Table 2. Initial stroke features

	All patients			Atherothrombotic stroke		
	recurrent	nonrecurrent	p value	recurrent	nonrecurrent	p value
Patients	395	7,641		174	2,471	
Admission NIHSS	4 (0–36) (368)	4 (0–36) (7,155)	0.8779	4 (0–36) (164)	5 (0–36) (2,361)	0.0218
Admission NIHSS ≥8, %	38.0 (368)	33.7 (7,155)	0.0890	28.7 (164)	34.1 (2,361)	0.1514
Vertebrobasilar infarcts, %	20.3 (370)	19.6 (7,191)	0.7646	22.4 (161)	25.1 (2,367)	0.4308
White matter lesions, grade	0.96 ± 0.85 (273)	0.94 ± 0.87 (4,907)	0.8029	1.08 ± 0.89 (119)	1.04 ± 0.85 (1,561)	0.6814
Periventricular hyperintensity, grade	1.16 ± 1.00 (273)	1.11 ± 1.03 (4,878)	0.4416	1.32 ± 1.08 (120)	1.22 ± 1.00 (1,558)	0.2930
Admission systolic blood pressure, mm Hg	161.2 ± 29.1 (395)	160.1 ± 27.8 (7,641)	0.4264	163.7 ± 28.2 (169)	160.8 ± 27.0 (2,391)	0.1856
Admission diastolic blood pressure, mm Hg	86.5 ± 17.8 (395)	87.3 ± 16.5 (7,641)	0.3161	87.5 ± 16.2 (169)	86.7 ± 15.7 (2,391)	0.5431
Acute progression within 48 h after admission, %	31.8 (384)	18.1 (7,606)	<0.0001	30.8 (169)	23.9 (2,460)	0.0429

Numbers in parentheses indicate the number of patients whose data were available.

Table 3. Multivariate analysis of independent predictors for acute recurrence

Items	p value	OR	(95% CI)
Total (n = 8,036)			
Previous ischemic stroke	0.1089	1.205	(0.959–1.513)
Hypertension	0.0110	1.348	(1.071–1.696)
Atrial fibrillation	0.0011	1.503	(1.177–1.918)
Admission NIHSS ≥8	0.8162	1.029	(0.811–1.305)
Atherothrombotic (n = 2,645)			
Previous ischemic stroke	0.5258	1.117	(0.793–1.574)
Hypertension	0.0788	1.383	(0.963–1.984)
Diabetes mellitus	0.0222	1.485	(1.058–2.085)
Atrial fibrillation	0.0051	1.998	(1.231–3.244)
Admission NIHSS	0.0664	1.022	(0.999–1.046)
Cardioembolic (n = 2,318)			
Hypertension	0.0255	1.529	(1.053–2.218)
Aortic aneurysm	0.0147	4.070	(1.318–12.566)
Peripheral artery disease	0.0868	3.955	(0.820–19.092)
Vertebrobasilar infarcts	0.0749	1.579	(0.955–2.610)
Lacunar (n = 2,443)			
Alcohol consumption	0.0822	2.377	(0.895–6.313)
Admission SBP	0.2602	0.993	(0.981–1.005)
Admission NIHSS ≥8	0.2866	1.646	(0.658–4.116)

SBP = Systolic blood pressure.

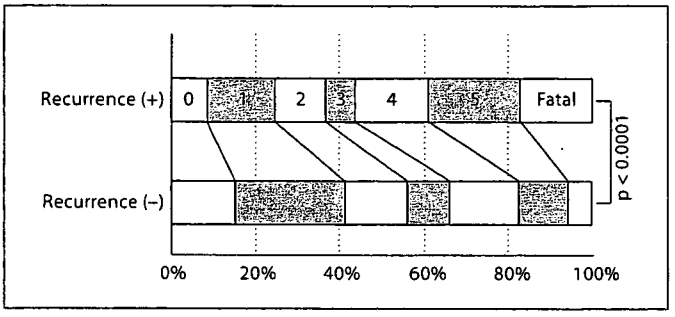

Fig. 2. Modified Rankin Scale score on discharge.

Among stroke subtypes, atherothrombotic stroke and stroke of other etiology were positive independent predictors for early recurrence after adjustment for age, gender, previous ischemic stroke, hypertension, atrial fibrillation, and a National Institute of Health Stroke Scale (NIHSS) score on admission ≥8; the last four characteristics showed a statistically significant ($p < 0.05$) or a marginally significant relationship ($p < 0.15$) with overall recurrence on univariate analyses (table 4). Lacunar stroke was a negative independent predictor for early recurrence. After adjustment, cardioembolic stroke was no longer positively related to recurrence, since, in this stroke subtype, atrial fibrillation has a strong association with recurrence.

At discharge, the median mRS score was higher in patients with recurrence than in those without a recurrence (3 vs. 2, $p < 0.0001$, fig. 2); for each stroke subtype, the median mRS score was also higher in patients with recurrence than in those without a recurrence (atherothrombotic stroke, 4 vs. 2, $p = 0.0021$; cardioembolic stroke, 5 vs. 3, $p < 0.0001$; lacunar stroke, 2 vs. 1, $p = 0.0286$; stroke of other etiology, 4 vs. 2, $p = 0.0064$).

Discussion

This study, which was based on a large sample of Japanese patients, confirmed the previous consensus that atherothrombotic stroke, hypertension, and atrial fibril-

Cardioembolic stroke			Lacunar stroke			Stroke of other etiology		
recurrent	nonrecurrent	p value	recurrent	nonrecurrent	p value	recurrent	nonrecurrent	p value
143	2,175		42	2,401		36	594	
9 (0–36) (132)	10 (0–36) (1,980)	0.9271	3 (0–13) (39)	3 (0–30) (2,279)	0.1028	4 (0–28) (33)	4 (0–35) (535)	0.4583
58.3 (132)	60.4 (1,980)	0.6378	20.5 (39)	11.7 (2,279)	0.0921	24.2 (33)	27.1 (535)	0.7192
15.9 (138)	10.7 (2,079)	0.0584	23.7 (38)	20.5 (2,249)	0.6247	24.2 (33)	27.0 (496)	0.7277
0.80 ± 0.81 (97)	0.84 ± 0.85 (1,415)	0.6727	0.89 ± 0.80 (27)	0.97 ± 0.90 (1,548)	0.6236	1.03 ± 0.81 (30)	0.78 ± 0.87 (383)	0.1260
0.99 ± 0.94 (96)	0.98 ± 0.99 (1,407)	0.8946	1.00 ± 0.83 (27)	1.18 ± 1.08 (1,533)	0.4009	1.20 ± 1.00 (30)	0.89 ± 1.04 (380)	0.1121
158.3 ± 29.7 (134)	157.6 ± 28.6 (2,061)	0.7619	170.0 ± 32.7 (40)	162.7 ± 27.4 (2,320)	0.0968	150.4 ± 23.1 (35)	154.9 ± 27.7 (552)	0.3455
85.3 ± 18.8 (134)	86.6 ± 17.3 (2,061)	0.4107	90.2 ± 21.5 (40)	89.1 ± 16.5 (2,320)	0.6704	81.8 ± 15.7 (35)	85.6 ± 16.1 (552)	0.1728
33.6 (140)	16.3 (2,163)	<0.0001	35.0 (40)	14.3 (2,391)	0.0003	25.7 (35)	15.5 (592)	0.1116

Table 4. Stroke subtypes as predictors for acute recurrence

Stroke subtype	Unadjusted (n = 8,036)		Adjusted 1 (n = 8,026)		Adjusted 2 (n = 7,318)	
	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)
Atherothrombotic stroke	<0.0001	1.647 (1.343–2.021)	<0.0001	1.631 (1.328–2.003)	<0.0001	1.942 (1.537–2.453)
Cardioembolic stroke	0.0010	1.426 (1.155–1.761)	0.0018	1.407 (1.135–1.743)	0.2390	1.213 (0.879–1.674)
Lacunar stroke	<0.0001	0.260 (0.188–0.359)	<0.0001	0.263 (0.190–0.364)	<0.0001	0.246 (0.172–0.352)
Stroke of other etiology	0.3345	1.190 (0.836–1.693)	0.2440	1.235 (0.866–1.762)	0.0343	1.502 (1.030–2.190)

Adjusted 1 = Adjusted for age and gender; adjusted 2 = adjusted for age, gender, previous ischemic stroke, hypertension, atrial fibrillation, and admission NIHSS ≥8.

lation contribute to early ischemic stroke recurrence [1–3]. In addition, the analysis by each stroke subtype clarified that diabetes mellitus was independently related to early recurrence in patients with an atherothrombotic stroke.

It has been reported that within 30 days after the initial attack, stroke recurs in 1.5–6% of patients overall [1, 2, 10–12], and in up to 18.5% of atherothrombotic patients [2]. The wide variance in the risk of recurrent stroke might be partly due to differences in subjects' background characteristics, including ethnic differences, in the various studies. In this study, as well as in previous studies, recurrent stroke was found to be mostly ischemic and to occur mainly within the initial week [1, 11, 13].

The predictors of recurrent stroke may be time-specific and may differ between early and late recurrences [1]. In particular, diabetes mellitus is an established predictor of a late (>3 months after onset) recurrence [10, 11, 13]; however, its contribution to early recurrence has not been established. In this study, only among atherothrombotic stroke patients was diabetes mellitus more frequent in patients with a recurrent stroke than in patients without a recurrent stroke; in patients with the other three stroke subtypes, the prevalence of diabetes mellitus was equal or relatively less in patients with a recurrence than in patients without a recurrence. It would seem to be difficult to explain these apparently paradoxical results. However, diabetes increases intrinsic platelet activation, decreases endogenous inhibitors of platelet activity [14, 15], augments blood coagulability, and impairs fibrinolysis [16, 17]; these hemostatic abnormalities enhance local thrombus formation in the carotid and large cerebral arteries, which would appear to trigger the recurrence of atherothrombotic stroke. In addition, autonomic diabetic neuropathy is strongly related to stroke [18, 19]; patients with autonomic neuropathy appear to have a high risk of hemodynamic accidents, including orthostatic hypotension [20]. Thus, diabetes may enhance the risk of hemodynamic stroke recurrence in patients with large artery disease.

