Author's personal copy

Table 1 – Characteristics of diabetic patients with normoalbuminuria, microalbuminuria, and overt nephropathy

Parameter	Stage of nephropathy		
	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
n	121	71	25
Age (years)	62 ± 9	65 ± 8	66 ± 7
Men/women	76/45	34/37	12/13
Duration of diabetes (years)	12 ± 8	14 ± 8	18 ± 8*
BMI (kg/m^2)	25.0 ± 3.7	25.1 ± 3.7	25.1 ± 3.9
SBP (mmHg)	128 ± 13	133 ± 15	141 ± 19*
DBP (mmHg)	74 ± 10	73 ± 9	76 ± 10
FBS (mmol/l)	7.4 ± 1.4	7.5 ± 1.5	7.5 ± 1.9
HbA1c (%)	8.3 ± 1.5	8.9 ± 1.7*	8.8 ± 1.4
HOMA-IR	1.62 ± 0.98	1.71 ± 2.06	2.29 ± 1.47
Total cholesteroyl (mmol/l)	4.86 ± 0.90	4.86 ± 0.90	4.73 ± 0.75
Serum creatinine (μmol/l)	70 ± 20	60 ± 20	110 ± 40
Urinary albumin (mg/day)	10 ± 7	85 ± 79**	583 ± 576**
Creatinine clearance (ml/s)	1.43 ± 0.52	1.50 ± 0.63	0.73 ± 0.43**
ACEI or ARB (yes/no)	36/85	24/47	11/14*
Statin (yes/no)	45/76	25/46	10/15
Current smoker (yes/no)	11/110	7/64	6/19

*$p < 0.05$, **$p < 0.01$ vs. normoalbuminuria, mean ± S.D.

strength of correlation between variables was tested by linear correlation and multiple regression analysis. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Patients characteristics

Table 1 shows the clinical characteristics of three groups. There was no significant difference in age, gender, BMI, FBS and total cholesterol among the three groups. HbA1c of diabetic patients with microalbuminuric patients was significantly higher than normoalbuminuric patients. Systolic blood pressure of macroalbuminuric patients was significantly higher than normo- and micro-albuminuric patients. Creatinine clearance was significantly decreased in macroalbuminuric patients compared with normo- and micro-albuminuric patients. There is no significant difference in rate of patients taking ACE/ARB between normo- and micro-albuminuric patients whereas the rate of patients taking ACE/ARB of macroalbuminuric patients were significantly large compared with other two groups. On the other hand, there is no significant difference in rate of patients taking statin among three groups.

3.2. %FMD of diabetic patients

We studied the endothelial function by FMD using brachial artery echography. %FMD (Δhyperemia) of diabetic patients with microalbuminuria (4.5 ± 3.7%) and macroalbuminuria (4.2 ± 2.4%) was apparently decreased compared with those of diabetic patients with normoalbuminuria (6.6 ± 3.7%) (Fig. 1A). Moreover, %FMD was significantly correlated with UAER in normo- and micro-albuminuric patients independent of age, HbA1c, and systolic blood pressure by multiple regression analysis ($r = -0.38$, $p < 0.05$) (Fig. 2). Dilatation of brachial artery by NTG (ΔNTG) showed no difference among three groups (Fig. 1B).

3.3. vWF, hsCRP, and ADMA of diabetic patients

We studied other atherosclerotic markers, that is, vWF, hsCRP, and ADMA. There was no significant difference of the levels of vWF and hsCRP between normoalbuminuric and microalbu-

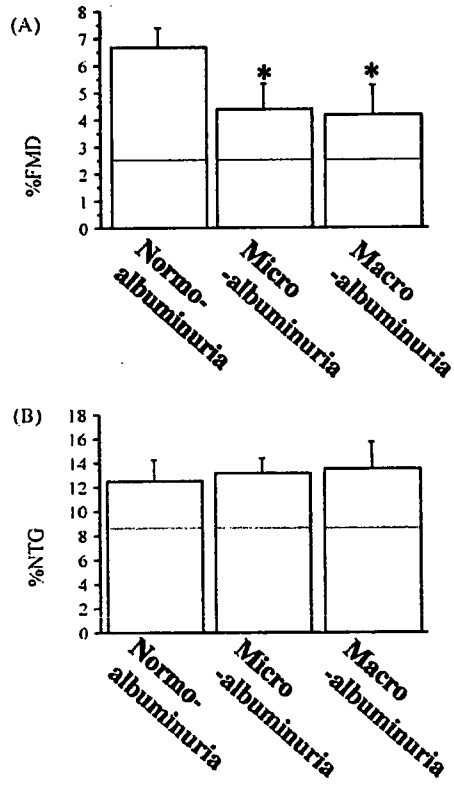

Fig. 1 – %FMD (A) and %NTG (B) in diabetic patients with normoalbuminuria, microalbuminuria and macroalbuminuria. Each value means (means ± S.D.), *$p < 0.001$.

Author's personal copy

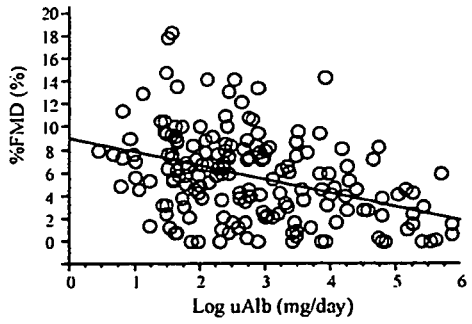

Fig. 2 – Correlation between degree of UAE and %FMD in normo- and micro-albuminuric diabetic patients. There was a significant correlation between both variables ($r = -0.38$, $p < 0.05$, $n = 192$).

minuric patients (Table 2). Although the levels of ADMA in microalbuminuric patients did not show significant difference compared with normoalbuminuric patients (Table 2), the levels of ADMA in macroalbuminuric patients were significantly elevated compared with normoalbuminuric patients (Table 2).

3.4. *Insulin sensitivity of diabetic patients*

We studied the insulin sensitivity by SSPG method. The levels of SSPG had weak but significant correlation with both %FMD ($r = -0.175$, $p < 0.05$) and UAER ($r = 0.181$, $p < 0.05$) independent of age, HbA1c, and systolic blood pressure (Fig. 3A, B).

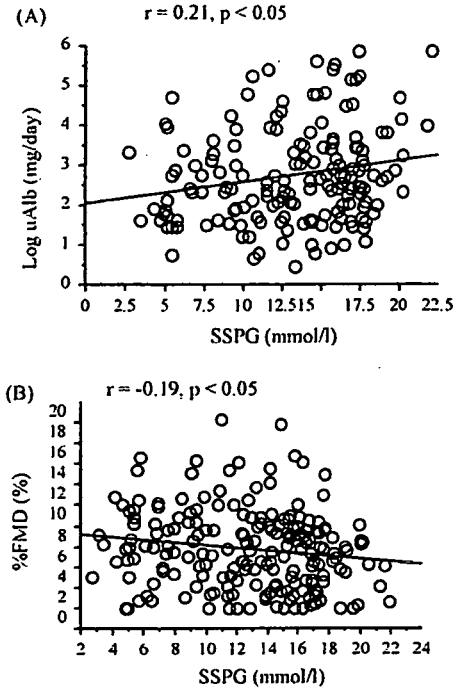

Fig. 3 – Correlation between SSPG and UAE (A), and correlation between SSPG and %FMD (B) in normo- and micro-albuminuric patients.

4. Discussions

There were two main findings from this investigation in type 2 diabetic patients. First, diabetic micro- and macro-albuminuric patients showed significant reduction of %FMD compared with normoalbuminuric patients. This finding suggests that the endothelial dysfunction may account for the association between atherosclerosis and albuminuria in diabetic patients. Second, the level of SSPG was significantly associated with both UAER and %FMD. This finding suggests that insulin resistance may play a role in both atherosclerosis and nephropathy in type 2 diabetic patients.

