Original Article

Genetic Influences of β-Adrenoceptor Polymorphisms on Arterial Functional Changes and Cardiac Remodeling in Hypertensive Patients

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Three subtypes of β-adrenoceptor, β_1, β_2 and β_3, are involved in the sympathetic nervous system, which plays an important role in the development of hypertension and hypertensive complications. These complications can include left ventricular hypertrophy and arterial stiffness, which are reported risk factors for cardiovascular diseases. We designed clinical trials to clarify the association between hypertensive complications and β-adrenoceptor single nucleotide polymorphisms in essential hypertension. Using Taqman PCR methods, we detected five polymorphisms of three β-adrenoceptors: Ser49Gly and Arg389Gly for the β_1-adrenoceptor; Gly16Arg and Glu27Gln for the β_2-adrenoceptor; and Trp64Arg for the β_3-adrenoceptor. We included 300 subjects and measured pulse wave velocity, vasodilator response to hyperemia, left ventricular hypertrophy (by electrocardiogram and echocardiography), and cardiac enlargement (by chest X-ray). We found that pulse wave velocity and nitroglycerin-induced hyperemia were both closely associated with the Ser49Gly polymorphism ($p<0.05$), and Glu27Gln was found by both electrocardiogram and echocardiography to be significantly associated with left ventricular hypertrophy ($p<0.05$). These data suggested that two polymorphisms of different β-adrenoreceptor subtypes are the genetic influences on the development of arterial stiffness and left ventricular hypertrophy in essential hypertension. (*Hypertens Res* 2006; 29: 875–881)

***Key Words*: β-adrenoreceptor, polymorphisms, hypertensive complications, arterial stiffness**

Introduction

The human β-adrenoceptor (βADR) is a member of the family of seven-transmembrane G-protein–coupled receptors, encoded by a gene on chromosome 5 (*1*). Previous studies have shown that sympathetic nervous activity *via* βADRs regulates numerous physiological events and modulates a wide range of physiological responses, including cardiac chronotropy and inotropy, vascular and smooth muscle tone, carbohydrate and lipid metabolism (*2*). Sympathetic nervous activity plays an important role in the development of hyper-

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Table 1. Probes and Primers for β-Adrenoceptor Polymorphism

		Probe	Primer
β_1	Ser49Gly	FAM CCAGCGAAAGCCCCGAGCC	Forward GTCGCCGCCCGCCTCGTT
		VIC CCAGCGAAGGCCCCGAGC	Reverse CCATGCCCGCTGTCCACTGCT
	Arg389Gly	FAM AGGCCTTCCAGGGACTGCTCTGCT	Forward GGCCTTCAACCCCATCATCTA
		VIC AGGCCTTCCAGCGACTGCTCTGC	Reverse CCGGTCTCCGTGGGTCGCGT
β_2	Gly16Arg	FAM CGCATGGCTTCTATTGGGTGC	Forward GGAACGGCAGCGCCTTCT
		VIC CGCATGGCTTCCATTGGGTGC	Reverse CAGGACGATGAGAGACATGACGAT
	Glu27Gln	FAM CTCGTCCCTTTGCTGCGTGACGT	Forward GGAACGGCAGCGCCTTCT
		VIC CTCGTCCCTTTCCTGCGTGACGT	Reverse CAGGACGATGAGAGACATGACGAT
β_3	Trp64Arg	FAM TCTCGGAGTCCAGGCGATGGCCA	Forward GGAGGCAACCTGCTGGTCAT
		VIC CTCGGAGTCCGGGCGATGGCC	Reverse CACGAACACGTTGGTCATGGT

In each probe, the polymorphic nucleotide is underlined.

tension and its complications (*3*). Three isotypes of human βADRs, β_1, β_2, and β_3, are involved in this system. The classical subdivision of βADRs defines β_1 as the subtype that stimulates cardiac muscle (*4*) and β_2 as the subtype that relaxes smooth muscle (*5*). The expression of the β_3 subtype is essentially limited to adipose tissue (*6*).