The contribution of an aortic aneurysm to cardioembolic stroke recurrence may be partly due to the statistical

limitation of this study, as there was only a small number of cardioembolic patients who had aortic aneurysms (only 20 of 2,318 patients).

A major limitation of this study was that around 20% of the acute stroke patients registered in the JSSRS were not included because their data regarding the presence of early stroke recurrence were incomplete. As well, for some patients included in this study, data on baseline characteristics and stroke features were missing, which affected the multivariate analyses. Another limitation was that some of the subanalyses related to recurrent stroke types (ischemic or hemorrhagic) were not done due to lack of information on the types for some of the patients. However, the present results primarily reflect the characteristics of ischemic recurrent strokes, since most (95.2%) of the 294 patients whose recurrent stroke type was documented had an ischemic stroke. Finally, in this study, we did not assess vascular lesions (extracranial or intracranial) or serological markers of hemostasis and infection, because the database did not require them, though early recurrence may be dependent on these conditions.

The management of risk factors during the acute stage of stroke may be essential for the prevention of stroke recurrence. In hypertensive patients, acute treatment with an angiotensin type 1 receptor blocker has been shown to decrease late (>12 months) recurrent stroke and cardiovascular events [21]. Likewise, acute blood glucose control may help prevent stroke recurrence. For example, the European Stroke Initiative Guidelines proposed that temporary insulin treatment may be necessary for acute ischemic stroke patients [22]. A randomized, controlled pilot study involving 25 patients indicated that rigorous glycemic control using sliding scale insulin is feasible and well tolerated after acute ischemic stroke [23]. Prospective studies are needed to establish the best acute management of the risk factors that are associated with early stroke recurrence.

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Clinical Aspects of Type-1 Long-QT Syndrome by Location, Coding Type, and Biophysical Function of Mutations Involving the KCNQ1 Gene

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Background—Type-1 long-QT syndrome (LQTS) is caused by loss-of-function mutations in the KCNQ1-encoded I_{Ks} cardiac potassium channel. We evaluated the effect of location, coding type, and biophysical function of KCNQ1 mutations on the clinical phenotype of this disorder.

Methods and Results—We investigated the clinical course in 600 patients with 77 different KCNQ1 mutations in 101 proband-identified families derived from the US portion of the International LQTS Registry (n=425), the Netherlands' LQTS Registry (n=93), and the Japanese LQTS Registry (n=82). The Cox proportional hazards survivorship model was used to evaluate the independent contribution of clinical and genetic factors to the first occurrence of time-dependent cardiac events from birth through age 40 years. The clinical characteristics, distribution of mutations, and overall outcome event rates were similar in patients enrolled from the 3 geographic regions. Biophysical function of the mutations was categorized according to dominant-negative (>50%) or haploinsufficiency (≤50%) reduction in cardiac repolarizing I_{Ks} potassium channel current. Patients with transmembrane versus C-terminus mutations (hazard ratio, 2.06; $P<0.001$) and those with mutations having dominant-negative versus haploinsufficiency ion channel effects (hazard ratio, 2.26; $P<0.001$) were at increased risk for cardiac events, and these genetic risks were independent of traditional clinical risk factors.

Conclusions—This genotype–phenotype study indicates that in type-1 LQTS, mutations located in the transmembrane portion of the ion channel protein and the degree of ion channel dysfunction caused by the mutations are important independent risk factors influencing the clinical course of this disorder. (***Circulation.* 2007;115:2481-2489.**)

Key Words: electrocardiography ■ genetics ■ long-QT syndrome

The hereditary long-QT syndrome (LQTS) is characterized by prolonged ventricular repolarization on the ECG and arrhythmia-related syncope and sudden death.[1] Mutations in 1 or more of several ion channel genes are known to cause this disorder,[2] with mutations in the KCNQ1 gene causing the type-1 long-QT syndrome.[3,4] The KCNQ1 gene codes for the potassium channel protein responsible for the slow component of the delayed rectifier repolarizing current (I_{Ks}). Mutations involving this gene result in reduction of the repolarizing I_{Ks} current and lengthening of the QT interval.[3]

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Functional I_{Ks} channels result from the coassembly of 4 subunits into a tetrameric protein channel that is transported to the myocyte membrane. Each subunit contains 6 membrane-spanning domains (S1 to S6) flanked by amino (N)- and carboxyl (C)-terminus regions. Two distinct biophysical mechanisms mediate the reduced I_{Ks} current in patients with KCNQ1 mutations: (1) coassembly or trafficking defects in which mutant subunits are not transported

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TABLE 1. KCNQ1 Mutations by Location and Coding, Type of Mutation, and Functional Effect

Location and Coding*	No. of Subjects†	Type of Mutation	Functional Effect‡
N-terminus			
M1V	1	Missense	Unknown
G57V	1	Missense	Unknown
Transmembrane			
W120C	2	Missense	Unknown
T144A	7	Missense	Unknown
A150fs/133 [del CT 451-452]	2	Frameshift	Haploinsufficiency
E160K	3	Missense	Unknown
G168R	44	Missense	Unknown
Y171X [513 C>G]	6	Nonsense	Haploinsufficiency
R174H	2	Missense	Unknown
A178P	5	Missense	Dominant-negative effect (a)
Y184S	18	Missense	Unknown
G185S	10	Missense	Unknown
G189E	2	Missense	Unknown
G189R	4	Missense	Dominant-negative effect (b)
R190Q	4	Missense	Haploinsufficiency (b, c)
L191fs/90 [del TGCGC 572-576]	8	Frameshift	Haploinsufficiency
R195fs/40 [del G 585]	2	Frameshift	Haploinsufficiency
S225L	13	Missense	Dominant-negative effect (d)
A226V	3	Missense	Unknown
R237P	1	Missense	Unknown
D242N	3	Missense	Unknown
R243C	13	Missense	Haploinsufficiency (e)
V254 mol/L	59	Missense	Dominant-negative effect (b, f)
R258C	1	Missense	Haploinsufficiency
R259C	1	Missense	Haploinsufficiency (g)
L266P	15	Missense	Unknown
G269D	35	Missense	Dominant-negative effect (h)
G269S	25	Missense	Haploinsufficiency (i)
L273F	6	Missense	Dominant-negative effect (a)
I274V	1	Missense	Unknown
S277L	3	Missense	Unknown
Y278H	2	Missense	Unknown
E284K	2	Missense	Unknown
G292D	3	Missense	Unknown
F296S	2	Missense	Unknown
G306R	2	Missense	Dominant-negative effect (b, j)
V310I	1	Missense	Unknown
T312I	14	Missense	Dominant-negative effect (a)
G314S	8	Missense	Dominant-negative effect (h, k, l, m)
Y315C	10	Missense	Dominant-negative effect (d, n)
Y315S	1	Missense	Dominant-negative effect (h, m)
D317G	3	Missense	Unknown
P320H	1	Missense	Unknown
T322 mol/L	2	Missense	Unknown
G325R	3	Missense	Unknown
delF340 [del CTT 1017-1019]	7	In-frame deletion	Haploinsufficiency
A341E	9	Missense	Dominant-negative effect (b)
A341V	20	Missense	Dominant-negative effect (o)

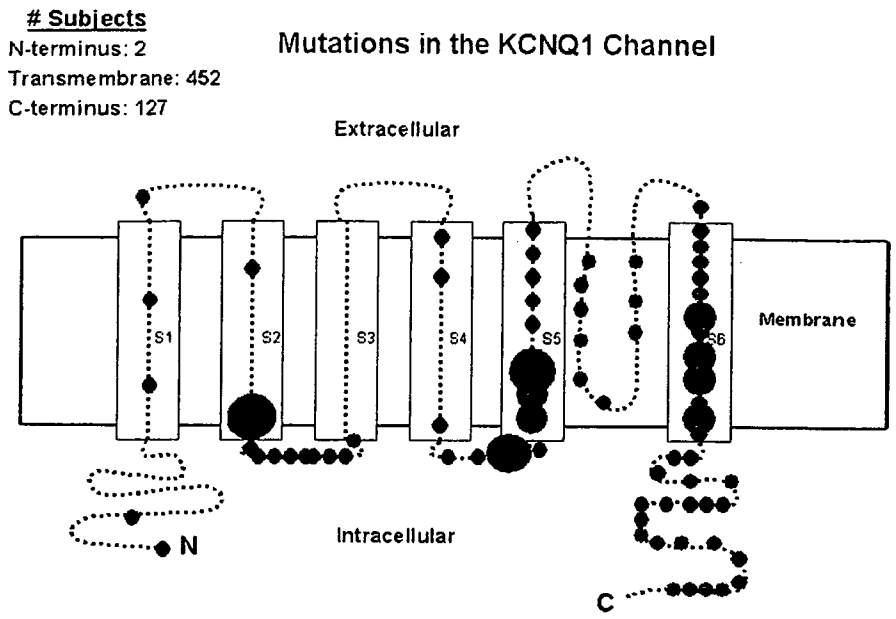

Figure 1. Frequency and location of 74 different mutations in the KCNQ1 potassium channel involving 581 subjects. The 19 subjects with 3 intron mutations are not included in this diagram. The α subunit involves the N-terminus (N), 6 membrane-spanning segments, and the C-terminus portion (C). The size of the circles reflect the number of subjects with mutations at the respective locations, with the small circles indicating <15, medium-sized circles 15 to 30, and large circles >30 subjects.