In diabetic patients, %FMD is decreased compared with healthy control [13,14]. These reports indicated that diabetes mellitus is associated with endothelial dysfunction due to impaired NO production. However the involvement of endothelial dysfunction in diabetic nephropathy has been unclarified. We demonstrated that microalbuminuric and macroalbuminuric patients showed significant decreased %FMD compared with normoalbuminuric patients. In contrast, there was no significant difference of vWF between normoalbuminuric patients and microalbuminuric patients. vWF is a product of vascular endothelial cell, and induces coagulation and platelet aggregation [15]. These findings suggest that endothelial dysfunction due to impaired NO production is specifically induced in micro- and macroalbuminuric patients. One recent report showed that coronary endothelium-dependent dilatation was impaired in a rat model of spontaneous albuminuria [16] supporting this hypothesis. It has been reported that renal NO production was decreased in rodent diabetic model [17]. This report suggests that decrease of NO production may play a role in the

Table 2 – Parameters of atherosclerosis in diabetic patients with normoalbuminuria, microalbuminuria, and overt nephropathy

Parameter	Stage of nephropathy		
	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
von Willebrand factor (%)	147 ± 44	146 ± 44	143 ± 41
High-sensitive CRP (ng/ml)	976 ± 1401	951 ± 1110	1113 ± 1187
ADMA (nmol/ml)	0.45 ± 0.06	0.47 ± 0.07	0.55 ± 0.11*

*$p < 0.001$ vs. normoalbuminuria, mean ± S.D.

progression of diabetic nephropathy as well as atherosclerosis. We investigated serum ADMA levels in diabetic patients. There was no significant difference of ADMA levels between normo- and micro-albuminuric patients, suggesting that the reduction of %FMD in microalbuminuric patients might not be resulted from the elevation of ADMA. However, in macroalbuminuric patients, ADMA level was significantly higher than normoalbuminuric patients. Vallance et al. reported that the level of ADMA was elevated in patients with chronic renal failure and suggested the involvement of this in coronary artery disease [18]. They indicate that the elevation of ADMA might be associated with atherosclerosis in patients with chronic renal disease [18]. Thus, this finding suggests that the elevation of ADMA might be associated with atherosclerotic change in diabetic patients with macroalbuminuria.

An association between chronic low-grade inflammation and development of atherosclerotic disease has been observed in basic and clinical studies [7,19–21]. Furthermore, diabetic patients have higher CRP levels than normal subjects, suggesting that chronic inflammation may contribute diabetic atherosclerotic complication [22]. An association between micro- and macro-albuminuria and inflammation has also been reported [23,24]. However, several other studies showed that inflammatory molecules were not associated with micro- and macro-albuminuria [25–27]. Thus the knowledge of this association is still controversial. Also we could not demonstrate the association between CRP and development of microalbuminuria in this study. Our data suggested that chronic low-grade inflammation might not be involved in the association between atherosclerosis and microalbuminuria. However, since this study was performed by cross-sectional analysis and other inflammatory marker was not measured, further study is necessary for demonstrating this hypothesis.

Insulin resistance has been reported to play an important role in the development and progression of atherosclerotic coronary disease [8,9]. Recently the association between insulin resistance and microalbuminuria was also reported [10]. Nakamura et al. demonstrated that administration of pioglitazone to diabetic patients attenuated UAER [28]. In this study, we showed that both the UAER and %FMD were significantly correlated to the level of SSPG. These findings suggest that insulin resistance may be involved in both the elevated urinary albumin excretion and endothelial dysfunction due to impaired NO production. However, HOMA-IR, another insulin sensitivity marker which reflects insulin sensitivity in both the liver and the periphery, did not show significant difference among three groups, suggesting that particularly peripheral insulin resistance may be important for the pathogenesis of atherosclerosis and diabetic nephropathy.

In summary, we showed that %FMD of micro- and macro-albuminuric patients was decreased compared with those of normoalbuminuric patients, without showing significant difference in other various atherosclerotic markers. Furthermore, the level of SSPG was significantly correlated to UAER and %FMD. These findings suggest that endothelial dysfunction which may be due to impaired NO production underlies the mechanism of association between elevated urinary albumin excretion and atherosclerosis in diabetic patients, and that peripheral insulin resistance might be possibly involved in both diabetic nephropathy and atherosclerosis.

Acknowledgement

This work was supported by the Research Grant for Cardiovascular Diseases (16C-2) from the Ministry of Health, Labour and Welfare.

REFERENCES

[1] S.F. Dinneen, H.C. Gerstein, The association of microalbuminuria and mortality in non-insulin dependent diabetes mellitus: a systematic overview of the literature, Arch. Intern. Med. 157 (1997) 1413–1418.
[2] J.S. Yudkin, R.D. Forrest, C.A. Jackson, Microalbuminuria as predictor of vascular disease in non-diabetic subjects: Islington diabetes survey, Lancet 2 (1988) 530–533.
[3] R.H. Boger, E.S. Ron, L-Arginine improves vascular function by overcoming the deleterious effects of ADMA, a novel cardiovascular risk factor, Altern. Med. Rev. 10 (2005) 14–23.
[4] I. Fleming, R. Busse, Significant transduction of eNOS activation, Cardiovasc. Res. 43 (1999) 532–541.
[5] R. Busse, I. Fleming, Pulsatile stretch and shear stress: physical stimuli determining the production of endothelium-derived relaxing factors, J. Vasc. Res. 35 (1998) 73–84.
[6] D.S. Celemajer, K.E. Sorensen, V.M. Gooch, D.J. Spiegelhalter, O.I. Miller, I.D. Sullivan, et al., Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis, Lancet 340 (1992) 1111–1115.
[7] R. Ross, Atherosclerosis: an inflammatory disease, N. Engl. J. Med. 340 (1999) 115–126.
[8] R.A. De Fronzo, E. Ferrannini, Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and atherosclerotic cardiovascular disease, Diabetes Care 14 (1991) 173–194.
[9] S. Inchiostro, G. Bertoli, G. Zanette, Evidence of higher insulin resistance in NIDDM patients with ischemic heart disease, Diabetologia 37 (1994) 597–603.
[10] M. Emoto, Y. Nishizawa, K. Maekawa, T. Kawagishi, K. Kogawa, Y. Hiura, et al., Insulin resistance in non-insulin-dependent diabetic patients with diabetic nephropathy, Metabolism 46 (1997) 1013–1018.
[11] M. Suzuki, I. Takamizawa, K. Suzuki, A. Hiuge, T. Horio, Y. Yoshimasa, et al., Close association of endothelial dysfunction with insulin resistance and carotid wall thickening in hypertension, Am. J. Hypertens. 17 (2004) 228–232.
[12] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (1985) 412–419.
[13] N. Ihlemann, K.H. Stokholm, P.C. Eskildsent, Impaired vascular reactivity is present despite normal levels of von Willebrand factor in patients with uncomplicated type 2 diabetes, Diabetes Med. 19 (2002) 476–481.
[14] R.V. Hogikyan, A.T. Galecki, B. Pitt, J.B. Halter, D.A. Greene, M.A. Supiano, Specific impairment of endothelium-dependent vasodilatation in subjects with type 2 diabetes independent of obesity, J. Clin. Endocrinol. Metab. 83 (1998) 1946–1952.

[15] B.M. Ewenstein, Vascular biology of von Willebrand factor, in: G.V.R. Born, C.J. Schwartz (Eds.), Vascular Endothelium, Schattauer, Stuttgart, 1997, pp. 107–123.
[16] S. Gschwend, S.J. Pinto-Siersma, H. Buikema, Y.M. Pinto, W.H. Van Gilst, A. Schulz, et al., Impaired coronary endothelial function in a rat model of spontaneous albuminuria, Kidney Int. 62 (2002) 181–191.
[17] A. Erdely, G. Freshour, D.A. Maddox, J.L. Olson, L. Samsell, C. Baylis, Renal disease in rats with type 2 diabetes is associated with decreased renal nitric oxide production, Diabetologia 47 (2004) 1672–1676.
[18] P. Vallance, A. Leone, A. Calver, J. Collier, S. Moncada, Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure, Lancet 339 (1992) 572–575.
[19] J. Torzewski, M. Torzewski, D.E. Bowyer, M. Frohlich, W. Koenig, J. Waltenberger, et al., C-reactive protein frequently colocalizes with the terminal component complex in the intima of early atherosclerotic lesions of human coronary arteries, Arterioscler. Thromb. Vasc. Biol. 18 (1998) 1386–1392.
[20] P.M. Ridker, M. Cushman, M.J. Stampfer, R. Tracy, C.H. Hennekens, Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men, N. Engl. J. Med. 336 (1997) 973–979.
[21] F. Haverkate, S.G. Thompson, S.D.M. Pyke, J.R. Gallimore, M.B. Pepys, The European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group, Production of C-reactive protein and risk of coronary events in stable and unstable angina, Lancet 349 (1997) 462–466.
[22] E.S. Ford, Body mass index, diabetes, and C-reactive protein among U.S. adults, Diabetes Care 22 (1999) 1971–1977.
[23] C.D.A. Stehouwer, M.A. Gall, J.W.R. Twisk, E. Knudsen, J.J. Emeis, H.H. Parving, Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes. Progressive, interrelated, and independently associated with risk of death, Diabetes 51 (2002) 1157–1165.
[24] K.E. Paisley, M. Beaman, J.E. Tooke, V. Mohamed-Ali, G.D.O. Lowe, A.C. Shore, Endothelial dysfunction and inflammation in asymptomatic proteinuria, Kidney Int. 63 (2003) 624–633.
[25] B. Mirup, M. Demaat, P. Rossing, J. Gram, C. Kluft, J. Jespersen, Elevated fibrinogen and the relation to acute phase response in diabetic nephropathy, Thromb. Res. 81 (1996) 485–490.
[26] M.A. Crook, P. Tutt, J.C. Pickup, Elevated serum sialic acid concentration in NIDDM and its relationship to blood pressure and retinopathy, Diabetes Care 16 (1993) 57–60.
[27] O. Ortega, I. Rodriguez, P. Gallar, A. Carreno, M. Ortiz, A. Molina, et al., Significance of high C-reactive protein levels in pre-dialysis patients, Nephrol. Dial. Transplant. 17 (2002) 1105–1109.
[28] T. Nakamura, C. Ushiyama, S. Osada, M. Hara, N. Shimada, H. Koide, Pioglitazone reduces urinary podocyte excretion in type 2 diabetes patients with microalbuminuria, Metabolism 50 (2001) 1193–1196.

