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ORIGINAL ARTICLE

β-Adrenergic receptor gene polymorphism is a genetic risk factor for cardiovascular disease: a cohort study with hypertensive patients

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Single-nucleotide polymorphisms (SNPs) of the β-adrenergic receptor (*βADR*) subtypes are related to hypertension and obesity. This hospital-based cohort study with hypertensive patients evaluated five *βADR* SNPs in association with cardiovascular events. The cohort included 357 hypertensive patients (male=181; mean age=61.5 ± 11.8 years) seen between January 1998 and June 2004. The SNPs (Ser49Gly and Arg389Gly for *β1ADR*; Gly16Arg and Glu27Gln for *β2ADR*; Trp64Arg for *β3ADR*) were identified by PCR. We used Kaplan–Meier curves to assess the prognostic effect of these SNPs on cardiovascular disease (CVD). The SNP frequencies were Ser/Ser:Ser/Gly:Gly/Gly=243:104:10; Arg/Arg:Arg/Gly:Gly/Gly=256:95:6; Gly/Gly:Gly/Arg:Arg/Arg=71:201:85; Gln/Gln:Glu/Gln=308:49; and Trp/Trp:Trp/Arg:Arg/Arg=265:89:3. A total of 17 stroke and 15 coronary artery disease cases were recorded. By Kaplan–Meier analysis, the Ser/Ser SNP in Ser49Gly (*P*=0.0398), the Glu/Gln SNP in Glu27Gln (*P*=0.0390) and the Trp/Trp SNP in Trp64Arg (*P*=0.0132) were associated with lower event-free CVD survival (log-rank, Mantel–Cox model). A Cox proportional hazards model revealed that only the Trp/Trp SNP (*P*=0.0321) and age (*P*=0.0186) were independently related to lower event-free survival for CVD, adjusted for gender, diabetes, dyslipidemia, blood pressure, body mass index, medication and hypertensive complications. Combination Kaplan–Meier analysis of these three positive SNPs indicated a higher frequency of CVD among patients with the combination of Ser/Ser in Ser49Gly of *β1*, Glu/Gln in Glu27Gln of *β2* and Trp/Trp in Trp64Arg of *β3* (*P*=0.0209). These three SNPs, especially the Trp64Arg SNP of *β3ADR*, might be risk factors for CVD in hypertensive patients.
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Keywords: β-adrenoreceptor; cohort study; hypertensive complication; polymorphism

INTRODUCTION

The human β-adrenergic receptor (*βADR*) is a member of the family of seven-transmembrane G-protein–coupled receptors and is encoded by a gene on chromosome 5.[1] Previous studies have shown that sympathetic nervous system activity by βADRs has an important role in the development of hypertension and its complications.[2] Three isotypes of human *βADR*, β_1, β_2 and β_3, are involved in this system. The classical subdivision of *βADR*s defines β_1 as the subtype that stimulates cardiac muscle[3] and β_2 as the subtype that relaxes smooth muscle.[4] The expression of the β_3 subtype is essentially limited to adipose tissue.[5] A number of single-nucleotide polymorphisms (SNPs) of the *βADR* subtypes have recently been reported to be positional candidate genes for cardiovascular diseases (CVDs).[6] For instance, the genotypes of the Ser49Gly and Arg389Gly polymorphisms in the human *β1ADR* gene have been reported to be associated with arterial stiffness[7] and acute myocardial infarction,[8] respectively. An association study[9] has suggested that the gene encoding *β2ADR* is associated with essential hypertension, and the Arg64 allele of the *β3ADR* gene is associated with obesity-related phenotypes, insulin resistance, hypertension, coronary artery disease and earlier age of onset of diabetes.[10] As noted, many studies have reported cross-sectional effects of these SNPs, and several cohort studies[11] have focused on one of these SNPs, but no cohort study on SNPs of *βADR* subtypes has focused on evaluating cardiovascular morbidity.

The aim of the present hospital-based cohort study, therefore, was to investigate the potential prognostic impact of the *βADR* SNPs that result in amino acid substitutions on the occurrence of CVDs and stroke in patients with essential hypertension.

