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ORIGINAL ARTICLE
Arrhythmia/Electrophysiology

Pronounced Shortening of QT Interval With Mexiletine Infusion Test in Patients With Type 3 Congenital Long QT Syndrome

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Background: Mexiletine is often used for medical therapy in LQT3 patients, however, the usefulness of mexiletine infusion test for LQT3 patients has not been reported. The aim of this study was to evaluate the usefulness of mexiletine infusion test for detecting LQT3 patients.

Methods and Results: We analyzed response in 12-lead electrocardiogram parameters measured in II or V5 to i.v. mexiletine infusion (2 mg/kg) during sinus rhythm among 31 genotype-positive LQT patients (29±18 years, 12 male). Change in QTc interval after mexiletine was compared between LQT3 (n=15, 24±21 years, 9 male) and other LQT patients (4 LQT1 and 12 LQT2; 34±14 years, 3 male). Baseline RR, QT, and QTc interval were not different between the 2 groups (981±182 vs. 1,023±192 ms; 550±94 vs. 524±75 ms; 556±66 vs. 520±62 ms, respectively). While QTc interval was shortened with mexiletine in both groups (P<0.0001 vs. baseline), degree of QTc shortening (ΔQTc) was significantly larger in LQT3 than in LQT1/LQT2 patients (99±39 vs. 48±32 ms; P=0.0004). The sensitivity, specificity and predictive accuracy of mexiletine infusion test for differentiating LQT3 from LQT1/LQT2 were 86.7%, 81.3% and 81.3%, respectively, and the optimal cut-off for ΔQTc was 69 ms on receiver operating characteristic analysis. No pro-arrhythmic event was observed.

Conclusions: Pronounced shortening of QT interval with mexiletine may facilitate genetic testing in patients with LQT3 syndrome. (*Circ J* 2016; **80:** 340–345)

Key Words: Diagnosis; Gene; Long QT syndrome; Mexiletine; Ventricular arrhythmia

Congenital long QT syndrome (LQTS), characterized by prolongation of the QT interval, T wave abnormalities, and torsade de pointes (TdP), is a genetic heart disorder that may cause sudden cardiac death. Congenital LQTS is clinically diagnosed by QT prolongation on standard 12-lead electrocardiogram (ECG), clinical history of syncope or cardiac arrest, and a family history of LQTS.[1,2] Molecular genetics is now available to identify LQTS forms by mutations in genes encoding ion channels (*KCNQ1*, *KCNH2*, and *SCN5A* etc), and has enabled risk stratification and treatment of LQTS patients according to genotype. In congenital LQTS type 3 (LQT3), a gain-of-function mutation in *SCN5A* encoding the α subunit of cardiac voltage-dependent sodium channel may increase persistent (late) sodium current (late-I_{Na}) resulting in prolongation of action potential duration, leading to life-threatening ventricular arrhythmia.[3,4] Beta-blockers have been proved to be the first choice of pharmacological therapy in patients with congenital LQTS type 1 (LQT1) and type 2 (LQT2),[5–9] but it has been believed to be less effective in LQT3 patients, who often experience cardiac events at rest or during sleep.[10] In contrast, the late-I_{Na} inhibitor, mexiletine, has been shown to be effective in shortening QT interval in

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Table 1. Patient Baseline Characteristics

	LQT3 (n=15)	LQT1/LQT2 (n=16)	P-value
Age (years)	24±21	34±14	NS
Male	9 (60.0)	3 (18.8)*	<0.05
History of syncope	8 (53.3)	16 (100)*	<0.05
Bradycardia	6 (40.0)	5 (31.3)	NS
Torsades de pointes	4 (26.7)	5 (31.3)	NS
VF/aborted cardiac arrest	3 (20.0)	6 (40.0)	NS

Data given as mean±SD or n (%). *P<0.05 vs. LQT3. LQT1–3, long QT syndrome types 1–3; QTc, corrected QT interval; VF, ventricular fibrillation.

LQT3 rather than in LQT1 or LQT2, both clinically and experimentally.[11–13] This indicates that mexiletine may be able to differentiate LQT3 from other major forms of LQTS such as LQT1 and LQT2. Therefore, the aim of this study was to investigate the usefulness of intra-venous injection of mexiletine for differentiating LQT3 from LQT1/LQT2.

Methods

Study Design and Subjects

The subjects consisted of genotype-positive LQT patients, including 4 with LQT1, 12 with LQT2, and 15 with LQT3. All the patients were informed about congenital LQTS and the importance of diagnosis, including drug infusion test, and mutual agreement was obtained regarding this study. Clinical characteristics are listed in **Table 1**. After baseline 12-lead ECG recording was completed, 2 mg/kg mexiletine was infused for 10 min during sinus rhythm. The 12-lead ECG was continuously monitored and recorded during infusion and for 5 min after infusion.

We analyzed change in 12-lead ECG parameters after mexiletine infusion in all patients, and compared them between LQT3 and other LQTS patients (LQT1 and LQT2), given that ECG response to mexiletine was similar between the LQT1 and LQT2 patients. All LQTS patients had neither oral β-blockers nor anti-arrhythmic agents at mexiletine infusion test.

Measurement of 12-Lead ECG

Measurement of the obtained 12-lead ECG parameters was manually performed (25 mm/s, 10 mm/mV). QT was defined as the interval between onset of QRS morphology and the point at which an isoelectic line intersected a tangential line drawn at the point of minimum dV/dt of a positive T wave or at the point of maximum dV/dt of a negative T wave. Secondary T wave or bifurcated T wave was included in the QT interval except when the second wave (positive U wave) was clearly separated from the T wave. QT interval was measured at 10-ms increments in the V5 lead by 2 electrophysiologists (M.F. and W.S.), and inter-observer variability was 3.2±3.1 ms. If it was difficult to define QT interval in the V5 lead, then the V4 and limb lead II were used for measuring QT interval.[14] Corrected QT (QTc) interval was used to correct the effects of heart rate. Bazett's formula was applied as follows:

$$QTc = QT\ interval / RR^{1/2},$$

where RR is the interval between 2 continuous R waves.

Genetic Analysis

The protocol for genetic analysis was approved by the Institutional Ethics Committee and carried out according to the guidelines (M24-031-4). All patients provided informed consent before genetic analysis. Genomic DNA was isolated from whole blood using a DNA analyzer (QIAGEN GmbH, Hilden, Germany).[15] Genetic screening for *KCNQ1*, *KCNH2*, and *SCN5A* was carried out using the direct sequencing method (ABI 3730 DNA Analyzer, Life Technologies, Carlsbad, CA, USA). cDNA sequence numbering was based on the GenBank reference sequence NM_000218, NM_000238 and AY038064 for *KCNQ1*, *KCNH2*, and *SCN5A*, respectively.

Statistical Analysis

All data are expressed as mean±SD. In order to compare the ECG parameters before and after mexiletine infusion test, repeated-measure 2-way ANOVA was used (JMP ver. 8.0, SAS Institute). Differences in frequencies were analyzed with the chi-squared test, and 2-sided $P<0.05$ was considered statistically significant. Receiver operating characteristic (ROC) analysis was used for non-parametric data distribution; correlation between baseline QTc interval and ΔQTc was analyzed using Pearson's product moment correlation coefficient.

Results

Baseline RR interval, QT interval and QTc interval were not different between the LQT3 and the LQT1/ LQT2 patients (**Table 2**). All of the patients with LQT1/LQT2 had a history of syncope and all of the LQT1 patients were VF survivors (**Table 1**). Two out of 4 LQT1 patients had mutation of A341V, reported as severe phenotype.[16] On the other hand, LQT3 patients except for those with the specific mutations of *SCN5A* (E1784K, Y1795C) were symptomatic (**Table 3**).

Change in ECG Parameters With Mexiletine Infusion Test

Figure 1 shows the change in QTc interval in V5 after mexiletine infusion in LQT1, LQT2 and LQT3 patients, demonstrating that mexiletine shortened QTc interval more in LQT3 compared with LQT1 and LQT2. As shown in **Table 2**, intravenous mexiletine significantly shortened the RR interval in both LQT3 and LQT1/LQT2 patients, however, the change in RR interval (ΔRR) was not different between the 2 groups. Although mexiletine significantly shortened the QT and QTc intervals in both groups ($P<0.0001$), the change in both QT (ΔQT) and QTc (ΔQTc) interval was significantly larger in the LQT3 than in LQT1/LQT2 patients (116±50 ms vs. 66±32 ms; $P=0.0021$, and 99±39 ms vs. 48±32 ms; $P=0.0004$, respectively; **Table 2**; **Figure 2A**). No significant difference was observed in the ΔRR, ΔQT and ΔQTc intervals between the LQT1 and LQT2 patients (data not shown). The sensitivity, specificity and predictive accuracy of ΔQTc for differentiating LQT3 from LQT1/LQT2 with mexiletine were 86.7%, 81.3%, and

Table 2. Response of ECG Parameters to Mexiletine

	LQT3 (n=15)		LQT1/LQT2 (n=16)	
	Baseline	Mexiletine	Baseline	Mexiletine
RR interval (ms)	981±182	913±189*,†	1,023±192	958±206*,†
ΔRR (ms)	(68±61)		(65±55)	
QT interval (ms)	550±94	434±77*,†,‡	524±75	458±59*,†
ΔQT (ms)	(116±50)†		(66±32)	
QTc interval (ms)	556±66	457±69*,†,‡	520±62	472±58*,†
ΔQTc (ms)	(99±39)*,‡		(48±32)	

Data given as mean±SD *P<0.05 †vs. baseline; ‡vs. LQT1/LQT2. ECG, electrocardiography. Other abbreviations as in Table 1.