Circulating CD34-Positive Cell Number Is Associated With Brain Natriuretic Peptide Level in Type 2 Diabetic Patients

SADANORI OKADA, MD[1]
HISASHI MAKINO, MD, PHD[1]
AYAKO NAGUMO, MD[1]
TAKAKO SUGISAWA, MD, PHD[1]
MUNEYA FUJIMOTO, MD, PHD[1]
ICHIRO KISHIMOTO, MD, PHD[1]
YOSHIHIRO MIYAMOTO, MD, PHD[1]
AKIE KIKUCHI-TAURA[2]
TOSHIHIRO SOMA, MD[2]
AKIHIKO TAGUCHI, MD, PHD[3]
YASUNAO YOSHIMASA, MD, PHD[1]

Patients with type 2 diabetes often suffer from asymptomatic left ventricular (LV) injury, including increased LV mass, without apparent myocardial ischemia. The mechanisms underlying diabetic LV injury remain unclear; however, it has been suggested that endothelial dysfunction plays a role. Accumulating evidence indicates that bone marrow–derived endothelial progenitor cells (EPCs) contribute to neovascularization of ischemic tissue and endothelialization of denuded endothelium. Recent studies have shown that circulating bone marrow–derived immature cells, including $CD34^+$ cells, contribute to the maintenance of the vasculature, both as a pool of EPCs and as the source of growth/angiogenesis factors (1). We hypothesized that circulating $CD34^+$ cells might be associated with LV dysfunction in patients with type 2 diabetes. Therefore, we studied the correlation between circulating $CD34^+$ cell levels and plasma brain natriuretic peptide (BNP) levels, an LV dysfunction marker, in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

The institutional review board of the National Cardiovascular Center approved this study, and all subjects provided informed consent. We examined 26 patients with type 2 diabetes (12 men and 14 women, duration of diabetes 16.1 ± 10.7 years) who were over 60 years of age (70.5 ± 6.4 years). Statin was given to nine subjects. ACE inhibitor or angiotensin receptor blocker was given to nine subjects, and thiazolidinedione was given to two subjects. Subjects were excluded from the study if they had known cardiovascular disease or chronic renal failure (defined as serum creatinine ≥180 μmol/l). No study subject showed hypokinesis by echocardiography or electrocardiogram change, indicating myocardial ischemia. Systolic (SBP) and diastolic (DBP) blood pressure and anthropometric parameters were determined. Blood samples were taken after 12-h fasting to measure circulating $CD34^+$ cells, plasma BNP, fasting plasma glucose (FPG), and A1C. Circulating $CD34^+$ cells were quantified by flow cytometry according to the manufacturer's protocol (ProCOUNT; Becton Dickinson Biosciences) as previously reported (2). BNP was quantified by enzyme immunoassay (Tohso, Tokyo, Japan). We further examined LV fractional shortening (LVFS), LV mass index (LVMI) (3), and peak flow velocity of the early filling wave (E), the late filling wave (A), and the E/A-wave ratio (E/A) by echocardiography. All echocardiograms were performed by several expert physicians who were blinded to $CD34^+$ cell level.

All statistical analyses were performed using JMP version 5.1.1 software (SAS Institute). Data are expressed as means ± SD. Comparisons of number of $CD34^+$ cells by sex were made using the two-tailed unpaired *t* test. Correlations between number of $CD34^+$ cells and clinical parameters were assessed by univariate liner regression analysis and multiple regression analysis. LVMI and plasma BNP concentrations were analyzed after logarithmic transformation.

RESULTS

FPG levels, A1C levels, and BMIs in the study subjects were measured to be 9.5 ± 2.6 mmol/l, 9.2 ± 1.8%, and 26.4 ± 4.3 kg/m^2, respectively. A total of 88% of the patients had hypertension (SBP 142 ± 18 mmHg, DBP 75.7 ± 13.5 mmHg). Plasma BNP levels were measured to be 95 ± 319 pg/ml. Although it has been reported that the level of BNP ≥100 pg/ml has a sensitivity of 90% of diagnosing congestive heart failure (CHF) in patients with CHF symptoms (4), none of the subjects in this study, including subjects with ≥100 pg/ml of BNP, showed symptoms of CHF. The level of circulating $CD34^+$ cells was measured to be 0.76 ± 0.39 cells/μl, and there was no significant difference between sexes. The range of LVMI was 73.3–340.2, and 11 subjects applied to the definition of LV hypertrophy (LVMI ≤131 in men and ≤100 in women) (3).

Plasma BNP levels had a significant inverse correlation with the number of circulating $CD34^+$ cells (Fig. 1*A*), whereas FPG, A1C, BMI, SBP, DBP, and age showed no significant correlations. There was a significant correlation between the number of circulating $CD34^+$ cells and LVMI by echocardiography (Fig. 1*B*). LVFS and E/A were not associated with circulating $CD34^+$ cell numbers (LVFS $r = -0.07$, $P = 0.72$; E/A $r = -0.11$, $P = 0.59$). There was also a significant correlation between BNP levels and LVMI ($r = 0.59$, $P = 0.001$).

In multiple regression analysis, the

From the [1]Department of Atherosclerosis, National Cardiovascular Center, Suita, Japan; the [2]Department of Hematology, Osaka Minami National Medical Center, Osaka, Japan; and the [3]Department of Cerebrovascular Disease, National Cardiovascular Center, Suita, Japan.

Address correspondence and reprint requests to Hisashi Makino, MD, Atherosclerosis, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan. E-mail: makinoh@hsp.ncvc.go.jp.

Received for publication 14 June 2007 and accepted in revised form 13 October 2007.

Published ahead of print at http://care.diabetesjournals.org on 24 October 2007. DOI: 10.2337/dc07-1125.

Abbreviations: BNP, brain natriuretic peptide; CHF, congestive heart failure; DBP, diastolic blood pressure; EPC, endothelial progenitor cell; FPG, fasting plasma glucose; LV, left ventricular; LVFS, LV fractional shortening; LVMI, LV mass index; SBP, systolic blood pressure.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

© 2008 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

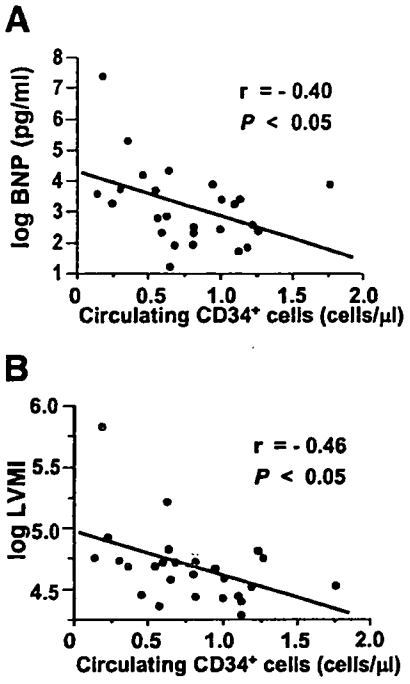

Figure 1—*Correlation between CD34$^+$ cell numbers and plasma BNP levels (A) and correlation between CD34$^+$ cell numbers and LVMI (B) in type 2 diabetic patients* (n = 26).

level of CD34$^+$ cells was an independent correlate of both BNP ($\beta = -1.64$, $P = 0.017$) and LVMI ($\beta = -0.337$, $P = 0.031$) in the model including age, A1C, SBP, BMI, and medication (ACE inhibitor/angiotensin receptor blocker, statin, and thiazolidinedione).

