SAGE-Hindawi Access to Research
International Journal of Hypertension
Volume 2010, Article ID 196307, 7 pages
doi:10.4061/2010/196307

Research Article

Promoter Polymorphism of RGS2 Gene Is Associated with Change of Blood Pressure in Subjects with Antihypertensive Treatment: The Azelnidipine and Temocapril in Hypertensive Patients with Type 2 Diabetes Study

Ken Sugimoto,[1] Tomohiro Katsuya,[2] Kei Kamide,[1] Tomomi Fujisawa,[1] Izumi Shimaoka,[1] Mitsuru Ohishi,[1] Ryuichi Morishita,[2] Toshio Ogihara,[3] and Hiromi Rakugi[1]

[1] *Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, 2-2 B6, Yamada-oka, Suita, Osaka 565-0871, Japan*
[2] *Department of Clinical Gene Therapy, Osaka University Graduate School of Medicine, Osaka 565-0871, Japan*
[3] *Osaka General Medical Center, Osaka Prefectural Hospital Organization, Osaka, Japan*

Correspondence should be addressed to Kei Kamide, kamide@geriat.med.osaka-u.ac.jp

Received 3 July 2010; Accepted 23 July 2010

Academic Editor: Stephen B. Harrap

Copyright © 2010 Ken Sugimoto et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

We performed a prospective study to examine the genetic effect on the response to a calcium (Ca) channel blocker, azelnidipine and an ACE inhibitor, temocapril treatment in patients with hypertension, as a part of the prior clinical trial, the Azelnidipine and Temocapril in Hypertensive Patients with Type 2 Diabetes Study (ATTEST). *Methods and Results.* All subjects who gave informed consent for genetic research were divided into two groups: the subjects treated with azelnidipine or temocapril, for 52 weeks. We selected 18 susceptible genes for hypertension and determined their genotypes using TaqMan PCR method. RNA samples were extracted from peripheral blood, and quantitative real time PCR for all genes was performed using TaqMan method. One of the polymorphisms of the RGS2 gene was extracted as being able to influence the effect of these treatments to reduce BP. At eight weeks, BP change showed a significant interaction between the A-638G polymorphism of Regulator of G protein signaling-2 (RGS2) gene and treatment with azelnidipine or temocapril. There was no gene whose expression was associated with BP phenotypes or the polymorphisms of each gene. *Conclusions.* A-638G polymorphism of the RGS-2 gene could be a predictive factor for therapeutic performance of Ca channel blockers.

1. Introduction

Genetic approaches may provide a powerful tool for clarifying the pathogenesis of essential hypertension. Many reports have demonstrated that gene polymorphisms of the renin-angiotensin system (RAS) are associated with hypertension. There have been some reliable reports about susceptible genes for hypertension including the results from "The Millennium Genome Project for Hypertension in Japan (2000~2005) [1, 2]; however, no convincing gene has yet been detected. Some of the genes regulating blood pressure might also be related to the response to antihypertensive medication [3, 4]. Indeed, we and other collaborators have investigated several susceptible genes related to hypertension [5–15], including genes of not only the renin-angiotensin system and sodium handling but also insulin resistance, oxidative stress, and sympathetic nervous system (described in the Section 2); however, the genes involved in the response to antihypertensive medication have not yet been identified. In addition, exhaustive gene expression analysis (transcriptome analysis) for lifestyle-related diseases has not been performed thus far. We performed a large collaboration with the study group led by Professor Katayama at Saitama Medical University to perform a randomized controlled trial

called "Azelnidipine (a calcium (Ca) channel blocker) and Temocapril (an ACE inhibitor) in Hypertensive Patients with Type 2 Diabetes Study (ATTEST) [16]", which included genetic analysis to evaluate the therapeutic effects of azelnidipine and temocapril.

The goals of this study were, first, to assess the association between polymorphisms of susceptible genes for hypertension (18 genes) and each treatment or phenotype and, second, to assess the association between expression in peripheral blood of susceptible genes for hypertension and each treatment or phenotype.

2. Methods

2.1. Study Subjects. ATTEST Study, a multicenter, randomized, open-label study, was originally performed to investigate the efficacy and safety of combination therapy using the calcium channel blocker (CCB) azelnidipine and angiotensin-converting enzyme (ACE) inhibitor temocapril in hypertensive diabetics, led by Professor Katayama of Saitama Medical University. All of the subjects fulfilled the following inclusion criteria: (1) age: 30~70 years, outpatients, (2) mean systolic BP (BP): 140~180 mmHg and/or mean diastolic BP: 90~110 mmHg in washout period (four weeks) and stable BP without fluctuation in systolic BP of more than 30 mmHg or diastolic BP of more than 15 mmHg, and (3) fasting blood glucose: more than 126 mg/dl or HbA1c: more than 6.5% at 6 months before entry. These mild or moderate hypertensive subjects with diabetes were treated with the following medication: CCB: azelnidipine and ACE inhibitor: temocapril in accordance with the protocol (Figure 1). Each medication was started at a dose of 8 mg azelnidipine or 2 mg temocapril and increased to 16 mg azelnidipine or 4 mg temocapril until BP of less than 130/80 mmHg was achieved. All subjects were measured for body height, body weight, systolic BP, diastolic BP, fasting blood glucose level, triglyceride level, low density lipoprotein (LDL) cholesterol level, and serum creatinine (Cr) level.

Informed consent for genetic analysis was obtained from all subjects, and finally a total of 44 subjects were recruited in this study.

2.2. Selection of Susceptible Genes for Hypertension and Genotyping and Quantitative Real Time PCR (RT-PCR). The 18 genes shown in Table 1 were selected for the current study. All of the genes were previously reported to be susceptible genes for hypertension. The genotypes of the 22 polymorphisms of the 18 susceptible genes (ACE Ins/del, ADD1 Gly460Trp (rs4961), ADIPOQ Ile164Thr, ADRB1 Ser49Gly (rs1801252) and Arg389Gly (rs1801253), ADRB2 Gly16Arg (rs1042713) and Glu27Gln (rs1042714), ADRB3 Trp64Arg (rs4994), AGT Met235Trp (rs699), AGTR1 A1166C (rs17231380), ALDH2 Glu487Lys (rs671), ARHGAP8 Arg338Gly (rs6007334), BDKRB2 T-58C, FASL G-670A, GRK4 Ala486Val (rs1801058) and Arg65Leu (rs2960306), hOGG1 Ser326Cys (rs1052133), MTHFR C677T (rs1801133), RGS2 A to G in promoter (rs3767489), RGS2 A-638G (rs2746071), SLC12A3 Arg904Gln (rs11643718), and TGFB1 Leu10Pro (rs1982073)) for which positive associations with hypertension were previously reported were successfully determined using TaqMan PCR method (Applied Biosystems Inc., Foster City, CA, USA).

Table 1: Susceptible genes for hypertension.

(1)	Angiotensin converting enzyme (ACE)
(2)	α-adducin (ADD1)
(3)	Adiponectin (ADIPOQ)
(4, 5, 6)	$\beta1/\beta2/\beta3$ adrenergic receptor (ADRB1/2/3)
(7)	Angiotensinogen (AGT)
(8)	Angiotensin II type1 receptor (AGTR1)
(9)	Aldehyde dehydrogenase 2 (ALDH2)
(10)	Rho-GTPase activating protein-8 (ARHGAP8)
(11)	Bradykinin receptor $\beta2$ (BDKRB2)
(12)	Fas ligand (FASL)
(13)	G protein-coupled receptor kinase 4 (GRK4)
(14)	Human 8-hydroxyguanine DNA-glycosylase (hOGG1)
(15)	Methylenetetrahydrofolate reductase (MTHFR)
(16)	Regulator of G protein signaling-2 (RGS2)
(17)	Solute carrier family 12 member 3 (SLC12A3)
(18)	Transforming growth factor-β (TGFB1)

According to quantitative RT-PCR analysis using cDNA extracted from peripheral blood, 11 genes were expressed in peripheral blood: ACE, ADD1, ADRB1, ADRB2, ARHGAP8, FASL, GRK4, MTHFR, RGS2, SLC12A3, and TGFB1. RNA samples were extracted from the peripheral blood of the subjects, and cDNA was made from RNA using reverse transcriptase. Quantitative real-time PCR (RT-PCR) for all the genes was performed using TaqMan PCR method to determine their expression levels.

2.3. Statistical Analysis. The associations between polymorphisms and clinical variables were analyzed using one-way analysis of variance (ANOVA). The difference in each genotype or allele distribution was examined by χ^2 analysis. Odd ratios were calculated as an index of the association of each genotype with the prevalence of hypertension. To assess the contribution of confounding factors, we performed multiple logistic regression analysis using the computer software application, JMP 7.0 (SAS Institute Inc., Cary, NC, U.S.A.). We focused on testing for SNP medication and gene-expression medication interactions (i.e., whether the effects of SNPs on systolic BP or diastolic BP differed between hypertensives on azelnidipine and temocapril). Multivariate analysis of variance (MANOVA) was performed to determine whether BP change showed a significant interaction of each antihypertensive medication with the genotypes of the susceptible genes.

3. Results

3.1. Baseline Characteristics of Subjects Treated with Azelnidipine and Temocapril. Baseline characteristics of the subjects treated with azelnidipine and temocapril are shown in

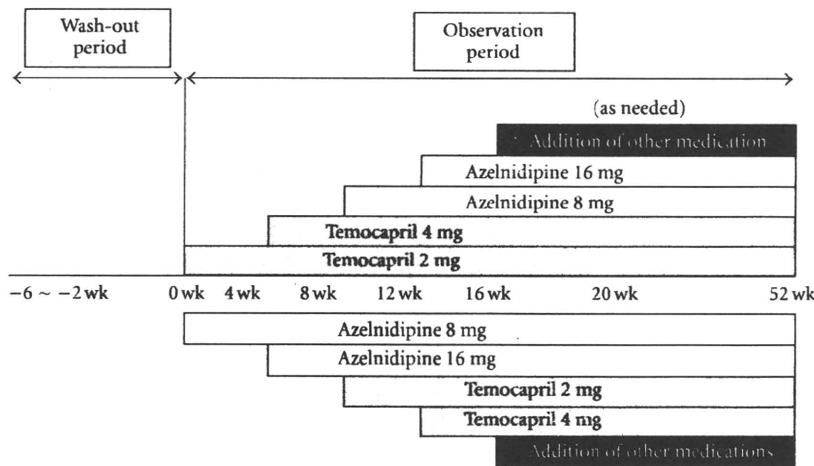

FIGURE 1: Study protocol; wk: week.

Table 2. There was no difference in sex, age, body mass index (BMI), affected period of hypertension, systolic BP, triglyceride level, LDL cholesterol level, and serum creatinine level between the azelnidipine and temocapril groups; however, fasting glucose level was higher in the azelnidipine group than in the temocapril group at the baseline.

3.2. BP Reduction with Azelnidipine and Temocapril. In the current study, the same level of BP reduction was observed in both the azelnidipine and temocapril groups, and both groups achieved a mean BP of less than 130/80 mmHg at 52 weeks. There was no difference in the dose of each treatment at 52 weeks (group started with azelnidipine: BP at 52 weeks 121.9 ± 11.4/74.7 ± 7.1 mmHg, final dose of azelnidipine 15.0 ± 2.7 mg, final dose of temocapril 3.8 ± 0.6 mg; group started with temocapril: BP at 52 weeks 125.6 ± 10.3/74.7 ± 6.1 mmHg, final dose of azelnidipine 14.9 ± 2.8 mg, final dose of temocapril 3.7 ± 0.7 mg, Figure 2).

3.3. Susceptible Gene Polymorphisms for Hypertension. The genotypes of 22 polymorphisms of the 18 genes were successfully determined at each time point (0, 8, 16, and 52 weeks). Only the Ile164Thr polymorphism of the adiponectin gene has no mutant genotype, and the other genotype frequencies were not significantly different from the values of Hardy-Weinberg's expectation (Figure 3).

According to the analysis of the association between BP, changes of BP, and all genotypes at each time point (0, 8, 16, and 52 weeks), only the A-638G polymorphism of the regulator of G protein signaling-2 gene (*RGS2*) showed a significant association with changes in BP (ΔBP: BP at 8 weeks—BP at 0 week). As shown in Figure 3, there was a significant relationship between the A-638G polymorphism of the RGS2 gene and the changes in BP between 0 and 8 weeks in subjects with azelnidipine (Δsystolic BP: *AA* − 28.0 ± 10.1 mmHg, *AG* − 15.5 ± 12.6 mmHg, *GG* + 7.0 ± 12.2 mmHg, $P = .0013$; Δdiastolic BP: *AA* − 17.2 ± 9.8 mmHg, *AG* − 8.1 ± 9.0 mmHg, *GG* − 4.0 ± 11.0 mmHg, $P = .067$), but not in subjects with temocapril (Δsystolic BP: *AA* − 5.3 ± 11.8 mmHg, *AG* − 8.4 ± 13.0 mmHg, *GG* − 9.9 ± 10.0 mmHg, $P = .81$; Δdiastolic BP: *AA* − 0.5 ± 7.2 mmHg, *AG* − 4.4 ± 3.5 mmHg, *GG* − 10.0 ± 4.5 mmHg, $P = .34$). At 8 weeks, changes in BP showed a significant interaction between *A-638G* and treatment with azelnidipine and temocapril (Δsystolic BP, Δdiastolic BP: $P = .0014$, $P = .036$, resp., by MANOVA, after adjustment for age, sex, and BMI) (Table 3).

There was no gene whose expression was associated with BP phenotypes or the polymorphisms of each gene through analysis of gene expression in peripheral blood. In terms of the RGS2 gene, the A-638G polymorphism was not related to the change of RGS2 expression between 0 and 8 weeks either in subjects with azelnidipine (ΔRGS2/18sRNA with azelnidipine: *AA* − 1.06 ± 2.1, *AG* + 0.31 ± 0.85, *GG* + 0.57 ± 0.44, $P = .13$, Figure 4) or in subjects with temocapril (ΔRGS2/18sRNA with temocapril: *AA* + 0.75 ± 1.3, *AG* − 0.10 ± 0.62, *GG* + 0.38 ± 0.44, $P = .24$, Figure 4).

4. Discussion

This study demonstrated the importance of pharmacogenetic research. Previously, Lynch et al. reported that the *NPPA* T2238C variant was associated with modification of antihypertensive medication effects on cardiovascular disease and BP, and TT allele carriers had more favorable outcomes when randomized to receive a CCB (amlodipine) [17]. Beitelshees et al. also demonstrated that the KCNMB1 genotype influenced responsiveness to verapamil SR and risk of adverse cardiovascular outcomes [18]. These reports could support the possibility of the existence of genes influencing drug efficacy.