20 years. In patients with transmembrane-localized mutations, the event rates for patients with mutations localized to the pore region (S5-pore-S6) were nearly identical to those with nonpore mutations (data not shown).

The findings from the Cox regression analysis for location and type of mutation are presented in Table 3. The clinical risk factors associated with first cardiac events involved males before age 13 years, females after age 13

TABLE 2. Phenotypic Characteristics by Source of Subjects, Location of Mutation, and Type of Mutation

	Source of Subjects			Location of Mutation		Missense Mutation		
Characteristics	United States (n=425)	Netherlands (n=93)	Japan (n=82)	Trans Membrane (n=452)	C-Terminus (n=127)	Yes (n=483)	No (n=98)	Intron Mutation (n=19)
Female, %	57	53	54	57	51	54	62	63
ECG at enrollment								
QTc†‡, ms	488±58	450±45	472±46	485±53	460±61	481±59	471±38	478±60
Therapy, %								
β-Blockers†‡	45	34	26	45	28	42	38	37
Pacemaker	2.4	0	0	1.5	2.4	1.4	3.1	0
Sympathectomy	0.5	0	0	0.4	0	0.4	0	0
Defibrillator	6.4	3.2	0	5.8	3.1	5.2	5.1	0
First cardiac event*‡§, %	41	37	38	45	21	43	26	42
Syncope‡ (n=200)	35	31	29	38	17	36	21	32
Aborted cardiac arrest (n=15)	1.9	1.1	7.3	2.9	0.8	2.5	2.0	5.3
Death (n=23)	4.0	5.5	1.2	4.0	3.1	4.2	2.0	5.3
Ever cardiac event, %								
Syncope‡§	35	31	31	39	17	37	21	33
Aborted cardiac arrest†	2.4	15	8.8	5.3	3.2	5.4	2.0	11
Death	11	14	2.4	10	6.3	11	4.1	26

Plus-minus values are mean±SD. Percentages >10 are rounded to a whole number. The 600 subjects in this table include 51 subjects who died suddenly at a young age, were from families with known KCNQ1 mutation, and were assumed to have the family mutation. Patients with intron mutations are categorized separately and are not included in the location or missense categories. Seven subjects with transmembrane mutations and 1 with C-terminus mutations had missing data about the date of the first cardiac event. Eight subjects with missense mutations had missing data about the date of the first cardiac event. Numbers in parentheses indicate the total number of specific first cardiac events from the 3 sources of patients.

*First cardiac event was syncope, aborted cardiac arrest, or sudden death, whichever occurred first.

†$P<0.01$ for the comparison of characteristics among the 3 sources of subjects.

‡$P<0.01$ for the comparison of characteristics between the 2 locations of the mutations.

§$P<0.01$ for the comparison of characteristics between missense yes and no.

TABLE 5. Cox Regression With Multiple Predictor Variables Including Ion Channel Dysfunction for First Cardiac Events

Variable	Hazard Ratio	95% CI	P
Netherlands:United States	2.78	1.48-5.23	<0.01
Japan:United States	1.63	1.02-2.63	0.04
Male <13 y:female <13 y	1.94	1.29-2.91	<0.01
Female 13-40 y:male 13-40 y	1.95	0.99-3.87	0.06
QTc 500–530 ms:QTc <500 ms	1.88	1.18-2.99	<0.01
QTc >530 ms:QTc <500 ms	3.22	2.06-5.05	<0.001
QTc missing*:QTc <500 ms	2.07	1.29-3.33	<0.01
Dominant-negative:haploinsufficiency	2.26	1.56-3.25	<0.001
Time-dependent β-blocker use	0.21	0.09-0.48	<0.001

The analysis involved 354 subjects with known or suspected ion channel dysfunction; 2 subjects were not included because of missing data about the date of their first cardiac event.

*The QTc missing category involves 26 patients who died suddenly at a young age without a prior ECG.

dominant-negative functional effects experienced a significantly greater risk for cardiac events than those with haploinsufficiency (hazard ratio, 2.26; 95% CI, 1.56 to 3.25; $P<0.001$) after adjustment for relevant covariates including QTc and gender effects by age group. β-Blocker use was associated with a significant 79% reduction in first cardiac events in this subset of patients. Because substantial colinearity exists for transmembrane mutations, missense mutations, and mutations with dominant-negative biophysical function, the individual effects of these 3 mutation parameters could not be ascertained reliably in the same Cox model.

Discussion

The main results of the present study from 600 patients having a spectrum of KCNQ1 mutations derived from 3 LQTS registries are significantly higher cardiac event rates in patients with transmembrane mutations and in patients with mutations having a putative dominant-negative effect on the repolarizing I_{Ks} current. The effect of these genetically determined factors is independent of traditional clinical risk factors and of β-blocker therapy.

Since 1995, when the first 2 genes responsible for LQTS were identified,[11,12] molecular genetic studies have revealed a total of 9 forms of congenital LQTS caused by mutations in genes involving potassium channel (LQT-1, -2, -5, -6, and -7), sodium channel (LQT-3, -9), and calcium channel proteins (LQT-8) as well as a membrane-adapter protein (LQT-4).[2,13] Genotype–phenotype studies have enabled us to stratify risk and to treat more specifically patients with LQT-1, LQT-2, and LQT-3 subtypes of this genetic disorder. LQT-1, the most common form of LQTS, accounts for ≈50% of genotyped patients[4,14] and has more variable expressivity and incomplete penetrance than the other forms.[15] Mutation location and knowledge of the functional effects of the mutation provide additional risk information beyond the clinical risk factors and the genotype, at least for LQT-1, and this information should contribute to improved risk stratification and more focused management of these higher-risk patients.

Mutations in KCNQ1 are responsible for defects in the slowly activating component of the delayed rectifier current I_{Ks}.[16] This current is the main repolarizing current at increased heart rate and is highly sensitive to catecholamines.[3] We speculate that I_{Ks} channels with transmembrane mutations might have reduced responsiveness to the regulatory β-adrenergic signaling of the ion-conduction pathway with more impairment of shortening of the QTc with exercise-related tachycardia than mutations in the C-terminus region.

Functional I_{Ks} channels result from the coassembly of 4 KCNQ1-encoded subunits. A mutated gene encodes a protein with aberrant function, and the presence of both normal and abnormal proteins in the ion channel contributes to a >50% reduction in ion channel function (dominant-negative effect). An alternative mechanism of reduced repolarizing KCNQ1 K^+ current is the inability of mutated subunits to coassemble with normal gene products, such as occurs with a trafficking defect, resulting in a ≤50% reduction in channel function (haploinsufficiency). With only 1 exception,[17] this is the case for all studied truncating mutations leading to incomplete proteins. Our assumption that truncated proteins (based on frameshift nonsense mutations) lead to haploinsufficiency seems justified. The biophysical effect of missense mutations is unpredictable, and both haploinsufficiency and dominant-negative effects have been described. In the absence of reported biophysical studies, missense mutations were classified as unknown.

Previous attempts to identify a genotype–phenotype relationship for KCNQ1 mutations failed to reach consensus on the clinical outcome of the type and site of mutations.[7,8] Relatively small numbers and different ethnic background of the previously reported patients with the LQT-1 genotype might be responsible for the discrepant results. The present larger study allows us to demonstrate for the first time that the biophysical effect clearly affects the clinical outcome (ie, dominant-negative mutations are associated with a more severe phenotype than are mutations conferring haploinsufficiency [Figure 2C], even after adjustment for relevant covariates [Table 5]). The risk observed in 19 subjects with 3 different intron mutations was not quite significant ($P=0.06$), possibly because of small numbers, but the magnitude of the risk effect was similar to the risk accompanying transmembrane mutations. Although these intron mutations produced splice-site alterations predicted to affect the transmembrane portion of the ion channel, we used a separate categorization of intron mutations in view of the limited understanding of the structural alterations and functional effects resulting from these exon-skipping intron mutations.

A few additional findings from this large genotype–phenotype study of type-1 LQTS patients emphasize high risk for first cardiac events during adolescence, a crossover in risk by sex at approximately age 13 years, and a lower rate of first cardiac events in the adult years than in the younger years. These findings are not especially new,[18,19] but the present study highlights their presence in type-1 LQTS.

Study Limitations

The present study used the biophysical function of mutations reported in the literature in only a portion of the mutations

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CLINICAL PERSPECTIVE

Type-1 long-QT syndrome is caused by loss-of-function mutations in the KCNQ1-encoded I_{Ks} cardiac potassium channel. In the present study involving 600 patients having a spectrum of KCNQ1 mutations derived from 3 long-QT syndrome registries, we found that cardiac event rates are increased significantly in patients with mutations located in the transmembrane region of the potassium channel and in patients with mutations having a putative dominant-negative effect on the repolarizing I_{Ks} current. The effects of these genetically determined factors are independent of traditional clinical risk factors and of β-blocker therapy. Mutation location and knowledge of functional effects of the mutation provide additional risk information beyond the clinical risk factors and the genotype, at least for type-1 long-QT syndrome, and this information should contribute to improved risk stratification and more focused management of these higher-risk patients.