CONCLUSIONS — In this study, circulating CD34$^+$ cell number was found to significantly correlate with plasma BNP level, a marker of LV dysfunction. To the best of our knowledge, this is the first report that circulating bone marrow–derived cells are associated with diabetic LV abnormality. Circulating CD34$^+$ cell numbers also significantly correlated with LVMI, whereas they did not correlate with LVFS (an LV systolic function marker) or E/A (an LV diastolic function marker). LV hypertrophy is a well-known predictor of cardiovascular events independent of coronary artery disease. The Framingham Heart Study identified an association between diabetes and increased LV wall thickness and mass (5). Although the precise mechanisms underlying the association between diabetes and LV hypertrophy remain unknown, our results suggest that reduced circulating CD34$^+$ cell numbers may be involved in the progression of LV hypertrophy in diabetic patients. However, further investigations are necessary to demonstrate this hypothesis.

We measured the level of CD34$^+$ cells in this study but not the levels of circulating CD34$^+$/kinase insert domain receptor (KDR)$^+$ cells that are regarded as EPCs. Circulating CD34$^+$ cell levels are associated with ischemic stroke (6), and administration of CD34$^+$ cells ameliorates cerebral ischemia in mice (7). This indicates that CD34$^+$ cells may be involved in cardiovascular disease. Indeed, another recent report indicated that levels of circulating CD34$^+$ cells are more strongly correlated with cardiovascular risk than levels of EPCs (8). Therefore, our results suggest that measurement of CD34$^+$ cells may provide an indicator for diabetic LV hypertrophy.

Our study had several limitations. First, the study was performed only by cross-sectional analysis; therefore, a prospective study is needed to clarify whether circulating CD34$^+$ cell numbers predict LV injury in diabetic patients. Second, although systemic blood pressure did not significantly associate with CD34$^+$ cell numbers, further investigation of normotensive diabetic patients is needed to exclude the possible effects of hypertension on circulating CD34$^+$ cell numbers, as most of the subjects in this study were hypertensive. Despite this caveat, these results may be of practical use in elderly patients with type 2 diabetes, as hypertension is a very common comorbid condition in this population.

In conclusion, reduced circulating CD34$^+$ cell numbers are significantly associated with plasma BNP concentration and LVMI in elderly patients with type 2 diabetes. These results suggest that decreased circulating CD34$^+$ cells may be involved in LV hypertrophy and that measurement of circulating CD34$^+$ cell numbers may be useful for the identification of diabetic patients at high risk of LV injury.

References

1. Majka M, Janowska-Wieczorek A, Ratajczak J, Ehrenman K, Pietrzkowski Z, Kowalska MA, Gewirtz AM, Emerson SG, Ratajczak MZ: Numerous growth factors, cytokines, and chemokines are secreted by human CD34(+) cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. *Blood* 97:3075–3085, 2001
2. Kikuchi-Taura A, Soma T, Matsuyama T, Stern DM, Taguchi A: A new protocol for quantifying CD34(+) cells in peripheral blood of patients with cardiovascular disease. *Tex Heart Inst J* 33:427–429, 2006
3. Devereux RB, Reichek N: Echocardiographic determination of left ventricular mass in man: anatomic validation of the method. *Circulation* 55:613–618, 1977
4. McCullough PA, Nowak RM, McCord J, Hollander JE, Herrmann HC, Steg PG, Duc P, Westheim A, Omland T, Knudsen CW, Storrow AB, Abraham WT, Lamba S, Wu AHB, Perez A, Clopton P, Krishnaswamy P, Kazanegra R, Maisel AS, BNP multinational study investigators: B-type natriuretic peptide and clinical judgment in emergency diagnosis of heart failure: analysis from Breathing Not Properly (BNP) Multinational Study. *Circulation* 106: 416–422, 2002
5. Galderisi M, Anderson KM, Wilson PW, Levy D: Echocardiographic evidence for existence of a distinct diabetic cardiomyopathy (the Framingham Heart Study). *Am J Cardiol* 68:85–89, 1991
6. Taguchi A, Matsuyama T, Moriwaki H, Hayashi T, Hayashida K, Nagatsuka K, Todo K, Mori K, Stern DM, Soma T, Naritomi H: Circulating CD34-positive cells provide an index of cerebrovascular function. *Circulation* 109:2972–2975, 2004
7. Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Stern DM, Naritomi H, Matsuyama T: Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J Clin Invest* 114:330–338, 2004
8. Fadini GP, de Kreutzenberg SV, Coracina A, Baesso I, Agostini C, Tiengo A, Avogaro A: Circulating CD34+ cells, metabolic syndrome, and cardiovascular risk. *Eur Heart J* 27:2247–2255, 2006

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

WATARU SHIMIZU, M.D., PH.D.,* KIYOTAKA MATSUO, M.D., PH.D.,† YOSHIHIRO KOKUBO, M.D., PH.D.,‡ KAZUHIRO SATOMI, M.D., PH.D.,* TAKASHI KURITA, M.D., PH.D.,* TAKASHI NODA, M.D., PH.D.,* NORITOSHI NAGAYA, M.D., PH.D.,* KAZUHIRO SUYAMA, M.D., PH.D.,* NAOHIKO AIHARA, M.D.,* SHIRO KAMAKURA, M.D., PH.D.,* NOZOMU INAMOTO, M.D., PH.D.,‡ MASAZUMI AKAHOSHI, M.D., PH.D.,¶ and HITONOBU TOMOIKE, M.D., PH.D.*

From the *Division of Cardiology, Department of Internal Medicine; ‡Department of Preventive Cardiology, National Cardiovascular Center, Suita, Japan; †Department of Cardiovascular Medicine, Nagasaki University Graduate School of Medicine; and ¶Department of Clinical Studies, Radiation Effects Research Foundation, Nagasaki, Japan

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

Dr. W. Shimizu was supported by the Hoansha Research Foundation, Japan Research Foundation for Clinical Pharmacology, Ministry of Education, Culture, Sports, Science, and Technology Leading Project for Biosimulation, and health sciences research grants (H18—Research on Human Genome—002) from the Ministry of Health, Labor, and Welfare, Japan. Drs. Y. Kokubo, N. Inamoto, and H. Tomoike were supported by the Program for the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research of Japan (MPJ-3).

Address for correspondence: Wataru Shimizu, M.D., Ph.D., Division of Cardiology, Department of Internal Medicine, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka, 565-8565 Japan. Fax: 81-6-6872-7486; E-mail: wshimizu@hsp.ncvc.go.jp

Manuscript received 11 September 2006; Revised manuscript received 2 November 2006; Accepted for publication 15 November 2006.

doi: 10.1111/j.1540-8167.2006.00743.x

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.

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.

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.