Signaling by G-protein-coupled neurotransmitter receptors in the autonomic nervous system and vasoregulatory factor receptors in the periphery governs both blood pressure, by controlling the constriction and dilatation of resistance arterioles, and electrolyte and fluid balance by the kidney [19, 20]. The recently identified regulator of G-protein signaling (RGS) proteins is important in regulating

TABLE 2: Baseline characteristics of subjects treated with azelnidipine and temocapril.

	Azelnidipine	Temocapril	P value
n	23	21	
Male/Female (n), (%)	18/5 (78/22)	15/6 (71/29)	.60
Age (years)	60.8 ± 7.9	61.0 ± 8.5	.96
BMI (Kg/m^2)	25.6 ± 4.3	25.8 ± 3.3	.85
Period of HT (yr)	5.3 ± 5.7	9.0 ± 12.8	.21
Systolic BP (mmHg)	155.1 ± 11.1	157.2 ± 10.2	.52
Diastolic BP (mmHg)	97.8 ± 5.7	95.2 ± 5.4	.14
Fasting BG (mg/dL)	147.4 ± 27.5	132.3 ± 19.4	.04
TG (mg/dL)	127.3 ± 54.7	129.1 ± 61.3	.92
LDL-cholesterol (mg/dL)	136.1 ± 39.5	137.0 ± 39.0	.94
Serum Cr (mg/dL)	0.75 ± 0.12	0.77 ± 0.15	.58

HT: hypertension, BP: blood pressure, BG: blood glucose, TG: triglyceride, LDL: low density lipoprotein, and Cr: creatinine (mean±SD).

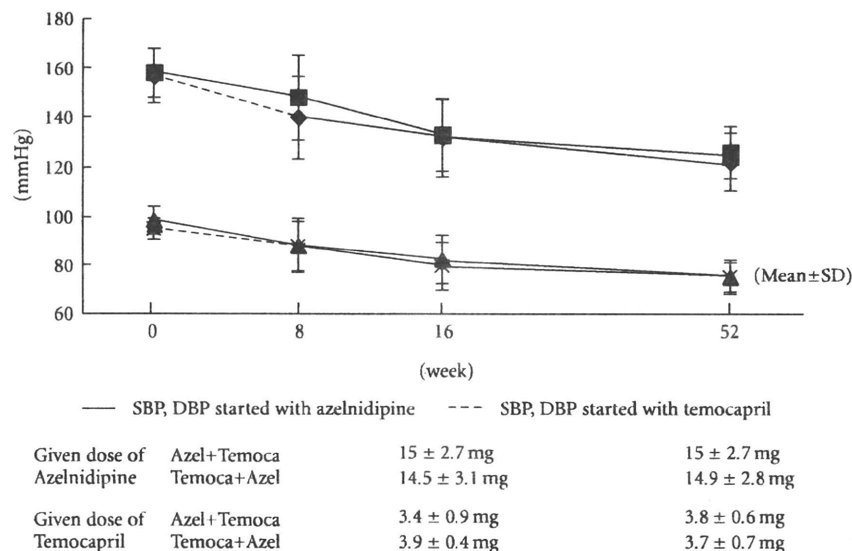

FIGURE 2: BP reduction with azelnidipine and temocapril; BP: blood pressure, SBP: systolic BP, and DBP: diastolic BP.

signaling cascades initiated by G-protein-coupled receptors (GPCRs) activation [21]. RGS proteins facilitate the intrinsic inactivating guanosine triphosphatase reaction of G-protein a-subunits , and thereby serve as effector channel blockers. RGS2 is unique among the RGS proteins in its apparent selectivity towards Gpa, which mediates the action of mouse physiological vasoconstrictors, including norepinephrine, angiotensin II, endothelin-1, and thrombin. RGS2 can also attenuate Gi- and Gs-mediated pathways [22, 23], which can also affect blood pressure via other physiologically important agonists such as serotonin, dopamine, and bradykinin. It was recently reported that mice lacking *RGS2* exhibit a strong hypertensive phenotype (increase in SBP of 50 mmHg) and resistance vasculature [24, 25]. Both heterozygous and homozygous *RGS2*-null mice exhibited a similar level of marked hypertension, suggesting that a naturally occurring mutation that affects the level of RGS2 protein may have a significant impact on blood pressure regulation. Recently there have been two reports that genetic changes in *RGS2* are associated with a hypertensive phenotype [26, 27]. A-638G, T1026A, and 1891-1892 del TC polymorphisms were extracted on the condition the allele frequencies of these polymorphisms were >0.1, and the T1026A and 1891-1892 del TC polymorphisms of this gene were associated with hypertension in women. These findings suggest that some functional variants of the RGS2 gene might be involved in regulating blood pressure in humans.

This ATTEST gene study revealed that one possible gene related to the effect of antihypertensive agents. In the current study, the A-638G polymorphism of the RGS2 gene was a predictive factor for therapeutic performance of a CCB, azelnidipine. Other classical candidate genes including genes associated with the renin-angiotensin system, sodium handling, vasodilatation and vasoconstriction, and the sympathetic nervous system did not show significant association between their polymorphisms and the effect of a Ca channel blocker or ACE inhibitor. In addition, there was no significant relationship between RGS2 polymorphisms and

Table 3: Genotype frequencies of 22 SNPs.

SNP Name	Major Homo (n)		Hetero (n)		Minor Homo (n)		Hardy-Weinberg Expectation (o value)
ACE Ins/Del (I/D)	II	15	ID	23	DD	6	0.54
ADD1 Gly460Trp (G/T)	GG	10	GT	24	TT	10	0.55
ADIPOQ Ile164Thr (T/C)	TT	44	TC	0	CC	0	-
ADRB1 Ser49Gly (G/A)	GG	1	GA	6	AA	37	0.24
ADRB1 Arg389Gly (G/C)	GG	3	GC	14	CC	27	0.53
ADRB2 Gly16Arg (A/G)	AA	14	AG	18	GG	12	0.23
ADRB2 Glu27Gln (C/G)	CC	41	CG	3	GG	0	0.81
ADRB3 Trp64Arg (T/C)	TT	29	TC	13	CC	2	0.73
AGT Met235Trp (T/C)	TT	31	TC	12	CC	1	0.90
AGTR1 A1166C (A/C)	AA	36	AC	7	CC	1	0.38
ALDH2 Glu487Lys (G/A)	GG	29	GA	13	AA	2	0.73
ARHGAP8 Arg338Gly (C/G)	CC	9	CG	20	GG	15	0.63
BDKRB2 T-58C (T/C)	TT	7	TC	23	CC	14	0.63
FASL G-670A (G/A)	GG	16	GA	17	AA	11	0.15
GRK4 Ala486Val (C/T)	CC	12	CT	20	TT	12	0.55
GRK4 Arg65Leu (G/T)	GG	33	GT	9	TT	2	0.21
hOGG1 Ser326Cys (G/C)	GG	7	GC	24	CC	13	0.46
MTHFR C677T (C/T)	CC	16	CT	24	TT	4	0.24
RGS2 A to G in promoter (A/G)	AA	9	AG	22	GG	13	0.96
RGS2 A-638G (A/G)	AA	9	AG	23	GG	12	0.74
SLC12A3 Arg904Gln (G/A)	GG	39	GA	5	AA	0	0.69
TGFB1 Leu10Pro (T/C)	TT	12	TC	24	CC	8	0.51

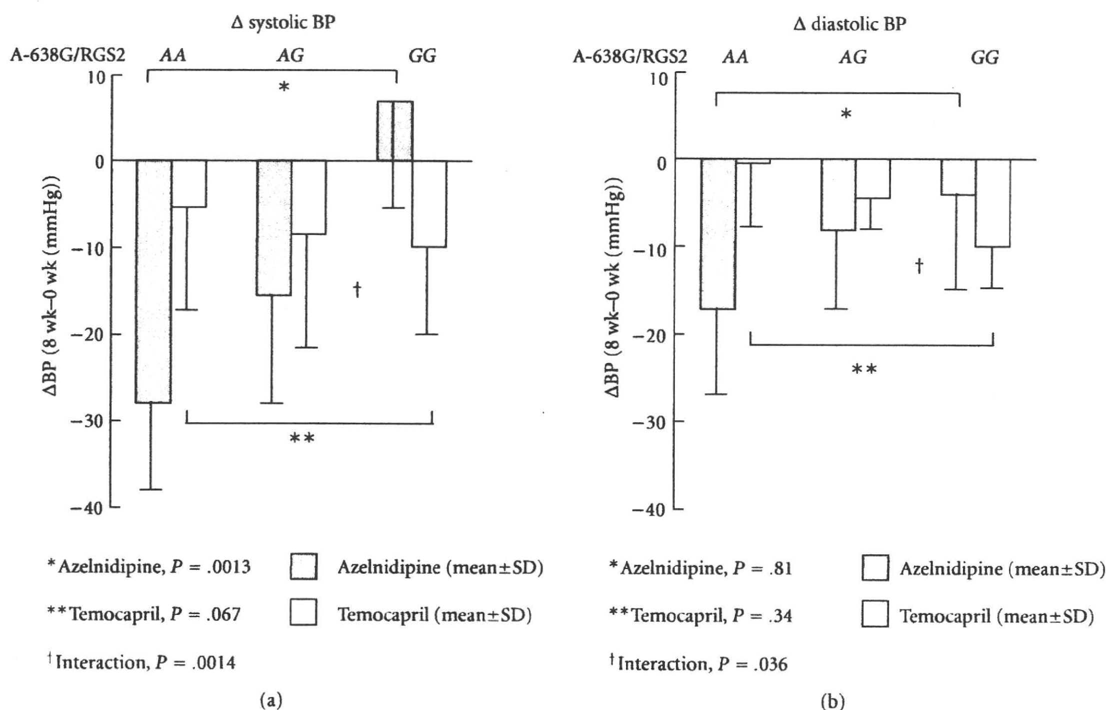

Figure 3: Relationship between A-638G polymorphism of RGS2 gene and BP change.

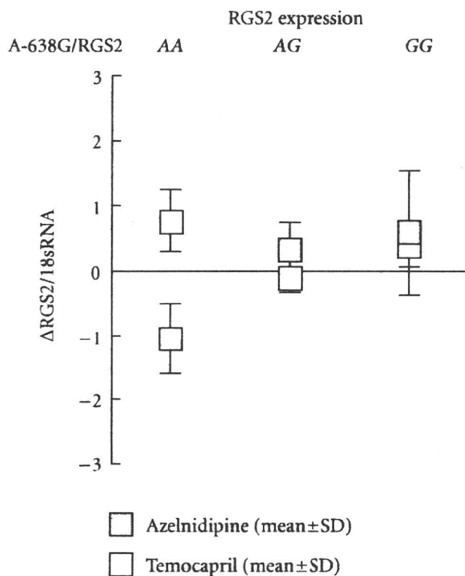

FIGURE 4: Relationship between A-638G polymorphism of RGS2 gene and RGS2 gene expression in peripheral blood.

BP-related phenotypes in this study, unlike previous reports. It has not yet been reported that the promoter variant A-638G of the RGS2 gene may change RGS2 function; however, this polymorphism has the possibility to change RGS2 protein production because this polymorphism is in the promoter region. There has been no evidence supporting the idea that RGS2 could be involved in the effect of CCBs. In several previous reports, genes without a drug-metabolizing effect, such as ACE [3] or AGTR1 [4], showed a significant association between their polymorphisms and response to antihypertensive medication; however, the mechanisms have not been clarified in any of these reports. In terms of the present study, RGS2 is suggested to function as a switch to turn on or off the G protein-associated pathway, and RGS2 can regulate blood pressure through smooth muscle cells. From this viewpoint, RGS2 might have the possibility of changing the effect of CCBs, because those mainly act through SMC; however, the detailed mechanism merits further investigation in the future.

Peripheral blood mainly contains white blood cells, red blood cells, platelets, and other circulating hormones, so the distribution of genes expressed in peripheral blood has to be investigated to clarify the significance of disease susceptibility gene expression in peripheral blood. In the present study, 11 genes were expressed in peripheral blood. There was no significant relationship between gene expression including the RGS2 gene and the effect of antihypertensives and phenotypes. From this viewpoint, RGS2 expression in peripheral blood does not seem to do anything and might not be a marker for BP regulation. However, further study is needed on its clinical application as a marker of drug efficacy.

As the limitation of this study, we could not exclude the possibility of the false positive association (type I error) due to the use of small number of subjects. However, this study was carried out under the strict protocol of a clinical trial so that the reliability of the results obtained seems to be high. To confirm the effect of RGS2 gene in the tailored medicine of hypertension, further study using another panel of hypertensive subjects should be required.

In conclusion, the A-638G polymorphism of the RGS2 gene could be a predictive factor for therapeutic effectiveness of CCBs such as azelnidipine. Further research is needed to determine the optimal approach for personalizing antihypertensive medication treatment regimens according to genotype information and for achieving the best blood pressure control.

Acknowledgments

The authors on behalf of the Azelnidipine and Temocapril in Hypertensive Patients with Type 2 Diabetes Study Group, are very grateful to Professor Katayama and his colleagues at Saitama Medical University. The authors are also grateful to Mr. Hironori Nakagami, Mr. Hidetoshi Yamashita, Ms. Katsuko Iwasa, and Mr. Yasutaka Fukuda for their professional assistance with research work. The present study was supported by a Grant-in-Aid for Scientific Research (H17-pharmaco-common-003) from the Japanese Ministry of Health, Labor, and Welfare Grants-in-Aid for Scientific Research (18590265, 18590811, 19650188, 21390223) from the Ministry of Education, Science, Sports and Culture of Japan and research grants from Takeda Science Foundation, the Japan Research Foundation for Clinical Pharmacology, and the Japan Intractable Disease Research Foundation.

References

[1] K. Kohara, Y. Tabara, J. Nakura et al., "Identification of hypertension-susceptibility genes and pathways by a systemic multiple candidate gene approach: the millennium genome project for hypertension," *Hypertension Research*, vol. 31, no. 2, pp. 203–212, 2008.

[2] Y. Watanabe, H. Metoki, T. Ohkubo et al., "Accumulation of common polymorphisms is associated with development of hypertension: a 12-year follow-up from the Ohasama study," *Hypertension Research*, vol. 33, no. 2, pp. 129–134, 2010.

