Brugada syndrome. *Circ J*. 2003;67:312–316.
4. Coronel R, Casini S, Koopmann TT, Wilms-Schopman FJ, Verkerk AO, de Groot JR, Bhuiyan Z, Bezzina CR, Veldkamp MW, Linnenbank AC, van der Wal AC, Tan HL, Brugada P, Wilde AA, de Bakker JM. Right ventricular fibrosis and conduction delay in a patient with clinical signs of Brugada syndrome: a combined electrophysiological, genetic, histopathologic, and computational study. *Circulation*. 2005;112:2769–2777.
5. Aiba T, Shimizu W, Hidaka I, Uemura K, Noda T, Zheng C, Kamiya A, Inagaki M, Sugimachi M, Sunagawa K. Cellular basis for trigger and maintenance of ventricular fibrillation in the Brugada syndrome model: high-resolution optical mapping study. *J Am Coll Cardiol*. 2006;47:2074–2085.
6. Nagase S, Kusano KF, Morita H, Fujimoto Y, Kakishita M, Nakamura K, Emori T, Matsubara H, Ohe T. Epicardial electrogram of the right ventricular outflow tract in patients with the Brugada syndrome: using the epicardial lead. *J Am Coll Cardiol*. 2002;39:1992–1995.
7. Hisamatsu K, Kusano KF, Morita H, Takenaka S, Nagase S, Nakamura K, Emori T, Matsubara H, Mikouchi H, Nishizaki Y, Ohe T. Relationships between depolarization abnormality and repolarization abnormality in patients with Brugada syndrome: using body surface signal-averaged electrocardiography and body surface maps. *J Cardiovasc Electrophysiol*. 2004;15:870–876.
8. Berbari EJ. High-resolution electrocardiography. In: Zipes DP, Jalife J, eds. *Cardiac Electrophysiology From Cell to Bedside*. 4th ed. Philadelphia, Pa: Saunders; 2004:793–802.
9. Das MK, Khan B, Jacob S, Kumar A, Mahenthiran J. Significance of a fragmented QRS complex versus a Q wave in patients with coronary artery disease. *Circulation*. 2006;113:2495–2501.
10. Das MK, Saha C, El Masry H, Peng J, Dandamudi G, Mahenthiran J, McHenry P, Zipes DP. Fragmented QRS on a 12-lead ECG: a predictor of mortality and cardiac events in patients with coronary artery disease. *Heart Rhythm*. 2007;4:1385–1392.
11. Michael MA, El Masry HE, Khan BR, Das MK. Electrocardiographic signs of remote myocardial infarction. *Prog Cardiovasc Dis*. 2007;50:198–208.
12. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, Gussak I, LeMarec H, Nademanee K, Perez Riera AR, Shimizu W, Schulze-Bahr E, Tan H, Wilde A. Brugada syndrome: report of the second consensus conference [published correction appears in *Heart Rhythm*. 2005;2:905]. *Heart Rhythm*. 2005;2:429–440.
13. Morita H, Morita ST, Nagase S, Banba K, Nishii N, Tani Y, Watanabe A, Nakamura K, Kusano KF, Emori T, Matsubara H, Hina K, Kita T, Ohe T. Ventricular arrhythmia induced by sodium channel blocker in patients with Brugada syndrome. *J Am Coll Cardiol*. 2003;42:1624–1631.
14. Morita H, Fukushima-Kusano K, Nagase S, Takenaka-Morita S, Nishii N, Kakishita M, Nakamura K, Emori T, Matsubara H, Ohe T. Site-specific arrhythmogenesis in patients with Brugada syndrome. *J Cardiovasc Electrophysiol*. 2003;14:373–379.
15. Kusano KF, Taniyama M, Nakamura K, Miura D, Banba K, Nagase S, Morita H, Nishii N, Watanabe A, Tada T, Murakami M, Miyaji K, Hiramatsu S, Nakagawa K, Tanaka M, Miura A, Kimura H, Fuke S, Sumita W, Urakawa S, Iwasaki J, Sakuragi S, Ohe T. Atrial fibrillation in patients with Brugada syndrome: relationships of gene mutation, electrophysiology and clinical backgrounds. *J Am Coll Cardiol*. 2008;51:1169–1175.
16. Morita H, Zipes DP, Lopshire J, Morita ST, Wu J. T wave alternans in an in vitro canine tissue model of Brugada syndrome. *Am J Physiol Heart Circ Physiol*. 2006;291:H421–H428.
17. Morita H, Zipes DP, Morita ST, Wu J. Differences in arrhythmogenicity between the canine right ventricular outflow tract and anteroinferior right ventricle in a model of Brugada syndrome. *Heart Rhythm*. 2007;4:66–74.
18. Wu J, Biermann M, Rubart M, Zipes DP. Cytochalasin D as excitation-contraction uncoupler for optically mapping action potentials in wedges of ventricular myocardium. *J Cardiovasc Electrophysiol*. 1998;9:1336–1347.
19. Wu J, Zipes DP. Transmural reentry during acute global ischemia and reperfusion in canine ventricular muscle. *Am J Physiol Heart Circ Physiol*. 2001;280:H2717–H2725.