Sex Hormone and Gender Difference—Role of Testosterone on Male Predominance in Brugada Syndrome

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Testosterone in Brugada Syndrome. ***Introduction:*** **The clinical phenotype is 8 to 10 times more prevalent in males than in females in patients with Brugada syndrome. Brugada syndrome has been reported to be thinner than asymptomatic normal controls. We tested the hypothesis that higher testosterone level associated with lower visceral fat may relate to Brugada phenotype and male predominance.**

Methods and Results: **We measured body-mass index (BMI), body fat percentage (BF%), and several hormonal levels, including testosterone, in 48 Brugada males and compared with those in 96 age-matched control males. Brugada males had significantly higher testosterone (631 ± 176 vs 537 ± 158 ng/dL; P = 0.002), serum sodium, potassium, and chloride levels than those in control males by univariate analysis, and even after adjusting for age, exercise, stress, smoking, and medication of hypertension, diabetes, and hyperlipidemia, whereas there were no significant differences in other sex and thyroid hormonal levels. Brugada males had significantly lower BMI (22.1 ± 2.9 vs 24.6 ± 2.6 kg/m^2; P < 0.001) and BF% (19.6 ± 4.9 vs 23.1 ± 4.7%; P < 0.001) than control males. Testosterone level was inversely correlated with BMI and BF% in both groups, even after adjusting for the confounding variables. Conditional logistic regression models analysis showed significant positive and inverse association between Brugada syndrome and hypertestosteronemia (OR:3.11, 95%CI:1.22–7.93, P = 0.017) and BMI (OR:0.72, 95%CI:0.61–0.85, P < 0.001), respectively.**

Conclusions: **Higher testosterone level associated with lower visceral fat may have a significant role in the Brugada phenotype and male predominance in Brugada syndrome.** *(J Cardiovasc Electrophysiol, Vol. 18, pp. 415-421, April 2007)*

Brugada syndrome, gender, sex hormones, testosterone, body mass index

Introduction

Brugada syndrome is characterized by coved-type ST-segment elevation in the right precordial electrocardiographic (ECG) leads (V1–V3) and an episode of ventricular fibrillation (VF) in the absence of structural heart disease.[1-5] The prevalence of the disease is estimated to be up to 5 per 10,000 inhabitants and is one of the important causes of sudden cardiac death of middle-aged males, particularly in Asian countries including Japan.[4]

More than eight dozen distinct mutations in *SCN5A*, the gene encoding the α subunit of the sodium channel, have been so far identified in patients with Brugada syndrome and all mutations display an autosomal-dominant mode of transmission.[6,7] Therefore, males and females are expected to inherit the defective gene equally. However, more than 80% of patients in Western countries and more than 90% of patients in Asian countries affected with Brugada syndrome are males.[8] Recent experimental studies have unveiled the cellular mechanism of Brugada phenotype. The male predominance in the Brugada syndrome is suggested to be due, at least in part, to intrinsic differences in ventricular action potential (AP) between males and females.[9]

A male hormone, testosterone is reported to increase net outward currents[10-12] and is expected to accentuate Brugada phenotype, such as ST-segment elevation and subsequent episodes of VF in patients with Brugada syndrome. Testosterone is also known to decrease visceral fat.[13-15] Since patients with Brugada syndrome have been reported to be

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thinner than asymptomatic normal controls by Matsuo et al.,[16] we speculated that higher testosterone level associated with lower visceral fat may modulate Brugada phenotype and may relate to male predominance in patients with Brugada syndrome.

Methods

Patient Population and Data Collection

The study population consisted of 48 males with Brugada syndrome who agreed to participate in this study and showed Type 1 "coved" ST-segment elevation in V1–V3 leads[17] ranging in age from 30 to 69 years with a mean age of 50 ± 11 years (mean ± SD). Brugada males who were less than 30 years old and more than 70 years old were excluded from this study to minimize the influence of age on the basal sex hormonal levels including testosterone. Forty of the forty-eight Brugada males have been included in our previous clinical studies.[18-20] In all patients, physical examination, chest roentgenogram, laboratory values, echocardiography with wall motion analysis, and Doppler screening excluded structural heart diseases. The clinical, electrocardiographic, and electrophysiologic characteristics of the 48 Brugada males are shown in Table 1. Average age of the 48 Brugada males at diagnosis was 47 ± 12 years old. Aborted cardiac arrest or VF was documented in 21 males (44%), syncope alone in 11 males (23%), and 16 males (33%) were asymptomatic. Family history of sudden cardiac death (SCD) was observed in eight males (17%). An *SCN5A* coding region mutation was identified in seven (17%) of 42 males in whom genetic screening was conducted. Implantable cardioverter defibrillator (ICD) was implanted in all 32 symptomatic males with documented VF and/or syncope. ICD was also implanted in nine of 16 asymptomatic males due to induction of VF during the electrophysiologic study. Type 1 ST-segment elevation was recorded spontaneously in 43 males (90%) and was induced by sodium channel blockers in five males (10%). Complete right bundle branch block was observed in three males (6%). Late potential was recorded by a signal-average ECG system in 27 (59%) of 46 males. During the electrophysiologic study, VF requiring direct cardioversion for termination was induced in 32 (73%) of 44 males. Average HV interval was 46 ± 11 msec.

We first obtained data, such as the hormonal levels, visceral fat parameters, and ECG parameters in the 48 Brugada males prospectively between January and July in 2003, mainly at regular outpatient clinics for checking ICD. Only a Brugada male refused to participate during the recruitment of the case.

Thereafter, age-matched control males were randomly selected from the municipal population registry in Suita City. The hormonal and visceral fat data were collected sequentially between August and December in 2003. The municipal population registry in Suita City included 5,846 control subjects, among whom 1,052 males were age-matched to the 48 Brugada males. The 96 control males with a mean age of 50 ± 11 years were sequentially recruited from the age-matched 1,052 males. None of the recruited 96 control males refused to participate in this study. There were no significant differences in the clinical characteristics between the 96 control males and the remaining 956 age-matched males. Therefore, we had no way of knowing the body weight of the individuals who were selected to serve as controls from a very large database. Although K. Matsuo is a co-author of this study, none of the Brugada males and control males who appeared in the article by Matsuo[16] are included in the present study population.

All protocols were approved by the Ethical Review Committee in the National Cardiovascular Center. Written informed consent was obtained from all subjects.

Sex and Thyroid Hormonal Levels and Serum Electrolytes

Blood samples for analysis of basal hormone levels and serum electrolytes were obtained between 8:00 and 9:00 AM after an overnight fast. Plasma sex hormonal levels including testosterone, estradiol, DHEA-S, LH, and FSH were measured using commercially prepared immunoassay kits (testosterone, LH, and FSH: Chemiluminescent immunoassay [Bayer HealthCare, New York, NY, USA]; estradiol: Electrochemiluminescent immunoassay [Roche Diagnostics GmbH, Mannheim, Germany]; DHEA-S: Radioimmunoassay [Diagnostic Products Corporation, Los Angeles, CA, USA]). Thyroid hormonal levels including free T3, T4, and TSH, and serum electrolyte levels including sodium, potassium, and chloride were also measured.

Body Mass Index and Body Fat Percentage

Body weight (BW) was measured to the nearest 0.1 kg and height to the nearest cm. Body-mass index (BMI) was calculated as weight/height^2 (kg/m^2) as a parameter of visceral fat. We also measured body-fat percentage (BF%) by using body composition analyzer (Biospace Co., Ltd. Tokyo, Japan). These visceral fat parameters were measured just after blood sampling. In the 32 symptomatic Brugada males who had had documented VF and/or syncope, the BW and BMI were also measured within 48 hours after their clinical events during admission in our hospital or other emergent hospitals.

TABLE 1
Clinical, Electrocardiographic, and Electrophysiologic Characteristics in the 48 Brugada Males

Clinical characteristics	
Age at diagnosis (years)	47 ± 12
Aborted cardiac arrest or VF (%)	21/48 (44%)
Syncope alone (%)	11/48 (23%)
Asymptomatic (%)	16/48 (33%)
Family history of SCD	8/48 (17%)
SCN5A mutation	7/42 (17%)
ICD implantation	41/48 (85%)
Follow-up period (month)	41 ± 2
Arrhythmic event (%)	9/48 (19%)
Electrocardiographic characteristics	
Spontaneous coved-type ST elevation	43/48 (90%)
CRBBB (%)	3/48 (6%)
RR (msec)	939 ± 113
PQ interval (II) (msec)	186 ± 34
QRS duration (V2) (msec)	104 ± 18
Corrected QT interval (V5) (msec)	394 ± 27
ST amplitude at J point (V2) (mV)	0.32 ± 0.16
Late potential (%)	27/46 (59%)
Electrophysiologic characteristics	
Induction of VF	32/44 (73%)
Mode (Triple/Double/Single)	16/15/1
HV interval (msec)	46 ± 11

CRBBB = complete right bundle branch block; ICD = implantable cardioverter defibrillator; SCD = sudden cardiac death; VF = ventricular fibrillation.

ECG Parameters

In the 48 males with Brugada syndrome, 12-lead ECG was recorded just before blood sampling, and ECG parameters were assessed by an investigator (WS) blinded to clinical information. The ECG parameters included RR interval, PQ interval measured in lead II, QRS interval measured in lead V2, QT interval, corrected QT (QTc) interval measured in leads V5, and ST amplitude at J point measured in lead V2.