[3] A. B. Chapman, G. L. Schwartz, E. Boerwinkle, and S. T. Turner, "Predictors of antihypertensive response to a standard dose of hydrochlorothiazide for essential hypertension," *Kidney International*, vol. 61, no. 3, pp. 1047–1055, 2002.

[4] A. Benetos, F. Cambien, S. Gautier et al., "Influence of the angiotensin II type 1 receptor gene polymorphism on the effects of perindopril and nitrendipine on arterial stiffness in hypertensive individuals," *Hypertension*, vol. 28, no. 6, pp. 1081–1084, 1996.

[5] J. Higaki, S. Baba, T. Katsuya et al., "Deletion allele of angiotensin-converting enzyme gene increases risk of essential hypertension in Japanese men: the Suita study," *Circulation*, vol. 101, no. 17, pp. 2060–2065, 2000.

[6] T. Asai, T. Ohkubo, T. Katsuya et al., "Endothelin-1 gene variant associates with blood pressure in obese Japanese subjects: the Ohasama study," *Hypertension*, vol. 38, no. 6, pp. 1321–1324, 2001.

[7] K. Ishikawa, S. Baba, T. Katsuya et al., "T+31C polymorphism of angiotensinogen gene and essential hypertension," *Hypertension*, vol. 37, no. 2, pp. 281–285, 2001.

[8] T. Katsuya, Y. Iwashima, K. Sugimoto et al., "Effects of antihypertensive drugs and gene variants in the renin-angiotensin system," *Hypertension Research*, vol. 24, no. 4, pp. 463–467, 2001.

[9] K. Sugimoto, A. Hozawa, T. Katsuya et al., "Alpha-adducin Gly460Trp polymorphism is associated with low renin hypertension in younger subjects in the Ohasama study," *Journal of Hypertension*, vol. 20, no. 9, pp. 1779–1784, 2002.

[10] Y. Fu, T. Katsuya, A. Matsuo et al., "Relationship of bradykinin B2 receptor gene polymorphism with essential hypertension and left ventricular hypertrophy," *Hypertension Research*, vol. 27, no. 12, pp. 933–938, 2004.

[11] A. Matsuo, T. Katsuya, K. Ishikawa et al., "G2736A polymorphism of thiazide-sensitive Na-Cl cotransporter gene predisposes to hypertension in young women," *Journal of Hypertension*, vol. 22, no. 11, pp. 2123–2127, 2004.

[12] K. Ohashi, N. Ouchi, S. Kihara et al., "Adiponectin I164T mutation is associated with the metabolic syndrome and coronary artery disease," *Journal of the American College of Cardiology*, vol. 43, no. 7, pp. 1195–1200, 2004.

[13] K. Sugimoto, T. Katsuya, T. Ohkubo et al., "Association between angiotensin II type 1 receptor gene polymorphism and essential hypertension: the Ohasama study," *Hypertension Research*, vol. 27, no. 8, pp. 551–556, 2004.

[14] K. Masuo, T. Katsuya, Y. Fu, H. Rakugi, T. Ogihara, and M. L. Tuck, "$\beta 2$- and $\beta 3$-adrenergic receptor polymorphisms are related to the onset of weight gain and blood pressure elevation over 5 years," *Circulation*, vol. 111, no. 25, pp. 3429–3434, 2005.

[15] H. Hanasaki, Y. Takemura, K. Fukuo et al., "Fas promoter region gene polymorphism is associated with an increased risk for myocardial infarction," *Hypertension Research*, vol. 32, no. 4, pp. 261–264, 2009.

[16] S. Katayama, R. Kawamori, Y. Iwamoto, I. Saito, and K. Kuramoto, "In half of hypertensive diabetics, co-administration of a calcium channel blocker and an angiotensin-converting enzyme inhibitor achieved a target blood pressure of <130/80 mmHg: the azelnidipine and temocapril in hypertensive patients with type 2 diabetes (ATTEST) study," *Hypertension Research*, vol. 31, no. 8, pp. 1499–1508, 2008.

[17] A. I. Lynch, E. Boerwinkle, B. R. Davis et al., "Pharmacogenetic association of the NPPA T2238C genetic variant with cardiovascular disease outcomes in patients with hypertension," *Journal of the American Medical Association*, vol. 299, no. 3, pp. 296–307, 2008.

[18] A. L. Beitelshees, Y. Gong, D. Wang et al., "KCNMB1 genotype influences response to verapamil SR and adverse outcomes in the INternational VErapamil SR/Trandolapril STudy (INVEST)," *Pharmacogenetics and Genomics*, vol. 17, no. 9, pp. 719–729, 2007.

[19] N. Takahashi and O. Smithies, "Gene targeting approaches to analyzing hypertension," *Journal of the American Society of Nephrology*, vol. 10, no. 7, pp. 1598–1605, 1999.

[20] R. P. Lifton, A. G. Gharavi, and D. S. Geller, "Molecular mechanisms of human hypertension," *Cell*, vol. 104, no. 4, pp. 545–556, 2001.

[21] L. De Vries, B. Zheng, T. Fischer, E. Elenko, and M. G. Farquhar, "The regulator of G protein signaling family," *Annual Review of Pharmacology and Toxicology*, vol. 40, pp. 235–271, 2000.

[22] S. Sinnarajah, C. W. Dessauer, D. Srikumar et al., "RGS2 regulates signal transduction in olfactory neurons by attenuating activation of adenylyl cyclase III," *Nature*, vol. 409, no. 6823, pp. 1051–1055, 2001.

[23] T. Ingi, A. M. Krumins, P. Chidiac et al., "Dynamic regulation of RGS2 suggests a novel mechanism in G-protein signaling and neuronal plasticity," *Journal of Neuroscience*, vol. 18, no. 18, pp. 7178–7188, 1998.

[24] S. P. Heximer, R. H. Knutsen, X. Sun et al., "Hypertension and prolonged vasoconstrictor signaling in RGS2-deficient mice," *Journal of Clinical Investigation*, vol. 111, no. 4, pp. 445–452, 2003.

[25] M. Tang, G. Wang, P. Lu et al., "Regulator of G-protein signaling-2 mediates vascular smooth muscle relaxation and blood pressure," *Nature Medicine*, vol. 9, no. 12, pp. 1506–1512, 2003.

[26] J. Yang, K. Kamide, Y. Kokubo et al., "Genetic variations of regulator of G-protein signaling 2 in hypertensive patients and in the general population," *Journal of Hypertension*, vol. 23, no. 8, pp. 1497–1505, 2005.

[27] E. L. Riddle, B. K. Rana, K. K. Murthy et al., "Polymorphisms and haplotypes of the regulator of G protein signaling-2 gene in normotensives and hypertensives," *Hypertension*, vol. 47, no. 3, pp. 415–420, 2006.

Hypertension Research (2010) 33, 1244–1249
© 2010 The Japanese Society of Hypertension All rights reserved 0916-9636/10 $32.00
www.nature.com/hr

ORIGINAL ARTICLE

Impact of RGS2 deficiency on the therapeutic effect of telmisartan in angiotensin II-induced aortic aneurysm

Sachiko Matsumoto[1], Kei Kamide[1,2], Fumiaki Banno[1], Nobutaka Inoue[1,3], Naoki Mochizuki[1], Yuhei Kawano[4] and Toshiyuki Miyata[1]

Regulator of G-protein signaling 2 (RGS2) negatively regulates the signaling of G-protein-coupled receptors, such as the angiotensin II (AngII) type 1 receptor by accelerating the inactivation of Gαq. Rgs2-deficient mice show increased sensitivity and prolonged responsiveness to vasoconstrictors, and genetic variations in the *RGS2* gene are associated with hypertension in humans. This study aimed to clarify whether Rgs2 deficiency contributes to the development of vascular remodeling and therapeutic efficacy of the angiotensin receptor blocker telmisartan on atherosclerotic vascular damage. We treated *Rgs2*$^{+/+}$, *Rgs2*$^{+/-}$ and *Rgs2*$^{-/-}$ mice with saline (control group), AngII (1000 ng per kg per min, AngII group) or low-dose telmisartan (0.3 mg per kg per day) with AngII infusion (AngII+Telmi group) for 4 weeks. For all genotypes, the AngII groups exhibited significantly higher blood pressure, a higher mortality rate and a higher incidence of aortic aneurysm than the respective control group. Interestingly, aneurysm incidence was decreased in the AngII+Telmi group compared with the AngII group in *Rgs2*$^{-/-}$ mice (6.7 *vs.* 42.9%, $P<0.05$), but not in *Rgs2*$^{+/+}$ mice (38.9 *vs.* 40.0%). Moreover, in *Rgs2*$^{-/-}$ mice, the AngII+Telmi group exhibited significant improvement in survival, reduction of enlarged aortic diameter, inhibition of superoxide production and suppression of NAD(P)H oxidase activity compared with the AngII group. Thus, Rgs2 deficiency potentiates the vascular protection effect of low-dose telmisartan. Our results suggest that angiotensin receptor blocker may be useful for protection from cardiovascular events in hypertensive subjects with risk alleles in the *RGS2* gene.
Hypertension Research (2010) **33**, 1244–1249; doi:10.1038/hr.2010.184; published online 30 September 2010

Keywords: angiotensin II; aortic aneurysm; oxidative stress; RGS2; telmisartan

INTRODUCTION

The renin–angiotensin system has an important role in the regulation of blood pressure and vascular structure. Angiotensin II (AngII) is a potent vasoconstrictor that elevates blood pressure through a G-protein-coupled receptor, angiotensin type 1 receptor (AT1R). AngII generates aldosterone at the adrenal gland and activates the sympathetic nervous system, leading to blood pressure elevation. In addition to the effects of AngII on the elevation of blood pressure, evidence has revealed that it has a role in atherogenesis. In animal models, chronic infusion of AngII promotes the formation of atherosclerotic lesions and aneurysms.[1,2] It is widely known that AngII-induced NAD(P)H oxidase activation increases the production of reactive oxygen species from various cell types,[3] including endothelial cells, vascular smooth muscle cells and monocytes/macropharges, and promotes inflammation in atherosclerotic lesions.[4]

Regulator of G-protein signaling 2 (RGS2) is present in many cardiovascular tissues, including the heart, kidney and blood vessels, and it is required for normal vascular function and regulation of blood pressure.[5] RGS2 negatively regulates the signaling of G-protein-coupled receptors, such as AT_1R, by accelerating the inactivation of Gαq by its guanosine triphosphatase-activating protein activity. RGS2 also mediates the nitric oxide–cyclic guanosine monophosphate pathway to decrease vascular resistance and attenuate vasoconstrictor signaling in vascular smooth muscle cells.[5,6] Patients with Bartter's and Gitelman's syndromes have hypotension with an enhancement of RGS2 expression.[7] Taken together, silencing of the *RGS2* gene disrupts these pathways and enhances the vasoconstrictor signaling.

The first reported phenotypes of Rgs2-deficient mice were the reduction of T-cell activation, the control of synapse development in the hippocampus and an increase in anxiety responses.[8] With respect to the blood pressure regulation, Rgs2-deficient mice exhibit a hypertensive phenotype and persistent constriction of the resistance vasculature.[5,9] This hypertensive phenotype differs in degree according to conditions such as age in weeks, anesthesia, postoperative recovery, restrained stress, time zone and apparatus for blood pressure measurement.[5,9,10] Together with another group, we have reported that genetic polymorphisms within the human *RGS2* gene are associated with hypertension.[11–14] It has been speculated that genetic variations

[1]Research Institute, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan; [2]Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [3]Department of Cardiovascular Medicine, Kobe Rosai Hospital, Kobe, Hyogo, Japan and [4]Division of Hypertension and Nephrology, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan
Correspondence: Dr K Kamide, Department of Geriatric Medicine and Nephrology, Osaka University Graduate School of Medicine, 2-2, Yamadaoka, Suita city, Osaka 565-0871, Japan.
E-mail: kamide@geriat.med.osaka-u.ac.jp
Received 28 May 2010; revised 21 June 2010; accepted 28 June 2010; published online 30 September 2010

may reduce RGS2 function.[15,16] Because hypertension contributes to the pathogenesis of cardiovascular disease, it is hypothesized that genetic variations in RGS2 might be a risk factor for the development of atherosclerosis through enhancement of AngII signaling.

Telmisartan is an angiotensin receptor blocker (ARB) with a longer half-life and higher lipophilicity than other ARBs,[17] and it is a commonly used medication for the treatment of hypertension. In a recent clinical trial,[18,19] telmisartan resulted in the prevention of vascular events such as myocardial infarction and stroke. Therefore, telmisartan is expected to be effective for cardiovascular protection in Rgs2-deficient mice that have enhanced AngII signaling.

In this study, we investigated the effects of RGS2 deficiency on the development of vascular remodeling and the therapeutic efficacy of low-dose telmisartan on atherosclerotic vascular damage resulting from excessive stimulation of AT_1R by RGS2 deficiency.

METHODS

Mice

Rgs2-deficient mice on the C57BL/6 background were provided by Dr Michael E Mendelsohn (Tufts University School of Medicine).[8] Mice were kept in a specific pathogen-free barrier under constant temperature conditions and housed on a 12-h light/12 h dark cycle. All experiments were approved by the Animal Care and Use Committees of the National Cardiovascular Center, Japan, and they were performed in accordance with the guidelines.

Drug administration

We used 18-week old male Rgs2-deficient ($Rgs2^{-/-}$, $Rgs2^{+/-}$) and wild-type ($Rgs2^{+/+}$) mice and divided them into three treatment groups. Mice were subcutaneously infused with AngII (1000 ng per kg per min, AngII group), or saline containing 0.3% bovine serum albumin (control group) using an ALZET Micro-Osmotic Pump (model 1004, Durect, Cupertino, CA, USA) for 4 weeks. Mice were also treated with AngII (1000 ng per kg per min) and low-dose telmisartan (0.3 mg per kg per day, AngII+Telmi group). Telmisartan was administered in drinking water. We adopted a low dosage of telmisartan that does not affect blood pressure.[20]

Hemodynamic analysis

Systolic blood pressure and heart rate were measured in conscious, pre-warmed, and restrained mice by the tail-cuff method using a non-invasive blood pressure measuring device (BP98-A, Softron, Tokyo, Japan) before treatment and on days 7, 14, 21 and 28 after treatment just around the same time of day. For stable measurement, tail-cuff pressures were obtained after a 2-week-training period to acclimatize the mice to the restraining device and cuff inflation. The pulse waveform was monitored in real time using the BP98AW software (version 2.12, Softron). The first 10 measurements were excluded from the analysis, and at least 5 measurements with an untroubled pulse waveform were collected.