Table 3. Mutation of *SCN5A* and ECG Response to Mexiletine Infusion in LQT3 Patients

Patient ID no.	*SCN5A* mutation	Proband/ Family	Symptom status	Baseline				After mexiletine				ΔQTc (ms)	ΔQTc/ QTc-baseline
				RR (ms)	QT (ms)	QT peak-end (ms)	QTc (ms)	RR (ms)	QT (ms)	QT peak-end (ms)	QTc (ms)		
1	E1784K	Proband	Asymptomatic	760	470	60	539	700	360	40	430	109	0.20
2	E1784K	Proband	Asymptomatic	920	470	80	490	840	370	60	404	86	0.18
3	E1784K	Family of 2	Asymptomatic	980	480	80	485	880	390	60	416	69	0.14
4	E1784K	Proband	Asymptomatic	1,080	560	60	539	1,060	440	40	427	112	0.21
5	E1784K	Family of 4	Asymptomatic	1,080	520	60	500	1,000	400	40	400	100	0.20
6	E1784K	Family of 4	Asymptomatic	1,320	620	80	540	1,320	440	60	383	157	0.29
7	A1746T	Proband	Symptomatic	940	520	100	536	840	420	60	458	78	0.15
8	P1509-I1510 ins QKP	Proband	Symptomatic	1,000	480	160	480	1,000	440	140	440	40	0.08
9	Y1795C	Proband	Asymptomatic	740	460	60	535	740	380	40	442	93	0.17
10*	I1771M	Proband	Symptomatic	1,000	670	80	670	920	540	70	563	107	0.16
11*	R1623Q	Proband	Symptomatic	780	530	100	600	580	400	40	525	75	0.13
12	V411M	Proband	Symptomatic	1,080	590	140	568	940	400	60	413	155	0.27
13	F1617/ fs/400	Proband	Symptomatic	700	440	100	526	720	370	60	436	90	0.17
14	Q1507-P1509 del QKP	Proband	Symptomatic	1,260	740	60	659	1,140	520	50	487	172	0.26
15*	A1186T	Proband	Symptomatic	1,080	700	60	674	1,020	640	40	634	40	0.06

Patients 2,3, and 4–6, were in the same family group, respectively. *Insensitive to mexiletine. Abbreviations as in Tables 1,2.

81.3% respectively, when the best ΔQTc cut-off of 69 ms (calculated using ROC analysis) was used (**Figure 2B**).

Mutation Site-Specific Difference in QTc Shortening

Ten different mutations in the *SCN5A* gene were confirmed in the 15 LQT3 patients (**Figure 3**), in whom 6 asymptomatic patients had E1784K mutation (**Table 3**). The location of *SCN5A* mutations and the change in ECG parameters including ΔQTc with mexiletine infusion are shown in **Figure 3A**, **Table 3**. The extent of QTc shortening in response to mexiletine varied with location of *SCN5A* mutation. Therefore, we classified the *SCN5A* mutations as sensitive or insensitive to mexiletine infusion according to QTc interval after mexiletine (<500 ms or ≥500 ms), respectively.[17] Three mutations, A1186T, R1623Q and I1771M, were defined as insensitive to mexiletine, whereas the remaining 7 mutations were sensitive. In patients with mexiletine-sensitive mutation, ΔQTc was significantly correlated with baseline QTc (r^2=0.79, P=0.0074, **Figure 3B**). In contrast, patients with mexiletine-insensitive mutation had smaller ΔQTc even in the longer baseline QTc interval, thus baseline ΔQTc/QTc tended to be larger in patients with mexiletine-sensitive mutations compared with those with mexiletine-insensitive mutations (0.19±0.06 vs. 0.11±0.05, P=0.055).

Oral Mexiletine After Mexiletine Infusion Test

In the present study, 2 patients (patients 7 and 12; **Table 3**) out of 15 with LQT3 were treated with oral mexiletine after mexiletine infusion test, and ECG data after oral mexiletine were compared to those before mexiletine infusion test. Patient 7 was treated with 450 mg per day oral mexiletine. The RR, QT, and QTc interval were changed from 940 ms, 520 ms, and 536 ms to 1,000 ms, 460 ms, and 460 ms, respectively. Blood concentration of mexiletine was 0.60 μg/mg at 1 month after initiation of oral mexiletine. Patient 12 was treated with 300 mg oral mexiletine and the RR, QT, and QTc interval were changed from 1,080 ms, 590 ms, and 568 ms to 1,160 ms, 560 ms, and 520 ms, respectively. Blood concentration of mexiletine was not available in patient 12. QT and QTc interval were shortened by oral mexiletine therapy in both patients.

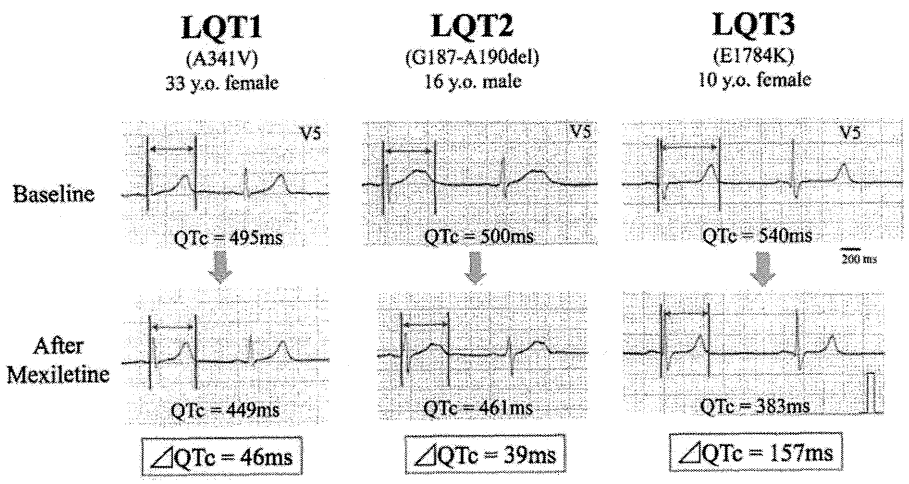

Figure 1. Representative electrocardiograms (lead V5) of the patients with long QT syndrome type 1 (LQT1), LQT2 and LQT3 syndrome before (baseline) and after mexiletine infusion test. Although corrected QT (QTc) interval was shortened after mexiletine in all patients, the change in QTc (ΔQTc) was more pronounced (157 ms) in the LQT3 patient compared with the LQT1 (46 ms) and LQT2 patients (39 ms).

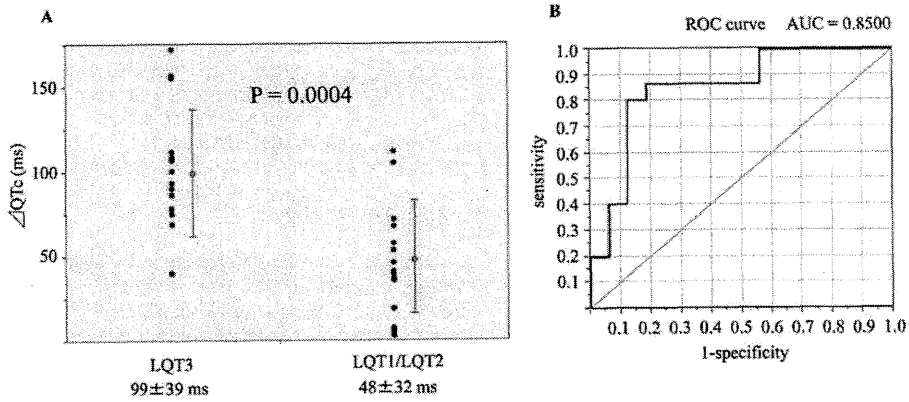

Figure 2. (**A**) Change in corrected QT (QTc) interval after mexiletine infusion test in long QT syndrome type 1 (LQT1)/LQT2 and LQT3 patients. (**B**) Receiver operating characteristic (ROC) analysis for differentiating LQT3 from LQT1/LQT2. AUC, area under the curve.

Complications of Mexiletine Infusion

There were no pro-arrhythmic complications including TdP, ventricular tachyarrhythmias, or premature ventricular contractions related to mexiletine infusion test.

Discussion

Main Findings

The main findings of the present study are that (1) mexiletine shortened QTc interval in the LQT3 patients more than in the LQT1, LQT2 patients, suggesting that mexiletine infusion test is a useful tool to distinguish LQT3 from LQT1 or LQT2; and (2) the difference in QTc shortening between mutation sites in response to mexiletine infusion was observed in LQT3 patients.

Usefulness of Mexiletine Infusion Test for LQT3

Clinical diagnosis of LQTS has been based on evaluation of the specific clinical setting with regard to history of syncope with sympathetic stimulation, cardiac events, and symptomatic