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

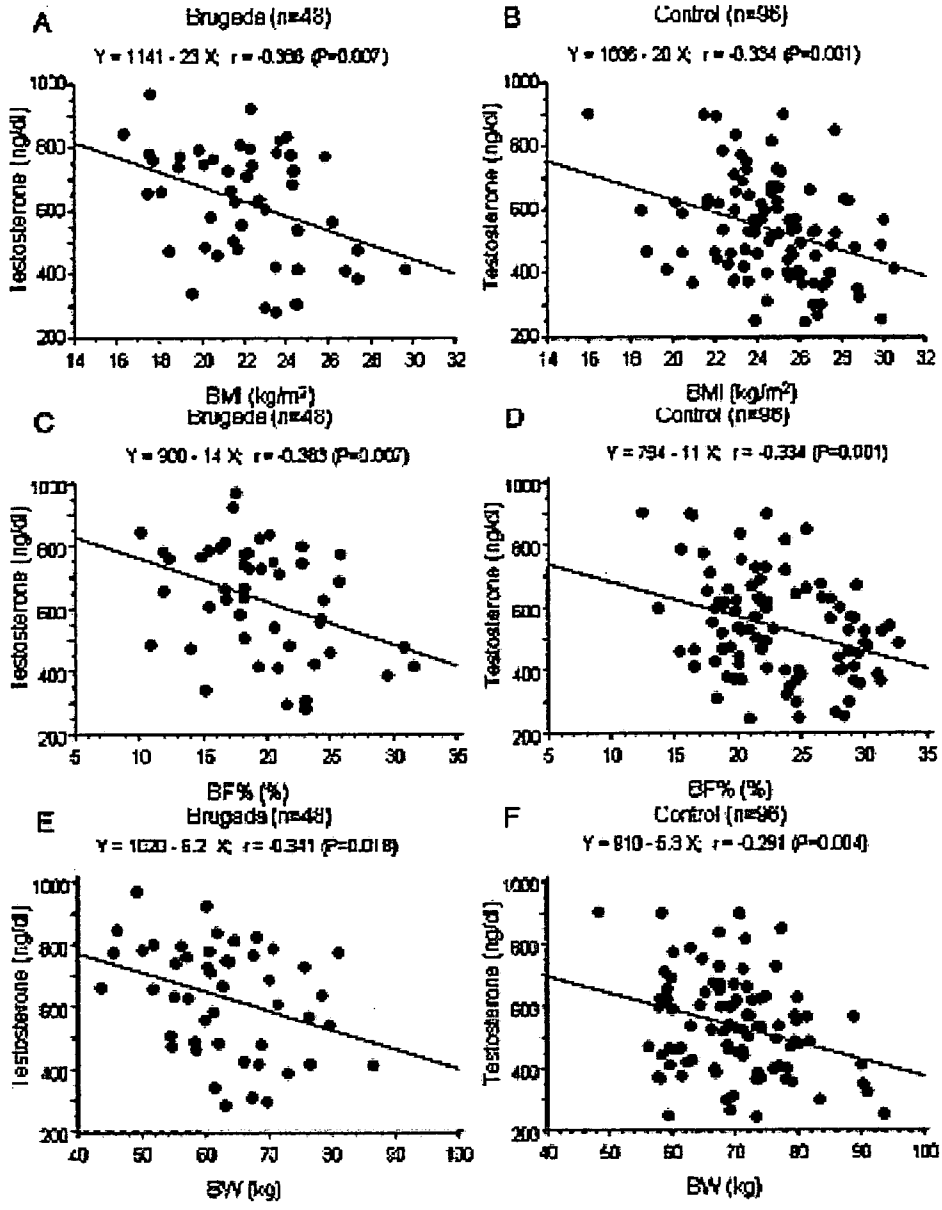

Figure 1. *Correlation between testosterone level and visceral fat parameters; body mass index (BMI) (A and B), body fat percentage (BF%) (C and D), and body weight (BW) (E and F) in the 48 Brugada males and the 96 age-matched control males. Testosterone level was inversely correlated with the BMI, BF%,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

Acknowledgment: We gratefully acknowledge the helpful suggestions of Yoshihiro Miyamoto, Laboratory of Molecular Genetics, National Cardiovascular Center, Suita, Japan.

References

1. Brugada P, Brugada J: Right bundle branch block, persistent ST segment elevation and sudden cardiac death: A distinct clinical and

electrocardiographic syndrome: A multicenter report. J Am Coll Cardiol 1992;20:1391-1396.
2. Brugada J, Brugada R, Brugada P: Determinants of sudden cardiac death in individuals with the electrocardiographic pattern of Brugada syndrome and no previous cardiac arrest. Circulation 2003;108:3092-3096.
3. Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Giordano U, Bloise R, Giustetto C, De Nardis R, Grillo M, Ronchetti E, Faggiano G, Nastoli J: Natural history of Brugada syndrome: Insights for risk stratification and management. Circulation 2002;105:1342-1347.
4. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, Gussak I, Lemarec H, Nademanee K, Perez Riera AR, Shimizu W, Schulze-Bahr E, Tan H, Wilde A: Brugada syndrome. Report of the second consensus conference. Endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. Circulation 2005;111:659-670.
5. Eckardt L, Probst V, Smits JP, Bahr ES, Wolpert C, Schimpf R, Wichter T, Boisseau P, Heinecke A, Breithardt G, Borggrefe M, Lemarec H, Bocker D, Wilde AA: Long-term prognosis of individuals with right precordial ST-segment-elevation brugada syndrome. Circulation 2005;111:257-263.
6. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, Potenza D, Moya A, Borggrefe M, Breithardt G, Ortiz-Lopez R, Wang Z, Antzelevitch C, O'Brien RE, Schulze-Bahr E, Keating MT, Towbin JA, Wang Q: Genetic basis and molecular mechanisms for idiopathic ventricular fibrillation. Nature 1998;392:293-296.
7. Shimizu W, Aiba T, Kamakura S: Mechanisms of disease: Current understanding and future challenges in Brugada syndrome. Nat Clin Pract Cardiovasc Med 2005;2:408-414.
8. Shimizu W: Editorial comment, gender difference and drug challenge in Brugada syndrome. J Cardiovasc Electrophysiol 2004;15:70-71.
9. Di Diego JM, Cordeiro JM, Goodrow RJ, Fish JM, Zygmunt AC, Perez GJ, Scornik FS, Antzelevitch C: Ionic and cellular basis for the predominance of the Brugada syndrome phenotype in males. Circulation 2002;106:2004-2011.
10. Shuba YM, Degtiar VE, Osipenko VN, Naidenov VG, Woosley RL: Testosterone-mediated modulation of HERG blockade by proarrhythmic agents. Biochem Pharmacol 2001;62:41–49.
11. Liu XK, Katchman A, Whitfield BH, Wan G, Janowski EM, Woosley RL, Ebert SN: In vivo androgen treatment shortens the QT interval and increases the densities of inward and delayed rectifier potassium currents in orchiectomized male rabbits. Cardiovasc Res 2003;57:28-36.
12. Bai CX, Kurokawa J, Tamagawa M, Nakaya H, Furukawa T: Nontranscriptional regulation of cardiac repolarization currents by testosterone. Circulation 2005;112:1701-1710.
13. Glass AR, Swerdloff RS, Bray GA, Dahms WT, Atkinson RL: Low serum testosterone and sex-hormone-binding-globulin in massively obese men. J Clin Endocrinol Metab 1977;45:1211-1219.
14. Marin P, Holmang S, Jonsson L, Sjostrom L, Kvist H, Holm G, Lindstedt G, Bjorntorp P: The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. Int J Obes Relat Metab Disord 1992;16:991-997.
15. Tsai EC, Boyko EJ, Leonetti DL, Fujimoto WY: Low serum testosterone level as a predictor of increased visceral fat in Japanese-American men. Int J Obes Relat Metab Disord 2000;24:485-491.
16. Matsuo K, Akahoshi M, Nakashima E, Seto S, Yano K: Clinical characteristics of subjects with the Brugada-type electrocardiogram: A case control study. J Cardiovasc Electrophysiol 2004;15:653-657.
17. Wilde AA, Antzelevitch C, Borggrefe M, Brugada J, Brugada R, Brugada P, Corrado D, Hauer RN, Kass RS, Nademanee K, Priori SG, Towbin JA: Study group on the molecular basis of arrhythmias of the European Society of Cardiology. Proposed diagnostic criteria for the Brugada syndrome: Consensus report. Circulation 2002;106:2514-2519.
18. Shimizu W, Matsuo K, Takagi M, Tanabe Y, Aiba T, Taguchi A, Suyama K, Kurita T, Aihara N, Kamakura S: Body surface distribution and response to drugs of ST segment elevation in the Brugada syndrome: Clinical implication of 87-leads body surface potential mapping and its application to 12-leads electrocardiograms. J Cardiovasc Electrophysiol 2000;11:396-404.
19. Kanda M, Shimizu W, Matsuo K, Nagaya N, Taguchi A, Suyama K, Kurita T, Aihara N, Kamakura S: Electrophysiologic characteristics and implication of induced ventricular fibrillation in symptomatic patients with Brugada syndrome. J Am Coll Cardiol 2002;39:1799-1805.
20. Yokokawa M, Takaki H, Noda T, Satomi K, Suyama K, Kurita T, Kamakura S, Shimizu W: Spatial distribution of repolarization and depolarization abnormalities evaluated by body surface potential mapping in patients with Brugada syndrome. Pacing Clin Electrophysiol 2006;29:1112-1121.
21. Matsuo K, Akahoshi M, Seto S, Yano K: Disappearance of the Brugada-type electrocardiogram after surgical castration: A role for testosterone and an explanation for the male preponderance. Pacing Clin Electrophysiol 2003;26:1551-1553.
22. Litovsky SH, Antzelevitch C: Transient outward current prominent in canine ventricular epicardium but not endocardium. Circ Res 1988;62:116-126.
23. Furukawa T, Myerburg RJ, Furukawa N, Bassett AL, Kimura S: Differences in transient outward currents of feline endocardial and epicardial myocytes. Circ Res 1990;67:1287-1291.
24. Yan GX, Antzelevitch C: Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST segment elevation. Circulation 1999;100:1660-1666.
25. Mok NS, Priori SG, Napolitano C, Chan NY, Chahine M, Baroudi G: A newly characterized SCN5A mutation underlying Brugada syndrome unmasked by hyperthermia. J Cardiovasc Electrophysiol 2003;14:407-411.
26. Song M, Helguera G, Eghbali M, Zhu N, Zarei MM, Olcese R, Toro L, Stefani E: Remodeling of Kv4.3 potassium channel gene expression under the control of sex hormones. J Biol Chem 2001;276:31883-31890.
27. Wickenden AD, Kaprielian R, You XM, Backx PH: The thyroid hormone analog DITPA restores I(to) in rats after myocardial infarction. Am J Physiol Heart Circ Physiol 2000;278:H1105-H1116.
28. Sunagawa M, Yamakawa M, Shimabukuro M, Higa N, Takasu N, Kosugi T: Electrophysiologic characteristics of atrial myocytes in levothyroxine-treated rats. Thyroid 2005;15:3-11.
29. Shimizu W: Acquired forms of Brugada syndrome (Antzelevitch C review). In: *The Brugada Syndrome: From Bench to Bedside*, Chapter 14. Blackwell Futura, UK, 2004;166-177.
30. Myers GB: Other QRS-T patterns that may be mistaken for myocardial infarction. IV. Alterations in blood potassium; myocardial ischemia; subepicardial myocarditis; distortion associated with arrhythmias. Circulation 1950;2:75-93.
31. Collins S, Kuhn CM, Petro AE, Swick AG, Chrunyk BA, Surwit RS: Role of leptin in fat regulation. Nature 1996;380:677.
32. Nagaya N, Moriya J, Yasumura Y, Uematsu M, Ono F, Shimizu W, Ueno K, Kitakaze M, Miyatake K, Kangawa K: Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. Circulation 2004;110:3674-3679.