Biochemical examination

After 4 weeks of treatment, overnight fasting blood was collected from anesthetized mice and put into capillary blood collection tubes including a gel/clot activator (Capiject tube, Terumo Medical, Somerset, NJ, USA). Serum was obtained by the manufacturer's instructions and stored at −80 °C in aliquots before use. Serum levels of blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, low-density lipoprotein-cholesterol and glucose were measured using a Hitachi clinical analyzer (model 7180, Hitachi High-Technologies, Tokyo, Japan).

Aortic tissue collection and morphometric analysis

Dissection was performed under anesthesia after blood collection. After thoracolaparotomy, the inferior vena cava was cut for exsanguination and the aorta was perfused with ice-cold saline through the left ventricle. The aortic root and heart were subsequently eviscerated, and the periadventitial tissue was dissected away under a stereomicroscope. The external diameters in the middle of the suprarenal abdominal aorta between the diaphragm and renal artery bifurcation were measured using the ImageJ software (version 1.40, National Institute of Health, Bethesda, MD, USA). After taking the images, the aorta was cut into thoracic and abdominal regions. The thoracic aorta was immediately frozen by liquid nitrogen and stored at −80 °C for the measurement of NAD(P)H oxidase activity. The suprarenal abdominal aorta was cut into two pieces. For detection of superoxide production, the superior half was immediately embedded in OCT compound (Sakura Finetek Japan, Tokyo, Japan) in liquid nitrogen and stored at −80 °C. For immunohistochemical staining, the inferior half was fixed with 4% paraformaldehyde overnight and paraffin embedded.

Detection of aortic superoxide production

Aortic superoxide levels were measured with dihydroethidium (Invitrogen Molecular Probes, Carlsbad, CA, USA) on cross sections (9 μm) obtained from the unfixed frozen blocks of abdominal aorta, as previously described.[21] The unfixed frozen sections were stained by dihydroethidium (2 μM, 30 min, 37 °C) in a dark humidified chamber and washed briefly. The images were captured with a laser scanning confocal fluorescent microscope (FLUOVIEW system, Olympus, Tokyo, Japan). For the quantification of ethidium fluorescence, the mean fluorescence intensity (fluorescence intensity per unit area) in the aortic wall was calculated using the ImageJ software on high-power (x300) images.

Measurement of NAD(P)H oxidase activity

The frozen aortic segments were homogenized using a Sample Grinding Kit (GE Healthcare UK, Buckinghamshire, England). After centrifugation, the supernatant was stored at −80 °C until use. Protein concentrations were measured by the BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA). The enzymatic activity of NAD(P)H oxidase of the homogenates was measured by lucigenin-enhanced chemiluminescence, as previously described.[20] The assay solution contained lucigenin (250 μM) as an electron acceptor and NADH (100 μM) or NADPH (100 μM) as a substrate. After pre-incubation at 37 °C for 20 min, the reaction was started by adding 50 μg of homogenate. Photon emissions were continuously recorded for 15 min with a chemiluminescence reader (BLR-201, ALOKA, Tokyo, Japan). The chemiluminescent signals observed in the absence of homogenates were subtracted from the signals of the samples. The signal was corrected for the protein concentration of each homogenate and expressed as counts per minute by mg protein for a 15-min period. In some experiments, the homogenates were pre-incubated with 10 μM diphenyleneiodium, a selective NADPH oxidase inhibitor, for 20 min before the lucigenin-enhanced chemiluminescence measurements.

Statistical analysis

Data are expressed as mean ± s.e.m. All statistical analyses were performed using the Prism software (version 5.0, GraphPad Software, La Jolla, CA, USA). Hemodynamic changes were analyzed by two-way analysis of variance during the period of administration. Differences between multiple groups were analyzed by one-way analysis of variance or Kruskal–Wallis test in the case of a non-Gaussian distribution, followed by the Bonferroni *post-hoc* test for comparison between treatment groups or genotype groups. The log-rank (Mantel–Cox) test was used for statistical analysis of survival curves, and the χ^2-test was used to compare the incidence of aneurysm. Values of $P<0.05$ were considered statistically significant.

RESULTS

Changes in blood pressure

To elucidate the direct role of AngII signaling in Rgs2-deficient mice, we divided $Rgs2^{+/+}$, $Rgs2^{+/-}$ and $Rgs2^{-/-}$ mice into three treatment groups: the control group, the AngII group and the AngII+Telmi group. Drug administration was performed for 4 weeks, and hemodynamic changes were measured. In all Rgs2 genotypes, the AngII group exhibited ∼50 mm Hg higher systolic blood pressure than the control group ($P<0.001$) (Figure 1). Rgs2 dysfunction was expected to enhance the AngII signaling, leading to higher blood pressure in

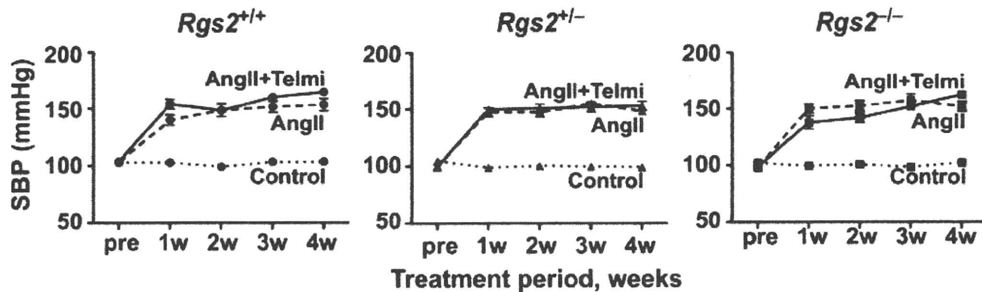

Figure 1 Blood pressure changes in response to AngII or AngII plus telmisartan. Systolic blood pressure (SBP) variations were shown in $Rgs2^{+/+}$, $Rgs2^{+/-}$ and $Rgs2^{-/-}$ mice. Mice were divided into three groups, control group (saline), AngII group (AngII, 1000 ng per kg per min) and AngII+Telmi group (AngII, 1000 ng per kg per min, telmisartan, 0.3 mg per kg per day). Results are expressed as mean ± s.e.m. in the control group ($Rgs2^{+/+}$: n=8, $Rgs2^{+/-}$: n=8, $Rgs2^{-/-}$: n=8), the AngII group ($Rgs2^{+/+}$: n=17, $Rgs2^{+/-}$: n=25, $Rgs2^{-/-}$: n=15) and the AngII+Telmi group ($Rgs2^{+/+}$: n=14, $Rgs2^{+/-}$: n=15, $Rgs2^{-/-}$: n=15). The AngII and AngII+Telmi groups showed significantly higher SBP than the control group (P<0.001).

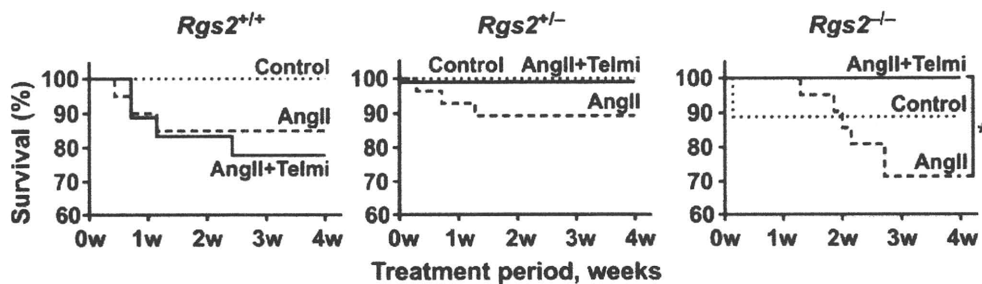

Figure 2 Comparison of survival curves in mice treated with AngII or AngII plus telmisartan. The treatment design was the same as that in Figure 1. The survival rate is expressed as a percent of animals in the control group ($Rgs2^{+/+}$: n=8, $Rgs2^{+/-}$: n=8, $Rgs2^{-/-}$: n=9), the AngII group ($Rgs2^{+/+}$: n=20, $Rgs2^{+/-}$: n=28, $Rgs2^{-/-}$: n=21) and the AngII+Telmi group ($Rgs2^{+/+}$: n=18, $Rgs2^{+/-}$: n=15, $Rgs2^{-/-}$: n=15). *P<0.05.

$Rgs2^{-/-}$ mice, but there were no blood pressure differences among the Rgs2 genotypes in the AngII group. Moreover, the AngII+Telmi group did not show reduced blood pressure in any of the genotypes. Before drug administration, there were no significant differences in blood pressure among the Rgs2 genotypes in our experimental condition using the tail-cuff method. Both the AngII and the AngII+Telmi groups showed no differences in heart rate among the genotypes (data not shown). Although $Rgs2^{-/-}$ mice tended to show a lower body weight than the other genotypes, drug administration did not affect the body weight (data not shown).

Survival rates and blood biochemical examinations

Some mice died during the period of administration mainly because of the cardiovascular events, including the rupture of aneurysms. Therefore, we compared the survival curves in mice with all Rgs2 genotypes and with the three treatment groups. As shown in Figure 2, AngII treatment decreased the survival rate for all the Rgs2 genotypes compared with the control group, but there were no significant differences in survival rate among the Rgs2 genotypes in the AngII group. Interestingly, $Rgs2^{-/-}$ mice treated with AngII+Telmi had significantly improved survival compared with $Rgs2^{-/-}$ mice treated with AngII alone (P<0.05).

As shown in the Table 1, blood urea nitrogen levels were significantly increased in the AngII and AngII+Telmi groups compared with the control group (P<0.05). Creatinine levels also tended to increase in the AngII group compared with the control group. These results indicated the exacerbation of renal function caused by AngII-induced hypertension in all Rgs2 genotypes. Recent studies have shown that telmisartan improves the metabolism of lipids and glucose.[22,23] Our study showed that low-density lipoprotein-cholesterol levels were significantly decreased in the AngII+Telmi group compared with the

Table 1 Biochemical examinations of serum samples

	Rgs2	*Control*	*AngII*	*AngII+Telmi*
BUN (mg dl^{-1})	+/+	27.2 ± 2.9	57.1 ± 4.6*	51.7 ± 1.8*
	+/−	23.8 ± 1.9	52.7 ± 2.2*	48.8 ± 3.7*
	−/−	29.4 ± 4.3	47.8 ± 2.3*	51.0 ± 2.6*
CRE (mg dl^{-1})	+/+	0.07 ± 0.00	0.12 ± 0.01*	0.09 ± 0.01*
	+/−	0.06 ± 0.01	0.13 ± 0.01*	0.10 ± 0.01*
	−/−	0.06 ± 0.01	0.10 ± 0.01	0.08 ± 0.01
LDL-C (mg dl^{-1})	+/+	2.0 ± 0.0	3.1 ± 0.3	4.9 ± 0.7
	+/−	2.4 ± 0.4	4.1 ± 0.4	2.8 ± 0.3
	−/−	5.0 ± 1.2	6.0 ± 1.4	3.1 ± 0.5†
GLU (mg dl^{-1})	+/+	108.4 ± 14.4	93.6 ± 11.7	98.6 ± 14.1
	+/−	112.4 ± 23.8	103.5 ± 10.4	109.5 ± 10.7
	−/−	70.8 ± 7.2	72.2 ± 9.7	88.9 ± 8.1

Abbreviations: AngII, angiotension II; BUN, blood urea nitrogen; CRE, creatinine, LDL-C, low-density lipoprotein-cholesterol; GLU, glucose, Rgs2, regulator of G-protein signaling 2; Telmi, telmisartan.
Results are expressed as mean ± s.e.m. in the control group ($Rgs2^{+/+}$: n=5, $Rgs2^{+/-}$: n=5, $Rgs2^{-/-}$: n=5), the AngII group ($Rgs2^{+/+}$: n=11, $Rgs2^{+/-}$: n=21, $Rgs2^{-/-}$: n=12) and the AngII+Telmi group ($Rgs2^{+/+}$: n=14, $Rgs2^{+/-}$: n=15, $Rgs2^{-/-}$: n=15). *P<0.05, *vs.* the control group. †P<0.05, *vs.* the AngII group.

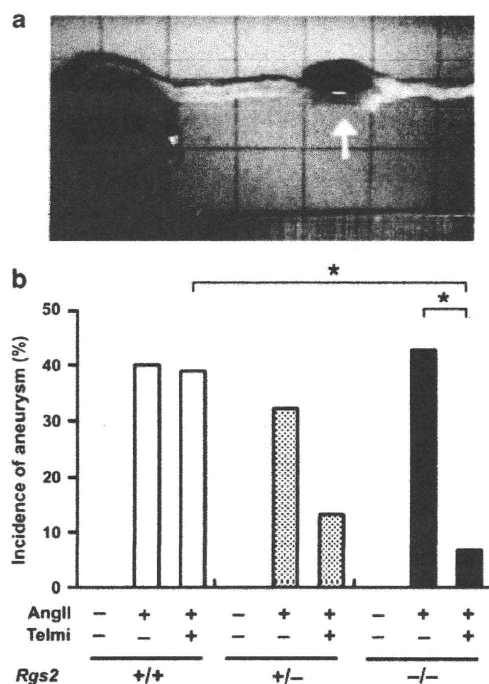

Figure 3 Gross morphology and incidence of aortic aneurysms observed in mice treated with AngII or AngII plus telmisartan. **(a)** Representative photograph showing the macroscopic features of aneurysm induced by AngII treatment. Aneurysm is indicated by arrow. **(b)** The incidence of aneurysms is expressed as a percent of animals in the control group (*Rgs2*$^{+/+}$: *n*=8, *Rgs2*$^{+/-}$: *n*=8, *Rgs2*$^{-/-}$: *n*=9), the AngII group (*Rgs2*$^{+/+}$: *n*=20, *Rgs2*$^{+/-}$: *n*=28, *Rgs2*$^{-/-}$: *n*=21) and the AngII+Telmi group (*Rgs2*$^{+/+}$: *n*=18, *Rgs2*$^{+/-}$: *n*=15, *Rgs2*$^{-/-}$: *n*=15). *$P<0.05$.

AngII group in *Rgs2*$^{-/-}$ mice ($P<0.05$), whereas no changes in glucose levels were observed. Liver function, as indicated by aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase levels, was normal (data not shown).