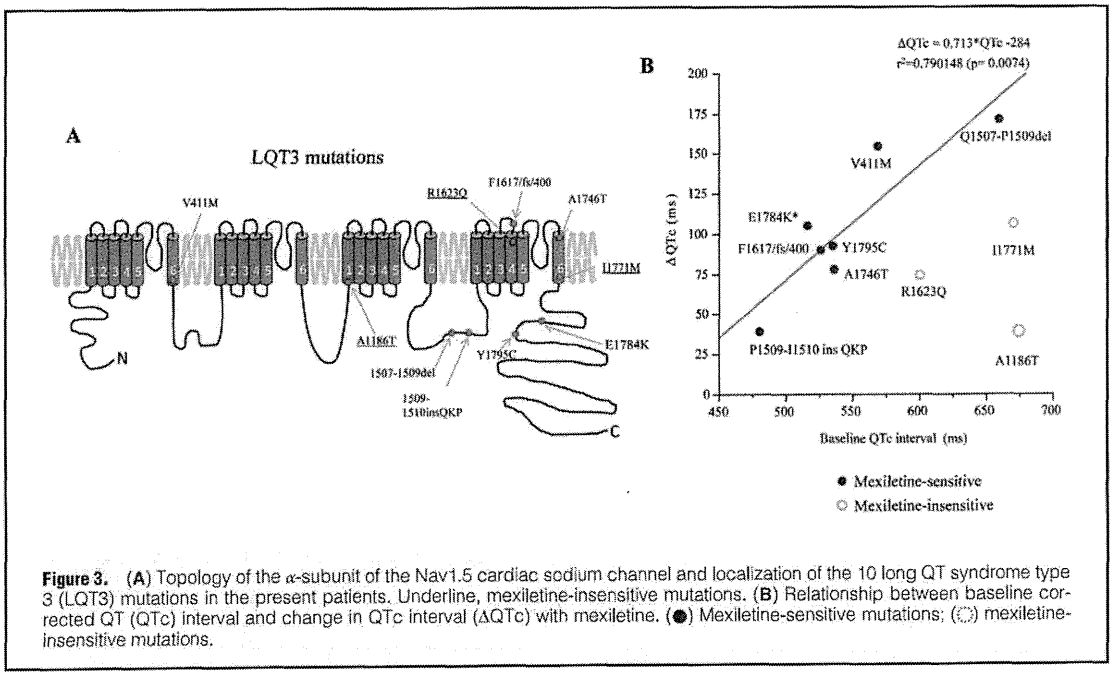

Figure 3. (**A**) Topology of the α-subunit of the Nav1.5 cardiac sodium channel and localization of the 10 long QT syndrome type 3 (LQT3) mutations in the present patients. Underline, mexiletine-insensitive mutations. (**B**) Relationship between baseline corrected QT (QTc) interval and change in QTc interval (ΔQTc) with mexiletine. (●) Mexiletine-sensitive mutations; (○) mexiletine-insensitive mutations.

or asymptomatic history of family members. Normal range of QT interval on 12-lead ECG varies between the sexes and between children and adults.[18] ECG diagnostic criteria involve QTc interval, T-wave morphology and QT dispersion,[18–20] but concealed or low-penetrance LQTS is difficult to detect on 12-lead ECG at rest.[21] Exercise stress testing such as treadmill testing or bicycle testing has been used for unmasking concealed or low-penetrance LQTS. Shimizu et al noted differential response in QTc interval on 12-lead ECG to epinephrine infusion test between LQT1, LQT2, and LQT3 patients.[22] Although both exercise and catecholamine challenge test were useful tools to predict LQT1 and LQT2 syndrome before genetic testing, the identification of LQT3 syndrome remained difficult. Genetic testing to identify specific mutations in LQTS is now commercially available for clinical use, but there are some difficulties with regard to cost and time to diagnosis. The combination of exercise, epinephrine infusion test and mexiletine infusion test may facilitate effective clinical diagnosis of LQTS. In the present study, we focused on the usefulness of mexiletine infusion test to differentiate LQT3 from LQT1 or LQT2, which has been previously reported by Schwartz et al clinically,[11] and by Shimizu et al and Priori et al experimentally.[13,23] Schwartz et al first reported that I_{Na} channel block with mexiletine shortened QT interval more in LQT3 than in LQT2 patients.[11] Shimizu et al and Priori et al demonstrated differential response in action potential duration or QT interval to mexiletine between experimental LQT2 and LQT3 models.[13,23] Furthermore, the QTc shortening with mexiletine infusion might be poor in the specific *SCN5A* mutations, as reported.[24] The present study first demonstrated the usefulness of mexiletine infusion test to distinguish LQT3 from LQT1 or LQT2 quantitatively. The correct diagnosis of LQT3 is important because some LQT3 patients are at high risk of sudden cardiac death and need implantation of cardioverter defibrillator. Mexiletine has fewer side-effects, such as TdP, sinus bradycardia or atrioventricular block compared with β-blockers. In the present study, 6 of 15 LQT3 patients had bradycardia and 1 patient had 2:1 atrioventricular block. Beta-blockers can worsen bradycardia or atrioventricular block. RR interval was slightly but significantly decreased after mexiletine infusion in the present LQTS patients, probably due to reactive response to slight decrease of blood pressure. Therefore, the lower number of side-effects with mexiletine infusion test is another advantage of this test, to facilitate genetic testing in LQT3 syndrome.

Mutation Site-Specific Differences in QT Shortening

Mexiletine shortens QTc interval in LQT3 rather than in LQT1 or LQT2 because of its suppression of late-I_{Na}, which may depend on the gating state of the sodium channel and the binding of local anesthetics. A previous study noted that some LQT3 patients were sensitive to mexiletine but the others were refractory to mexiletine.[24] Therefore, in this study, the 7 mutations E1784K, A1746T, 1509-1510insQKP, Y1795C, V411M, F1617/fs/400, and 1507-1509delQKP were defined as mexiletine sensitive and the remaining 3 mutations, I1771M, R1623Q and A1186T, as mexiletine insensitive according to QTc interval after mexiletine infusion test (<500 ms or ≥500 ms).[17] Although the extent of QTc shortening (ΔQTc) with mexiletine varied in the present LQT3 patients, ΔQTc was strongly correlated with baseline QTc interval in patients with the mexiletine-sensitive mutation (**Figure 3B**). Thus, prolonged QT interval due to increased late-I_{Na} can be normalized with mexiletine in these patients. In an in vitro study, Ruan et al suggested that mutations sensitive to mexiletine showed a negative shift in the steady-state inactivation curve and greater use of dependent block by mexiletine.[17] Makita et al reported that the E1784K mutant channel produced a negative shift in steady-state inactivation and enhanced slow inactivation, which may increase late-I_{Na}.[25] In contrast, steady-state

inactivation was not altered in the mexiletine-insensitive mutants, I1771M[26] and R1623Q.[27] On the other hand, mexiletine is known to bind to the sodium channel during the inactivated state.[28] These findings suggest that the negative shift in V1/2 of steady-state inactivation in E1784K and other mexiletine-sensitive mutant channels may be attributable to the greater shortening of QT interval in response to mexiletine infusion.[29] Thus, such functional heterogeneity of Na channels may account for the heterogeneous response of QT interval to mexiletine therapy in LQT3 patients.

Study Limitations

First, this study included a small number of patients with genotype-positive LQTS and therefore a selection bias may have been present. This study was also a cross-sectional study by a single center. Second, the reproducibility of the response of ECG parameters to mexiletine infusion was not evaluated. Third, the usefulness of mexiletine infusion was evaluated in this study, therefore, the chronic effect of oral mexiletine therapy on QT interval remains unknown.

Conclusions

Mexiletine infusion test with a ΔQTc cut-off of 69 ms was a safe and useful method to facilitate the genetic testing of LQT3 patients.

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原　著

母体抗 SS-A 抗体陽性の先天性完全房室ブロックの胎児における子宮内胎児死亡の危険因子

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Risk Factors for Intrauterine Death in Fetuses with Congenital Complete Atrioventricular Block and Positive Maternal Anti-SS-A Antibodies

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Background: There are few reports that focus specifically on the prognostic factors for fetal cases of congenital complete atrioventricular block (CCAVB) with positive maternal anti-SS-A antibodies, and the impact of maternal symptoms and anti-SS-A antibody levels on fetal prognosis remains unclear. The aim of this study was to elucidate the risk factors for intrauterine fetal death (IUFD) in fetuses with CCAVB and positive maternal anti-SS-A antibodies.

Method: We retrospectively analyzed 47 fetal cases of CCAVB born to mothers with positive anti-SS-A antibodies at 66 hospitals in Japan from 1996 to 2010. Clinical characteristics and measurements between the IUFD group ($n=7$) and the live-birth group ($n=40$) were compared.

Results: Cases of fetal heart rate <55 beats/minute at diagnosis of CCAVB (57% vs 17%, $p<0.05$) and development of hydrops fetalis during follow up (71% vs 20%, $p<0.05$) were more in the IUFD group than the live-birth group. Advanced maternal age was also associated with IUFD. Multivariate analysis showed that hydrops fetalis and advanced maternal age were independent risk factors for IUFD. There were no significant differences between the groups in the frequency of mothers with symptoms of connective tissue diseases, maternal anti-SS-A antibody levels, and the rate of transplacental administration of steroids.

Conclusion: Hydrops fetalis and advanced maternal age are independent risk factors for IUFD. Fetuses with CCAVB and positive maternal SS-A antibodies should be closely followed up for hydrops fetalis so that they can be delivered in a timely fashion.

Keywords: anti-SS-A antibody, complete atrioventricular block, intrauterine fetal death, hydrops fetalis

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背景：抗 SS-A 抗体陽性妊娠に限定した胎児先天性完全房室ブロック（CCAVB）の予後規定因子についての報告はほとんどなく，母体の膠原病症状や母体抗 SS-A 抗体価と CCAVB の胎児の予後の関連についても報告は少ない．本研究の目的は，母体抗 SS-A 抗体陽性の CCAVB の胎児における子宮内胎児死亡（IUFD）の危険因子を明らかにすることである．
方法：全国 66 施設で 1996～2010 年に娩出された母体抗 SS-A 抗体陽性の CCAVB 胎児 47 例を，IUFD 群（7 例）と live-birth 群（40 例）に分け，臨床データや各種検査値を後方視的に比較した．
結果：IUFD 群では，live-birth 群に比べ，診断時の胎児心拍数が 55 回／分未満であった症例が多く（57% vs 17%, $p<0.05$），経過中に胎児水腫を認める頻度が高く（71% vs 20%, $p<0.05$），さらに母体年齢が高かった．多変量解析では，胎児水腫と母体高年齢が IUFD の独立した危険因子であった．両群で母体膠原病の有症状率，母体抗 SS-A 抗体価，およびステロイドの経胎盤的投与率に有意差はなかった．
結論：母体抗 SS-A 抗体陽性の CCAVB の胎児では，胎児水腫と母体高年齢が IUFD の危険因子であり，胎児水腫について注意深く経過観察し，適切な娩出時期を検討する必要がある．