20. Morita H, Zipes DP, Morita ST, Wu J. Temperature modulation of ventricular arrhythmogenicity in a canine tissue model of Brugada syndrome. *Heart Rhythm*. 2007;4:188–197.
21. Yan GX, Antzelevitch C. Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST-segment elevation. *Circulation*. 1999;100:1660–1666.
22. Di Diego JM, Cordeiro JM, Goodrow RJ, Fish JM, Zygmunt AC, Perez GJ, Scornik FS, Antzelevitch C. Ionic and cellular basis for the predominance of the Brugada syndrome phenotype in males. *Circulation*. 2002;106:2004–2011.
23. Meregalli PG, Wilde AA, Tan HL. Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder, or more? *Cardiovasc Res*. 2005;67:367–378.
24. Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Giordano U, Bloise R, Giustetto C, De Nardis R, Grillo M, Ronchetti E, Faggiano G, Nastoli J. Natural history of Brugada syndrome: insights for risk stratification and management. *Circulation*. 2002;105:1342–1347.
25. Bezzina CR, Rook MB, Wilde AA. Cardiac sodium channel and inherited arrhythmia syndromes. *Cardiovasc Res*. 2001;49:257–271.

CLINICAL PERSPECTIVE

Brugada syndrome is characterized by coved-type ST elevation (type I ECG described in the consensus report on Brugada syndrome) in the right precordial leads, which represents abnormal repolarization in the right ventricle. Spontaneous type I ECGs and a history of syncope have been reported as prognostic indicators. Patients with Brugada syndrome also have a depolarization abnormality that can be detected as right bundle-branch block, HV-interval prolongation, late potentials by signal-averaged electrogram, and delayed potentials at the epicardium. The contribution of these abnormal depolarization indices to prognosis is controversial, and indeed, the existence of late potentials did not predict prognosis in the present study. We focused on a new marker of depolarization abnormality, fragmented QRS (f-QRS), which is the presence of multiple spikes within the QRS complex. f-QRS has been reported in patients with myocardial infarction and can detect myocardial damage and arrhythmia occurrence. Of 115 patients with Brugada syndrome, f-QRS existed in 43%, more frequently in patients with prior ventricular fibrillation (VF) than in patients with syncope or asymptomatic patients. Patients with f-QRS who had prior episodes of VF or syncope without detected VF often experienced recurrent VF within 4 years. These results suggest that patients who experienced syncope without detected VF who had f-QRS were at increased risk for a subsequent arrhythmic event and should be considered for an implantable cardioverter defibrillator. In patients with prior VF, the existence of f-QRS also indicates a risk of recurrent VF, including arrhythmic storm.

Assessment of QT Intervals and Prevalence of Short QT Syndrome in Japan

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ABSTRACT

Background: Long QT syndrome causes ventricular tachyarrhythmias and sudden death. Recently, a short QT interval has also been shown to be associated with an increased risk of tachyarrhythmia and sudden death. However, the prevalence of short QT syndrome is not well-known.

Hypothesis: The aim of this study was to assess the distribution of corrected QT intervals (QTc) and prevalence of short QT syndrome.

Methods: This study comprised 12,149 consecutive subjects who received a consultation at Kanazawa University Hospital, Kanazawa, Japan, and had an electrocardiogram (ECG) between February 2003 and May 2004. Of these subjects, 1,165 subjects were excluded because of inappropriate ECGs, while the remaining 10,984 subjects had their last-recorded ECGs analyzed.

Results: The QTc values showed a nearly normal distribution (408±25 $msec^{1/2}$), and were significantly longer in females (412±24 $msec^{1/2}$) than in males (404±25 $msec^{1/2}$) ($p<0.05$). Among 5,511 males, 69 subjects (1.25%) exhibited QTc <354 $msec^{1/2}$ (2 standard deviations [SDs] below the mean in males), and among 5,473 females, 89 subjects (1.63%) exhibited QTc <364 $msec^{1/2}$ (2 SDs below the mean in females). Only 3 subjects (0.03% in all subjects and 0.05% in males) exhibited QTc <300 $msec^{1/2}$, however, none had clinical symptoms of short QT syndrome.

Conclusions: Short QT syndrome may be very rare.