METHODS

Study population and study design

This hospital-based cohort study was designed as a part of the NOn-invasive Atherosclerotic evaluation in Hypertension study.[12] In the NOn-invasive Atherosclerotic evaluation in Hypertension study, 813 serial outpatients who had been diagnosed with essential hypertension were sequentially recruited between January 1998 and June 2004 at the Osaka University Hospital. The

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study protocol was approved by the hospital ethics committee, written informed consent was obtained from all participants, and additional written informed consent was obtained from 384 participants for the analysis of genotypes; seven patients opted not to participate in this study. A clinical survey was conducted for every patient, and 14 patients were excluded because of insufficient information about cardiovascular events and/or mortality. We also evaluated five representative *βADR* SNPs and could not detect these SNPs in 13 patients. Ultimately, our analysis included 357 hypertensive patients, as shown in Table 2. A total of 120 patients were not treated with any anti-hypertensive drugs, and 237 patients were treated with one or more anti-hypertensive drugs: 137 patients with a calcium antagonist, 90 patients with an angiotensin II receptor blocker, 69 patients with an angiotensin-converting enzyme inhibitor, 45 patients with a *β*-blocker, 23 patients with a diuretic and 20 patients with an α-blocker. In addition, 110 patients were also treated with a statin.

Follow-up evaluation

Clinical follow-up was conducted by clinical visits, mailed questionnaires and telephone contact every September from 2003. The questionnaire included events related to hypertensive complications (described below) and cause of death. We also confirmed responses in detail by comparing them against patient medical records. The primary endpoint of this study was stroke (paralysis and diagnosis with computed tomography and/or magnetic resource imaging); the onset of CVD, including angina pectoris (typical chest pain with ST segment changes of electrocardiogram), myocardial infarction (St segmental elevation with more than twofold elevation of creatinine kinase), or heart failure (diagnosed by American Heart Association criteria); or the rupture of an aortic aneurysm (diagnosed by ultrasound echography or computed tomography). The follow-up duration was considered to encompass the interval from the initial evaluation to the time of event onset or September 2006. The average follow-up period was 57.0 ± 23.7 months.

Table 1 Patient characteristics and β-adrenoceptor polymorphisms at baseline

		β1			
		Ser49Gly		*Arg389Gly*	
	Total	*Ser/Ser*	*Ser/Gly+GlyGly*	*Arg/Arg*	*Arg/Gly+GlyGly*
Number	357	243	114	256	101
Male/female	181/176	132/111	53/61	142/114	36/65
Age (years)	61.5 ± 11.8	61.2 ± 12.5	61.4 ± 10.9	60.7 ± 12.1	62.0 ± 11.5
BMI (kg m^{-2})	24.3 ± 3.3	24.3 ± 3.3	24.6 ± 3.4	24.3 ± 3.2	24.1 ± 3.9
DL (%)	53.2	50.5	52.6	51.9	51.3
Diabetes (%)	16.2	17.5	27.1	23.5	17.5
SBP (mm Hg)	140 ± 18	140 ± 18	141 ± 19	140 ± 19	140 ± 17
DBP (mm Hg)	83 ± 11	83 ± 11	83 ± 12	83 ± 12	83 ± 11
Heart rate (bpm)	67 ± 11	67 ± 11	67 ± 10	67 ± 10	67 ± 10
Total-C (mg dl^{-1})	207 ± 35	208 ± 34	207 ± 38	206 ± 35	213 ± 34
Triglyceride (mg dl^{-1})	145 ± 18	147 ± 90	142 ± 91	155 ± 98**	122 ± 57
HDL-C (mg dl^{-1})	58 ± 17	58 ± 15	58 ± 21	56 ± 15***	63 ± 20
FBG (mg dl^{-1})	104 ± 23	104 ± 21	104 ± 26	106 ± 24	101 ± 20
HbA1c (%)	5.4 ± 1.0	5.3 ± 0.8**	5.7 ± 1.3	5.4 ± 1.0	5.3 ± 1.1
Creatinine (mg dl^{-1})	0.81 ± 0.25	0.81 ± 0.25	0.80 ± 0.27	0.83 ± 0.25*	0.75 ± 0.24
Uric acid (mg dl^{-1})	5.4 ± 1.3	5.5 ± 1.3	5.4 ± 1.4	5.5 ± 1.4	5.3 ± 1.1