はじめに

抗 SS-A 抗体は，Sjögren 症候群患者の約 70%，全身性ループスエリテマトーデス患者の約 50%で検出される自己抗体であり[1]，抗 SS-A 抗体陽性妊娠の 1～2%で胎児に先天性完全房室ブロック（congenital complete atrioventricular block; CCAVB）が生じる[2,3]．CCAVB の出生前診断例では，子宮内胎児死亡（intrauterine fetal death; IUFD）も稀ではなく，また出生後も高い頻度でペースメーカ治療を要するなど，胎児期から厳重な管理が必要な疾患である．

CCAVB の予後不良因子についてはさまざまな報告[4,5]があるが，抗 SS-A 抗体陽性妊娠に限定した胎児 CCAVB の予後規定因子についての報告はほとんどない．また，母体の膠原病症状や母体抗 SS-A 抗体価と CCAVB の胎児の予後の関連についても報告は少ない．

目　的

母体抗 SS-A 抗体陽性の CCAVB の胎児における IUFD の危険因子を明らかにすること．

対象と方法

「自己抗体陽性女性の妊娠管理指針の作成及び新生児ループスの発症リスクの軽減に関する研究」研究班により作成された抗 SS-A 抗体陽性妊娠例のデータベースを用い，胎児期発症の CCAVB の予後規定因子について後方視的検討を行った．

研究班では，国立成育医療研究センターの倫理委員会で承認を得たうえで，全国 66 施設を対象とし，1996 年から 2010 年に分娩となった抗 SS-A 抗体陽性妊娠例のアンケート調査を行った．合計で 732 例の抗 SS-A 抗体陽性妊娠が登録され，このうち 50 例に胎児徐脈を認めた．うち 2 例は II 度房室ブロックであり，48 例が完全房室ブロックであった．1 例は児の周産期データが欠損していたために除外し，母体抗 SS-A 抗体陽性の完全房室ブロックの胎児 47 例を解析の対象とした（Fig. 1）．左側相同や修正大血管転位などの複雑心奇形に伴う完全房室ブロックは含まれていなかった．

アンケート調査で収集した主な項目は以下の通りである．胎児に関しては，完全房室ブロックと診断された時点の在胎週数，診断確定時の胎児心拍数，経過中の胎児水腫の有無，IUFD の有無，出生時週数，出生時体重，CCAVB を発症した同胞の有無，児の性別を調査した．膠原病内科医および産科医による調査であり，出生後の新生児の経過については，本データベースには登録されていない．母体に関しては，分娩時年齢，抗 SS-A 抗体陽性と判明した時期（妊娠前か妊娠後か），膠原病に関連した臨床症状（ドライアイ，ドライマウス，紅斑，紫斑，レイノー現象，発熱，関節痛，髄膜炎，間質性腎炎，間質性肺炎，肺高血圧，血栓症）の既往，膠原病の診断名（Sjögren 症候群もしくは全身性エリテマトーデス），妊娠前および妊娠中のステロイド投与の有無，β刺激薬の投与の有無，投与開始時期を調査した．母体抗 SS-A 抗体価については，妊娠 15 週に最も近い時期に測定された，二重免疫拡散法による力価もしくは酵素結合免疫吸着測定（enzyme-linked immunosorbent assay; ELISA）法による濃度の記入を求めた．また，抗 SS-A 抗体のサブタイプの一つである抗 Ro52 抗体の測定を行っている症例については，その測定値も調査した．

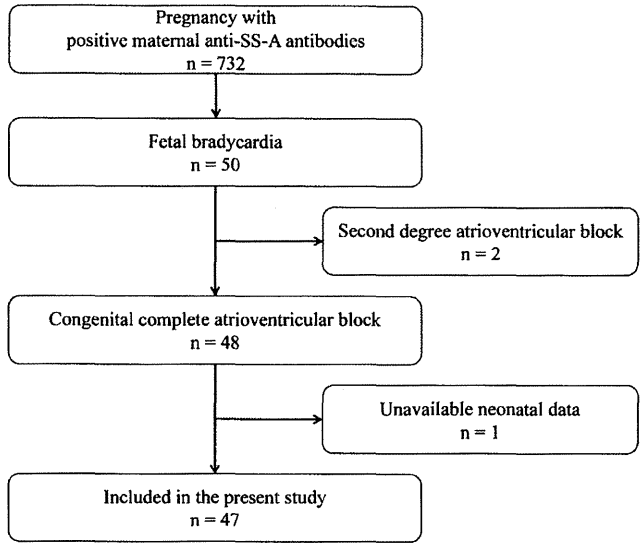

Fig. 1 Flow chart depicting the selection of fetal cases included in the present study

症例をIUFD群とlive-birth群に分け，臨床データ，各種検査値の比較を行った．データは，その性質に応じて，頻度(%)，平均値±標準偏差，中央値（最小値-最大値）で表示した．群間の比較は，名義変数はFisherの正確確率検定法を，連続変数はMann-Whitney U検定を用いて行った．さらに，IUFDの危険因子を見出すために，単変量解析でp値が0.1未満であった項目を説明変数とする重回帰分析を行った．p値が0.05未満を統計学的に有意とした．統計学的解析は，フリーソフトR version 2.13.0を用いた．

結　果

解析対象となった母体抗SS-A抗体陽性のCCAVB 47症例のうち，IUFD群は7例，live-birth群は40例であった．Table 1に両群の児および母体の臨床データを示す．IUFD群では在胎30（19～37）週でIUFDを来していた．live-birth群では，在胎37（25～40）週で出生し，出生体重は2.2±0.6kgであった．

完全房室ブロックと診断された時点の在胎週数は，IUFD群が21（18～26）週，live-birth群が23（18～34）週で，統計学的有意差はなかった（p=0.059）．診断時の胎児心拍数は，IUFD群が51（42～80）回／分，live-birth群が60（48～137）回／分であった（p=0.089）．胎児心拍数が55回／分未満であった症例は，IUFD群が7例中4例（57%），live-birth群が胎児心拍数データが確認できた30例中5例（17%）であり，IUFD群の方が胎児心拍数が55回／分未満である頻度が有意に多かった（p<0.05）．経過中に胎児水腫を来した症例は，IUFD群が71%，live-birth群が20%であり，IUFD群の方が胎児水腫を来した頻度が有意に多かった（p<0.05）．両群で，児の性別に有意差はなかった．また，CCAVBを発症した同胞を持つ症例が3症例あったが，全例がlive-birth群であった．

分娩時の母体年齢は，IUFD群で32（30～36）歳，live-birth群で28.5（22～37）歳であり，IUFD群の方が有意に年齢が高かった（p<0.05）．母体の膠原病症状はIUFD群の71%，live-birth群の48%で認められ，Sjögren症候群もしくは全身性ループスエリテマトーデスと診断されていた．妊娠前に母体抗SS-A抗体が陽性であると判明していたのは全47例中17例にすぎず，両群で有意差はなかった．残る30例は，妊娠後に母体抗SS-A抗体陽性であると診断されており，そのうち14例は，胎児がCCAVBを発症した後の精査で抗SS-A抗体陽性と判明した症例であった．

母体へのステロイド投与が行われていたのは，IUFD群で43%，live-birth群で53%であり，そのうち胎児CCAVBへの効果を期待してフッ化ステロイド（betamethasoneもしくはdexamethasone）のいわゆる経胎盤的投与が行われていたのは，IUFD群で29%，live-birth群で45%であった．いずれも両群で有意差は認めなかった．

両群における母体抗SS-A抗体の力価および濃度，

Table 1 Comparison of patient characteristics between the groups

	IUFD (*N*=7)	Live-birth (*N*=40)	*p*
Gestational age at diagnosis (weeks)	21 (18–26)	23 (18–34)	0.059
Fetal heart rate at diagnosis (beats/min)*	51 (42–80)	60 (48–137)	0.089
Fetal heart rate at diagnosis <55 beats/min*	4 (57%)	5 (17%)	<0.05
Hydrops fetalis	5 (71%)	8 (20%)	<0.05
Gestational age at birth (weeks)	—	37 (25–40)	
Gestational age at IUFD (weeks)	30 (19–37)	—	
Body weight at birth (kg)	—	2.2±0.6	
Boys	3 (43%)	11 (37%)	0.41
Older siblings with CCAVB	0	3 (8%)	1
Maternal age at delivery (years)	32 (30–36)	28.5 (22–37)	<0.05
Maternal symptoms of collagen diseases	5 (71%)	19 (48%)	0.416
Maternal diagnosis of Sjögren's syndrome	4 (57%)	14 (35%)	0.403
Maternal diagnosis of systemic lupus erythematosus	1 (14%)	5 (13%)	1
Maternal diagnosis of positive anti-SS-A antibody before conception	3 (43%)	14 (35%)	0.692
Transplacental administration of all steroids	3 (43%)	21 (53%)	0.701
Transplacental administration of fluorinated steroids	2 (29%)	18 (45%)	0.682
Transplacental administration of beta agonists	1 (14%)	9 (23%)	1

*Data available in 37 cases (7 cases from the IUFD group, 30 cases from the live-birth group). CCAVB=congenital complete atrioventricular block, IUFD = intrauterine fetal death.

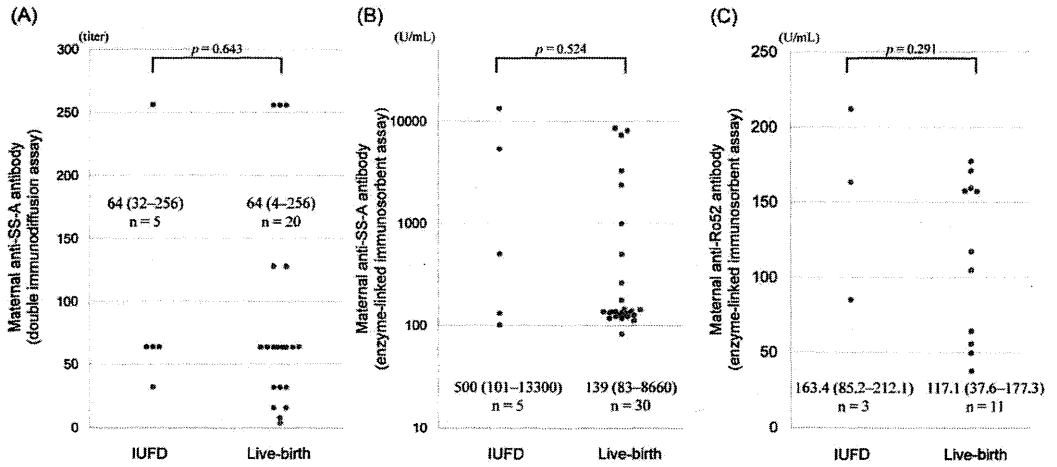

Fig. 2 Scatter plots of maternal antibody levels

(A) Maternal anti-SS-A antibody titer measured by double immunodiffusion assay. (B) Maternal anti-SS-A antibody concentration measured by enzyme-linked immunosorbent assay. (C) Maternal anti-Ro52 antibody concentration measured by enzyme-linked immunosorbent assay. IUFD=intrauterine fetal death.