Genetic polymorphisms may influence the development of disease states (*7*). Diseases such as hypertension could be based on genetic disorders as well as environmental factors, or may occur as a secondary product of either cardiac events such as left ventricular hypertrophy (LVH) and arterial stiffness, or interactions among them (*8*). LVH has been considered to be an intermediate phenotype of hypertensive heart diseases (*9*). Recently arterial stiffness was suggested to be an independent predictor of the presence of coronary artery disease (*10*), and arterial stiffness was associated with LVH in hemodialysis patients (*11*). Therefore, arterial stiffness was considered to be an intermediate phenotype of hypertensive complications. A number of single nucleotide polymorphisms (SNPs) of βADR subtypes have recently been reported to be positional candidate genes for cardiovascular diseases (*12*, *13*). For instance, the genotype of the Arg389Gly polymorphism in the human β_1-adrenoceptor (β_1ADR) gene is reported to be associated with acute myocardial infarction (*14*) and hypertensive status (*15*), an association study (*16*) suggested that the gene encoding the β_2-adrenoceptor (β_2ADR) was associated with essential hypertension; and the Arg64 allele of the β_3-adrenoceptor (β_3ADR) gene is associated with obesity-related phenotypes (*17*), insulin resistance, hypertension, coronary artery disease and earlier age of onset of diabetes (*18*). The essential effects of the three βADRs, however, need to be further clarified.

The aim of the present hospital-based observational study was to investigate potential genetic relationships between the βADR SNPs that result in amino acid substitutions and the cardiovascular risk factors of hypertension, such as LVH and aortic stiffness. We focused on five representative β-adrenoceptor SNPs in patients with essential hypertension.

Methods

Study Population

The present clinical study was designed as a hospital-based and cross-sectional study. We identified 331 Osaka University Medical Hospital patients with essential hypertension. Hypertension was defined as systolic blood pressure (SBP) of more than 140 mmHg and/or diastolic blood pressure (DBP) of more than 90 mmHg and/or administration of antihypertensive drugs. Twelve patients were excluded because of atrial fibrillation and/or frequent ventricular premature beats (more than 5% of the day), and we excluded nine patients whose echocardiography could not be evaluated. Four patients refused to undergo venepuncture. We also excluded six patients in whom we failed to detect the five target SNPs. Finally, 300 patients with essential hypertension for whom the target SNPs could be detected were enrolled. We diagnosed essential hypertension as defined above. The protocol of this study was approved by the hospital ethics committee (permission number: 80) and written informed consent was obtained from all participants. For this study, 115 patients not receiving antihypertensive drug treatment were included. The remaining 185 patients were treated with one or more antihypertensive drugs as follows: 128 patients with a calcium antagonist, 62 patients with an angiotensin receptor blocker, 47 patients with an angiotensin-converting enzyme (ACE) inhibitor, 37 patients with a β-blocker, 25 patients with a diuretic and 10 patients with an α-blocker.

Genotyping

Total genomic DNA was extracted from leukocytes obtained from samples of whole blood, following the standard techniques. In this study, the TaqMan PCR assay as described previously (*19*) was used to perform polymorphisms analysis of the three βADRs, β_1, β_2 and β_3 (*20*). We detected two poly-

Table 2. Patients' Characteristics and β-Adrenoceptor Polymorphism

	Total	β_1					
		Ser49Gly			Arg389Gly		
		Ser/Ser	Ser/Gly	Gly/Gly	Arg/Arg	Arg/Gly	Gly/Gly
Number	300	194	96	10	213	80	7
Male/female	164/136	105/89	56/40	3/7	123/90	38/42	3/4
Age (years old)	62.0±0.7	61.8±0.9	62.3±1.1	65.3±3.0	61.9±0.8	62.0±1.2	66.4±6.9
BMI (kg/m^2)	24.2±0.2	24.2±0.2	24.1±0.4	25.7±2.3	24.2±0.2	24.4±0.3	24.5±0.3
Hyperlipidemia (%)	51.5	50.5	52.6	60.0	51.9	51.3	42.9
Diabetes (%)	21.7	17.5	27.1	50.0	23.5	17.5	14.3%
SBP (mmHg)	141.4±1.1	140.9±1.3	142.4±2.0	141.7±4.1	141.6±1.3	140.9±1.9	140.7±6.6
DBP (mmHg)	83.4±0.7	83.6±0.8	83.3±1.3	79.3±2.2	83.3±0.8	83.7±1.3	80.7±3.7
HR (bpm)	67.8±0.7	67.9±0.9	67.8±1.1	64.3±4.3	67.1±0.8	69.1±1.2	72.4±7.1
TC (mmol/l)	5.3±0.1	5.3±0.1	5.2±0.1	5.0±0.2	5.3±0.1	5.3±0.1	4.8±0.3
HDL-C (mmol/l)	1.4±0.03	1.4±0.04	1.4±0.05	1.5±0.13	1.4±0.03	1.5±0.06	1.5±0.25
FBG (mmol/l)	6.0±0.1	5.9±0.1	6.0±0.2	6.4±0.6	6.1±0.1	5.9±0.2	5.7±0.3