	β2				*β3*	
	Gly16Arg		*Glu27Gln*		*Trp64Arg*	
	Gly/Gly	*Gly/Arg+ArgArg*	*Gln/Gln*	*Glu/Gln*	*Trp/Trp*	*Trp/Arg+ArgArg*
Number	71	286	308	49	265	92
Male/female	44/27	144/152	156/152	24/25	145/120*	36/56
Age (years)	61.1 ± 10.6	61.1 ± 10.6	60.7 ± 12.1	63.4 ± 10.3	61.7 ± 11.1	61.2 ± 13.3
BMI (kg m^{-2})	24.4 ± 3.5	24.3 ± 3.4	24.2 ± 3.4	24.8 ± 3.1	24.5 ± 3.4*	23.6 ± 3.1
DL (%)	51.4	52.7	49.4*	65.0	53.3*	47.8
Diabetes (%)	20.3	21.7	21.5	22.5	22.8	18.8
SBP (mm Hg)	139 ± 16	140 ± 19	140 ± 18	140 ± 19	140 ± 17	139 ± 21
DBP (mm Hg)	83 ± 13	83 ± 11	83 ± 11	83 ± 12	83 ± 11	83 ± 12
Heart rate (bpm)	67 ± 12	67 ± 9	67 ± 10	71 ± 15	67 ± 10	67 ± 9
Total-C (mg dl^{-1})	210 ± 34	207 ± 35	207 ± 36	214 ± 30	207 ± 36	206 ± 32
TG (mg dl^{-1})	139 ± 81	147 ± 92	147 ± 93	143 ± 68	148 ± 87	136 ± 83
HDL-C (mg dl^{-1})	60 ± 15	58 ± 18	58 ± 17	57 ± 15	58 ± 17	61 ± 18
FBG (mg dl^{-1})	101 ± 17	105 ± 24	104 ± 23	105 ± 20	105 ± 22	104 ± 26
HbA1c (%)	5.1 ± 0.7	5.4 ± 1.1	5.4 ± 1.1	5.3 ± 0.8	5.4 ± 1.1	5.2 ± 1.0
Crn (mg dl^{-1})	0.81 ± 0.25	0.80 ± 0.25	0.80 ± 0.24	0.86 ± 0.29	0.81 ± 0.24	0.79 ± 0.27
UA (mg dl^{-1})	5.3 ± 1.3	5.6 ± 1.4	5.5 ± 1.3	5.6 ± 1.5	5.5 ± 1.4	5.3 ± 1.3

Abbreviations: BMI, body mass index; Crn, creatinine; DBP, diastolic blood pressure; DL, dyslipidemia; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; Total-C, total cholesterol; TG, triglyceride; UA, uric acid. *$P<0.05$, **$P<0.01$, ***$P<0.001$ *vs.* other genotype.

Genotyping

Total genomic DNA was extracted from leukocytes obtained from samples of whole blood, following standard techniques. In this study, the TaqMan PCR assay as described previously was used to perform polymorphism analysis of the three *βADR*s, β_1, β_2 and β_3.[7] We detected two polymorphisms of β_1*ADR* that result in serine/glycine (Ser49Gly) and arginine/glycine (Arg389-Gly) amino acid substitutions at residues 49 and 389, respectively. We also detected two polymorphisms of β_2*ADR* that result in glycine/arginine (Gly16Arg) and glutamate/glutamine (Glu27Gln) amino acid substitutions at residues 16 and 27, respectively. Finally, we identified a β_3*ADR* polymorphism that results in a tryptophan/arginine (Trp64Arg) amino acid substitution at residue 64.

Statistical analysis

Data were analyzed using JMP ver. 4 (SAS, Cary, NC, USA) and are presented as the mean ± s.d. in Table 1. An event-free curve was estimated by the Kaplan–Meier method. Patients were stratified according to SNPs for the analysis of event-free rates. The log-rank test was used to compare the differences in event-free rates among these patient groups. Baseline clinical variables for these patients were analyzed with the Cox proportional hazards model, and the hazard ratio with a 95% confidence interval for each factor is given. Backward elimination techniques were used to identify variables independently associated with event onset. Analysis of variance and the Student's *t*-test were used to test for significant differences among the SNPs. A value of $P<0.05$ was regarded as statistically significant.

RESULTS

Table 1 presents the genotype frequencies and participant characteristics stratified by genotypes. With respect to the Gly16Arg β_2*ADR* polymorphism, systolic blood pressure values in patients with the Gly16Gly or Gly16Arg polymorphism were significantly lower than those of patients with the Arg16Arg polymorphism. With respect to the Trp64Arg β_3*ADR* polymorphism, the body mass index values of patients with the Arg64Arg or Trp64Arg polymorphism were significantly higher than those of patients carrying other genotypes.