母体抗 52-kD 抗体の濃度の分布を Fig. 2 に示した．力価については，IUFD 群が 64（32～256）倍，live-birth 群が 64（4～256）倍で，有意差は認めなかった（p=0.643）．濃度については，IUFD 群が 500 U/mL（101～13,300 U/mL），live-birth 群 が 139 U/mL（83～8,660 U/mL）であり，両群で有意差は認めなかった（p=0.524）．母体抗 52-kD 抗体の濃度が測定されていたのは 14 例（IUFD 群 3 例，live-birth 群 11 例）で，IUFD 群は 163.4 U/mL（85.2～212.1 U/mL），live-birth 群は 117.1 U/mL（37.6～177.3 U/mL）であり有意差は認めなかった（p=0.291）．

Table 2 に IUFD の危険因子の多変量解析結果を示した．在胎週数，胎児心拍数，胎児水腫の有無，母体年齢を説明変数として重回帰分析を行った結果，胎児水腫の有無および母体年齢が IUFD の独立した危険因子であった．

Table 2 Predictors of IUFD from multivariate analysis

Variables	Coefficient	Standard error	*p*
Gestational age at diagnosis	—	—	—
Fetal heart rate at diagnosis	—	—	—
Hydrops fetalis	0.282	0.107	0.0113
Maternal age at delivery	0.025	0.012	0.0431

CCAVB=congenital complete atrioventricular block, IUFD=intrauterine fetal death.

考　察

本研究で得られた知見は，(1)胎児水腫と母体年齢が，母体抗 SS-A 抗体陽性の CCAVB の胎児における IUFD の独立した危険因子であること，(2)母体膠原病の症状，母体抗 SS-A 抗体価，およびそのサブタイプの一つである抗 Ro52 抗体価は，CCAVB の胎児の予後と相関がないこと，である．

母体抗 SS-A 抗体陽性の CCAVB の胎児の予後を検討した結果，IUFD となった胎児では，生産に至った胎児に比べ，経過中に胎児水腫を来した症例が有意に多かった．診断時の胎児心拍数は，統計学的有意差はないものの IUFD 群の方が低い傾向にあり，胎児心拍数が 55 回／分未満であった症例は IUFD 群の方が有意に多かった．診断時の週数は IUFD 群の方が早い傾向があったが，有意差はなかった．また，IUFD 群では live-birth 群に比べ，母体年齢が有意に高かった．在胎週数，胎児心拍数，胎児水腫の有無，母体年齢を説明変数とした多変量解析では，胎児水腫と母体年齢が IUFD の独立した危険因子であった．

CCAVB は，左側相同に代表される複雑心奇形に伴うことがあり，この場合は胎児水腫や胎児心拍数によらず，予後が悪い[6,7]．複雑心奇形を背景としない CCAVB の胎児の予後不良因子については，これまでにも多くの報告がなされている．II 度ないし III 度の完全房室ブロックの胎児 175 例を対象とした欧州の多施設共同後方視的研究では，診断時の在胎週数が 20 週未満，胎児心拍数 50 回／分以下，胎児水腫，左室機能障害の 4 つが，胎児死亡の危険因子であるとしている[4]．CCAVB の胎児 61 例を対象とした，本邦における全国調査では，胎児期および新生児期の死亡の危険因子として有意であったものは胎児水腫のみであった[5]．さらに胎児心拍数と胎児水腫には有意な相関はなかったとしている．今回の検討は，母体抗 SS-A 抗体陽性の胎児症例のみを対象としたものであるが，従来の報告と同様に，胎児水腫が IUFD の危険因子であった．

母体抗 SS-A 抗体陽性の CCAVB を対象とした本研究では，母体年齢が高いことも IUFD の危険因子であるとの結果が得られた．CCAVB の胎児における母体年齢と IUFD との相関については，IUFD 群の方が母体年齢が若いという，今回の検討とは相反する報告[4]もみられる．現時点では，母体年齢と IUFD との関連について断定的な結論は出せないが，母体抗 SS-A 抗体が陽性かどうかで，母体年齢が CCAVB の胎児の予後に与える影響が異なる可能性もあると考えられ，さらなる研究が必要である．

本研究で用いたデータベースは，膠原病内科医および産科医による調査がもとになっており，膠原病に関連した臨床症状や，抗 SS-A 抗体価など，抗 SS-A 抗体陽性母体の詳細なデータを有している点が特徴である．母体膠原病の症状や診断の有無，抗 SS-A 抗体陽性が妊娠前に判明していたかどうかは，IUFD 群と live-birth 群で有意差はなく，CCAVB の胎児の予後とは関連がなかった．また，母体抗 SS-A 抗体価，およびそのサブタイプの一つである抗 Ro52 抗体価についても，IUFD 群と live-birth 群で有意差は認められず，CCAVB の胎児の予後規定因子とは考えられなかった．

抗 SS-A 抗体は，52-kDa と 60-kDa という 2 つのサブユニットからなるタンパク質とリボ核酸との複合体である Ro 抗原に対する自己抗体であり，Sjögren 症候群患者の約 70%，全身性ループスエリテマトーデス患者の約 50%で検出され[1]，抗 SS-A 抗体陽性妊娠の 1～2%で胎児に CCAVB が生じる[2,3]．近年，抗 SS-A 抗体のサブタイプである抗 Ro52 抗体と抗 Ro60 抗体のそれぞれについての基礎的な研究が進み，特に抗 Ro52 抗体が CCAVB の発症に強く関与していることが分かってきた．抗 Ro52 抗体は，L 型カルシウムチャネルと親和性が高く，内向きカルシウム電流を抑制することにより房室結節の心筋細胞の脱分極を阻害する[8]．母体から経胎盤的に移行した抗 Ro52 抗体は，胎児房室結節の働きを阻害して I 度房室ブロックを引き起こす．この時点では房室ブロックは可逆的であるが，高い抗体価の抗 Ro52 抗体に慢性的に曝露されると，心筋細胞のアポトーシスが誘発され，不可逆

的なCCAVBをきたすものと考えられている[9].

母体の抗SS-A抗体価が高いほど，胎児のCCAVB発症リスクが高まることが明らかにされている．Jaeggiら[10]は，抗SS-A抗体陽性妊娠186例の検討で，抗SS-A抗体に起因する胎児心病変（CCAVBの他，心収縮不良や心内膜線維弾性症も含む）をきたした40例は，全例が母体の抗SS-A抗体の濃度（ELISA法）が50U/mL以上であった一方，50U/mL未満の症例では心病変を来したものはなかったと報告した．Anamiら[11]は，抗SS-A抗体陽性妊娠189例（うち17例がCCAVBを発症）の後方視的解析で，母体抗SS-A抗体の力価（二重免疫拡散法）が32倍以上であることが，胎児房室ブロック発症の独立した危険因子であると報告した．

今回の我々の検討では，CCAVBを発症した胎児の予後と，母体抗SS-A抗体価に，関連は認められなかった．抗体価が測定されている症例数が限られており，さらなる症例の蓄積が必要であるが，現在のところ，母体抗SS-A抗体陽性妊娠における胎児CCAVBの診断確定時点で，胎児の予後を予測することは難しいと考えられた．CCAVBと胎児診断した場合，胎児予後不良因子である胎児水腫の出現について，注意深いフォローアップを行い，適切な娩出時期を検討する必要がある．

胎児診断されたCCAVBに対し，母体へのフッ化ステロイドおよびβ刺激薬の投与による経胎盤的治療が試みられているが，その予後改善効果を示す充分なエビデンスは存在しないのが現状である．非フッ化ステロイドであるprednisoloneは，胎盤に存在する11β-Hydroxysteroid dehydrogenaseにより代謝されて活性が減弱するため，ステロイドの経胎盤的投与にはbetamethasoneやdexamethasoneなどのフッ化ステロイドが用いられる[12,13]．Jaeggiら[14]は，dexamethasoneの経胎盤的治療が行われた21例と，行われなかった13例を後方視的に比較し，live-birth率（95% vs 77%）および一年生存率（90% vs 46%）とも，dexamethasone投与群で有意に高かったと報告したが，dexamethasone非投与群はより旧い時代の症例が多い影響があるかもしれない．一方，欧州の多施設共同後方視的研究[4]では，フッ化ステロイド投与の有無によるIUFDおよび新生児期の死亡の頻度に有意差はなかった．また，β刺激薬の経胎盤的投与は，胎児心拍数を上昇させる効果があるものの，予後を改善させる効果は示されなかった[5]．母体抗SS-A抗体陽性の症例のみを対象とした今回の検討でも，IUFD群とlive-birth群で，母体へステロイド投与の頻度，フッ化ステロイドおよびβ刺激薬の経胎盤的投与の頻度に有意差はなかった．ただし，ステロイドやβ刺激薬の経胎盤的投与の方針が施設により異なっていること，および，本研究が経胎盤的治療の効果を証明するためにデザインされた研究ではないことに注意を要する．

本研究で用いたデータベースに登録された抗SS-A抗体陽性妊娠732例のうち，48例（6.6%）の胎児にCCAVBを認めた．一般に，抗SS-A抗体陽性妊娠の胎児にCCAVBが発生する頻度は1～2%でされており[2,3]，本研究で用いたデータベースではCCAVBの発生率が高かった．これは，全ての妊婦で抗SS-A抗体価が測定されているわけではなく，抗SS-A抗体陽性が見逃されている妊娠が相当数あるためと思われる．実際，本研究のデータベースで，胎児がCCAVBを発症した後の精査で抗SS-A抗体陽性と判明した症例が14例認められており，CCAVBを発症した症例ほど母体の抗SS-A抗体陽性が発見されやすいことがうかがわれる．

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CLINICAL RESEARCH
Arrhythmia/electrophysiology

Significance of electrocardiogram recording in high intercostal spaces in patients with early repolarization syndrome

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Aims Published reports regarding inferolateral early repolarization (ER) syndrome (ERS) before 2013 possibly included patients with Brugada-pattern electrocardiogram (BrP-ECG) recorded only in the high intercostal spaces (HICS). We investigated the significance of HICS ECG recording in ERS patients.