Statistical Analysis

We first conducted univariate analysis by using unpaired *t*-test to compare each data between the Brugada males and the control males. Since several confounding variables, such as age, exercise (none, sometimes, regularly), stress (none, sometimes, regularly), current smoking (no, yes), and medication (no, yes) of hypertension, diabetes, and hyperlipidemia may affect the hormonal levels including testosterone level and the visceral fat parameters, analysis of covariance (ANCOVA) was used to compare least square mean values between the Brugada males and the control males adjusting for these confounding variables. Pearson's correlation coefficients were calculated between the testosterone level and the visceral fat parameters. Partial correlation coefficients were calculated between the testosterone level and the visceral fat parameters after adjusting for age, exercise, stress, current smoking, and medication. Moreover, conditional logistic regression models were used to calculate odds ratios and 95% confidence intervals adjusting for age, BMI, exercise, stress, current smoking, hypertension, diabetes, and hyperlipidemia. Hypertestosteronemia was defined as serum testosterone levels $\geq$700 ng/dL, which is 75 percentiles of testosterone levels among case and control combined groups. In the 32 Brugada males with documented VF and/or syncope, a paired *t*-test was used to compare the visceral fat parameters at the clinical cardiac events and at the measurement of hormonal and visceral fat data. A two-sided P value below 0.05 was considered to indicate significance. All statistical analyses were performed by using SAS software, Ver 8.2.

Results

Hormonal Levels, Serum Electrolytes, and Visceral Fat

Table 2 illustrates univariate analysis for comparing sex and thyroid hormonal levels, serum electrolytes, and visceral fat parameters between the two groups. Testosterone level was significantly higher in the Brugada males than in the control males, whereas there were no significant differences in other sex hormonal levels; estradiol, DHEA-S, LH, FSH, and thyroid hormonal levels; T3, T4, and TSH. Serum sodium, potassium, and chloride levels were all significantly higher in the Brugada males than in the control males. BMI, BF%, and BW were all significantly lower in the Brugada males than in the control males. All variables followed normal distribution, both in the 48 Brugada and 96 control males.

The comparison of the confounding variables that may affect the hormonal levels and the visceral fat parameters between the 48 Brugada males and the 96 control males was shown in Table 3. Even after adjusting for age, exercise, stress, current smoking, and medication (hypertension, diabetes, and hyperlipidemia), the testosterone level, serum sodium, potassium, and chloride levels were all significantly higher, and the visceral fat parameters were significantly lower in the 48 Brugada males than in the 96 control males (Table 4). There were also significant differences in these parameters between the 24 definite Brugada males with documented VF and/or *SCN5A* mutations and the 96 control males after adjusting for the confounding variables (Table 4).

Correlation between Testosterone, Visceral Fat, and Serum Electrolytes

Testosterone level was inversely correlated with all visceral fat parameters, BMI, BF%, or BW in both the Brugada males and the control males, even after adjusting for age,

TABLE 2

Sex and Thyroid Hormonal Levels, Serum Electrolytes, and Visceral Fat Parameters in the 48 Brugada Males and the 96 Age-Matched Control Males

	Brugada Males (n = 48)	Control Males (n = 96)	P Value
Sex hormones			
Testosterone (ng/dL)	631 ± 176	537 ± 158	0.002
Estradiol (pg/mL)	28.9 ± 7.6	31.1 ± 12.6	0.263
DHEA-S (ng/mL)	1,901 ± 850	1,966 ± 861	0.668
LH (mIU/mL)	4.6 ± 2.6	3.9 ± 2.0	0.073
FSH (mIU/mL)	6.2 ± 4.9	5.0 ± 2.9	0.066
Thyroid hormones			
Free T3 (pg/mL)	3.3 ± 0.4	3.4 ± 0.3	0.360
Free T4 (ng/dL)	1.3 ± 0.1	1.3 ± 0.2	0.089
TSH (μIU/mL)	1.9 ± 1.4	1.7 ± 1.4	0.619
Serum electrolytes			
Sodium (mEq/L)	143.7 ± 2.0	142.6 ± 2.0	0.003
Potassium (mEq/L)	4.6 ± 0.3	4.3 ± 0.3	<0.001
Chloride (mEq/L)	105.1 ± 2.1	103.6 ± 2.1	<0.001
Viceral fat			
BMI (kg/m^2)	22.1 ± 2.9	24.6 ± 2.6	<0.001
BF% (%)	19.6 ± 4.9	23.1 ± 4.7	<0.001
BW (kg)	62.9 ± 9.7	70.0 ± 8.6	<0.001

Values are mean ± SD where indicated.
BMI = body-mass index; BF% = body-fat percentage; BW = body weight.

TABLE 3

Comparison of the Confounding Variables Between the 48 Brugada Males and the 96 Age-Matched Control Males

	Brugada Males (n = 48)	Control Males (n = 96)	P Value
Exercise			
None (%)	39.6	44.8	
Sometimes (%)	41.6	43.8	
Regularly (%)	18.8	11.5	0.482
Stress			
None (%)	27.1	21.9	
Sometimes (%)	54.2	54.2	
Regularly (%)	18.8	24.0	0.684
Current smoking (%)	25.0	27.1	0.789
Medication			
Hypertension (%)	20.8	19.8	0.883
Diabetes (%)	2.1	13.5	0.028
Hyperlipidemia (%)	10.4	5.2	0.246

TABLE 4
Testosterone, Serum Electrolytes, and Visceral Fat Parameters in the Brugada Males and the 96 Age-Matched Control Males after Adjusting for Confounding Variables

	Brugada Males	Control Males (n = 96)	P Value
ALL Case (n = 48)			
Testosterone (ng/dL)	631 ± 44	538 ± 40	0.003
Sodium (mEq/L)	144.2 ± 0.5	143.2 ± 0.5	0.007
Potassium (mEq/L)	4.6 ± 0.1	4.3 ± 0.1	<0.001
Chloride (mEq/L)	105.5 ± 0.5	103.9 ± 0.5	<0.001
BMI (kg/m^2)	22.3 ± 0.7	24.9 ± 0.7	<0.001
BF% (%)	20.0 ± 1.3	23.9 ± 1.1	<0.001
BW (kg)	63.4 ± 2.4	70.1 ± 2.1	0.001
Definite Brugada case with VF and/or *SCN5A* (n = 24)			
Testosterone (ng/dL)	656 ± 59	550 ± 48	0.009
Sodium (mEq/L)	143.9 ± 0.7	142.9 ± 0.6	0.042
Potassium (mEq/L)	4.7 ± 0.1	4.4 ± 0.1	<0.001
Chloride (mEq/L)	105.2 ± 0.7	103.9 ± 0.6	0.006
BMI (kg/m^2)	21.5 ± 1.0	24.5 ± 0.8	<0.001
BF% (%)	19.9 ± 1.7	24.1 ± 1.4	<0.001
BW (kg)	60.5 ± 3.1	69.2 ± 2.5	0.001

Values are mean ± SE adjusted for age, exercise, stress, current smoking, and medication of hypertension, diabetes and hyperlipidemia. BMI = body-mass index; BF% = body-fat percentage; BW = body weight; VF = ventricular fibrillation.

exercise, stress, current smoking, and medication (Brugada: BMI, r = –0.394, P = 0.011; BF%, r = –0.390, P = 0.012; BW, r = –0.335, P = 0.032; Control: BMI, r = –0.333, p = 0.002; BF%, r = –0.333, P = 0.001; BW, r = –0.305, P = 0.004), suggesting that Brugada males had higher testosterone level associated with lower visceral fat compared with control males (Fig. 1). No significant correlations were observed between other serum electrolytes and testosterone level or visceral fat parameters. Testosterone level was not correlated with age, even after adjusting for exercise, stress, current smoking, and medication (r = 0.007, P = 0.947).

Conditional Logistic Regression Models Analysis

Conditional logistic regression models analysis showed significant positive and inverse association between Brugada syndrome, hypertestosteronemia (Odd Ratio (OR): 3.11, 95%CI: 1.22–7.93, P = 0.017), and BMI (OR: 0.72, 95%CI: 0.61–0.85, P < 0.001), respectively (Table 5). Other variables did not significantly increase or decrease risks of Brugada syndrome (Table 5).

Visceral Fat at Clinical Cardiac Events in Brugada Males

In the 32 symptomatic Brugada males with documented VF and/or syncope, the time-span between the clinical cardiac events and the measurement of hormonal and the visceral fat data was 42 ± 32 months (mean ± SD, 1–99 months). The BMI and BW at the clinical cardiac events (VF or syncope) were significantly lower than those at the measurement of hormonal and visceral fat data (BMI, 21.0 ± 2.6 vs 22.1 ± 2.9 kg/m^2; BW, 60.0 ± 8.9 vs 62.9 ± 9.7 kg: P < 0.001, respectively).

Testosterone versus ECG Parameters, Symptoms or SCN5A Mutation in Brugada Males

Baseline electrocardiographic data of the 48 Brugada males are shown in Table 1. No significant correlations were observed between testosterone level and ECG parameters, including ST amplitude (r = –0.123, P = 0.406) and QTc interval (r = –0206, P = 0.160), in the 48 Brugada males. There was no significant difference in testosterone level between 32 symptomatic and 16 asymptomatic Brugada males (649 ± 185 vs 593 ± 157 ng/dL: P = 0.298). No significant difference was observed in testosterone level between 43 Brugada males with spontaneous Type 1 ST-segment elevation and five Brugada males with sodium channel blocker-induced Type 1 ST-segment elevation (624 ± 171 vs 688 ± 230 ng/dL: P = 0.448). Testosterone level was also no different between seven Brugada males with *SCN5A* mutation and 41 Brugada males without *SCN5A* mutation (700 ± 198 vs 619 ± 172 ng/dL: P = 0.261).

Follow-Up

Arrhythmic events occurred in nine (19%) of 48 Brugada males during average follow-up periods of 41 ± 2 months after blood sampling for the present study (Table 1). In more detail, arrhythmic events appeared in eight (38%) of 21 Brugada males with a history of aborted cardiac arrest or VF, in one (9%) of 11 Brugada males with syncope alone, but did not appear in any (0%) of 16 asymptomatic Brugada males.