Vascular remodeling

In the AngII group, ~30–40% of mice had aortic aneurysms, as shown in Figure 3a. The incidence of aortic aneurysm was not different among Rgs2 genotypes in the AngII group. *Rgs2*$^{-/-}$ mice treated with AngII+Telmi had a significantly lower rate of aneurysm formation than the AngII group ($P<0.05$, Figure 3b). In *Rgs2*$^{+/-}$ mice, the AngII+Telmi group had a lower rate of aneurysm than the AngII group. Moreover, the incidence of aortic aneurysms in *Rgs2*$^{-/-}$ mice was significantly lower than that in *Rgs2*$^{+/+}$ mice in the AngII+Telmi group ($P<0.05$).

Next, we compared the diameters of the abdominal aorta (Figure 4). The AngII group showed significantly more enlarged aortic diameters than the control group for all Rgs2 genotypes (*Rgs2*$^{+/+}$: $P<0.05$, *Rgs2*$^{+/-}$, *Rgs2*$^{-/-}$: $P<0.001$). Although AngII+Telmi treatment did not reduce blood pressure in any of the genotypes, it significantly reduced enlargement of the aortic diameter compared with the AngII treatment of *Rgs2*$^{-/-}$ mice ($P<0.01$). In addition, in *Rgs2*$^{+/-}$ mice the AngII+Telmi treatment tended to reduce the enlargement of the aortic diameter compared with AngII treatment alone.

Oxidative stress in vascular walls

To assess oxidative stress in the aortic wall, we measured superoxide production and NAD(P)H oxidase activity. Figure 5A shows the *in situ*

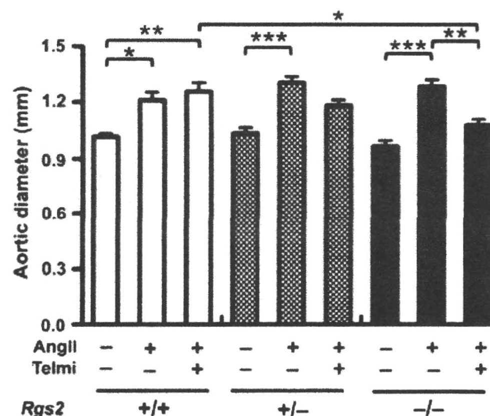

Figure 4 Comparison of abdominal aortic diameters in mice treated with AngII or AngII plus telmisartan. Diameter was measured in the middle of the abdominal aorta between the diaphragm and the renal artery bifurcation, and was compared. Results are expressed as mean±s.e.m. in the Control group (*Rgs2*$^{+/+}$: *n*=8, *Rgs2*$^{+/-}$: *n*=8, *Rgs2*$^{-/-}$: *n*=8), the AngII group (*Rgs2*$^{+/+}$: *n*=12, *Rgs2*$^{+/-}$: *n*=19, *Rgs2*$^{-/-}$: *n*=11) and the AngII+Telmi group (*Rgs2*$^{+/+}$: *n*=10, *Rgs2*$^{+/-}$: *n*=13, *Rgs2*$^{-/-}$: *n*=14). *$P<0.05$, **$P<0.01$, ***$P<0.001$.

detection of superoxide in the abdominal aorta using dihydroethidium staining in *Rgs2*$^{-/-}$ mice. The red fluorescence intensity of the aorta in *Rgs2*$^{-/-}$ mice tended to be more intense in the AngII group and was obviously suppressed in the AngII+Telmi group. Quantitative analysis showed that superoxide production of the aorta in *Rgs2*$^{-/-}$ mice was significantly decreased in the AngII+Telmi group compared with the AngII group ($P<0.001$), whereas production in the other Rgs2 genotypes was not statistically different (Figure 5B). Furthermore, the NAD(P)H oxidase activity of aorta in *Rgs2*$^{-/-}$ mice was also decreased in the AngII+Telmi group compared with the AngII group ($P<0.001$, Figure 5c).

DISCUSSION

This study showed that the AngII group exhibited higher systolic blood pressure, a higher mortality rate, a higher aortic aneurysm incidence, and a more enlarged aortic diameter than the control group for all Rgs2 genotypes. Interestingly, in *Rgs2*$^{-/-}$ mice, the AngII+Telmi group showed a significant improvement in the survival rate as well as in the suppression of vascular remodeling compared with the AngII group, although blood pressure was not changed. In parallel with this improvement of vascular phenotypes in *Rgs2*$^{-/-}$ mice, the NAD(P)H oxidase activity and superoxide production of the aorta in *Rgs2*$^{-/-}$ mice was decreased in the AngII+Telmi group. Thus, low-dose telmisartan could prevent AngII-induced vascular remodeling via the suppression of oxidative stress in the vascular wall of *Rgs2*$^{-/-}$ mice.

Rgs2 dysfunction is expected to enhance the AngII signaling through AT_1R, leading to blood pressure elevation, atherosclerotic vascular remodeling and organ damage. However, our results did not show significant differences among Rgs2 genotypes in the AngII group, including blood pressure, mortality rate, aneurysmal formation, aortic diameter and aortic oxidative stress (Figures 1–5). The reason for the lack of differences among the Rgs2 genotypes may have been because the concentrations and dosing period of AngII may be excessive and outside the capability of Rgs2 regulation. Nevertheless, AngII was given in doses sufficient to cause the

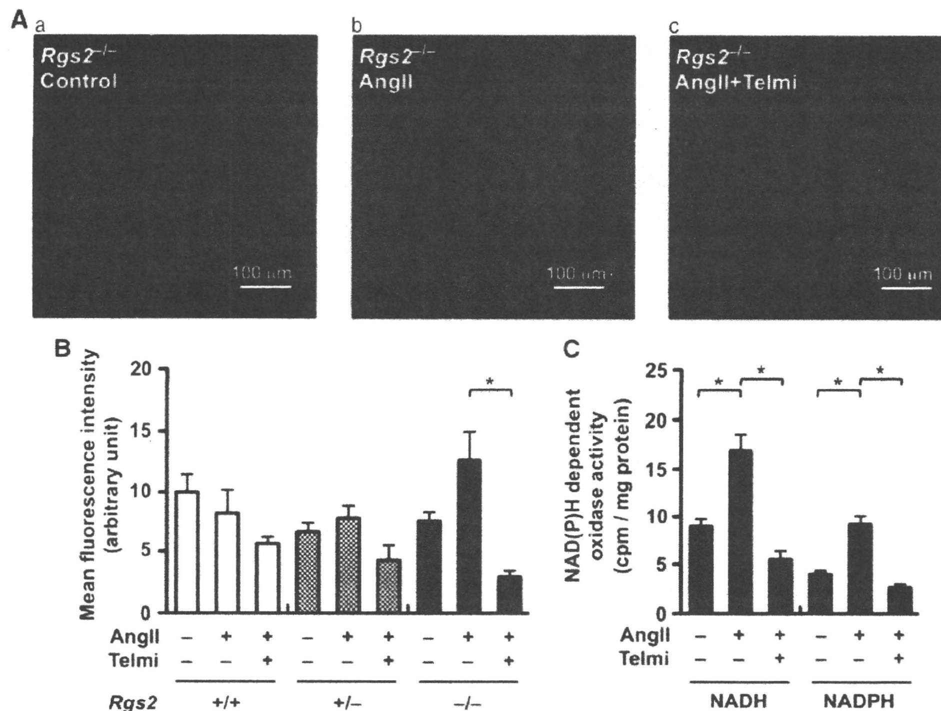

Figure 5 Comparison of oxidative stress in the aortic wall in mice treated with AngII or AngII plus telmisartan. (**A**), Representative photographs of *in situ* superoxide production in the aortic wall using dihydroethidium staining in $Rgs2^{-/-}$ mice of the Control group (a), the AngII group (b) and the AngII+Telmi group (c). (**B**), Quantitative analysis of superoxide production in the aortic wall. For quantification of ethidium fluorescence at the aortic wall, the fluorescence intensity was calculated using the ImageJ software and is expressed in arbitrary units. (**C**), Effect of telmisartan on NAD(P)H dependent oxidase activity of aorta homogenates using lucigenin-enhanced chemiluminescence in $Rgs2^{-/-}$mice. NAD(P)H oxidase activity was measured as described in Methods section. As a control, NADPH oxidase inhibitor, diphenyleneiodium, reduced NADH and NADPH oxidase activities below measurable limits. Results are expressed as mean ± s.e.m. in the control group ($Rgs2^{+/+}$: n=5, $Rgs2^{+/-}$: n=5, $Rgs2^{-/-}$: n=5), the AngII group ($Rgs2^{+/+}$: n=11, $Rgs2^{+/-}$: n=21, $Rgs2^{-/-}$: n=12) and the AngII+Telmi group ($Rgs2^{+/+}$: n=14, $Rgs2^{+/-}$: n=15, $Rgs2^{-/-}$: n=15). *P<0.001.

exacerbation of vascular phenotypes in all genotypes under our experimental condition. Thus, we examined the impact of Rgs2 deficiency on the therapeutic effect of low-dose telmisartan in AngII-infused mice.

The most interesting aspect of our study was the observed therapeutic efficacy of telmisartan. Low-dose telmisartan significantly improved survival, inhibited vascular remodeling such as aneurysmal formation and enlargement of aortic diameter, and decreased aortic oxidative stress in $Rgs2^{-/-}$ mice. These effects, mostly observed in the aorta, were independent of blood pressure reduction and were not observed in $Rgs2^{+/+}$ mice (Figures 2–5). In $Rgs2^{+/-}$ mice, low-dose telmisartan exhibited partial therapeutic effects such as improvement of survival, inhibition of aneurysm formation and reduction of enlarged aortic diameter, although there were no significant differences (Figures 2–4). These Rgs2 deficiency-dependent vascular protective effects of low-dose telmisartan could be explained by the following mechanisms. Some reports have shown that telmisartan and another ARB, valsartan, prevent vascular remodeling through inhibition of oxidative stress, inflammation and degradation of the extracellular matrix independent of their antihypertensive effects.[20,24–26] Heximer *et al.*[9] have reported that responsiveness to another ARB, candesartan, is more sensitive in $Rgs2^{-/-}$ mice than $Rgs2^{+/+}$ mice with regard to its antihypertensive and organ protection effects. Thus, vascular protective effects through inhibition of oxidative stress by low-dose ARB may be exaggerated in $Rgs2^{-/-}$ mice as a result of its antagonism for excessive AT_1R signaling. Moreover, some reports have characterized new functions of telmisartan as a partial agonist for peroxisome proliferator-activated receptors (PPARs).[22,23,27] Activation of PPARα by agonists or telmisartan induces an anti-inflammatory response through the repression of nuclear factor-κB signaling in umbilical vein endothelial cells and aortic smooth muscle cells *in vitro*[27,28] and inhibits macrophage infiltration and reduces aortic dilatation in a mouse model of aortic aneurysm.[29] PPARα and PPARγ improve lipid and glucose metabolism, respectively.[30] Low-dose telmisartan in $Rgs2^{-/-}$ mice improved lipid metabolism but did not affect glucose metabolism, as shown in the Table 1. Therefore, these protective effects of telmisartan in $Rgs2^{-/-}$ mice might be dependent on the anti-inflammatory response via PPARα activation, and Rgs2 deficiency might affect enhancement of the anti-inflammatory effect of telmisartan. Taken together, these results show that the therapeutic effect of low-dose telmisartan might be higher in the aorta of $Rgs2^{-/-}$ mice than in that of $Rgs2^{+/+}$ mice through both AT_1R blockade and PPARα activation.

Hypertension is a major risk factor of cardiovascular disease. Human *RGS2* genetic polymorphism is associated with the pathogenesis of hypertension in different races[11–14] that may result from Gαq-signal acceleration by RGS2 dysfunction. Our mouse study did not indicate a relationship between Rgs2 deficiency and the development of atherosclerosis and aneurysm *in vivo*. Instead, we found that low-dose telmisartan would be beneficial in AngII-induced vascular remodeling, dependent on Rgs2 deficiency and dysfunction. This

study suggests that ARB might be more useful for protection from cardiovascular events in hypertensive subjects with risk alleles in the *RGS2* gene than other antihypertensive drugs. This concept might be applicable for personalized medicine on the basis genetic information.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Boehringer Ingelheim (Germany) for providing telmisartan. We are grateful to Dr Michael E Mendelsohn (Tufts University School of Medicine) for providing the Rgs2-deficient mice. This work was supported in part by grants-in-aids from the Program for the Promotion of Fundamental Studies in the National Institute of Biochemical Innovation of Japan; the Ministry of Health, Labor and Welfare of Japan; the Ministry of Education, Culture, Sports, Science and Technology of Japan; and the Japan Cardiovascular Research Foundation.