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

Miki Yokokawa, MD, Takashi Noda, MD, PhD, Hideo Okamura, MD, Kazuhiro Satomi, MD, PhD, Kazuhiro Suyama, MD, PhD, Takashi Kurita, MD, PhD, Naohiko Aihara, MD, Shiro Kamakura, MD, PhD, and Wataru Shimizu, MD, PhD*

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. © 2007 Elsevier Inc. All rights reserved. (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,

Division of Cardiology, Department of Internal Medicine, National Cardiovascular Center, Suita, Osaka, Japan. Manuscript received February 6, 2007; revised manuscript received and accepted March 15, 2007.

Dr. Shimizu was supported by the Uehara Memorial Foundation, Tokyo; the Hoansha Research Foundation, Osaka; Japan Research Foundation for Clinical Pharmacology, Tokyo; Ministry of Education, Culture, Sports, Science and Technology Leading Project for Biosimulation, Tokyo; and health sciences research grants (H18-Research on Human Genome-002) from the Ministry of Health, Labor and Welfare, Tokyo, Japan.

*Corresponding author: Tel: 81-6-6833-5012; fax: 81-6-6872-7486.
E-mail address: wshimizu@hsp.ncvc.go.jp (W. Shimizu).

0002-9149/07/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.amjcard.2007.03.078

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

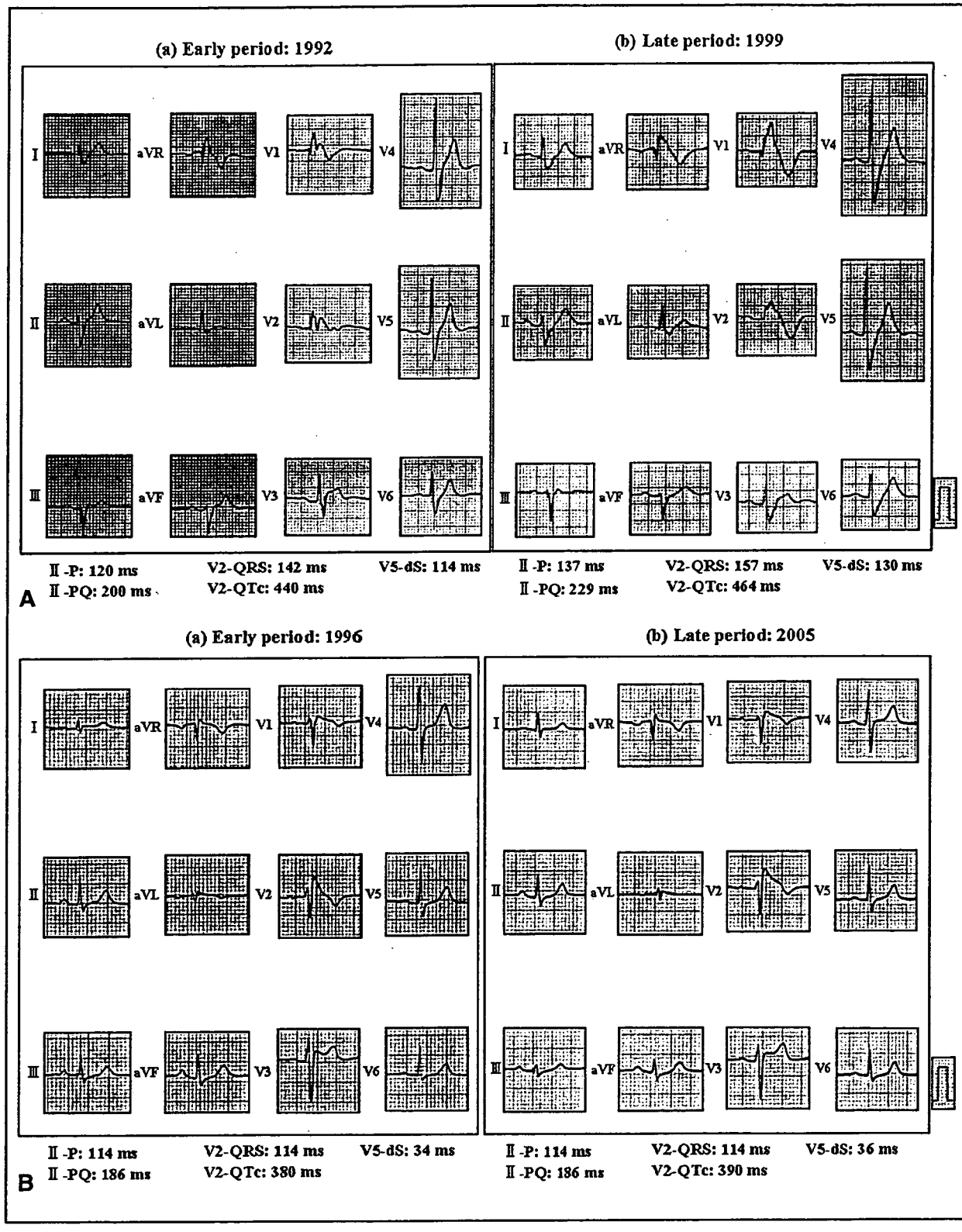

Figure 1. Standard 12-lead ECG at early and late periods during follow-up in representative cases of BS. *A*, in an *SCN5A*-positive proband (follow-up period, 7 years), the P-wave (lead II), QRS (lead V_2), and S-wave (lead V_5) durations and PQ interval (lead II) were prolonged even at the early period (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.