Discussion

The major findings of the present study were: (1) Brugada males had significantly higher testosterone level, serum sodium, potassium, and chloride level, and significantly lower BMI, BF%, and BW than those in control males by univariate analysis, even after adjusting for age, exercise, stress, current smoking, and medications related to hypertension, diabetes and hyperlipidemia. (2) Testosterone level was inversely correlated with the BMI, BF%, and BW in both Brugada males and control males, even after adjusting for the confounding variables. (3) Conditional logistic regression models analysis showed strong positive association between Brugada syndrome and higher testosterone level (hypertestosteronemia) and strong inverse association between Brugada syndrome and BMI.

Testosterone in Brugada Phenotype and Male Predominance

For the past decade, numerous clinical, experimental, and molecular genetic studies have elucidated Brugada syndrome as a distinct clinical entity.[1-5,17] However, several problems remain unresolved, such as genetic heterogeneity, ethnic difference, and gender difference.[7] Di Diego and Antzelevitch recently suggested the cellular basis for male predominance in Brugada syndrome by using arterially perfused canine right ventricular wedge preparations.[9] Transient outward current (I_{to})-mediated phase 1 AP notch was larger in male dogs than in female dogs in the right ventricular epicardium, but not in the left ventricular epicardium, responsible for the male predominance in the Brugada phenotype. Recent clinical studies suggested that male hormone testosterone might be attributable to gender difference of the prevalence in this

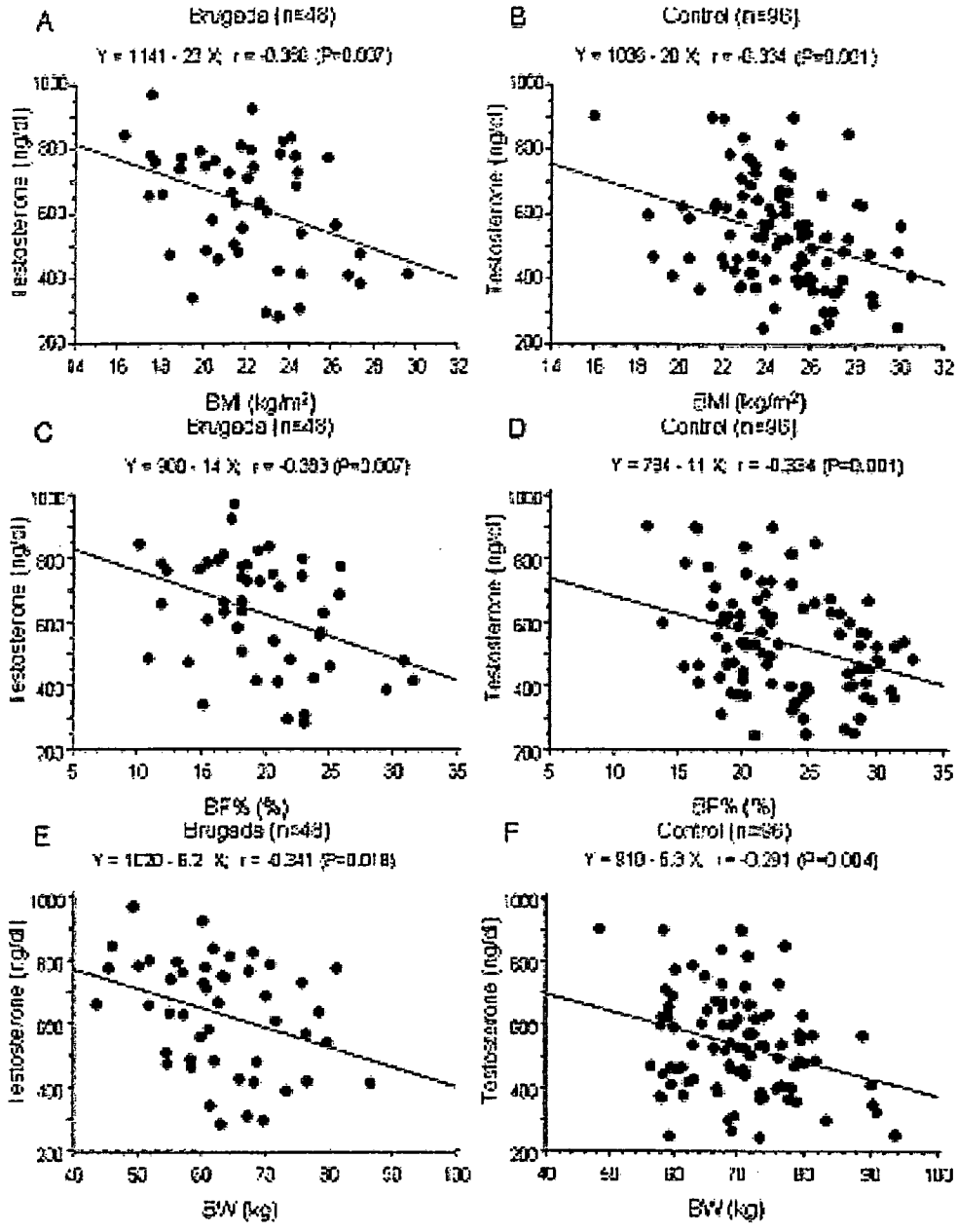

Figure 1. *Correlation between testosterone level and visceral fat parameters; body mass index (BMI) (A and B), body fat percentage (BF%) (C and D), and body weight (BW) (E and F) in the 48 Brugada males and the 96 age-matched control males. Testosterone level was inversely correlated with the BMI, BF%, or BW in both Brugada males and control males.*

syndrome. Matsuo et al. reported two cases of asymptomatic Brugada syndrome in whom typical coved ST-segment elevation disappeared following orchiectomy as therapy for prostate cancer,[21] indicating that testosterone may contribute to the Brugada phenotype in these two cases. Several experimental studies reported that testosterone increased outward potassium currents, such as the rapidly activating component (I_{Kr})[10,11] and the slowly activating component (I_{Ks})[12] of the delayed rectifier potassium current, and the inward rectifier potassium current (I_{K1}),[11] or decreased inward L-type calcium current (I_{Ca-L}).[12] Since the maintenance of the AP dome is determined by the fine balance of currents active at the end of phase 1 of the AP (principally I_{to} and I_{Ca-L}),[22,23] any agents that increase outward currents or decrease inward currents can increase the magnitude of the AP notch, leading to loss of the AP dome (all-or-none repolarization) in the epicardium, but not in the endocardium, contributing to a significant voltage gradient across the ventricular wall during ventricular activation, thus augmenting ST-segment elevation, the Brugada phenotype.[24] Therefore, testosterone would be expected to accentuate the Brugada phenotype. In the present study, males with Brugada syndrome had significantly higher testosterone level than age-matched control males, even after adjusting for age, exercise, stress, current smoking, and medication (hypertension, diabetes, and hyperlipidemia), which may affect the testosterone level. Moreover, conditional logistic regression models analysis showed strong positive association between Brugada syndrome and higher testosterone level (OR: 3.11). Our data suggest a significant role of testosterone, male hormone, in the Brugada phenotype. The

TABLE 5
Odds Ratios of Presence of Hypertestosteronemia and Confounding Risk Factors for Brugada Syndrome in Males

Variable	Odd Ratio	95% Confidence Interval	P Value
Hypertestosteronemia	3.11	1.22–7.93	0.017
Age	0.99	0.95–1.03	0.637
BMI	0.72	0.61–0.85	<0.001
Exercise	1.57	0.87–2.83	0.135
Stress	0.69	0.35–1.35	0.277
Current smoking	0.71	0.26–1.90	0.493
Hypertension	3.12	0.85–11.45	0.087
Diabetes	0.13	0.01–1.27	0.079
Hyperlipidemia	2.14	0.44–10.49	0.348

Hypertestosteronemia was defined as serum testosterone levels ≥ 700 ng/dL.

data also indicate that the male predominance in the Brugada phenotype is at least in part due to testosterone, which is present only in males.

Lower Visceral Fat May Be a Predictor for Brugada Phenotype

Matsuo et al. recently reported in their epidemiologic study that cases with the Brugada-type ECG had significantly lower BMI than that in control subjects.[16] Similarly, in the present study, males with Brugada syndrome had significantly lower visceral fat parameters, BMI, BF%, and BW than those in age-matched control males, even after adjusting for several confounding variables. Moreover, conditional logistic regression models analysis showed strong inverse association between Brugada syndrome and BMI (OR: 0.72). All of the visceral fat parameters were inversely correlated with testosterone level in both Brugada and control males, even after adjusting for the confounding variables. It has been well demonstrated that testosterone level in obese males is decreased compared to normal males of similar age.[13] Tsai et al. reported that lower baseline total testosterone level independently predicted an increase in visceral fat in the Japanese-American male cohort for 7.5 years.[15] Reversely, Marin et al. reported that testosterone treatment of middle-aged abdominally obese males was followed by a decrease of visceral fat mass measured by computerized tomography.[14] These data suggest that primarily higher level of testosterone in Brugada males compared to that in control males may result in lower visceral fat in Brugada males, which would be an "innocent bystander" sign of Brugada phenotype. In reverse, if primary lower visceral fat (body weight loss) would result in higher testosterone level, the weight loss could be a trigger for Brugada phenotype, just like fever is.[25] It is noteworthy that the visceral fat parameters at the clinical cardiac events (VF or syncope) in the 32 symptomatic Brugada males were significantly lower than those at the time of blood sampling for this study. This indicates that testosterone level is expected to be additively higher at the clinical cardiac events, which may contribute to spontaneous episodes of VF or syncope.

Other Hormonal Levels and Serum Electrolytes

Estradiol, female hormone, is reported to reduce the expression of Kv4.3 channels, which are important molecular components of I_{to} currents.[26] However, in contrast to testosterone, other sex hormonal levels including estradiol were not different between the Brugada males and the control males in the present study. Although thyroid hormones are also demonstrated to alter membrane currents, such as I_{to} and I_{Ca-L},[27,28] no significant differences were observed in the thyroid hormonal levels between the two groups in the present study.