Key words: short QT interval, sex difference of QT intervals, electrocardiogram, sudden death, arrhythmia

Introduction

The QT interval on an electrocardiogram (ECG) represents ventricular depolarization and repolarization. It is well-known that a prolonged QT interval is associated with an increased risk of tachyarrhythmias and sudden cardiac death.[1-4] Furthermore, there have been recent reports of a similar association between a short QT interval and tachyarrhythmias and sudden cardiac death.[5] The short QT syndrome is a new clinical entity that was first reported by Gussak et al.[5] in 2000. It is characterized by short QT intervals on the ECG (corrected QT interval [QTc]: QTc <300 $msec^{1/2}$), a high incidence of ventricular tachycardia and fibrillation, the absence of structural heart disease, a familial history of sudden cardiac death, resuscitated cardiac arrest, and syncope. The familial nature of this syndrome was confirmed by Gaita et al. in 2003.[6] Until now, focus has been given to the predominant prolonged QT interval, and an upper limit of normal has been proposed for QTc.[7] Nevertheless, the lower limit of normal for QTc, the prevalence of short QT intervals in the population, and the frequency of short QT syndrome have not been determined. Accordingly, we assessed the distribution of QTc interval and the frequency of short QT syndrome.

Materials and Methods

Subjects and ECG Analysis

We analyzed 26,350 consecutive ECGs that had been recorded at Kanazawa University Hospital, Kanazawa, Japan, for cardiac examination between February 2003 and May 2004. These ECGs had been obtained from 12,149 subjects, with a mean age±standard deviation (SD) of 51±21 y (range, birth to 96 y); 6,286 were male and 5,863 were female. For individual subjects with several ECGs, the last recorded ECG was selected for the analysis. All ECGs were recorded with an FCP-4266L instrument (Fukuda Denshi Co., Tokyo, Japan). The RR interval and QT interval were automatically measured with the same instrument. The RR interval is the average value across all beats, excluding premature beats measured in a 10-sec period. The QT interval is the average value across averaged 12-lead waveforms, excluding premature beats according to the setup of the instrument. As in the method of recognizing the end point of a T-wave, the first point of the differentiated average waveform that reached the baseline from the peak (i.e., became lower than the noise level of baseline) was defined as the end point of the T-wave. The QTc was calculated using Bazett's formula:[8] $QTc=QT/RR^{1/2}$. The averaged values of the QT and RR intervals as described previously were used.

Regarding ECGs with QTc ≤360 $msec^{1/2}$ or ≥440 $msec^{1/2}$, the QT interval was remeasured manually and was adopted

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for the analysis. The QT intervals were measured manually from a 12-lead ECG recorded at a paper speed of 25 mm/sec. The QT interval was defined as the time between the beginning of the QRS complex and the point at which the line of the maximal downslope of the T-wave crossed the isoelectric line. Predicted QT interval (QTp) was calculated using Rautaharju's formula:[9] QTp (msec) = 656/(1 + heart rate/100).

The following ECGs, with inappropriate findings for QTc analysis, were excluded: (1) irregular rhythms such as atrial fibrillation, frequent premature ventricular, and/or supraventricular contractions; (2) conduction disturbances such as sick sinus syndrome, advanced atrioventricular block, or bundle-branch block; and (3) wide QRS complexes such as pacemaker rhythm, Wolff-Parkinson-White syndrome, or marked left ventricular hypertrophy. Consequently, a total of 1,165 subjects were excluded and the remaining 10,984 subjects were analyzed. We also investigated the distribution of QTc values according to the sex of the subject, in order to determine if there was a difference between males and females. This study was approved by the Bioethical Committee on Medical Researches, School of Medicine, Kanazawa University, Kanazawa, Japan.

Statistics

Values are expressed as the mean±SD; 95% confidence intervals (CIs) were calculated. A normal distribution test was carried out by using a normal probability plot. A p-value of <0.05 was considered statistically significant. Comparisons of data were performed using the unpaired Student *t*-tests and Mann-Whitney U-tests. Statistical analyses were carried out with the computer software StatView for Windows version 5.0 and JMP for Windows version 5.1 (SAS Institute Inc., Cary, NC, USA).

Results

Distributions of QTc

The distribution of QTc showed a nearly normal distribution (408±25 $msec^{1/2}$), and was not statistically significant (Figure 1). The 95% CI for QTc ranged from 358 to 458 $msec^{1/2}$.

The results of ECG analysis in males and females are shown in Table 1. The study population, with a mean age of 52±21 y, was evenly distributed between males and females: 5,511 (50.2%) versus 5,473 (49.8%), respectively. The QTc intervals of the female subjects (412±24 $msec^{1/2}$) were significantly longer than those of the males (404±25 $msec^{1/2}$), ($p<0.05$) (Table 1). Heart rates, QT intervals, and QTc values in the various age groups are shown in Table 2. In both males and females, the highest heart rate was observed in the youngest age group (0–10 y) and the lowest heart rate was observed in the 11–20 y age group in both males and females. The QTc values of females were significantly longer than those of males in all age groups from 11 to 80 y.