We classified the SNPs into pairs: SerGly+GlyGly and SerSer for Ser49Gly β_1*ADR*; ArgGly+GlyGly and ArgArg for Arg389Gly β_1*ADR*; ArgArg+ArgGly and GlyGly for Gly16Arg β_2*ADR*; GlnGln and GluGln for Glu27Gln β_2*ADR*; and ArgArg+TrpArg and TrpTrp for Trp64Arg β_3*ADR*. Figure 1 shows the results of the Kaplan–Meier analysis for event-free survival with respect to CVD and stroke classified by SNP group. Patients with SerSer in the Ser49Gly β_1*ADR* polymorphism showed lower event-free survival compared with those with SerGly and GlyGly polymorphisms (P=0.0398) (Figures 1a and 1b); however, there was no statistical significance for the Arg389Gly $\beta\beta_1$*ADR* polymorphism. Analysis of β_2*ADR* polymorphisms showed that patients with the GluGln polymorphism in Glu27Gln β_2*ADR* had lower event-free survival compared with patients with GlnGln polymorphisms (P=0.0390); however, there was no statistical significance for the Gly16Arg β_2*ADR* polymorphism. Finally, patients with the

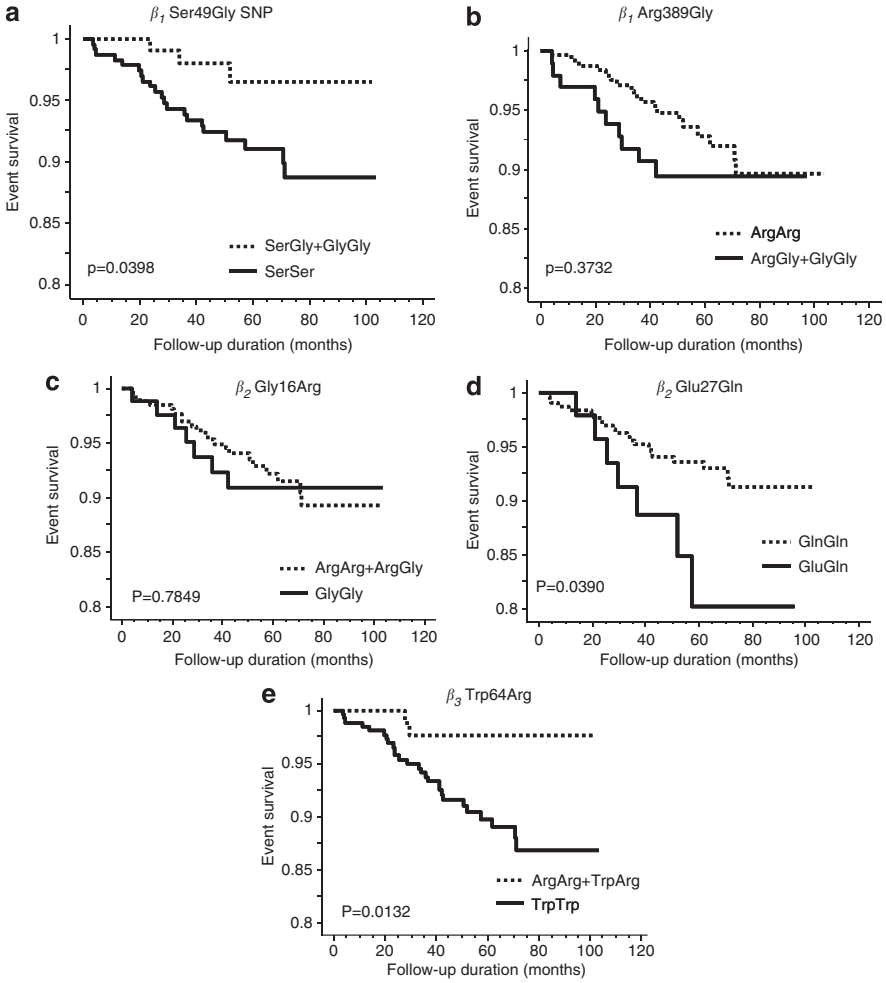

Figure 1 Kaplan–Meier analysis for stroke and CVD in each SNP group. (**a**) β_1 Ser49Gly SNP; (**b**) β_1 Arg389Gly; (**c**) β_2 Gly16Arg; (**d**) β_2 Glu27Gln; and (**e**) β_3 Trp64Arg.