β_2					β_3		
Gly16Arg			Glu27Gln		Trp64Arg		
Gly/Gly	Gly/Arg	Arg/Arg	Gln/Gln	Glu/Gln	Trp/Trp	Trp/Arg	Arg/Arg
74	166	60	260	40	228	69	3
44/30	86/80	34/26	146/114	18/22	129/99	32/37	3/0
62.0±1.3	62.3±0.9	61.5±1.9	62.3±0.6	63.8±1.1	62.8±0.6	61.6±1.6	58.3±0.8
24.1±0.4	24.3±0.2	24.3±0.4	24.2±0.2	24.6±0.5	24.5±0.2	23.5±0.3	26.3±0.4*
51.4	52.7	48.3	49.4	65.0	53.3	47.8	0
20.3	21.7	23.3	21.5	22.5	22.8	18.8	0
137.9±1.8	141.4±1.5	145.9±2.8*	140.7±0.9	139.9±2.7	140.5±1.0	141.4±2.0	124.7±12.7
82.3±1.7	83.4±0.9	84.7±1.6	83.0±0.6	82.7±1.5	82.8±0.6	83.7±1.2	74.3±14.3
66.5±1.4	68.0±0.9	68.5±1.4	68.2±0.7	68.9±1.5	68.3±0.7	68.2±1.1	64.5±2.5
5.3±0.12	5.2±0.07	5.3±0.11	5.3±0.05	5.5±0.10	5.3±0.05	5.3±0.08	4.9±0.30
1.5±0.05	1.4±0.04	1.4±0.06	1.5±0.02	1.6±0.06	1.5±0.02	1.5±0.04	1.5±0.21
6.2±0.3	6.1±0.1	5.7±0.1	5.9±0.8	6.2±0.3	6.0±0.8	6.0±0.2	5.7±0.1

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; FBG, fasting blood glucose. *$p<0.05$ *vs*. other genotype.

morphisms of β_1ADR that result in serine/glycine (Ser49Gly) and arginine/glycine (Arg389Gly) amino acid substitutions at residues 49 and 389, respectively. We also detected two polymorphisms of β_2ADR that result in glycine/arginine (Gly16Arg) and glutamate/glutamine (Glu27Gln) amino acid substitutions at residues 16 and 27, respectively. Finally, we identified a polymorphism in the β_3ADR that results in a tryptophan/arginine (Trp64Arg) amino acid substitution at residue 64. The primers and probes for the five representative SNPs are shown in Table 1.

Arterial Functional Changes

We non-invasively evaluated pulse wave velocity (PWV) and vasodilator response to hyperemia as arterial functional changes. PWV was measured using a model FCP-4731 (Fukuda Denshi Co., Tokyo, Japan), which allowed on-line pulse wave recording and automatic calculation as previously reported (*21*, *22*), and the details of this procedure have been reported previously (*23*). The intra-observer coefficient of variation was 2.8±1.2%. We measured the post-ischemic vasodilator response to reactive hyperemia by strain-gauge plethysmography (EC5R; DE Horkkanson, Inc., Bellevue, USA), using a previously published protocol (*24*). To calculate the reactive hyperemia/baseline value of forearm blood flow, we used the reactive hyperemia ratio, and we used the nitroglycerin (NTG)-induced hyperemia ratio as the NTG-induced hyperemia/baseline value. The intra-observer coefficient of variation was 3.4±1.4%.