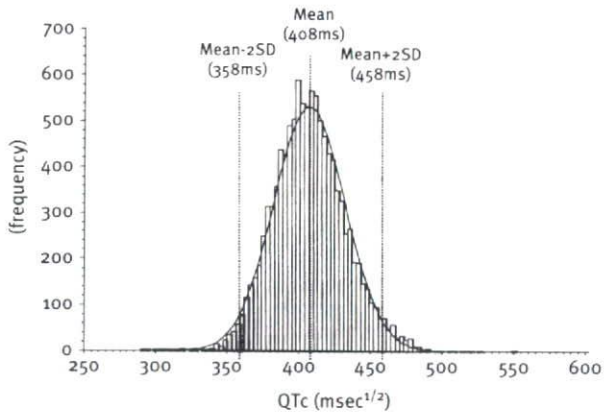

Figure 1: The QTc values show a nearly normal distribution (408±25 $msec^{1/2}$, mean ± SD).

TABLE 1: Baseline characteristics of study subjects

Data	Male	Female	p-value
Number (%)	5,511 (50.2)	5,473 (49.8)	—
Age (y)	51.8±21.8	50.2±21.6	<0.001
ECG			
HR (bpm)	69.2±15.2	70.8±15.3	<0.001
QT (msec)	380±36	383±36	<0.001
QTc ($msec^{1/2}$)	404±25	412±24	<0.001

Data are shown as mean±SD. *Abbreviations*: ECG = electrocardiogram; HR = heart rate; QT = QT interval; QTc = corrected QT interval.

Short QT Interval

The minimum QTc recorded in this study was 290 $msec^{1/2}$. Among the 5,511 male subjects, 69 subjects (1.25%) exhibited QTc <354 $msec^{1/2}$ (2 SDs below the mean in males). Whereas, 89 (1.63%) of the 5,473 female subjects exhibited QTc <364 $msec^{1/2}$ (2 SDs below the mean in females) (Figure 2). The ECGs that showed the shortest QTc in male and female subjects are shown in Figures 3 and 4, respectively. Only 3 subjects, all males, exhibited QTc <300 $msec^{1/2}$ (0.03% in all subjects and 0.05% in male subjects). These were as follows: (1) 23-y-old male, QTc = 290 $msec^{1/2}$, 74% of QTp; (2) 53-y-old male, QTc = 295 $msec^{1/2}$, 81% of QTp; and (3) 25-y-old male, QTc = 299 $msec^{1/2}$, 78% of QTp. None had a history of syncope, ventricular tachyarrythmias, or a family history of sudden cardiac death.

Discussion

Long QT syndrome is associated with a risk of life-threatening events, but little has previously been known

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TABLE 2: Heart rates, QT intervals, and QTc values in various age groups

	Males				Females				
Age (y)		Heart rate				Heart rate			
	number	(bpm)	QT (msec)	QTc (msec$^{1/2}$)	number	(bmp)	QT (msec)	QTc (msec$^{1/2}$)	p-value[a]
0–10	258	95±24	338±39	417±20	212	100±29	332±47	417±22	0.9547
11–20	433	65±15	385±33	397±24	437	65±14	394±35	408±24	<0.0001
21–30	507	65±13	374±31	387±23	625	69±16	378±36	401±21	<0.0001
31–40	390	68±14	371±30	392±21	533	70±13	379±31	405±24	<0.0001
41–50	476	68±14	376±33	398±23	547	70±14	382±32	409±23	<0.0001
51–60	1028	68±13	381±33	403±24	991	70±13	385±34	412±23	<0.0001
61–70	1202	68±13	386±35	408±24	1021	70±12	388±33	416±24	<0.0001
71–80	1028	69±14	388±36	411±24	893	71±13	388±25	419±25	<0.0001
81–90	182	70±13	391±39	418±23	204	71±12	390±37	422±25	0.0738
<90	7	70±12	390±40	418±30	10	68±12	393±35	416±27	0.8579

Data are shown as mean±SD. [a]p-value of QTc values in males versus in females.

about the significance of a short QT interval. Short QT intervals may be related to an increase in heart rate, ionic changes (i.e., hypercalcemia, hyperkalemia), acidosis, autonomic nervous system imbalance, or the effect of a particular drugs (e.g., digitalis).[10–13] In 1933, with an analysis of 6,693 consecutive Holter recordings, Algra et al.[14] reported that an increased risk of sudden death was presented not only in patients with long QT interval but also in those with short QT interval (<400 msec). Both abnormalities were associated with a 2-fold increase in the risk of sudden death. Their report was the first to point out the risk associated with short QT intervals. Short QT is defined by an interval that is always <300 msec, with no display of significant dynamic changes during heart rate variations or on exertion.[6] That is, an individual is regarded as having a short QT when the intervals are consistently <300 msec.[6] On the other hand, Rautaharju et al.[9] investigated the QT intervals in 14,379 healthy subjects, and established a formula by which the QT interval could be predicted: QTp (msec) = 656/(1 + heart rate/100). In their study, the prevalence of a QT interval shorter than 88% of predictive value was 2.5% (360 out of 14,379). Since 2 SDs below the mean is 88% of QTp (with only 2.5% of the population having a shorter QT interval), this value could reasonably be set as the lower limit of normal QT interval.[5] In their study, a QT interval <80% of QTp occurred in only 0.03% of subjects (3 out of 14,379). Therefore, we tried to identify the patients with short QT syndrome using these 2 indices: QTc <300 msec and QTc <88% of QTp. Consequently, only 3 subjects (0.03% in all subjects and 0.05% in male subjects) exhibited QTc <300 msec$^{1/2}$ and QTc <88% of QTp. However, they had no clinical symptoms of short QT syndrome, such as palpitation or syncope, no family history of sudden death, and no distinctive ECG features, such as tall, peaked, and symmetrical T-waves. These results suggest that short QT syndrome may be very rare.