Methods and results Fifty-six patients showing inferolateral ER in the standard ECG and spontaneous ventricular fibrillation (VF) not linked to structural heart disease underwent drug provocation tests by sodium channel blockade with right precordial ECG (V_1–V_3) recording in the 2nd–4th intercostal spaces. The prevalence and long-term outcome of ERS patients with and without BrP-ECG in HICS were investigated. After 18 patients showing type 1 BrP-ECG in the standard ECG were excluded, 38 patients (34 males, mean age; 40.4 ± 13.6 years) were classified into four groups [group A (n = 6;16%):patients with ER and type 1 BrP-ECG only in HICS, group B (n = 5;13%):ERS with non-type 1 BrP-ECG only in HICS, group C (n = 8;21%):ERS with non-type 1 BrP-ECG in the standard ECG, and group D (n = 19;50%):ERS only, spontaneously or after drug provocation test]. During follow-up of 110.0 ± 55.4 months, the rate of VF recurrence including electrical storm was significantly higher in groups A (4/6:67%), B (4/5:80%), and C (4/8:50%) compared with D (2/19:11%) (A, B, and C vs. D, $P < 0.05$).

Conclusions Approximately 30% of the patients with ERS who had been diagnosed with the previous criteria showed BrP-ECG only in HICS. Ventricular fibrillation mostly recurred in patients showing BrP-ECG in any precordial lead including HICS; these comprised 50% of the ERS cohort.

Keywords Early repolarization • Ventricular fibrillation • Brugada syndrome • Electrocardiogram • High intercostal recording

Clinical perspective

This study shows evidence that ventricular fibrillation mostly recurs in patients with early repolarization syndrome (ERS) showing Brugada-pattern electrocardiogram (ECG) in any of the right precordial leads including high intercostal spaces (HICS), and that ~30% of them had Brugada-pattern ECG (BrP-ECG) only in the HICS. These results indicate the importance of a systematic search for BrP-ECG with high intercostal ECG recording for the risk stratification of patients with ERS.

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Introduction

Early repolarization syndrome (ERS) is diagnosed in patients with inferolateral early repolarization (ER) on the standard 12-lead electrocardiogram (ECG), who had been resuscitated from idiopathic ventricular fibrillation (VF) in the absence of other causes of cardiac arrest such as Brugada syndrome (BrS).[1] It has been proposed that patients with ERS should not have type 1 Brugada-pattern ECG (BrP-ECG) in the right precordial leads on the standard ECG.[1] So far, an additional ECG recording has not been required for the diagnosis of ERS. Even in the expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes, which was published in 2013, there is no mention with regard to recording sites in ERS.[2] However, in patients with BrS, the new consensus statement recommends that ECG recordings be taken in the higher intercostal spaces, because it has been recognized since 2005 that the high intercostal recordings showed better sensitivity and specificity in the ECG diagnosis of BrS.[3–6]

Therefore, there is a possibility that patients with ERS in previous reports might have had a type 1 or non-type 1 BrP-ECG only in the high intercostal spaces (HICS). The purpose of this study was to investigate the prevalence of Brugada ECG pattern recorded in the HICS in patients with ERS and the prognosis of patients with and without BrP-ECG.

Methods

Study population

The study population consisted of 56 consecutive patients with inferolateral ER and spontaneous VF who were admitted to National Cerebral and Cardiovascular Center, Suita, Japan, between 1996 and 2014 (52 men, mean age: 39.9 ± 13.0 years). None of the patients had structural heart disease, including arrhythmogenic right ventricular cardiomyopathy, which was confirmed by noninvasive studies (physical examination, 12-lead ECG, 87-lead body surface ECG, exercise stress test, signal-averaged electrocardiography, and cardiac magnetic resonance imaging or computed tomography), and invasive studies consisting of coronary angiography including ergonovine/acetylcholine injection and right or left ventricular cineangiography. Patients with coronary artery spasm, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia, commotio cordis, drug-induced VF, and hypothermia were excluded. This study was approved by the Institutional Research Board of National Cerebral and Cardiovascular Center.

Classification of each group

Of the total of 56 patients, 18 patients showing type 1 BrP-ECG in the standard ECG spontaneously or after drug provocation tests by a sodium channel blocker were excluded, and 38 patients with inferolateral ER and a prior VF (34 males, mean age: 40.4 ± 13.6 years) were classified into four groups (A–D) based on ST-T morphology in the right precordial leads with the results of the drug provocation tests by a sodium channel blocker (group A: patients with an inferolateral ER and type 1 BrP-ECG only in the high intercostal recording, group B: patients with an inferolateral ER and non-type 1 BrP-ECG only in the high intercostal recording spontaneously or after drug test, group C: patients with an inferolateral ER and non-type 1 BrP-ECG in the standard ECG spontaneously or after drug test, group D: patients with inferolateral ER without BrP-ECG in any of the right precordial leads). Electrocardiograms recorded at a higher intercostal space were evaluated by 87-lead body surface maps before 1998,[7] and by leads V_1 to V_3 placed in the second and third intercostal spaces in addition to the standard intercostal space after 1998.[8] Patients were seen routinely every 3–6 months for clinical review and device interrogation. Both the standard and high costal ECGs were recorded at every visit. Patients in group A never showed type 1 BrP-ECG at a standard intercostal space spontaneously or after drug provocation test in the repeated ECG recordings during follow-up. None of the ECGs of patients in groups B and C revealed type 1 BrP-ECG during follow-up including in the high costal recordings.

The presence of inferolateral ER, which was defined as an elevation of the J point in at least 2 leads, was evaluated by baseline 12-lead ECGs (25 mm/s and 10 mm/mV). The amplitude of the inferolateral J-wave or J-point elevation had to be at least 1 mm or 0.1 mV above the baseline level, either as QRS slurring or notching in any of the inferior (II, III, and aVF), lateral (V_4, V_5, and V_6), and high lateral (I and aVL) leads in at least one ECG recording.[1]

BrS was diagnosed when a type 1 ST-segment elevation was observed either spontaneously or after intravenous administration of a sodium channel blocking agent in at least one right precordial lead (V_1 and V_2), which was placed in a standard or a superior position (up to the second intercostal space).[2] Type 1 ECG was defined as a coved-type J-point or ST elevation ≥2 mm followed by a negative T-wave.[9]

Non-type 1 BrP-ECG was defined as type 2 BrP-ECG, type 3 BrP-ECG or upward/downward notching or downward slurring with an amplitude ≥1 mm at the end of QRS to early ST segment in any of the anterior leads (V_1, V_2, and V_3) in the baseline standard or high costal (second and third) ECG recordings or in those ECGs after drug provocation tests.[10] The upward/downward notch in the anterior leads should have appeared between the late QRS and early ST period in the same timeframe as J-waves in other leads in the same 12-lead ECG.

Drug provocation test

Drug provocation tests were conducted with pilsicainide (up to 1 mg/kg body weight injected at a rate of 5–10 mg/min), disopyramide (1.5 mg/kg, 10 mg/min), or flecainide (2 mg/kg, 10 mg/min) during standard and high costal (second and third) ECG recordings. All ECGs were recorded at 25 mm/s and 10 mm/mV. First, the ECG recordings were independently analyzed by two cardiologists (T.K. and S.K.), and consensus was reached about the diagnosis. A third trained cardiologist (K.K.) independently evaluated all of the ECGs with no knowledge of the other observers' judgment or the clinical information to test for inter-observer variability, and consensus was established.

Clinical data, electrocardiogram, and electrophysiological testing

Clinical data including age at the first episode of VF, sex, family history of sudden cardiac death at <45 years of age, patients' activity at VF, prognosis, and drug therapy were collected on all patients. We defined the patients' state at VF as sleep when VF occurred in a state of sleeping, as near sleep when VF occurred in a resting state just after waking,[10] and as arousal at rest when VF occurred at rest in an awake state without active body movement. During follow-up, patients were considered to have an arrhythmic event if VF was documented by implantable cardioverter-defibrillator (ICD) interrogation. An electrical storm was defined as ≥3 VF episodes within 24 h. The beginning of the follow-up period was at the time of the first VF event. In patients with recurrent arrhythmias, the choice of antiarrhythmic drugs was decided by the patient's physician.

Electrophysiological study (EPS) was conducted in 19 patients as previously described.[11] Genetic testing for mutations in the SCN5A gene was performed in 22 patients (A: 3, B: 3, C: 4, D: 12), as previously described.[12]

Clinical profiles, electrocardiographic characteristics, and VF recurrences during 110.0 ± 55.4 months of follow-up were compared among the four groups.

Statistical analysis

Data were analyzed with JMP10 software (SAS Institute Inc., Cary, NC, USA). Numeric values are presented as mean ± standard deviation. The χ^2 test, Student's *t*-test, or one-way analysis of variance was performed as appropriate to test for statistically significant differences. Survival curves were constructed by the Kaplan–Meier method and compared using the log-rank test. *P*-value of <0.05 was considered statistically significant.