1 Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J Clin Invest* 2000; **105**: 1605–1612.
2 Deng GG, Martin-McNulty B, Sukovich DA, Freay A, Halks-Miller M, Thinnes T, Loskutoff DJ, Carmeliet P, Dole WP, Wang YX. Urokinase-type plasminogen activator plays a critical role in angiotensin II-induced abdominal aortic aneurysm. *Circ Res* 2003; **92**: 510–517.
3 Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 1994; **74**: 1141–1148.
4 Marchesi C, Paradis P, Schiffrin EL. Role of the renin-angiotensin system in vascular inflammation. *Trends Pharmacol Sci* 2008; **29**: 367–374.
5 Tang KM, Wang GR, Lu P, Karas RH, Aronovitz M, Heximer SP, Kaltenbronn KM, Blumer KJ, Siderovski DP, Zhu Y, Mendelsohn ME. Regulator of G-protein signaling-2 mediates vascular smooth muscle relaxation and blood pressure. *Nat Med* 2003; **9**: 1506–1512.
6 Sun X, Kaltenbronn KM, Steinberg TH, Blumer KJ. RGS2 is a mediator of nitric oxide action on blood pressure and vasoconstrictor signaling. *Mol Pharmacol* 2005; **67**: 631–639.
7 Calo LA, Pagnin E, Ceolotto G, Davis PA, Schiavo S, Papparella I, Semplicini A, Pessina AC. Silencing regulator of G protein signaling-2 (RGS-2) increases angiotensin II signaling: insights into hypertension from findings in Bartter's/Gitelman's syndromes. *J Hypertens* 2008; **26**: 938–945.
8 Oliveira-Dos-Santos AJ, Matsumoto G, Snow BE, Bai D, Houston FP, Whishaw IQ, Mariathasan S, Sasaki T, Wakeham A, Ohashi PS, Roder JC, Barnes CA, Siderovski DP, Penninger JM. Regulation of T cell activation, anxiety, and male aggression by RGS2. *Proc Natl Acad Sci USA* 2000; **97**: 12272–12277.
9 Heximer SP, Knutsen RH, Sun X, Kaltenbronn KM, Rhee MH, Peng N, Oliveira-dos-Santos A, Penninger JM, Muslin AJ, Steinberg TH, Wyss JM, Mecham RP, Blumer KJ. Hypertension and prolonged vasoconstrictor signaling in RGS2-deficient mice. *J Clin Invest* 2003; **111**: 445–452.
10 Hercule HC, Tank J, Plehm R, Wellner M, da Costa Goncalves AC, Gollasch M, Diedrich A, Jordan J, Luft FC, Gross V. Regulator of G protein signalling 2 ameliorates angiotensin II-induced hypertension in mice. *Exp Physiol* 2007; **92**: 1014–1022.
11 Yang J, Kamide K, Kokubo Y, Takiuchi S, Tanaka C, Banno M, Miwa Y, Yoshii M, Horio T, Okayama A, Tomoike H, Kawano Y, Miyata T. Genetic variations of regulator of G-protein signaling 2 in hypertensive patients and in the general population. *J Hypertens* 2005; **23**: 1497–1505.
12 Riddle EL, Rana BK, Murthy KK, Rao F, Eskin E, O'Connor DT, Insel PA. Polymorphisms and haplotypes of the regulator of G protein signaling-2 gene in normotensives and hypertensives. *Hypertension* 2006; **47**: 415–420.
13 Kohara K, Tabara Y, Nakura J, Imai Y, Ohkubo T, Hata A, Soma M, Nakayama T, Umemura S, Hirawa N, Ueshima H, Kita Y, Ogihara T, Katsuya T, Takahashi N, Tokunaga K, Miki T. Identification of hypertension-susceptibility genes and pathways by a systemic multiple candidate gene approach: the millennium genome project for hypertension. *Hypertens Res* 2008; **31**: 203–212.
14 Watanabe Y, Metoki H, Ohkubo T, Katsuya T, Tabara Y, Kikuya M, Hirose T, Sugimoto K, Asayama K, Inoue R, Hara A, Obara T, Nakura J, Kohara K, Totsune K, Ogihara T, Rakugi H, Miki T, Imai Y. Accumulation of common polymorphisms is associated with development of hypertension: a 12-year follow-up from the Ohasama study. *Hypertens Res* 2010; **33**: 129–134.
15 Bodenstein J, Sunahara RK, Neubig RR. N-terminal residues control proteasomal degradation of RGS2, RGS4, and RGS5 in human embryonic kidney 293 cells. *Mol Pharmacol* 2007; **71**: 1040–1050.
16 Gu S, Tirgari S, Heximer SP. The RGS2 gene product from a candidate hypertension allele shows decreased plasma membrane association and inhibition of Gq. *Mol Pharmacol* 2008; **73**: 1037–1043.
17 Wienen W, Entzeroth M, van Meel JCA, Stangier J, Busch U, Ebner T, Schmid J, Lehmann H, Matzek K, Kempthorne-Rawson J, Gladigau V, Hauel NH. A review on telmisartan: a novel, long-acting angiotensin II-receptor antagonist. *Caldiovasc Drug Rev* 2000; **18**: 127–154.
18 Yusuf S, Teo K, Anderson C, Pogue J, Dyal L, Copland I, Schumacher H, Dagenais G, Sleight P. Effects of the angiotensin-receptor blocker telmisartan on cardiovascular events in high-risk patients intolerant to angiotensin-converting enzyme inhibitors: a randomised controlled trial. *Lancet* 2008; **372**: 1174–1183.
19 Verdecchia P, Angeli F, Mazzotta G, Ambrosio G, Reboldi G. Angiotensin receptor blockers in hypertension. New insights from Japan. *Hypertens Res* 2010; **33**: 394–397.
20 Takaya T, Kawashima S, Shinohara M, Yamashita T, Toh R, Sasaki N, Inoue N, Hirata K, Yokoyama M. Angiotensin II type 1 receptor blocker telmisartan suppresses superoxide production and reduces atherosclerotic lesion formation in apolipoprotein E-deficient mice. *Atherosclerosis* 2006; **186**: 402–410.
21 Terashima M, Ohashi Y, Azumi H, Otsui K, Kaneda H, Awano K, Kobayashi S, Honjo T, Suzuki T, Maeda K, Yokoyama M, Inoue N. Impact of NAD(P)H oxidase-derived reactive oxygen species on coronary arterial remodeling: a comparative intravascular ultrasound and histochemical analysis of atherosclerotic lesions. *Circ Cardiovasc Interv* 2009; **2**: 196–204.
22 Schupp M, Janke J, Clasen R, Unger T, Kintscher U. Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor-γ activity. *Circulation* 2004; **109**: 2054–2057.
23 Clemenz M, Frost N, Schupp M, Caron S, Foryst-Ludwig A, Bohm C, Hartge M, Gust R, Staels B, Unger T, Kintscher U. Liver-specific peroxisome proliferator-activated receptor α target gene regulation by the angiotensin type 1 receptor blocker telmisartan. *Diabetes* 2008; **57**: 1405–1413.
24 Kaschina E, Schrader F, Sommerfeld M, Kemnitz UR, Grzesiak A, Krikov M, Unger T. Telmisartan prevents aneurysm progression in the rat by inhibiting proteolysis, apoptosis and inflammation. *J Hypertens* 2008; **26**: 2361–2373.
25 Fujiwara Y, Shiraya S, Miyake T, Yamakawa S, Aoki M, Makino H, Nishimura M, Morishita R. Inhibition of experimental abdominal aortic aneurysm in a rat model by the angiotensin receptor blocker valsartan. *Int J Mol Med* 2008; **22**: 703–708.
26 Satoh M, Haruna Y, Fujimoto S, Sasaki T, Kashihara N. Telmisartan improves endothelial dysfunction and renal autoregulation in Dahl salt-sensitive rats. *Hypertens Res* 2010; **33**: 135–142.
27 Nakano A, Hattori Y, Aoki C, Jojima T, Kasai K. Telmisartan inhibits cytokine-induced nuclear factor-κB activation independently of the peroxisome proliferator-activated receptor-γ. *Hypertens Res* 2009; **32**: 765–769.
28 Staels B, Koenig W, Habib A, Merval R, Lebret M, Torra IP, Delerive P, Fadel A, Chinetti G, Fruchart JC, Najib J, Maclouf J, Tedgui A. Activation of human aortic smooth-muscle cells is inhibited by PPARα but not by PPARγ activators. *Nature* 1998; **393**: 790–793.
29 Golledge J, Cullen B, Rush C, Moran CS, Secomb E, Wood F, Daugherty A, Campbell JH, Norman PE. Peroxisome proliferator-activated receptor ligands reduce aortic dilatation in a mouse model of aortic aneurysm. *Atherosclerosis* 2010; **210**: 51–56.
30 Marx N, Duez H, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. *Circ Res* 2004; **94**: 1168–1178.

Journal of the American College of Cardiology
Published by Elsevier Inc.
Vol. 56, No. 19, 2010
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2010.06.033
© 2010 by the American College of Cardiology Foundation

Augmented ST-Segment Elevation During Recovery From Exercise Predicts Cardiac Events in Patients With Brugada Syndrome

Hisaki Makimoto, MD,* Eiichiro Nakagawa, MD, PHD,† Hiroshi Takaki, MD, PHD,* Yuko Yamada MD,* Hideo Okamura, MD,* Takashi Noda, MD, PHD,* Kazuhiro Satomi, MD, PHD,* Kazuhiro Suyama, MD, PHD,* Naohiko Aihara, MD,* Takashi Kurita, MD, PHD,‡ Shiro Kamakura, MD, PHD,* Wataru Shimizu, MD, PHD*

Suita and Osaka, Japan

Objectives The goal of this study was to evaluate the prevalence and the clinical significance of ST-segment elevation during recovery from exercise testing.

Background During recovery from exercise testing, ST-segment elevation is reported in some patients with Brugada syndrome (BrS).

Methods Treadmill exercise testing was conducted for 93 patients (91 men), 46 ± 14 years of age, with BrS (22 documented ventricular fibrillation, 35 syncope alone, and 36 asymptomatic); and for 102 healthy control subjects (97 men), 46 ± 17 years of age. Patients were routinely followed up. The clinical end point was defined as the occurrence of sudden cardiac death, ventricular fibrillation, or sustained ventricular tachyarrhythmia.

Results Augmentation of ST-segment elevation ≥0.05 mV in V_1 to V_3 leads compared with baseline was observed at early recovery (1 to 4 min at recovery) in 34 BrS patients (37% [group 1]), but was not observed in the remaining 59 BrS patients (63% [group 2]) or in the 102 control subjects. During 76 ± 38 months of follow-up, ventricular fibrillation occurred more frequently in group 1 (15 of 34, 44%) than in group 2 (10 of 59, 17%; p = 0.004). Multivariate Cox regression analysis showed that in addition to previous episodes of ventricular fibrillation (p = 0.005), augmentation of ST-segment elevation at early recovery was a significant and independent predictor for cardiac events (p = 0.007), especially among patients with history of syncope alone (6 of 12 [50%] in group 1 vs. 3 of 23 [13%] in group 2) and among asymptomatic patients (3 of 15 [20%] in group 1 vs. 0 of 21 [0%] in group 2).

Conclusions Augmentation of ST-segment elevation during recovery from exercise testing was specific in patients with BrS, and can be a predictor of poor prognosis, especially for patients with syncope alone and for asymptomatic patients. (J Am Coll Cardiol 2010;56:1576–84) © 2010 by the American College of Cardiology Foundation

Brugada syndrome (BrS) is recognized as a clinical syndrome that leads to sudden cardiac death (SCD) in middle-aged persons due to ventricular fibrillation (VF) (1). Brugada syndrome is defined by a distinct 12-lead electrocardiogram (ECG) pattern in precordial leads (V_1 to V_3) presenting coved-type ST-segment elevation. Both depolarization and repolarization hypotheses have been reported for the pathogenesis of phenotype in BrS (2–5). Although several indexes have been reported as predictive factors of VF occurrence (6), the recent largest series of BrS patients suggested that there were no reliable predictors of cardiac events except for prior symptoms and spontaneous type 1 ECG (7). However, risk stratification remains disputable, especially for BrS patients without documented VF episodes.

See page 1585

Autonomic function has been suggested to relate to the occurrence of VF in BrS. It has also been shown that ST-segment elevation in patients with BrS was augmented

From the *Division of Arrhythmia and Electrophysiology, Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Suita, Japan; the †Department of Cardiology, Osaka City General Hospital, Osaka, Japan; and the ‡Division of Cardiology, Department of Internal Medicine, Kinki University School of Medicine, Osaka-Sayama, Osaka, Japan. Dr. Shimizu was supported in part by a research grant for the Cardiovascular Diseases (21C-8, 22-4-7) from the Ministry of Health, Labor and Welfare, Japan. All other authors have reported that they have no relationships to disclose.

Manuscript received April 21, 2010; revised manuscript received June 8, 2010, accepted June 15, 2010.

by selective stimulation of muscarinic receptors but mitigated by beta-adrenergic stimulation (8). Heart rate during exercise testing is considered as 1 parameter to evaluate cardiac autonomic function (9). Sympathetic withdrawal and parasympathetic activation occur at early recovery after exercise (10), which are expected to augment ST-segment elevation directly by inhibition of calcium-channel current or by decreasing heart rate (5,11). Two cases of BrS were reported in which ST-segment was augmented during and after exercise (12). Amin et al. (13) recently assessed the ECG responses to exercise in BrS patients with and without *SCN5A* mutations and control subjects. They reported that exercise resulted in an increase of peak J-point amplitude in all groups, including control subjects, and more QRS widening in BrS patients with *SCN5A* mutation. The peak J-point amplitude measured by Amin et al. (13) is thought to represent the depolarization parameter as QRS duration, or at least the combined parameter of both depolarization and repolarization. Therefore, in the present study, we measured several points of ST-segment as a repolarization parameter rather than a depolarization parameter, and tried to investigate the relationship between augmented ST-segment elevation during recovery from exercise testing and prognosis of BrS patients. We also evaluated parasympathetic reactivation by using heart rate recovery (HRR), which is defined as heart rate decay in the first minute after exercise cessation, and its relation with ST-segment change.

Methods

Study population. The study population consisted of 93 consecutive Japanese patients with BrS (91 males; mean age 46 ± 14 years) admitted to the National Cerebral and Cardiovascular Center in Suita, Japan, between 1994 and 2006. Ventricular fibrillation was documented in 22 BrS patients, syncope alone in 35 patients, and the remaining 36 patients were asymptomatic. As control subjects, 102 age-, sex-, and QRS duration-matched healthy subjects were randomly selected from persons who underwent treadmill exercise testing between 2002 and 2007 (97 males; mean age 46 ± 17 years). They included 55 normal subjects with normal QRS duration (<100 ms), 21 with incomplete right bundle branch block (RBBB) (100 ms ≤QRS duration <120 ms), and 26 with complete RBBB (120 ms ≤QRS duration) but without structural heart disease or any ventricular arrhythmias.

Brugada syndrome was diagnosed when a coved 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–blocking agent, and in conjugation with 1 of the following: documented VF, polymorphic ventricular tachycardia, family history of SCD <45 years of age, family history of BrS, inducibility of VF with programmed electrical stimulation, syncope, or an nocturnal agonal respiration (6). Structural heart diseases were carefully excluded by history taking, physical examinations, chest roentgenogram, ECG, and echocardiogram.

Abbreviations and Acronyms
AF = atrial fibrillation
BrS = Brugada syndrome
ECG = electrocardiogram
EPS = electrophysiologic study
HRR = heart rate recovery
ICD = implantable cardioverter-defibrillator
RBBB = right bundle branch block
RVOT = right ventricular outflow tract
SCD = sudden cardiac death
VF = ventricular fibrillation

Clinical, laboratory, electrocardiographic, and electrophysiologic study. The following clinical data were collected: family history of SCD (<45 years of age) or BrS, documented atrial fibrillation (AF), documented VF, syncope, age at the first cardiac event, and implantation of implantable cardioverter-defibrillator (ICD).