In this study, we investigated only subjects with normal sinus rhythm. However, there have been reports of short QT syndrome in patients presenting with other arrhythmias, such as atrial fibrillation.[5,6] Therefore, short QT syndrome might well exist in subjects who were excluded from our study. Although only a few cases have been reported until now, it is possible that its prevalence is underestimated because little attention has been focused to date on short QT interval. We need to pay more attention to the existence of short QT interval.

Gain of function mutations in the genes for outward potassium currents such as *KCNQ1*, *KCNH2*, and *KCNJ2* have been shown to underlie short QT syndrome.[15–17] In

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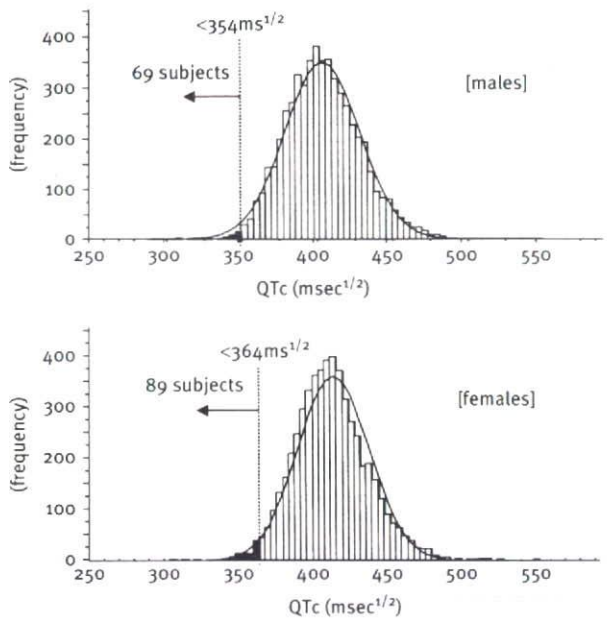

Figure 2: Distributions of QTc in males and females. Mean ± SDs of QTc in males and females are 404 ± 25 $msec^{1/2}$ and 412 ± 24 $msec^{1/2}$, respectively. The QTc intervals of females are significantly longer than those of males ($p < 0.05$).

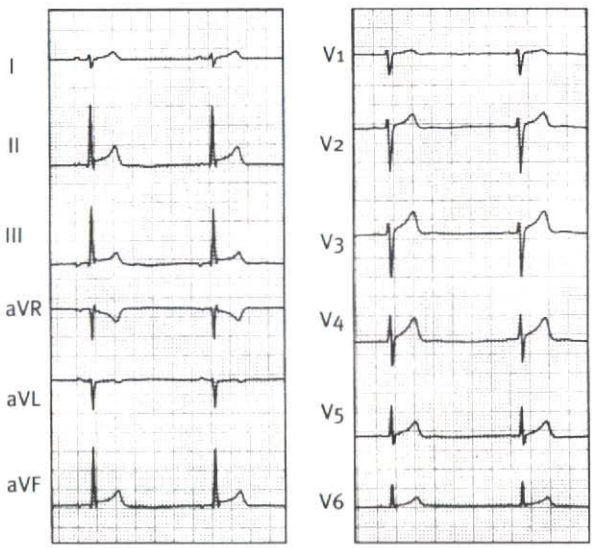

Figure 3: Electrocardiogram showing the shortest QTc interval in male subjects. The QTc is 290 $msec^{1/2}$.

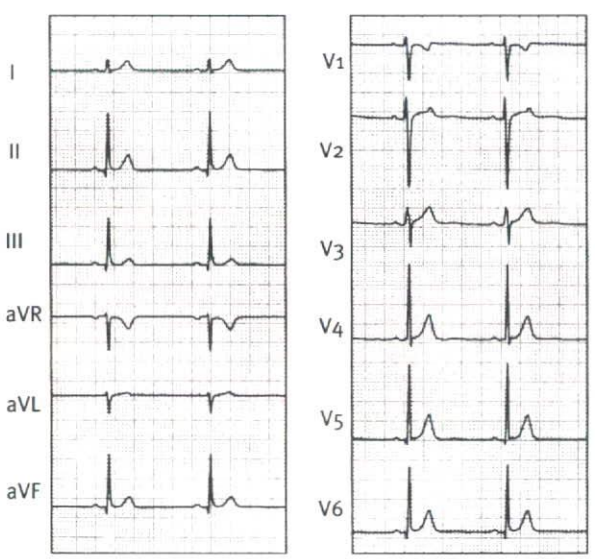

Figure 4: Electrocardiogram showing the shortest QTc interval in female subjects. The QTc is 304 $msec^{1/2}$.