Table 2 Cox's proportional hazard model for CVD+stroke

	Exp	*95% CI*	P-*value*
Model 1			
Gender (female)	1.185	0.455–3.087	0.7287
Age (/years)	1.080	1.023–1.141	0.0052
Diabetes (−)	2.710	0.336–21.86	0.3492
Dyslipidemia (−)	0.896	0.342–2.348	0.8226
Medication (−)	1.112	0.414–2.984	0.8336
Systolic blood pressure	1.007	0.974–1.042	0.6801
Diastolic blood pressure	1.036	0.973–1.102	0.2665
Pulse rate	0.941	0.508–1.032	0.9059
β_1 Ser49Gly (CC; SerSer)	2.017	0.554–7.344	0.2871
β_2 Glu27Gln (CG; GlnGlu)	2.358	0.812–6.847	0.1147
β_3 Trp64Arg (TT; TrpTrp)	9.067	1.141–72.07	0.0371
Model 2			
Gender (female)	1.443	0.429–4.855	0.5532
Age (/years)	1.045	0.984–1.110	0.1521
Dyslipidemia	1.436	0.430–4.794	0.5562
BMI	0.925	0.766–1.117	0.4194
HbA1c	1.790	1.050–3.050	0.0323
β_1 Ser49Gly (CC; SerSer)	10.67	1.059–107.9	0.0446
β_2 Glu27Gln (CG; GlnGlu)	5.272	1.499–18.55	0.0096
β_3 Trp64Arg (TT; TrpTrp)	4.480	0.512–39.22	0.1755

Abbreviations: BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease.

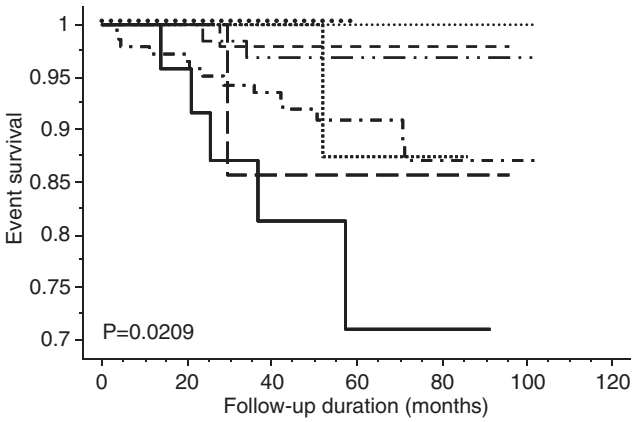

Figure 2 Kaplan–Meier analysis for stroke and CVD, combined analysis with β_1 Ser49Gly, β_2 Glu27Gln and β_3 Trp64Arg. ····· β_1 (SerGly+GlyGly)+β_2 GlnGln+β_3 (ArgArg+TrpArg); —·- β_1 (SerGly+GlyGly)+β_2 GlnGln+β_3 TrpTrp; ••••• β_1 (SerGly+GlyGly)+β_2 GlnGlu+β_3 (ArgArg+TrpArg); ····· β_1 (SerGly+GlyGly)+β_2 GlnGlu+β_3 TrpTrp; – – – β_1 SerSer+β_2 GlnGln+β_3 (ArgArg+TrpArg); –·– β_1 SerSer+β_2 GlnGln+β_3 TrpTrp; — — β_1 SerSer+β_2 GlnGlu+β_3 (ArgArg+TrpArg); —— β_1 SerSer+β_2 GlnGlu+β_3 TrpTrp.

TrpTrp polymorphism in Trp64Arg β_3*ADR* had a lower event-free survival compared with those with the ArgArg and ArgTrp polymorphisms (P=0.0132).

To clarify the influences of other risk factors on event-free survival with respect to stroke and CVD, we used the Cox's proportional hazards model (Table 2). In model 1, which incorporated common risk factors as confounding factors, TrpTrp in Trp64Arg β_3*ADR* SNP emerged as an independent risk factor along with age. In model 2, we analyzed several confounding factors that showed statistical positivity ($P<0.05$) in the analysis of patient characteristics and a *βADR* SNP at baseline. In this model, the SerSer polymorphism in Ser49Gly β_1*ADR* and the GluGln polymorphism in Glu27Gln β_2*ADR* emerged as independent risk factors in addition to age and HbA1c values.