On the other hand, serum sodium, potassium, and chloride levels were all significantly higher in the Brugada males than in the control males, even after adjusting for several confounding variables. Recently, many agents and conditions that cause an outward shift in current activity at the end of phase 1 AP have been known to unmask ST-segment elevation, as found in the Brugada syndrome, leading to the acquired form of this disorder.[4,29] Electrolyte abnormalities, such as hyperkalemia, are reported to amplify ST-segment elevation like that in Brugada syndrome.[30] The lower visceral fat found in the Brugada males is expected to decrease serum level of insulin, leptine, a novel adipocyte-derived hormone, or ghrelin, a novel growth hormone-releasing peptide, suppressing β-adrenergic receptor or plasma norepinephrine level, resulting in an increase of serum potassium level.[31,32] Further studies including measurement of levels of insulin, leptine, and ghrelin will be required to elucidate the precise mechanism.

Study Limitations

Although the testosterone level was significantly higher in the Brugada males than in the control males, no statistically significant correlations were observed between the testosterone level and the ST amplitude in the Brugada males. The degree of the ST-segment elevation is variable between Brugada patients because it is influenced by several factors other than sex hormonal levels or electrolytes levels, such as basal autonomic tone, presence of *SCN5A* mutation, or probably intrinsic current density of I_{to}, etc., in the right ventricular epicardial cells. The threshold of ST-segment elevation for spontaneous induction of VF also varies between Brugada patients. Therefore, the Brugada phenotype, such as ST-segment elevation or spontaneous induction of VF, may correlate with the testosterone level day to day individually (intra-personally) in each Brugada male, but may not correlate among the pooled data obtained from many Brugada males, probably due to inter-person difference of the ST-segment elevation.

There were no significant differences in testosterone level between symptomatic and asymptomatic Brugada males, between Brugada males with spontaneous ST elevation and those with sodium channel blocker-induced ST elevation, or between Brugada males with and without *SCN5A* mutation, all of which are probably due to a relatively small number of Brugada males in the present study. Further evaluation with increasing number of Brugada males will be required.

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Comparison of Long-Term Follow-Up of Electrocardiographic Features in Brugada Syndrome Between the SCN5A-Positive Probands and the SCN5A-Negative Probands

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To investigate changes of electrocardiographic parameters with aging and their relation to the presence of SCN5A mutation in probands with Brugada syndrome (BS), we measured several electrocardiographic parameters prospectively during long-term follow-up (10 ± 5 years) in 8 BS probands with SCN5A mutation (SCN5A-positive group, all men; age 46 ± 10 years) and 36 BS probands without SCN5A mutation (SCN5A-negative group, all men; age 46 ± 13 years). Throughout the follow-up period, depolarization parameters, such as P-wave (lead II), QRS (leads II, V_2, V_5), S-wave durations (leads II, V_5), and PQ interval (leads II) were all significantly longer and S-wave amplitude (II, V_5) was significantly deeper in the SCN5A-positive group than in the SCN5A-negative group. The SCN5A-positive group showed a significantly longer corrected QT interval (lead V_2) and higher ST amplitude (lead V_2) than those in the SCN5A-negative group. The depolarization parameters increased with aging during the follow-up period in both groups; however, the PQ interval (lead II) and QRS duration (lead V_2) were prolonged more prominently and the QRS axis deviated more to the left with aging in the SCN5A-positive group than in the SCN5A-negative group. In conclusion, conduction slowing was more marked and more progressively accentuated in Brugada probands with SCN5A mutation than in those without SCN5A mutation. (Am J Cardiol 2007; 100:649–655)

Brugada syndrome (BS) is characterized by a ST-segment elevation in the right precordial leads V_1 to V_3 and is associated with sudden cardiac death (SCD) secondary to a rapid polymorphic ventricular tachycardia (VT) or ventricular fibrillation (VF).[1–9] It has been suggested that a transient outward current-mediated action potential notch and a loss of action potential dome in the epicardium of the right ventricular outflow tract (RVOT) give rise to a transmural voltage gradient, resulting in ST-segment elevation in the right precordial lead in BS.[8] Conversely, the SCN5A gene encoding the cardiac sodium channel has been reported to be linked to BS,[10] and mild conduction abnormalities and QRS prolongation have been described.[5,11] Smits et al[12] have compared these electrocardiographic parameters between SCN5A mutation carriers and those who do not carry the mutation. Probst et al[13] meticulously studied aging-associated electrocardiographic parameters in SCN5A-related BS.[13] However, progressive changes of the depolarization and repolarization parameters on the electrocardiogram (ECG) with aging during long-term follow-up in relation to the SCN5A mutation have not been fully evaluated. In the present study, we prospectively measured several electrocardiographic parameters during long-term follow-up periods and compared them between patients with BS with and without SCN5A mutation.

Methods

The study population consisted of 44 probands with BS admitted to the National Cardiovascular Center in Suita, Japan, due to history of aborted SCD, syncope, or evaluation of electrocardiographic abnormality, who could be prospectively followed up for >5 years (average 10 ± 5 years) at regular outpatient clinics in our hospital. All probands were men, and their age on admission (i.e., at early period) ranged from 20 to 72 years (mean 46 ± 12 years). BS was diagnosed when a type 1 coved-type ST-segment elevation (≥0.2 mV at J point) was observed in >1 of the right precordial leads (V_1 to V_3) in the presence or absence of a sodium channel blocker in conjunction with 1 of the following: (1) documented VF or polymorphic VT, (2) a family history of SCD at <45 years of age, type 1 ECG in family members, (3) inducibility of VF or polymorphic VT with programmed electrical stimulation, and (4) history of aborted cardiac arrest with or without documentation of VF,

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Table 1
SCN5A mutations, common variants and promotor haplotype

Coding*	No. of Patients	Type	Coding	No. of Patients	Type
	SCN5A Positive Group (n = 8)			SCN5A Negative Group (n = 36)	
Mutation					
A735V	1	Missense			
P1719fsX1786	1	Frameshift			
L276Q	1	Missense			
V1764fsX1786	1	Frameshift			
L136P	1	Missense			
R367H	2	Missense			
T1709M	1	Missense			
Common variant					
H558R	1	Missense	H558R	4	Missense
Promotor haplotype					
AA	5		AA	12	
AB	2		AB	4	
BB	0		BB	1	

* The numbers and letters refer to the amino acid coding of the mutant channel protein.
AA = haplotype A (common alleles) homozygotes; AB = haplotype A/haplotype B (minor alleles) heterozygotes; BB = haplotype B homozygotes. See detail in Bezzina et al.[14]

syncopal episodes of unknown origin, or nocturnal agonal respiration.[4]

We divided the 44 Brugada probands into 2 groups according to the presence or absence of an SCN5A coding region mutation: SCN5A-positive group (n = 8) and SCN5A-negative group (n = 36).

The standard 12-lead ECGs were recorded at least every 6 months prospectively at regular outpatient clinics with a paper speed of 25 mm/s and an amplitude of 10 mm/mV. The ECGs were magnified to 150%, and several electrocardiographic parameters were measured manually by an investigator (MY) blinded to clinical and genetic information. As depolarization parameters, P-wave duration (lead II), PQ interval (lead II), QRS duration (leads II, V_2, and V_5), S-wave duration and amplitude (leads II, V_5), and QRS axis were measured. Conversely, corrected QT interval (QTc, leads II, V_2, and V_5), corrected JT interval (JTc, leads II, V_2, and V_5), and ST amplitude at the J point and 40 ms after theJ point (STJ and STJ40, lead V_2) were measured as repolarization parameters. The absolute values of these parameters and the change of each parameter between early and late periods were compared between the 8 probands in the SCN5A-positive group and the 36 in the SCN5A-negative group.

In all patients, we screened SCN5A mutation in all 28 exons of SCN5A gene by a direct sequencing method using an ABI 3700 system (Applied Biosystems, Foster City, California). An SCN5A mutation was defined when the mutation was not identified in any of the 100 control subjects. We also screened the SCN5A promoter haplotype, which we have recently identified in an Asian population,[14] in 7 recent SCN5A-positive probands and 17 SCN5A-negative probands.

Numeric values were expressed as means ± SD. Comparisons of each electrocardiographic parameter between the SCN5A-positive group and the SCN5A-negative group and between the early and the late periods were made using 2-way repeated-measures analysis of variance (ANOVA) followed by the Scheffe multiple-comparison test. Comparisons of changes in each parameter between the SCN5A-positive group and the SCN5A-negative group were made using 1-way ANOVA followed by Scheffe test. Comparisons of the clinical, electrophysiologic, and follow-up data between the SCN5A-positive group and the SCN5A-negative group were made using chi-square test or 1-way ANOVA followed by Scheffe test. A p value <0.05 was considered significant.

Results

The SCN5A mutations, which were identified at a coding region in the SCN5A-positive group, are shown in Table 1. Five missense mutations and 2 frameshift mutations were identified. A missense mutation, R367H, was identified in 2 unrelated Brugada probands. The common variant and SCN5A promoter haplotype[14] in both groups are also shown in Table 1. There were no significant differences in the frequency of the common variant and the promoter haplotype between the 2 groups.