contrast, loss of function mutations in these genes leads to long QT syndrome.[18] In an analysis of 2,008 individuals from the general population, Laetitia et al.[19] reported an interesting finding that subjects with borderline QTc prolongation were carriers of *KCNQ1* gene mutations that placed them at a potential risk of arrhythmia. Their report suggests that subjects with borderline short QTc values may be carriers of the gene mutation. With this in mind, studies should be carried out to investigate further this genetic involvement. In addition, it is desired that the genotype-phenotype correlation in short QT syndrome will be clarified in the future, as has been done in long QT syndrome.

It is known that the QTc values in females are generally longer than in males.[9] We confirmed this in our study, where the QTc values of females were significantly longer than that of males. This difference has been reported to be due to shortening in QTc values in adolescent males after puberty, whereas QTc values of females remain unchanged throughout the growth, maturation, and reproductive years.[9] The precise reasons for this difference are not well-known, and we should define the lower limits of QTc and the criteria of short QT syndrome in males and females separately. Unfortunately, until now there have been no reports that define short QT syndrome in males and females separately. Therefore, we introduced Gaita's criteria[6] of QTc <300 msec in the present study. All 3 subjects showing QTc <300 msec were male, and sex differences might affect the results. We should consider the difference in sex for diagnosing short QT syndrome as is done in long QT syndrome, and further investigations are necessary.

Study Limitations

This study had some limitations. First, subjects were selected from those who had come to the University Hospital for some medical advice; they did not include healthy individuals. The QTc distribution in the present

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study was almost comparable with past reports.[20,21] Second, we did not take into account in our analysis, the effect of the concomitant medications being taken by our subjects or the presence of any underlying illnesses. So, it is possible that short QT intervals were concealed by such factors. Third, the QTc can vary within the same day, or from day to day even in the same subject, and we analyzed ECGs at a single moment in time. So there is the possibility that transient short QT intervals may have been overlooked. And in several subjects, especially among children, shortening of QT intervals independent of heart rate have probably been concealed by tachycardia. The existence, or the degree of, QT variation in patients with short QT syndrome is not well-known. In the future, further studies should include gene analysis as well as such factors.

Conclusion

QTc <300 $msec^{1/2}$ was found in 0.03% of the presently examined population. Our results suggest that short QT syndrome may be very rare.

References

1. Moss AJ, Schwartz PJ, Crampton RS, Tzivoni D, Locati EH, et al.: The long QT syndrome: prospective longitudinal study of 328 families. *Circulation* 1991;84:1136–1144
2. Jervell A, Lange-Nielsen F: Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval and sudden death. *Am Heart J* 1957;54:59–68
3. Romano C, Gemme G, Pongiglione R: Aritmie cardiache rare dell'eta pediatrica. *Clin Pediatr* 1963;45:656–683
4. Ward OC: A new familial cardiac syndrome in children. *J Ir Med Assoc* 1964;54:103–106
5. Gussak I, Brugada P, Brugada J, Wright RS, Kopecky SL, et al.: Idiopathic short QT interval: a new clinical syndrome? *Cardiology* 2000;94:99–102
6. Gaita F, Giustetto C, Bianchi F, Wolpert C, Schimpf R, et al.: Short QT syndrome: a familial cause of sudden death. *Circulation* 2003;108:965–970
7. Goldberger AL: Electrocardiography. In: Braunwald E, ed. *Harrison's Principles of Internal Medicine* 15th ed. New York: Mcgraw-Hill; 2001; 1262–1271
8. Bazett HC: An analysis of the time-relations of electrocardiograms. *Heart* 1920;7:353–370
9. Rautaharju PM, Zhou SH, Wong S, Calhoun HP, Berenson GS, et al.: Sex differences in the evolution of the electrocardiographic QT interval with age. *Can J Cardiol* 1992;8:690–695
10. Nierenberg DW, Ransil BJ: Q-aTc interval as a clinical indicator of hypercalcemia. *Am J Cardiol* 1979;44:243–248
11. Nakagawa M, Takahashi N, Iwao T, Yonemochi H, Ooie T, et al.: Evaluation of autonomic influences on QT dispersion using the head-up tilt test in healthy subjects. *Pacing Clin Electrophysiol* 1999;22:1158–1163
12. DiFrancesco D, Ducouret P, Robinson RB: Muscarinic modulation of the cardiac rate at low acetylcholine concentrations. *Science* 1989;243:669–671
13. Cheng TO: Digitalis administration: an underappreciated but common cause of short QT interval. *Circulation* 2004;109:e152
14. Algra A, Tijssen JG, Roelandt JR, Pool J, Lubsen J: QT interval variables from 24-hour electrocardiography and the 2-y risk of sudden death. *Br Heart J* 1993;70:43–48
15. Brugada R, Hong K, Dumaine R, Cordeiro JM, Gaita F, et al.: Sudden death associated with short QT syndrome linked to mutations in HERG. *Circulation* 2004;109:30–35
16. Bellocq C, van Ginneken A, Bezzina CR, Alders M, Escande D, et al.: Mutation in the KCNQ1 gene leading to the short QT-interval syndrome. *Circulation* 2004;109:2394–2397
17. Priori SG, Pandit SV, Rivolta I, Berenfeil O, Ronchetti E, et al.: A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene. *Circ Res* 2005;96:800–807
18. Roden DM, Lazzara R, Rosen M, Schwartz PJ, Towbin J, et al.: Multiple mechanisms in the long-QT syndrome: current knowledge, gaps, and future directions. The SADS Foundation Task Force on LQTS. *Circulation* 1996;94:1996–2012
19. Laitilia G, Chloe B, Myram B, Franck P, Sophie D, et al.: New KCNQ1 mutations leading to haploinsufficiency in a general population: defective trafficking of a KvLQT1 mutant. *Cardiovasc Res* 2004;63:30–68
20. Simonson E, Cady LD, Woodbury M: The normal Q-T interval. *Am Heart J* 1962;63:747–753
21. Lepeschkin E: Components of Q-T and Q-U intervals of the electrocardiogram in normals. *J Appl Physiol* 1956;9:443–446