Table 3
Electrocardiographic parameters during follow-up period

ECG Parameter (leads)	Early Period			Late Period		
	SCN5A-Positive Group (n = 8)	SCN5A-Negative Group (n = 36)	p Value	SCN5A-Positive Group (n = 8)	SCN5A-Negative Group (n = 36)	p Value
Heart rate (beats/min)	66 ± 11	64 ± 10	0.924	60 ± 6	67 ± 12	0.194
P-wave duration (II) (ms)	137 ± 21	110 ± 12	<0.001	155 ± 19†	119 ± 16†	<0.001
PQ interval (II) (ms)	227 ± 31	179 ± 18	<0.001	257 ± 22*	190 ± 22†	<0.001
QRS duration (II) (ms)	125 ± 22	102 ± 18	<0.001	142 ± 41‡	111 ± 19‡	<0.001
QRS duration (V_2) (ms)	135 ± 15	110 ± 13	<0.001	157 ± 28*	115 ± 16	<0.001
QRS duration (V_5) (ms)	130 ± 28	101 ± 15	<0.001	147 ± 42	108 ± 17	<0.001
S-wave duration (II) (ms)	65 ± 38	35 ± 24	<0.001	77 ± 54	43 ± 26‡	<0.001
S-wave duration (V_5) (ms)	69 ± 40	37 ± 19	<0.001	78 ± 50	49 ± 17*	<0.001
S-wave amplitude (II) (mV)	0.37 ± 0.23	0.23 ± 0.24	0.005	0.43 ± 0.24	0.21 ± 0.17	<0.001
S-wave amplitude (V_5) (mV)	0.83 ± 0.47	0.34 ± 0.25	<0.001	0.88 ± 0.48	0.47 ± 0.27†	<0.001
QRS axis (°)	44 ± 81	49 ± 43	0.954	10 ± 76‡	43 ± 41	0.001
QTc interval (II) (ms)	409 ± 37	396 ± 28	0.535	432 ± 40	410 ± 34	0.164
QTc interval (V_2) (ms)	427 ± 51	392 ± 37	0.038	471 ± 38‡	405 ± 38	<0.001
QTc interval (V_5) (ms)	401 ± 43	389 ± 29	0.593	408 ± 39	398 ± 36	0.746
JTc interval (II) (ms)	279 ± 32	290 ± 30	0.554	292 ± 44	293 ± 34	0.100
JTc interval (V_2) (ms)	285 ± 39	279 ± 35	0.960	316 ± 42	283 ± 38	0.044
JTc interval (V_5) (ms)	265 ± 26	286 ± 30	0.108	262 ± 42	283 ± 32	0.105
STJ amplitude (V_2) (mV)	0.42 ± 0.19	0.29 ± 0.13	0.014	0.37 ± 0.23	0.24 ± 0.17	0.011
STJ40 amplitude (V_2) (mV)	0.38 ± 0.14	0.23 ± 0.12	<0.001	0.34 ± 0.17	0.21 ± 0.15	0.006

Data are presented as means ± SD.
* $p < 0.001$ versus early period.
† $p < 0.01$ versus early period.
‡ $p < 0.05$ versus early period.
ECG = electrocardiographic; JTc = corrected JT; QTc = corrected QT; STJ amplitude = ST amplitude at J point; STJ 40 amplitude = ST amplitude 40 ms after J point.

Table 4
Comparison of the change of electrocardiographic parameters during follow-up

Change in ECG Parameter (leads)	SCN5A-Positive Group (n = 8)	SCN5A-Negative Group (n = 36)	p Value
Heart rate (beats/min)	−7 ± 10	3 ± 13	0.046
P-wave duration (II) (ms)	19 ± 12	9 ± 13	0.077
PQ interval (II) (ms)	30 ± 22	11 ± 14	0.004
QRS duration (II) (ms)	17 ± 22	8 ± 15	0.163
QRS duration (V_2) (ms)	22 ± 20	6 ± 11	0.003
QRS duration (V_5) (ms)	17 ± 29	8 ± 14	0.161
S-wave duration (II) (ms)	12 ± 17	8 ± 13	0.423
S-wave duration (V_5) (ms)	9 ± 15	12 ± 14	0.604
S-wave amplitude (II) (mV)	0.06 ± 0.10	−0.02 ± 0.14	0.152
S-wave amplitude (V_5) (mV)	0.05 ± 0.27	0.13 ± 0.18	0.331
QRS axis (°)	−34 ± 55	−6 ± 16	0.010
QTc interval (II) (ms)	22 ± 32	15 ± 34	0.562
QTc interval (V_2) (ms)	44 ± 49	13 ± 40	0.064
QTc interval (V_5) (ms)	6 ± 37	9 ± 30	0.845
JTc interval (II) (ms)	13 ± 27	3 ± 28	0.339
JTc interval (V_2) (ms)	31 ± 48	5 ± 38	0.094
JTc interval (V_5) (ms)	−3 ± 29	−3 ± 29	0.990
STJ amplitude (V_2) (mV)	−0.05 ± 0.18	−0.05 ± 0.12	0.949
STJ40 amplitude (V_2) (mV)	−0.04 ± 0.16	−0.02 ± 0.11	0.642

Abbreviations as in Table 3.

SCN5A mutation.[5,11] Smits et al[12] observed significantly longer PQ and HV intervals at baseline and a larger increase in PQ and QRS intervals after administration of sodium channel blockers in patients with BS with SCN5A mutations than in those without SCN5A mutations. Age-dependent variability in the conduction parameters was evidenced in SCN5A-positive patients with BS.[13,15] Moreover, this concept has been mechanistically investigated in vivo in heterozygous SCN5A mice, which showed progressive impairment with aging of atrial and

ventricular conduction associated with myocardial rearrangements and fibrosis.[16] Meregalli et al[17] showed prolongation of S-wave duration in leads II and III after administration of sodium channel blockers. Their group suggested that these electrocardiographic signs included reciprocal changes in the inferior leads, mirroring the conduction slowing in the RVOT,[17,18] which may progress with aging and relate to the pathogenesis of BS. In the present study, the P-wave, QRS, S-wave durations, and PQ intervals were all significantly longer, and the S-wave amplitude was significantly deeper in the SCN5A-positive group than in the SCN5A-negative group. In addition, the PQ interval and QRS duration in lead V_2 were more markedly prolonged, and the QRS axis deviated more to the left with aging in the SCN5A-positive group than in the SCN5A-negative group during the follow-up period. The results of previous clinical studies and the present study suggest that progressive depolarization abnormalities (i.e., conduction slowing) with aging may play a key role in the pathogenesis of BS.

It has been argued recently that arrhythmic events may occur when a sufficient degree of cell damage has been reached as a result of the severity of ion channel protein mutation. Frustaci et al[19] showed that myocyte apoptosis at the right and left ventricular myocardium was significantly higher in patients with BS with SCN5A mutations than in control subjects on histologic study. They suggested that abnormalities in the function of sodium channels may lead to cellular damage because intracellular sodium homeostasis has a relevant role in myocellular function.[19] Experimentally, Aiba et al[20] used a high-resolution optical mapping system in a pharmacologic BS model and demonstrated that depolarization abnormalities (i.e., conduction slowing) is required for the maintenance of VF in BS, although the initiating premature beats were a result of a phase 2 reentry mechanism. These histologic and experimental studies also support that progressive conduction abnormalities with aging may explain why an initial VF episode appears at middle to older ages, usually 40 to 50 years, in BS. It is generally accepted that SCN5A mutation is not associated with a higher risk of cardiac events, suggesting that genetic analysis is a useful diagnostic parameter but is not helpful for risk stratification.[7] Similarly, in the present study, the presence of SCN5A mutation did not predict subsequent arrhythmic events (Table 2). Most clinical studies have reported that induction of VF by programmed electrical stimulation did not predict the clinical outcome or clinical severity in patients with BS.[6,21,22] If the progressive conduction slowing with aging often observed in patients with BS, especially SCN5A-positve patients, are really linked to VF appearance, conduction parameters, such as QRS widening, late potentials, or inducibility of VF, may still have a potential to predict new or subsequent cardiac events.[23] A much larger patient population is required to make a definitive conclusion regarding the predictive value of SCN5A mutation and the conduction parameters for cardiac events.

Several clinical studies have suggested a localized QT prolongation, a repolarization parameter, in the right precordial leads (mainly lead V_2) in patients with BS.[24,25] Castro Hevia et al[25] have suggested that a QTc >460 ms in lead V_2 was a significant risk factor for subsequent cardiac events. We recently used 87-lead body surface ECGs and reported that a corrected recovery time, another repolarization parameter, was significantly longer in the right precordial body surface ECGs, reflecting the potentials of the RVOT, than in other body surface ECGs.[26] Similarly, in the present study, the longest QTc interval was observed in lead V_2 in most patients with BS with SCN5A mutation, who usually also had a coved-type ST-segment elevation and a terminal negative T wave. The fact that the QTc interval in lead V_2 was significantly longer in the SCN5A-positive patients than in the SCN5A-negative patients at the early and late periods can be explained by more frequent and higher coved-type ST-segment elevation with a terminal negative T wave in the SCN5A-positive patients. The QTc interval in lead V_2 was significantly prolonged from the early period to the late period in the SCN5A-positive patients; however, the JTc interval in lead V_2 did not change from the early period to the late period, suggesting that the significant QTc prolongation in lead V_2 with aging occurred mainly as a result of a significant prolongation of the QRS duration in lead V_2.