A 12-lead ECG was recorded in all 93 BrS patients, and RR interval, PR interval (lead II), QRS duration (lead V_5), corrected QT interval (lead V_2), QRS axis, J-point amplitude (leads V_2), and amplitude of several points of ST-segment (leads V_1, V_2, V_3) were measured.

Signal-averaged ECG was recorded and analyzed in 91 patients by using a signal-averaged ECG system (1200EPX, Arrhythmia Research Technology, Milwaukee, Wisconsin). Three parameters were assessed using a computer algorithm: 1) total filtered QRS duration; 2) root mean square voltage of the terminal 40 ms of the filtered QRS complexes (V_{40}); and 3) duration of low-amplitude signals <40 μV of the filtered QRS complexes (T_{40}). Late potential was considered present when the 2 criteria (V_{40} <18 μV and T_{40} >38 ms) were fulfilled.

Electrophysiologic study (EPS) was performed in 79 BrS patients (21 documented VF patients, 30 syncope alone patients, and 28 asymptomatic patients). A maximum of 3 programmed ventricular extrastimuli were delivered from the right ventricular apex and RVOT, unless VF was induced. No patients received antiarrhythmic drugs before EPS. The atrio-His and His-ventricular intervals were measured during sinus rhythm. The EPS was conducted after all subjects gave written informed consent.

Genetic testing for the presence of an *SCN5A* mutation was also conducted.

Exercise testing. Treadmill exercise testing was conducted in all 93 patients with BrS and 102 control subjects. Neither BrS patients nor control subjects used antiarrhythmic agents. A symptom-limited or submaximal (up to 90% of the age-predicted maximum heart rate) graded treadmill exercise testing similar to modified Bruce protocol was used. All 93 BrS patients and 102 control subjects were in normal sinus rhythm, and none had atrioventricular block at the exercise testing. The standard 12-lead ECGs were recorded at rest, at the end of each exercise stage, at peak exercise, and at every minute during recovery. The amplitude of ST-segment from the isoelectric line at the right precordial leads (V_1 to V_3 leads) and QRS width at V_5 lead were manually measured. The ST-segment point was defined as the point

where the vertical line from the end point of QRS at V_5 lead intersected the precordial leads. We also measured peak J-point amplitude in lead V_2 as a depolarization parameter, and amplitude of the point, which was 40 and 80 ms later than the peak J-points (ST40, ST80) in lead V_2 as a repolarization parameter. Measurements of ECG parameters were performed as the mean of 3 beats by single electrocardiologist who knew nothing about the patients. Significant augmentation of ST-segment elevation was defined as ST-segment amplitude increase ≥0.05 mV in at least 1 of V_1 to V_3 leads at early recovery (1 to 4 min at recovery) compared with the ST-segment amplitude at baseline (pre-exercise). We also recorded heart rate and blood pressure during exercise testing.

The HRR was defined as decay of heart rate from peak exercise to 1 min at recovery.

Follow-up. Follow-up was started after undergoing treadmill exercise testing. All patients with BrS were routinely followed up at the outpatient clinic of our hospital. The ICD implantation was performed in 63 BrS patients (20 documented VF patients, 25 syncope alone patients, and 18 asymptomatic patients). Antiarrhythmic drugs were prescribed for 7 patients; 2 patients who had episodes of VF but refused implantation of ICD (disopyramide 300 mg daily for 1 patient, and amiodarone 200 mg daily for another patient), 2 patients who had AF (quinidine 300 mg daily), and 3 patients who had previous history of both VF and AF and implanted ICD (quinidine 300 mg daily for 1 patient, amiodarone 200 mg daily for 2 patients).

Cardiac events were defined as SCD or aborted cardiac arrest, and VF or sustained ventricular tachyarrhythmia documented by ICD or ECG recordings.

Statistical analysis. Data were analyzed with Dr. SPSS II for Windows software package (SPSS Inc., Chicago, Illinois). Numeric values are expressed as mean ± SD. The chi-square test, Student *t* test, or 1-way analysis of variance was performed when appropriate to test for statistical differences. All p values <0.05 were considered statistically significant. Event rate curves were plotted according to the Kaplan-Meier method, and were analyzed with the log-rank test. Univariate and multivariate Cox regression were performed to assess whether 7 indexes can be significant and independent predictors of subsequent cardiac events. We used the forward step-wise approach with p to enter a value of 0.05 for multivariate analysis. Augmentation of ST-segment elevation at early recovery, family history of SCD or BrS, spontaneous coved-type ST-segment elevation, presence of *SCN5A* mutation, late potential, VF inducibility during EPS, and previous episodes of VF were included as indexes.

Results

There were no significant differences between 93 BrS patients and 102 control subjects with respect to age at exercise testing, sex, QRS duration (lead V_5), and QTc interval (lead V_2), as summarized in Table 1. The RR interval and PR interval (lead II) were significantly longer in BrS patients than in control subjects.

Table 1 Initial Characteristics of Patients and Control Subjects

	Brugada Patients (n = 93)	Control Subjects (n = 102)	p Value
Age at exercise testing, yrs	46 ± 14	46 ± 17	NS
Sex, male	91 (98%)	97 (95%)	NS
Electrocardiographic characteristics, ms			
RR	952 ± 151	903 ± 140	0.020
PR	178 ± 30	165 ± 24	0.001
QRS duration	98 ± 16	98 ± 20	NS
QTc	416 ± 44	406 ± 30	NS

Values are mean ± SD or n (%).
QTc = corrected QT interval.

Response of ST-segment elevation during treadmill exercise testing. Among 93 BrS patients, significant augmentation of ST-segment elevation mostly associated with coved pattern at early recovery phase was observed in 34 BrS patients (37% [group 1]), but not in the remaining 59 BrS patients (63% [group 2]). Conversely, ST-segment augmentation was never observed in any of the 102 control subjects (34 of 93 [37%] vs. 0 of 102 [0%], p < 0.0001). Typical responses of ST-segment amplitudes of 3 groups are shown in Figure 1. Composite data of serial changes of ST-segment amplitude in V_1 and V_2 leads during exercise testing are illustrated in Figure 2A. The serial changes of ST-segment amplitude in V_3 lead showed the same trend (not shown). In group 1, ST-segment amplitude decreased at peak exercise and started to reascend at early recovery, and culminated at 3 min of recovery (Figs. 1A and 2A). In contrast, ST-segment amplitude of group 2 patients and control subjects decreased at peak exercise, and gradually returned to the baseline amplitude rather than showing augmentation (Figs. 1B to 1D and 2A). Significant differences were identified between group 1 and group 2 patients in the ST-segment amplitude in leads V_1 and V_2 from peak exercise to 6 min of recovery, whereas no major differences were observed between group 2 patients and control subjects (Fig. 2A). Composite data of serial changes of peak J-point amplitude, ST40, and ST80 amplitudes are presented in Figure 2B. The peak J-point amplitude and ST40 amplitude during recovery showed the same trend as the ST-segment amplitude in Figure 2A. Significant differences were identified between group 1 and group 2 patients in the peak J-point and ST40 amplitudes from peak exercise to 6 min of recovery. The ST80 amplitude showed significant differences between group 1 and group 2 patients at 2, 3, and 4 min of recovery. At peak exercise, the peak J-point amplitude increased in 34 (37%) of 93 Brugada patients and in 26 (26%) of 102 control subjects, although the ST-segment

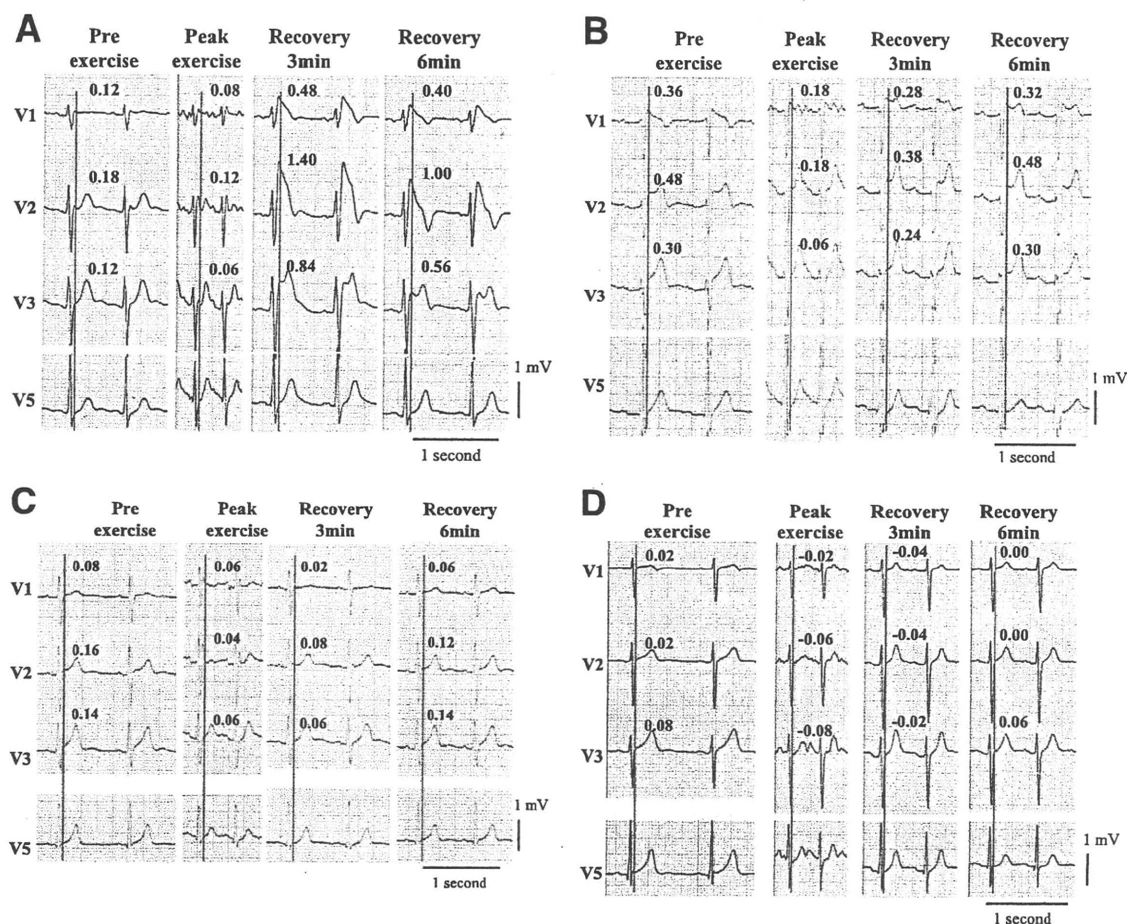

Figure 1 **Typical Responses of ST-Segment Amplitude in Leads V_1, V_2, V_3, and V_5 During Exercise Testing in Brugada Syndrome Patients**

(A) In the group 1 Brugada patient showing saddle-back type ST-segment (lead V_2) at baseline, ST-segment amplitude slightly decreased at peak exercise, but reascended at early recovery (3 min), resulting in typical coved-type ST-segment elevation. **(B, C)** In the group 2 Brugada patient and **(D)** in the control subject, ST-segment amplitude decreased at peak exercise and gradually recovered to the baseline at recovery. It is noteworthy that the peak J-point amplitude in lead V_2 was augmented despite not showing ST-segment augmentation in **A** and **C**. The ST-segment amplitudes are shown as numeric values expressed in millivolts (mV). The **red vertical line** indicates the line from the end point of the QRS interval at electrocardiography lead V_5.

amplitude and ST40 amplitude decreased in most patients of both groups.

Comparison of HRR is shown in Figure 3. The HRR of group 1 patients was significantly larger than that of group 2 patients (32 ± 15 vs. 23 ± 10, p = 0.0007) and control subjects (32 ± 15 vs. 26 ± 10, p = 0.021). The differences of HRR between group 2 patients and control subjects were also statistically significant (23 ± 10 vs. 26 ± 10, p = 0.026).

Although there were no sustained or nonsustained ventricular arrhythmias throughout exercise testing, single premature ventricular complexes were observed during exercise in 8 of the group 1 patients and in 11 of the group 2 patients, and at recovery in 10 of the group 1 patients and in 9 of the group 2 patients. There were no significant differences between groups 1 and 2 in incidences of premature ventricular complexes.

Clinical, laboratory, electrocardiographic, and electrophysiologic characteristics. Comparison of the clinical, laboratory, electrocardiographic, and electrophysiologic characteristics between groups 1 and 2 patients are shown in Table 2. There were no significant differences in these characteristics between groups 1 and 2 except for the presence of *SCN5A* mutation and late potential (*SCN5A* mutation, 17% vs. 5%, p = 0.048; late potential, 82% vs. 53%, p = 0.004).