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SCN5A variants in Japanese patients with left ventricular noncompaction and arrhythmia

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Abstract

Left ventricular noncompaction (LVNC) is a genetically heterogenous disorder. Mutations in the human cardiac sodium channel alpha-subunit gene (*SCN5A*) are involved in the pathophysiology of cardiac arrhythmias and cardiomyopathies. This study was performed to compare the frequency of *SCN5A* variants in LVNC patients with or without arrhythmias, and to investigate the relationship between variants and disease severity. DNA was isolated from the peripheral blood of 62 Japanese probands with LVNC, comprising 17 familial cases and 45 sporadic cases. Blood samples were screened for variants in *SCN5A* using single-strand conformational polymorphism analysis (SSCP) and DNA sequencing. Seven variants, rs6599230:G > A, c.453C > T, c.1141-3C > A, rs1805124:A > G (p.H558R), rs1805125:C > T (p.P1090L), c.3996C > T, and rs1805126:T > C were identified in 7 familial and 12 sporadic cases. The frequency of *SCN5A* variants was significantly higher in the patients with arrhythmias than those without (50% vs 7%: $P = 0.0003$), suggesting these variants represent a risk factor for arrhythmia and supporting the hypothesis that genes encoding ion channels are involved in LVNC pathophysiology. The LVNC patients with heart failure also had high occurence of *SCN5A* variants, suggesting the presence of *SCN5A* variants and/or arrhythmias increase the severity of LVNC.

Keywords: Noncompaction; *SCN5A* variants; Arrhythmia; Heart failure

Introduction

Left ventricular noncompaction (LVNC) is characterized by persistence of multiple prominent ventricular trabeculations and deep intertrabecular recesses in the left ventricle and is defined as an unclassified cardiomyopathy [1]. To date, this disorder is considered an arrest in the morphogenetic process of myocardial compaction [2]. The clinical manifestations are not specific for this form of cardiomyopathy, with clinical overlap with dilated and hypertrophic cardiomyopathies, and are highly variable, ranging from asymptomatic to severe cardiac dysfunction leading to heart transplantation or death. Most LVNC patients also present with some form of arrhythmia [2].

LVNC, without other morphologic cardiac abnormalities, was first described echocardiographically in 1984 [3].

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In recent years, defects in several genes have been associated with LVNC. Recently, we identified novel sequence variants in *DTNA*, *TAZ*, and *LDB3* in patients with LVNC [4–6]. However, like other forms of inherited cardiomyopathy, LVNC is a genetically heterogeneous disease and can be inherited as an autosomal dominant or X-linked recessive disorder [4–6].

Mutations in the human cardiac sodium channel alpha-subunit gene (*SCN5A*) have been identified in patients with a range of arrhythmias including the long QT syndrome (LQTS) [7], Brugada syndrome [8], sudden unexplained nocturnal death syndrome [9], idiopathic ventricular fibrillation [10], congenital sick sinus syndrome [11], and cardiac conduction defects (CCD) [12], as well as sudden infant death syndrome [13]. Recently, variants in *SCN5A* [14,15] and a cardiac K_{ATP} channel gene (*ABCC9*)[16] have been reported in patients with dilated cardiomyopathy (DCM). The sodium channel plays a central role in the excitability of myocardial cells, by establishing a subtle equilibrium between depolarizing and repolarizing currents determining the action potential (AP) duration. Thus, variations in *SCN5A* may influence this equilibrium, even by weak effects on activity and/or the expression level of channels subunits. Single nucleotide polymorphisms (SNPs) in *SCN5A* have been implicated not only as the causes of inherited arrhythmic syndromes, but also as genetic risk factors for some acquired arrhythmias [17–20].