There are several limitations to the present study. First, because a small number of patients with BS with SCN5A mutation could be included in a single-center study, a larger number of patients with SCN5A mutation will be required to make a definitive conclusion. Second, the study population included 44 Brugada probands who could be prospectively followed up for average of 10 ± 5 years in our hospital. Therefore, the probands represent a severely affected population, but not a consecutively referred population. Third, Veltmann et al[27] recently reported the prevalence of fluctuations between diagnostic and nondiagnostic ECGs in patients with BS, which may influence the measurement of some electrocardiographic parameters, especially QT, JT interval, and ST amplitude, and should be taken into account. However, the influence of the fluctuations on depolarization parameters such as QRS duration is expected to be less pronounced.

1. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome: a multicenter report. *J Am Coll Cardiol* 1992;20:1391–1396.
2. Brugada J, Brugada P. Further characterization of the syndrome of right bundle branch block, ST segment elevation, and sudden cardiac death. *J Cardiovasc Electrophysiol* 1997;8:325–331.
3. Brugada J, Brugada R, Brugada P. Right bundle-branch block and ST-segment elevation in V1 through V3. A marker for sudden death in patients without demonstrable structural heart disease. *Circulation* 1998;97:457–460.
4. Wilde AA, Antzelevitch C, Borggrefe M, Brugada J, Brugada R, Brugada P, Corrado D, Hauer RN, Kass RS, Nademanee K, Priori SG, Towbin JA. Study Group on the Molecular Basis of Arrhythmias of the European Society of Cardiology. Proposed diagnostic criteria for the Brugada syndrome: consensus report. *Circulation* 2002;106:2514–2519.
5. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, Gussak I, Lemarec H, Nademanee K, Perez Riera AR, et al. Brugada syndrome: report of the second consensus conference: endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. *Circulation* 2005;111:659–670.
6. Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Giordano U, Bloise R, Giustetto C, De Nardis R, Grillo M, Ronchetti E, Faggiano G, Nastoli J. Natural history of Brugada syndrome:

insights for risk stratification and management. *Circulation* 2002;105:1342–1347.
7. Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Brignole M, Giordano U, Giovannini T, Menozzi C, Bloise R, et al. Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: a prospective evaluation of 52 families. *Circulation* 2000;102:2509–2515.
8. Shimizu W, Aiba T, Kamakura S. Mechanisms of diseases: current understanding and future challenges in Brugada syndrome. *Nat Clin Pract Cardiovasc Med* 2005;2:408–414.
9. Nademanee K, Veerakul G, Nimmannit S, Chaowakul V, Bhuripanyo K, Likittanasombat K, Tunsanga K, Kuasirikul S, Malasit P, Tansupasawadikul S, Tatsanavivat P. Arrhythmogenic marker for the sudden unexplained death syndrome in Thai men. *Circulation* 1997;96:2595–2600.
10. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, Potenza D, Moya A, Borggrefe M, Breithardt G, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature* 1998;392:293–296.
11. Alings M, Wilde A. "Brugada" syndrome: clinical data and suggested pathophysiological mechanism. *Circulation* 1999;99:666–673.
12. Smits JP, Eckardt L, Probst V, Bezzina CR, Schott JJ, Remme CA, Haverkamp W, Breithardt G, Escande D, Schulze-Bahr E, LeMarec H, Wilde AA. Genotype-phenotype relationship in Brugada syndrome: electrocardiographic features differentiate SCN5A-related patients from non- SCN5A-related patients. *J Am Coll Cardiol* 2002;40:350–356.
13. Probst V, Allouis M, Sacher F, Pattier S, Babuty D, Mabo P, Mansourati J, Victor J, Nguyen JM, Schott JJ, et al. Progressive cardiac conduction defect is the prevailing phenotype in carriers of a Brugada syndrome *SCN5A* mutation. *J Cardiovasc Electrophysiol* 2006;17:270–275.
14. Bezzina CR, Shimizu W, Yang P, Koopmann TT, Tanck MWT, Miyamoto Y, Kamakura S, Roden DM, Wilde AAM. A common sodium channel promoter haplotype in Asian subjects underlies variability in cardiac conduction. *Circulation* 2006;113:338–344.
15. Probst V, Kyndt F, Potet F, Trochu JN, Mialet G, Demolombe S, Schott JJ, Baro I, Escande D, Le Marec H. Haploinsufficiency in combination with aging causes *SCN5A*-linked hereditary Lenegre disease. *J Am Coll Cardiol* 2003;41:643–652.
16. Royer A, van Veen TA, Le Bouter S, Marionneau C, Griol-Charhbili V, Leoni AL, Steenman M, van Rijen HV, Demolombe S, Goddard CA, et al. Mouse model of SCN5A-linked hereditary Lenegre's disease: age-related conduction slowing and myocardial fibrosis. *Circulation* 2005;11:1738–1746.
17. Meregalli PG, Ruijter JM, Hofman N, Bezzina CR, Wilde AA, Tan HL. Diagnostic value or flecainide test in unmasking *SCN5A*-related Brugada syndrome. *J Cardiovasc Electrophysiol* 2006;17:857–864.
18. Tukkie R, Sogaard P, Vleugels J, de Groot IK, Wilde AA, Tan HL. Delay in right ventricular activation contributes to Brugada syndrome. *Circulation* 2004;109:1272–1277.
19. Frustaci A, Priori SG, Pieroni M, Chimenti C, Napolitano C, Rivolta I, Sanna T, Bellocci F, Russo MA. Cardiac histological substrate in patients with clinical phenotype of Brugada syndrome. *Circulation* 2005;112:3680–3687.
20. Aiba T, Shimizu W, Hidaka I, Uemura K, Noda T, Zheng C, Kamiya A, Inagaki M, Sugimachi M, Sunagawa K. Cellular basis for trigger and maintenance of ventricular fibrillation in the Brugada syndrome model: High resolution optical mapping study. *J Am Coll Cardiol* 2006;47:2074–2085.
21. Kanda M, Shimizu W, Matsuo K, Nagaya N, Taguchi A, Suyama K, Kurita T, Aihara N, Kamakura S. Electrophysiologic characteristics and implications of induced ventricular fibrillation in symptomatic patients with Brugada syndrome. *J Am Coll Cardiol* 2002;39:1799–1805.
22. Eckardt L, Probst V, Smits JP, Bahr ES, Wolpert C, Schimpf R, Wichter T, Boisseau P, Heinecke A, Breithardt G, et al. Long-term prognosis of individuals with right precordial ST-segment-elevation Brugada syndrome. *Circulation* 2005;111:257–263.
23. Shimizu W. Does an overlap syndrome really exist between Brugada syndrome and progressive cardiac conduction defect (Lenegre syndrome)? *J Cardiovasc Electrophysiol* 2006;17:276–278.
24. Pitzalis MV, Anaclerio M, Iacoviello M, Forleo C, Guida P, Troccoli R, Massari F, Mastropasqua F, Sorrentino S, Manghisi A, Rizzon P. QT-interval prolongation in right precordial leads: an additional electrocardiographic hallmark of Brugada syndrome. *J Am Coll Cardiol* 2003;42:1632–1637.
25. Castro Hevia J, Antzelevitch C, Tornes Barzaga F, Dorantes Sanchez M, Dorticos Balea F, Zayas Molina R, Quinones Perez MA, Fayad Rodriguez Y. Tpeak-Tend and Tpeak-Tend dispersion as risk factors for ventricular tachycardia/ventricular fibrillation in patients with the Brugada syndrome. *J Am Coll Cardiol* 2006;47:1828–1834.
26. Yokokawa M, Takaki H, Noda T, Satomi K, Suyama K, Kurita T, Kamakura S, Shimizu W. Spatial distribution of repolarization and depolarization abnormalities evaluated by body surface potential mapping in patients with Brugada syndrome. *Pacing Clin Electrophysiol* 2006;29:1112–1121.
27. Veltmann C, Schimpf R, Echternach C, Eckardt L, Kuschyk J, Streitner F, Spehl S, Borggrefe M, Wolpert C. A prospective study on spontaneous fluctuations between diagnostic and non-diagnostic ECGs in Brugada syndrome: implications for correct phenotyping and risk stratification. *Eur Heart J* 2006;27:2544–2552.