The comparison of the clinical and electrophysiologic characteristics between the 8 SCN5A-positive probands and the 36 SCN5A-negative probands are shown in Table 2. There were no significant differences in the age on admission, when the clinical diagnosis of BS was made, between the 2 groups. No significant differences were observed in the incidence of spontaneous type 1 ECG, documented VF until the early period, family history of SCD, implantation of implantable cardioverter defibrillator, complete right bundle branch block (RBBB) at the early period and the latest follow-up period (i.e., late period), and late potentials. The HV interval during the electrophysiologic study was significantly longer in the SCN5A-positive group than in the SCN5A-negative group. There were no significant differ-

Table 2
Clinical and electrophysiologic characteristics and follow-up

Characteristic	SCN5A-Positive Group (n = 8)	SCN5A-Negative Group (n = 36)	p Value
Clinical characteristics			
Age on admission (yrs)	46 ± 10	46 ± 13	0.938
Spontaneous type 1 ECG	6 (75%)	25 (69%)	0.755
Documented VF until early period	2 (25%)	17 (47%)	0.251
Family history of SCD	3 (38%)	4 (11%)	0.065
ICD implantation	8 (100%)	26 (72%)	0.090
Complete RBBB at early period	1 (13%)	2 (5%)	0.481
Complete RBBB at late period	1 (13%)	6 (17%)	0.771
Late potentials	7/7 (100%)	24/33 (73%)	0.117
Electrophysiologic characteristics			
Induction of VF	5/8 (63%)	25/33 (76%)	0.658
Mode (triple/double/single)	1/3/1	12/11/2	-
HV interval (ms)	65±5 (n=7)	41±8 (n=27)	<0.001
Follow-up			
Follow-up period (yrs)	10 ± 5	10 ± 4	0.993
Arrhythmic events during follow-up periods	4/8 (50%)	12/36 (33%)	0.375
Previous VF	2/2 (100%)	8/17 (47%)	0.156
No previous VF	2/6 (33%)	4/19 (21%)	0.539

EPS = electrophysiological study; HV = His-ventricular interval; ICD = implantable cardioverter-defibrillator.

ences in the frequency and mode of VF induction between the 2 groups.

Figure 1 illustrates the standard 12-lead ECGs at early and late periods during the follow-up period in representative patients with BS in the SCN5A-positive group (Figure 1) and the SCN5A-negative group. Table 3 shows composite data of the electrocardiographic parameters at the early and late periods in the 8 SCN5A-positive probands and 36 SCN5A-negative probands during the follow-up period.

As depolarization parameters, the P-wave duration (lead II), PQ interval (lead II), and QRS duration (lead II) significantly increased with aging from early to late periods in both groups and were all significantly longer in the SCN5A-positive group than in the SCN5A-negative group at both early and late periods. The QRS duration (lead V_2) in the SCN5A-positive group and the S-wave duration (leads II and V_5) in the SCN5A-negative group significantly increased with aging. The QRS duration (leads V_2 and V_5) and the S-wave duration (leads II and V_5) were significantly longer, and the S-wave amplitude (leads II and V_5) was significantly deeper in the SCN5A-positive group at early and late periods. The QRS axis was not different between the 2 groups at the early period; however, it was significantly smaller (i.e., deviated to the left) at the late period in the SCN5A-positive group.

As a repolarization parameter, the corrected QT interval (lead V_2) was significantly prolonged from the early period to the late period in the SCN5A-positive group, and was significantly longer in the SCN5A-positive group than in the SCN5A-negative group at the early and late periods. However, the QTc intervals (leads II and V_5) did not change from the early period to the late period in both groups and were not different between groups at the early and late periods. Conversely, no JTc intervals (leads II, V_2, and V_5) changed from the early period to the late period in both groups, and the JTc interval (lead V_2) at the late period was significantly longer in the SCN5A-positive group. The STJ amplitude (lead V_2) and STJ40 amplitude (lead V_2) did not change throughout the follow-up period in both groups, but were significantly greater in the SCN5A-positive group than in the SCN5A-negative group at the early and late periods. Even if we eliminated probands with BS with complete RBBB (1 SCN5A-positive proband and 2 SCN5A-negative probands at the early period, 1 SCN5A-positive proband and 6 SCN5A-negative probands at the late period), the main results and statistical differences were not significant.

Table 4 depicts comparison of the change of the electrocardiographic parameters from early to late periods between the SCN5A-positive group and the SCN5A-negative group.

The changes in PQ interval (lead II) and QRS duration (lead V_2) were significantly longer in the SCN5A-positive group than in the SCN5A-negative group. The change in QRS axis was greater (i.e., deviated more to the left) in the SCN5A-positive group than in the SCN5A-negative group.

There were no significant differences in the duration of follow-up period and the incidence of arrhythmic events during the follow-up period between the 2 groups (Table 2). Because a history of documented VF (until the early period) was proven to be the strongest predictor for subsequent arrhythmic events, arrhythmic events were compared between the 2 groups separately in probands with previous VF and those without previous VF, but no significant differences were observed (Table 2).

Discussion

The present study includes what is, to our knowledge, the longest follow-up of changes of electrocardiographic parameters in SCN5A-positive probands and SCN5A-negative probands with BS.

Mild conduction abnormalities, such as widening of the P wave, prolongation of QRS duration and PQ and HV intervals, and higher incidence of RBBB, have been described in patients with BS, especially those with

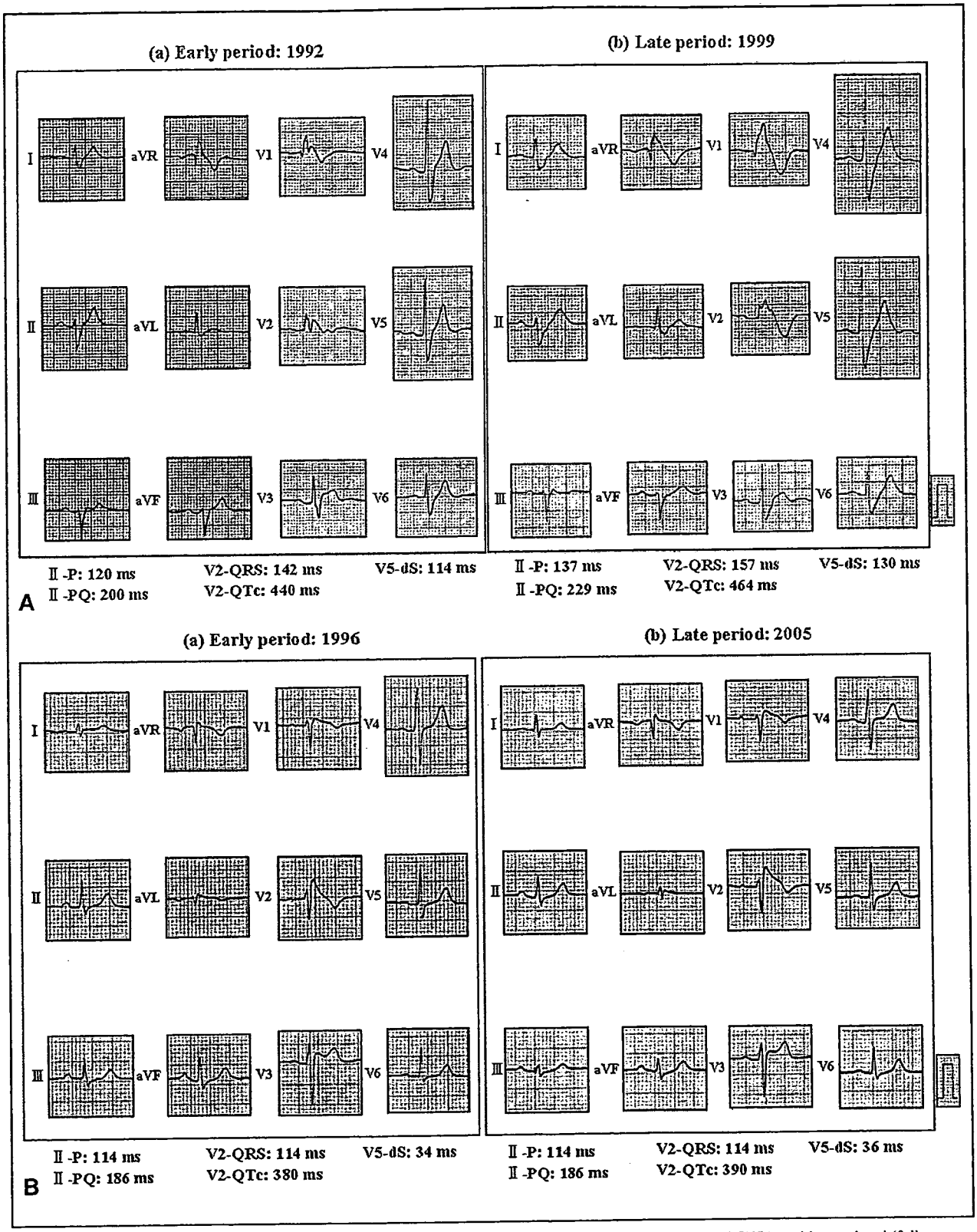

Figure 1. Standard 12-lead ECG at early and late periods during follow-up in representative cases of BS. *A*, in an *SCN5A*-positive proband (follow-up period, 7 years), the P-wave (lead II), QRS (lead V_2), and S-wave (lead V_5) durations and PQ interval (lead II) were prolonged even at the early period (47 years of age, *a*). The S-wave amplitude (lead V_5) was also deep, and the QRS axis deviated to the left. The QTc interval (lead V_2) was borderline prolonged. At the late period (*b*), all these parameters further increased. *B*, in an SCN5A-negative proband (follow-up period, 9 years), the P-wave (lead II), QRS (lead V_2), and S-wave (lead V_5) durations, PQ interval (lead II), and QTc interval (lead V_2) were less prolonged compared with those in an SCN5A-positive proband at the early period (51 years of age, *a*). At the late period (*b*), these parameters did not change significantly. V_5-dS = S-wave duration in lead V_5.