Therefore, we hypothesized that variations in genes encoding ion channels are implicated in the pathophysiology of LVNC in relation to the development of arrhythmias and the severity of disease. Here we report the analysis of the *SCN5A* gene in a large cohort of Japanese patients with LVNC, and present data supporting this hypothesis.

Methods

Subjects and clinical diagnostic criteria

LVNC was diagnosed by echocardiographic criteria, including: (1) LV hypertrophy with deep endomyocardial trabeculations in ≥ 1 ventricular wall segments, (2) reduced LV systolic function, (3) a two-layered endocardium with a noncompacted to compacted ratio of >2.0, and (4) deep recesses filled with blood from the ventricular cavity visualized on color Doppler imaging (Fig. 1) [4].

Initial clinical evaluations were performed without knowledge of genotype status. Once a proband was identified, a family history was obtained, and all potentially informative family members underwent clinical evaluation, including physical examination, chest radiograph, electrocardiogram (ECG), echocardiogram (2 dimensional and color Doppler) was used to evaluate the cardiac structure, LV size and function (shortening fraction and ejection fraction), and valve regurgitation (Fig. 1).

Peripheral blood samples were collected after written informed consent. Lymphoblastoid cell lines were established from the peripheral blood samples and then genomic DNA was isolated using QIAamp DNA extraction kits (Qiagen: Valencia, CA). The study was approved by the Research Ethics Committee of Toyama University Hospital.

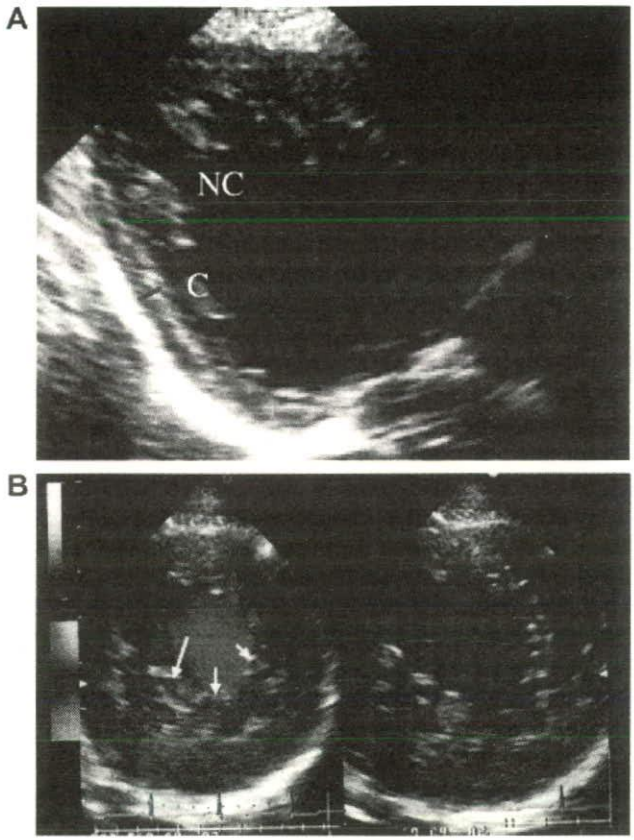

Fig. 1. (A) Two-dimensional echocardiogram of a patient with LVNC revealing noncompacted layer with intertrabecular recesses (NC) and outside the compacted layer (C), with an NC:C ratio >2.0. (B) Color Doppler echocardiogram of a LVNC patient demonstrating flow within deep intertrabecular recesses (arrow) in continuity with the left ventricular cavity. (For interpretation of the references to colours in this figure legend, the reader is referred to the web version of this paper.)

Molecular genetic studies

The *SCN5A* gene was amplified by PCR from the genomic DNA of each of the probands. The PCR primers were designed to amplify each of the coding exons, as well as flanking intronic sequences, using the online utility Primer 3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) and sequences from NCBI and Celera databases: the NCBI genomic DNA sequence file NT_022517.17 served as the primary source. PCR reactions were performed as previously described [6]: primer sequences and PCR conditions are available upon request. After PCR amplification, the samples were denatured by heating in a denaturing buffer and analyzed by SSCP [5].

Both normal and aberrant SSCP bands were cut directly from dried gels, purified, and sequenced according to the Applied Biosystems (ABI) Big Dye Terminator Cycle Sequencing protocol and analyzed using an ABI 310 Automated Sequencer. Sequencing results were compared with wild-type sequence published in NCBI by the BLAST comparative search algorithms (www.ncbi.nlm.nih.gov/BLAST/). Variations were confirmed by repeating the PCR from the genomic DNA template and sequencing the PCR products.

Statistical analysis

SNP frequencies in both of the groups were compared using the Chi square test. Relations between variants and arrhythmias were calculated by logistic regression. The differences were considered to be significant when $P < 0.05$.