We also used a combined Kaplan–Meier analysis incorporating the Ser49Gly β_1*ADR*, Glu27Gln β_2*ADR* and Trp64Arg β_3*ADR* SNPs (Figure 2). The shape of the Kaplan–Meier curve for each *βADR* SNP indicated statistical significance (P=0.0209). In patients with the SerSer polymorphism in the Ser49Gly β_1*ADR*, the GluGln polymorphism in the Glu27Gln β_2*ADR* and the TrpTrp polymorphism in the Trp64Arg β_3*ADR*, the event-free survival ratio was the lowest relative to the other combined SNPs.

DISCUSSION

In this hospital-based study, the frequencies of five SNPs of three *βADR*s in a patient population were determined, and the frequencies of each SNP were similar to those identified in previous reports.[13–15] Several functional analyses have addressed these SNPs; for example, participants with the Gly allele in the Ser49Gly polymorphism of β_1*ADR* have been reported to have a lower heart rate.[16] *In vitro* studies of isoproterenol stimulation showed that Arg389 in β_1*ADR* produces higher levels of adenylyl cyclase activity, resulting in enhanced cardiac sensitivity to catecholamines.[17] The Gly16 and Glu27 polymorphisms in β_2*ADR* have been associated with sympathetic overactivity, as reflected by high plasma norepinephrine levels.[18] Dietary sodium restriction blunts the increase in nitric oxide-mediated β_2*ADR* responsiveness of the forearm in Gly16 homozygotes following administration of normal dietary sodium, whereas baseline cardiac output decreases and peripheral resistance increases under sodium restriction.[19] In male twins with highly similar genetic and environmental backgrounds, the Arg64 variant of the β_3*ADR* polymorphism was found to be associated with insulin resistance and higher post-prandial hyperglycemia.[20]

As we noted in the introduction, many cross-sectional studies have focused on cardiovascular-related disease and these SNPs of the *βADR*s. Moreover, previous reports have suggested that *βADR*s mediate smooth muscle relaxation in small resistance arteries and large conduit arteries.[21] We previously found an association between a genetic polymorphism in Ser49Gly of the β_1*ADR* and aortic stiffness as measured by pulse wave velocity, and the Gly49Gly genotype showed a genetic association with nitroglycerin-induced hyperemia.[7] Although functional analysis is required, the Ser49Gly polymorphism of β_1*ADR* might influence arterial functional changes. The Glu27Gln polymorphism of β_2*ADR* has been identified by both electrocardiography and echocardiography to be significantly associated with left ventricular hypertrophy. Therefore, it is expected that these SNPs would affect prognostic outcomes of cardiovascular events in patients with essential hypertension.

There have been several reports about the prognostic impacts of Trp64Arg. In a multicenter study conducted in Spain, the Trp/Arg allele was associated with more severe insulin resistance,[22] and the Arg allele was associated with a higher incidence of cardiac diseases in a Chinese population as determined by an 8-year prospective cohort study of type 2 diabetes.[23] The results from these studies are different from those of our present investigation; however, the baseline disease states and ethnicities were different. A large number of cohort studies of hypertensive patients are required to confirm our results.

We also performed a combined Kaplan–Meier analysis of the various polymorphisms, which showed an association for three SNPs with cardiovascular prognosis. Ser49Gly β_1*ADR*, Glu27Gln β_2*ADR* and Trp64Arg β_3*ADR* SNPs showed an additive effect on stroke and cardiovascular events in patients with essential hypertension. Although

the present study had several limitations, described below, these results nonetheless underscore the possibility of the independent effects of these three *βADR* SNPs. These results do not directly impact common clinical practice; however, these findings might be important when considering the relationship between autonomic nervous system responses and hypertensive complications in patients with essential hypertension.

Study limitations

Our study was a hospital-based investigation that included patients with essential hypertension and had several limitations. First, a large number of cohort studies will be required to fully analyze the relationship between polymorphisms and cardiovascular events. Moreover, for combined analyses, the number of participants in this study was too small to yield definitive results. Furthermore, the participants were heterogeneous with respect to clinical background, which could influence cardiovascular events. For example, some patients were taking medications, and the patients had been sick for different periods of time. Finally, there may have been a selection bias because our study was a hospital-based study. In spite of these important limitations, the results showed a prognostic impact of *βADR* gene polymorphisms on cardiovascular events in hypertensive patients.

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