Follow-up. The mean follow-up period for the 93 BrS patients was 75.7 ± 38.4 months. During follow-up, 25 of all 93 BrS patients (27%) had cardiac events, and the incidence of cardiac events was significantly higher in group 1 than in group 2 patients (44% vs. 17%, p = 0.004). The period from exercise testing to cardiac events ranged from 1 to 78 months (median 12 months). One patient in group 2, who refused implantation of ICD and was taking disopyr-

JACC Vol. 56, No. 19, 2010
November 2, 2010:1576–84

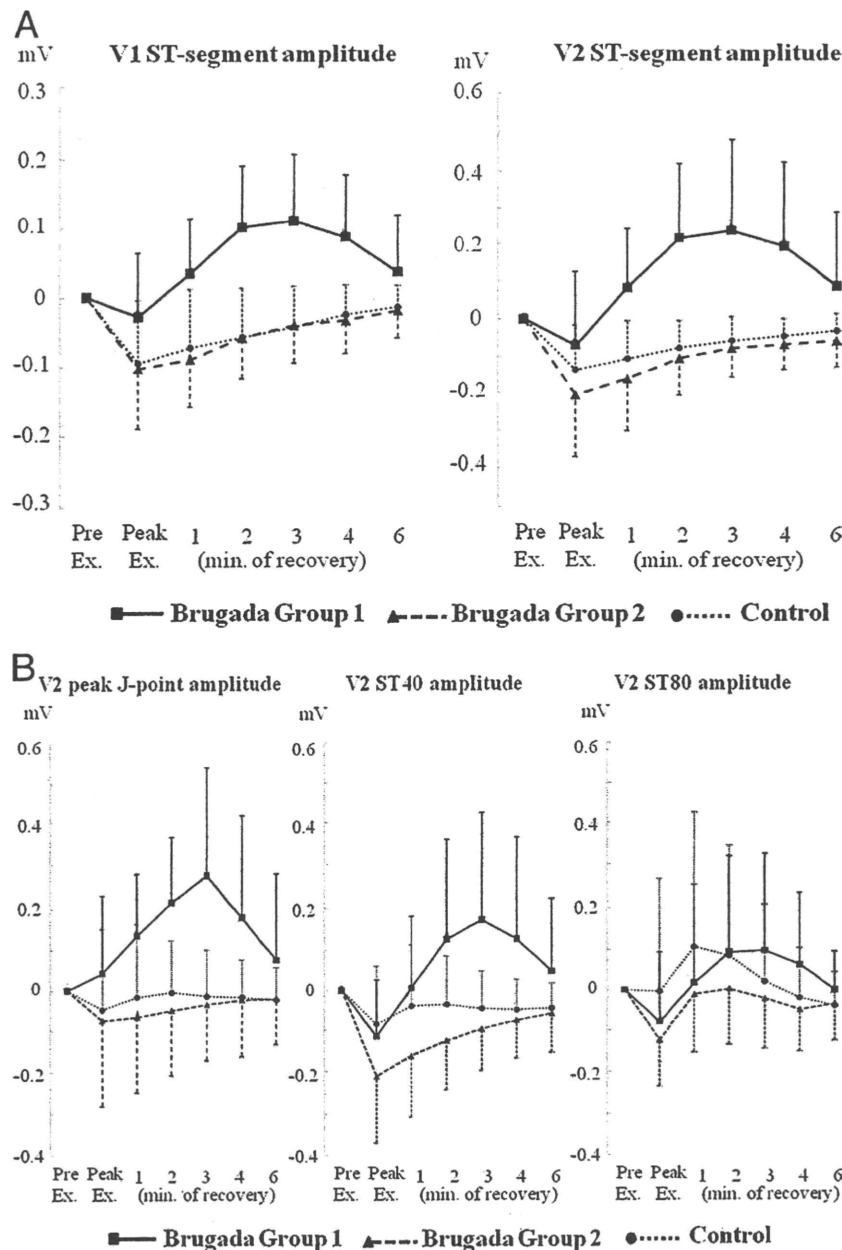

Figure 2 Composite Data of Serial Changes of ST-Segment Amplitude

(A) Composite data of serial changes of ST-segment amplitude in lead V_1 **(left)** and lead V_2 **(right)** during exercise (Ex.) testing in group 1 Brugada syndrome patients **(squares)** and group 2 Brugada syndrome patients **(triangles)**, and in control subjects **(circles)**. **(B)** Peak J-point amplitude **(left)**, ST40 amplitude **(middle)**, and ST80 amplitude **(right)** in lead V_2. The ST-segment amplitude decreased at peak exercise and started to reascend at early recovery, and culminated at 3 min of recovery in group 1 Brugada patients. In the group 2 Brugada patients and control subjects, the ST-segment amplitude decreased at peak exercise and gradually recovered to the baseline level during recovery. The peak J-point amplitude and ST40 amplitude during recovery showed the same trend as the ST-segment amplitude. Since ST80 amplitude was influenced by T wave, especially at rapid heart rate, the trends of the 3 groups were somewhat different from ST-segment amplitude or ST40 amplitude. The ST-segment amplitudes are shown as values compared to pre-exercise ST-segment amplitudes. $p < 0.05$.

amide 300 mg daily, died of VF. Three of 7 patients with medication had cardiac events, including 1 death.

Predictors of outcome. Kaplan-Meier analysis demonstrated significant differences in the time to the first cardiac event depending on the presence of ST-segment augmentation during recovery from exercise (Fig. 4A). Group 1 patients had a significantly higher cardiac event rate than group 2 patients (log-rank, $p = 0.0029$). Previous history of VF (Fig. 4B) and positive *SCN5A* mutation (Fig. 4C) also had significant values for occurrence of subsequent cardiac events ($p = 0.0013$ and $p = 0.028$, respectively); however, spontaneous coved-type ST-segment elevation did not predict cardiac events (p =

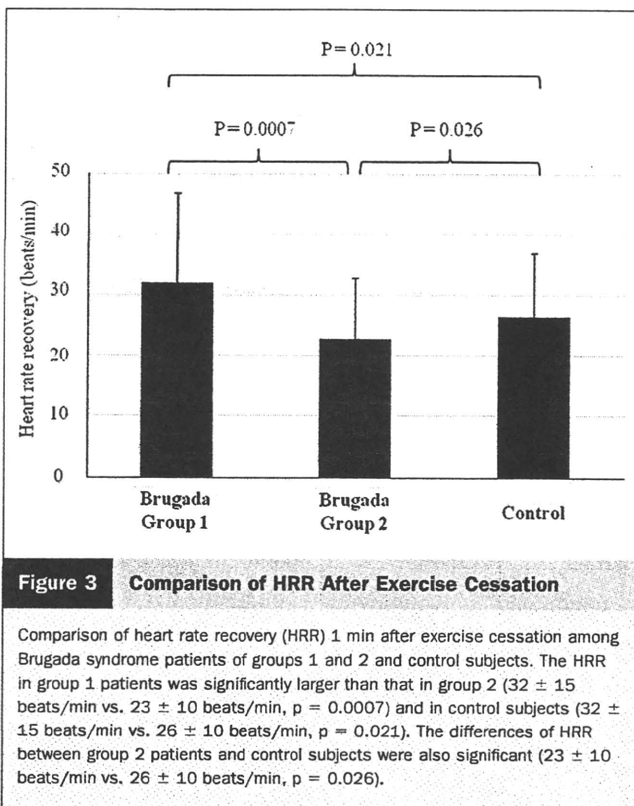

Figure 3 Comparison of HRR After Exercise Cessation

Comparison of heart rate recovery (HRR) 1 min after exercise cessation among Brugada syndrome patients of groups 1 and 2 and control subjects. The HRR in group 1 patients was significantly larger than that in group 2 (32 ± 15 beats/min vs. 23 ± 10 beats/min, p = 0.0007) and in control subjects (32 ± 15 beats/min vs. 26 ± 10 beats/min, p = 0.021). The differences of HRR between group 2 patients and control subjects were also significant (23 ± 10 beats/min vs. 26 ± 10 beats/min, p = 0.026).

0.068) (Fig. 4D). The results of Cox regression analysis are shown in Table 3. In univariate analysis, indexes predictive of cardiac events were previous episodes of VF (p = 0.003), ST-segment augmentation at early recovery (group 1; p = 0.005), and presence of *SCN5A* mutation (p = 0.037). In multivariate Cox regression analysis, previous episodes of VF and ST-segment augmentation at early recovery were significant and independent predictors of subsequent cardiac events (p = 0.005 and p = 0.007, respectively).

The incidence of cardiac events during follow-up in the subgroups according to symptoms before exercise testing is shown in Table 4. In the subgroup of 35 BrS patients with syncope alone, group 1 had a significantly higher cardiac event rate than group 2 (log-rank, 6 of 12 [50%] vs. 3 of 23 [13%], p = 0.016). Of note, among 36 asymptomatic patients, only 3 patients (9%) in group 1 experienced cardiac events. The log-rank test also demonstrated higher cardiac event risk in group 1 compared with group 2 (3 of 15 [20%] vs. 0 of 21 [0%], p = 0.039).

Discussion

The major findings of the present study were the following: 1) 37% of BrS patients showed ST-segment augmentation at early recovery during exercise testing; 2) ST-segment augmentation at early recovery was specific in BrS patients, and was significantly associated with a higher cardiac event rate, notably for patients with previous episode of syncope or for asymptomatic patients; and 3) BrS patients with ST-segment augmentation at early recovery showed significantly larger HRR. This is the first systematic report on the relationship between ST-segment augmentation during recovery from exercise and prognosis for BrS patients.

Augmentation of ST-segment elevation and possible mechanism. It is well known that autonomic function influences an extent of ST-segment elevation in BrS (8). The ST-segment elevation is mitigated by administration of β-adrenergic agonists and is enhanced by parasympathetic agonists such as acetylcholine in experimental and clinical investigations (5,14–16). Parasympathetic reactivation is thought to occur at early recovery after treadmill exercise testing, especially in the first minute after cessation of exercise (10,17). In the present study, we measured the ST-segment amplitude as a repolarization parameter rather than a depolarization parameter, and evaluated HRR to investigate the correlation between ST-segment augmentation and parasympathetic activity (9,18). The BrS patients who had ST-segment augmentation had significantly larger HRR compared with patients who did not, suggesting that the ST-segment augmentation was closely related to higher parasympathetic activity. However, it is still unclear whether ST-segment augmentation observed in the 34 BrS patients was simply due to more increased parasympathetic activity or to more increased susceptibility (hypersensitivity) to the parasympathetic reactivation.

Conversely, the *SCN5A* mutation was more frequently identified in group 1. Scornik et al. (19) reported that *SCN5A* mutation can accentuate parasympathetic activity toward the heart directly. It was also reported that specific mutations in the *SCN5A* gene may lead to augmentation of J-point amplitude or ST-segment amplitude during beta-adrenergic stimulation (20,21). Veldkamp et al. (20) demonstrated that a specific *SCN5A* mutation, 1795insD, augments slow inactivation, and delays recovery of sodium channel availability, thus reducing the sodium current and resulting in augmented peak J-point amplitude at rapid heart rate. Increased body temperature induced by exercise can be a risk of life-threatening arrhythmias in patients with BrS (22). A specific *SCN5A* missense mutation, T1620M, was reported to cause a faster decay of the sodium channel but slower recovery from inactivation, resulting in increased ST-segment elevation in precordial leads at higher temperatures during exercise. Although Amin et al. (13) reported that exercise induced augmentation of peak J-point amplitude, a depolarization parameter or at least combined parameter of both depolarization and repolarization, in all subjects tested, the incidence of increase in the peak J-point amplitude at peak exercise was lower (37%) in our Brugada patients. This is probably in part because only 9 (10%) of our 93 BrS patients had the *SCN5A* mutation. We could not identify significant differences in HRR, QRS duration, peak J-point amplitude (lead V_2), and ST-segment amplitude (leads V_1, V_2, V_3) at peak exercise between patients with and without *SCN5A* mutation (not shown), and that may be also due to the small number of BrS patients with *SCN5A* mutation.

Risk stratification in BrS. Implantation of an ICD is a first line of therapy for secondary prevention in patients with BrS who exhibited previous history of VF. The American College

Table 2 Clinical, Laboratory, Electrocardiographic, and Electrophysiologic Characteristics and Long-Term Follow-Up of Groups 1 and 2 Brugada Syndrome Patients

Characteristic	Group 1 (n = 34)	Group 2 (n = 59)	p Value
Clinical characteristics			
Age at exercise testing, yrs	42 ± 11	48 ± 15	NS
Men	34 (100%)	57 (97%)	NS
Family history of SCD at age <45 yrs or Brugada syndrome	7 (21%)	16 (27%)	NS
Documented AF	7 (21%)	12 (20%)	NS
Documented VF before exercise testing	7 (21%)	15 (25%)	NS
Syncope alone before exercise testing	12 (35%)	23 (39%)	NS
Asymptomatic before exercise testing	15 (44%)	21 (36%)	NS
Age at first cardiac event, yrs	42 ± 13	45 ± 15	NS
ICD implantation	25 (74%)	38 (64%)	NS
Laboratory characteristics			
SCN5A mutation	6 (17%)	3 (5%)	0.048
Electrocardiographic characteristics			
RR, ms	951 ± 170	953 ± 140	NS
PR, ms	184 ± 28	175 ± 31	NS
QRS, ms	98 ± 14	98 ± 17	NS
QTc, ms	418 ± 46	415 ± 43	NS
ST-segment amplitude (mV) at baseline			
V_1	0.14 ± 0.09	0.16 ± 0.12	NS
V_2	0.41 ± 0.22	0.38 ± 0.26	NS
V_3	0.22 ± 0.13	0.19 ± 0.14	NS
Spontaneous coved-type ST-segment elevation in right precordial leads	30 (88%)	43 (73%)	NS
Signal-averaged electrocardiogram			
TfQRS, ms	122 ± 15	118 ± 17	NS
Late potential	28/34 (82%)	30/57 (53%)	0.004
Premature ventricular complexes during exercise	8 (24%)	11 (19%)	NS
Premature ventricular complexes at recovery	10 (29%)	9 (15%)	NS
Electrophysiologic characteristics			
AH interval, ms	107 ± 24	98 ± 27	NS
HV interval, ms	45 ± 8	44 ± 11	NS
Induction of VF	26/31 (84%)	33/47 (70%)	NS
Follow-up			
Cardiac events	15 (44%)	10 (17%)	0.004
Follow-up period, months	74.1 ± 42.2	76.5 ± 36.4	NS

AF = atrial fibrillation; ICD = implantable cardioverter-defibrillator; SCD = sudden cardiac death; TfQRS = total filtered QRS duration; VF = ventricular fibrillation; other abbreviations as in Table 1.

of Cardiology/American Heart Association/Heart Rhythm Society guidelines refer to BrS patients who have had syncope as having Class IIa indication for ICD therapy (23). However, there is still much room for argument with respect to treatments for patients who have had only syncope, and for asymptomatic patients (24–28). Although inducibility of VF during EPS (25,26), family history of SCD (24), spontaneous type 1 ECG (25,27), and late potential (28) have been proposed as predictors of cardiac events, the availability of these indexes remains controversial (7,29).

In the present study, a previous episode of VF (or aborted cardiac arrest) was the strongest predictor of subsequent cardiac events, as in previous studies (7,30,31). Moreover, ST-segment augmentation at early recovery during exercise testing was a significant and independent predictor of subsequent cardiac events in the present study. The results suggested that parasympathetic activity plays an important role in both ST-segment augmentation and subsequent cardiac events. As previously noted, it remains unclear that the cause of ST-segment augmentation in our 34 patients was a result of more increased parasympathetic activity or of more increased susceptibility of the patients to the increased parasympathetic reactivation.

Study limitations. First, BrS patients were confined to those who were hospitalized in our hospital for close investigation. That indicates these patients can be biased toward relatively high risk. Second, the present study is based on data from a small population of 93 patients; hence, it was not sufficient to evaluate the prognosis, and there also was a small number of events. Although we adopted a step-wise approach, the limited number of events can lessen the precision of the consequences for multivariate Cox regression analysis.

Conclusions

The presence of *SCN5A* mutation was a significant predictor of subsequent cardiac events by univariate Cox regression analysis. However, multivariate Cox regression analysis showed it was not a significant predictor of prognosis.