Results

Electrocardiogram findings in groups A, B, C, and D

Figure 1 shows typical ECGs at baseline and after the drug provocation test, respectively, in patients in each group. Assignment to groups was performed according to ECG findings at screening: 6 patients (16%) were assigned to group A (*Figure 1A*), 5 patients (13%) to group B (*Figure 1B*), 8 patients (21%) to group C (*Figure 1C*), and 19 patients (50%) to group D (*Figure 1D*). Therefore, 16% of the

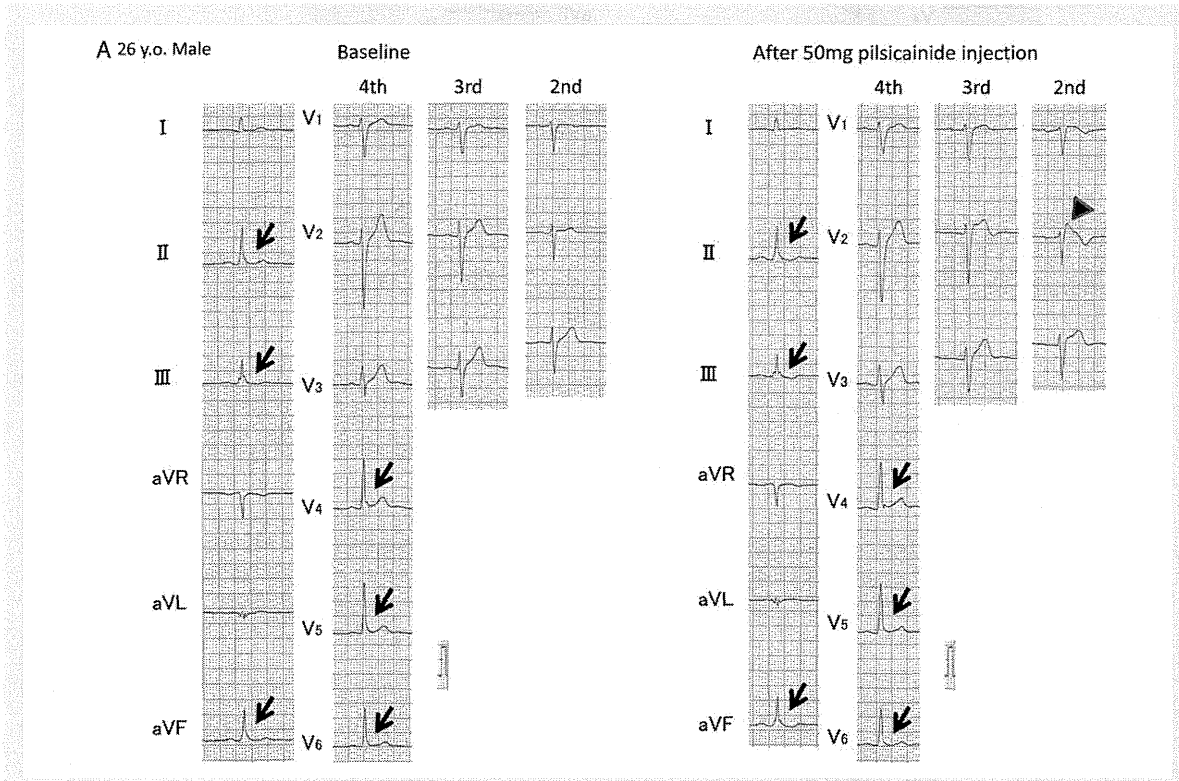

Figure 1 (*A*) At baseline, electrocardiograms of a 26-year-old male exhibited J-waves (arrows) followed by near horizontal ST segments in leads II, III, aVF, and V_6, and ascending ST segments in V_4 and V_5. There was no sign of coved or saddleback ST elevation in all chest leads. After injection of 50 mg pilsicainide, type 1 ST elevation (broad arrow) was noted in V_2 only in the second intercostal space. He experienced VF recurrence 4 years after implantable cardioverter-defibrillator implantation. (*B*) At baseline, electrocardiograms of a 42-year-old male exhibited J-waves (arrows) followed by ascending ST segments in leads I, aVL, V_4, and V_5 in the standard (4th) recording and in lead V_2 in the 2nd intercostal recording. After pilsicainide injection, all J-waves in limb lead disappeared with appearance of s-waves and an R-wave in lead aVR. Saddleback ST elevation with slightly augmented J-waves (broad arrows) was also noted in V_2 in the high (2nd and 3rd) intercostal spaces. He experienced an electrical storm 4 years after ICD implantation. (*C*) At baseline, electrocardiograms of a 27-year-old male exhibited J-waves followed by ascending ST segments in leads I, V_4, and V_5 (arrows), and non-type 1 (saddleback) ST elevation in leads V_2 and V_3 in the standard (4th), 3rd, and 2nd intercostal recordings (broad arrows). After pilsicainide injection, saddleback ST elevation with slightly augmented J-waves (broad arrows) was also noted in lead V_2 in the standard recording and in lead V_3 in the high intercostal spaces. (*D*) At baseline, electrocardiograms of 63-year-old male showed J-waves in leads II, III, aVF, V_5 and V_6 (arrows). After pilsicainide injection, they disappeared or attenuated with appearance of s-waves. Electrocardiograms in leads V_1–V_3 during standard and high costal recordings remained normal even after pilsicainide injection.

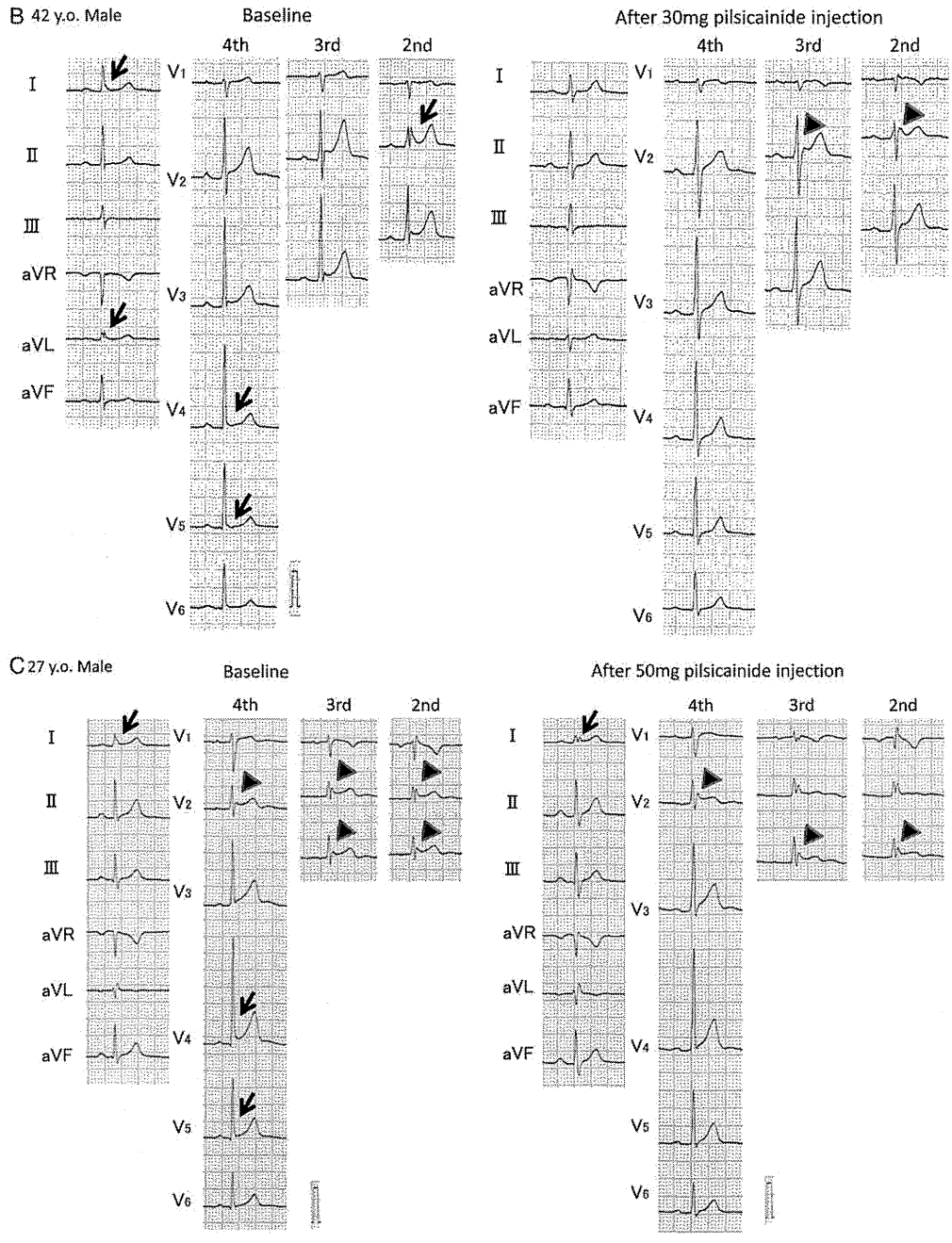

Figure 1 Continued

patients who had been diagnosed with ERS under the previous criteria were actually BrS patients with inferolateral ER. Nineteen patients (50%) had BrP-ECG in any of the right precordial leads and 11 patients (29%) had type 1 or non-type 1 BrP-ECG only in the HICS. In group A, type 1 BrP-ECG was observed in the high intercostal position spontaneously ($n = 3$) or only after the drug provocation test ($n = 3$). In group B, non-type 1 BrP-ECG was observed before ($n = 4$) or after ($n = 1$) the drug provocation test only in the HICS.

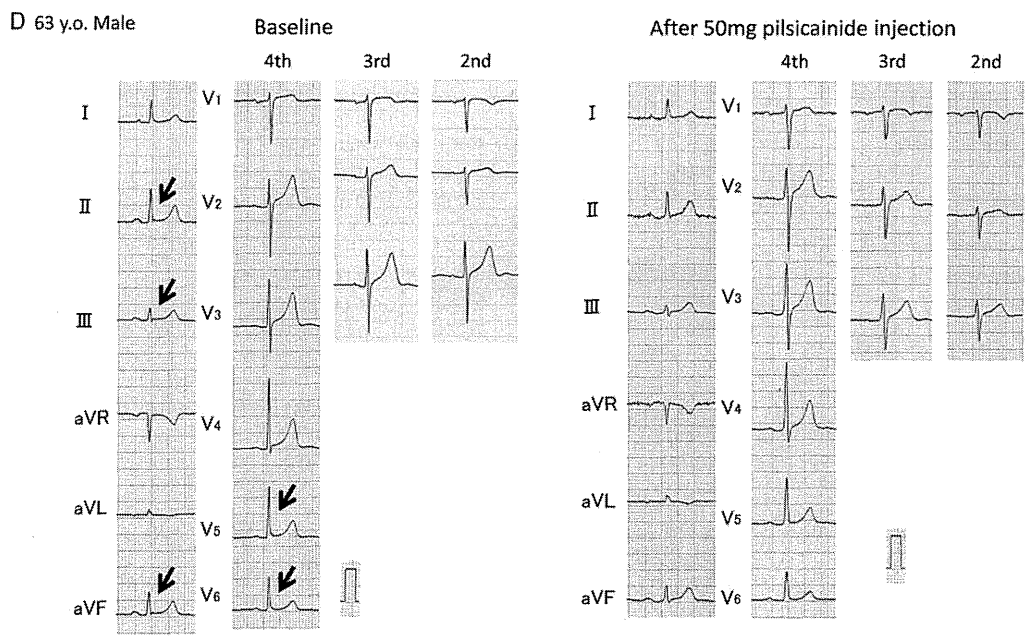

Figure 1 Continued

Clinical profiles in each group

Clinical characteristics of the patients in each group are shown in *Table 1*. Ventricular fibrillation was observed during sleep or near sleep in three patients (50%) in group A, five patients (100%) in group B, and six patients (75%) in group C; in contrast, only two patients (11%) in group D had VF during sleep or near sleep (B, and C vs. D, $P < 0.05$). Most patients in group D had VF in an awake state. The VF inducibility among the four groups was similar. Mutations of SCN5A were identified in one patient in group C, but in none of the patients in the other groups.

Clinical outcome

Mean follow-up period of group A, B, C, and D was 120 ± 51, 109 ± 61, 106 ± 67, and 108 ± 54 months, respectively. Thirty-seven of the 38 patients received an ICD. One patient in group D was followed without ICD implantation. No patients died during the follow-up period.

VF recurrence rates including electrical storm were significantly higher in group A (4/6:67%), group B (4/5:80%), and group C (4/8:50%), compared with group D (2/19:11%) (A, B, and C vs. D, $P < 0.05$). Kaplan–Meier curves of the four groups are illustrated in *Figure 2*. Patients in group A, B, and C exhibited significantly higher rates of arrhythmic events than those in group D (log rank, $P = 0.0019$). Type 1 and non-type 1 BrP-ECG were observed only in the HICS in 8 (type 1:4, non-type 1:4) of the 14 patients with VF recurrence. The incidence of BrP-ECG in any of the right precordial leads was significantly higher in patients with VF recurrence than in those without (12/14; 86% vs. 7/24; 29%, $P = 0.0019$) and the presence of BrP-ECG showed 86% sensitivity, 71% specificity, and 63% positive predictive value to identify VF recurrence in patients with ERS. Single or combination drug therapy consisting of isoproterenol, quinidine, cilostazol, and bepridil was effective in five patients with a VF recurrence in group A, B, and C.

Discussion

Main findings

This is the first study in which high intercostal ECGs of all ERS patients were evaluated on a long-term basis. Results showed that VF mostly recurred in patients showing BrP-ECG in any precordial lead including HICS, who comprised 50% of the cohort diagnosed with ERS under the previous criteria, in contrast to favorable prognosis in the remaining 50% of ERS patients without BrP-ECG in both the standard and HICS. A systematic search of the BrP-ECG with high intercostal ECG recordings and drug challenge test is considered requisite not only to exclude BrS but also to stratify the risk of ERS.

Diagnosis of early repolarization syndrome

Early repolarization syndrome is diagnosed when structural and nonstructural heart diseases including BrS are excluded as a cause of VF.[1,2] In the diagnostic criteria of BrS proposed in the consensus reports that were published in 2002[9] and 2005,[13] a diagnostic Brugada ECG was defined as the presence of a coved-type ST segment elevation ≥2 mm followed by a negative T wave in at least two right

Table 1 Patient characteristics of the four groups

	A (n = 6)	B (n = 5)	C (n = 8)	D (n = 19)	P-value comparing four groups	Total (n = 38)
Clinical characteristics						
Age at diagnosis (year)	35.2 ± 6.9	38.2 ± 8.7	40.8 ± 14.4	42.5 ± 15.9	0.83	40.4 ± 13.6
Men	6 (100%)	5 (100%)	5 (63%)	18(95%)	0.045	34 (89%)
FH of SCD (%)	0 (0%)	0 (0%)	1 (13%)	0 (0%)	0.28	1 (3%)
Activity at the time of initial SCA						
Sleep, near sleep (%)	3 (3, 0) (50%)	5 (4, 1) (100%)	6 (4, 2) (75%)	2 (2, 0) (11%)	0.0023	16 (13, 3) (42%)
Physical activity (%)	0 (0%)	0 (0%)	0 (0%)	6 (31%)		6 (16%)
Arousal at rest (%)	3 (50%)	0 (0%)	2 (25%)	11 (58%)		16 (42%)
Occurrence of initial VF						
Out of hospital (%)	5 (83%)	4 (80%)	7 (87%)	18 (95%)	0.73	34 (89%)
In hospital for syncope or other reason (%)	1 (17%)	1 (20%)	1 (13%)	1 (5%)		4 (11%)
Induction of VF by EPS (%)	2/3 (67%)	1/1 (100%)	2/5 (40%)	3/10 (30%)	0.44	8/19 (42%)
Clinical outcome						
Follow-up period (months)	120 ± 51	109 ± 61	106 ± 67	108 ± 54	0.95	110 ± 55
VF recurrence (%)	4 (67%)	4 (80%)	4 (50%)	2 (11%)	0.0057	14 (37%)
Electrical storm (%)	0 (0%)	2 (40%)	3 (38%)	0 (0%)	0.011	5 (13%)
ICD implantation	6 (100%)	5 (100%)	8 (100%)	18 (95%)	0.79	37 (97%)

Numeric values are expressed as mean ± standard deviation.
FH of SCD: family history of sudden cardiac death before 45 years of age.
SCA, sudden cardiac arrest; EPS, electrophysiological study; VF, ventricular fibrillation; ICD, implantable cardioverter defibrillator.
A: Patients with ER and type 1 BrP-ECG only in HICS; B: ERS with non-type 1 BrP-ECG only in HICS; C: ERS with non-type 1 BrP-ECG in the standard ECG; D: ERS without BrP-ECG.

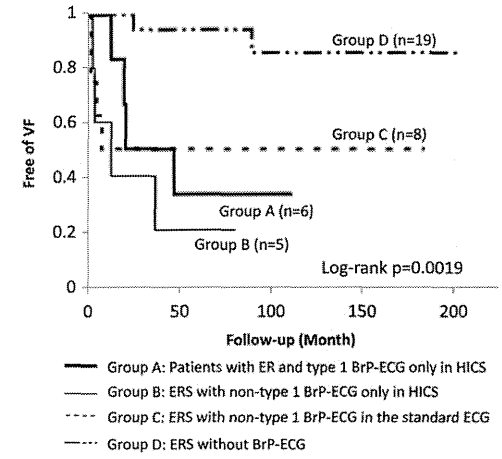

Figure 2 Kaplan–Meier analysis of freedom from lethal arrhythmic events (documented ventricular fibrillation) during follow-up in the four groups of patients with inferolateral early repolarization and a prior ventricular fibrillation. The incidence of ventricular fibrillation during follow-up was significantly higher in early repolarization syndrome patients with BrP-ECG (groups *A*, *B*, and *C*) than in those without BrP-ECG (group *D*).

precordial leads on the standard 12-lead ECG. Regarding the high intercostal ECG recordings, it was stated that it could increase sensitivity, but there were not enough data at that time to exclude the possibility of false positives. However, some studies addressed the diagnostic value of the high intercostal ECG recordings in the following years,[3–6] leading to revision of the diagnostic criteria for BrS in the expert consensus statement in 2013.[2] The new diagnostic criteria allow diagnosis of BrS when a type 1 ST-segment elevation is observed either spontaneously or after drug provocation tests in at least 1 lead among the right precordial leads (V_1 and V_2) positioned in the second, third, or fourth intercostal spaces. These criteria proposed in 2013 have been reported to increase diagnostic sensitivity without increasing specificity.[14]

Since inferolateral ER was reported in association with idiopathic VF in 2008 by Haïssaguerre *et al.*,[1] numerous studies on ERS have been published.[15–21] However, these were mostly reported before 2013 and excluded BrS according to the 2002[9] and 2005 criteria.[13] Therefore, there is a possibility that previously reported cases of ERS might have included patients with BrP-ECG in HICS only. This study showed that 6 (16%) of 38 ERS patients had a type 1 BrP-ECG only in the HICS. Savastano *et al.*[14] reported that 44% of BrS patients in their cohort showed a type 1 BrP-ECG exclusively in the HICS. Govindan *et al.*[5] indicated that 42% of the patients showed type 1 ECG only in the high costal spaces in their cohort of drug-induced BrS. Thus, unless high intercostal recordings with or without drug provocation test were conducted, some BrS