Cardiac Remodeling

To evaluate cardiac remodeling, we estimated LVH by electrocardiography and echocardiography, and also estimated cardiac enlargement using the cardio-thoracic-ratio (CTR) obtained from chest X-rays. Electrocardiographic LVH

Table 3. β-Adrenoceptor Genotypes and Arterial Functional Changes/Cardiac Remodeling

		PWV (m/s)	Hyperemia		ECGLVH (mm)	LVMI (g/m^2)	CTR (%)
			Reactive	NTG			
β_1	Ser49Ser	9.1±0.1	1.60±0.05	1.03±0.05	3.0±0.1	132±2.8	49.6±0.4
	Ser49Gly	9.0±0.2	1.55±0.08	0.92±0.03	2.9±0.1	134±3.7	49.0±0.6
	Gly49Gly	10.5±0.4*	1.32±0.12	0.74±0.06*	2.5±0.3	144±1.7	57.8±2.5**
	Arg389Arg	9.1±0.1	1.59±0.05	0.98±0.04	2.9±0.1	115±7.8	49.3±0.4
	Arg389Gly	9.2±0.1	1.54±0.08	1.00±0.08	2.9±0.1	126±3.8	50.5±0.5
	Gly389Gly	9.3±0.5	1.51±0.29	0.79±0.10	3.2±0.2	134±7.9	49.8±1.0
β_2	Gly16Gly	9.0±0.2	1.67±0.09	0.92±0.04	3.0±0.1	132±4.1	50.1±0.9
	Gly16Arg	9.2±0.1	1.55±0.06	0.99±0.05	2.9±0.1	131±3.2	49.7±0.4
	Arg16Arg	9.0±0.2	1.57±0.09	1.03±0.10	3.0±0.1	133±5.4	49.2±0.6
	Gln27Gln	9.3±0.2	1.54±0.04*	1.00±0.04	2.9±0.1*	129±2.4**	49.5±0.4
	Glu27Gln	9.1±0.1	1.82±0.16	0.87±0.04	3.2±0.2	147±7.0	50.5±1.0
β_3	Trp64Trp	9.2±0.1	1.56±0.05	0.95±0.03	2.9±0.1	135±2.6	50.2±4.9
	Trp64Arg	8.9±0.2	1.66±0.09	1.08±0.11	2.9±0.1	127±5.0	48.9±0.6
	Arg64Arg	8.6±0.8	1.57±0.34	1.02±0.02	2.8±0.4	158±4.8	49.9±0.4

PWV, pulse wave velocity; reactive, reactive hyperemia; NTG, nitroglycerin-induced hyperemia; ECGLVH, electrocardiographic left ventricular hypertrophy; LVMI, left ventricular mass index; CTR, cardio-thoracic-ratio. *$p<0.05$ and **$p<0.01$ *vs*. other genotype.

(ECGLVH) was determined by Sokolow-Lyon's criteria as the depth of SV_1 plus RV_5 (mV) (*25*). Left ventricular mass index (LVMI) was calculated by echocardiography following Devereux's methods (*26*). Additionally, CTR from chest X-rays was calculated as the directly measured ratio of the width of the heart to the thorax width.

Statistical Analysis

Data were analyzed using JMP ver. 4 (SAS Inc., Cary, USA) and presented as the mean±SEM. ANOVA and 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

Participant characteristics stratified by genotypes are presented in Table 2. With respect to the Gly16Arg β_2ADR polymorphism, SBP in patients with the Gly16Gly or Gly16Arg polymorphism was significantly lower than that of patients with the Arg16Arg polymorphism. With respect to the Trp64Arg β_3ADR polymorphism, the body mass index of patients with the Arg64Arg or Trp64Arg polymorphism was significantly higher than that of patients carrying other genotypes.

The relationship between βADR polymorphisms and arterial functional changes is shown in Table 3. We evaluated three different parameters for arterial changes, the carotid-femoral PWV as an index of aortic stiffness, reactive hyperemia as an index of post-ischemic vasodilatation to reactive hyperemia, and NTG-induced hyperemia as an index of endothelium-independent vasodilatation. PWV in patients with the Gly49Gly genotype of the β_1ADR polymorphism was significantly elevated compared to that of patients with other genotypes ($p<0.05$). In the patients with the Gly49Gly genotype, NTG-induced hyperemia was significantly lower compared to that of patients with other genotypes; however, reactive hyperemia showed no significant differences compared to the β_1ADR Ser49Gly polymorphism. Reactive hyperemia in patients with the Glu27Gln genotype of the β_2ADR polymorphism was significantly lower compared to that of patients with Gln27Gln genotype. Our results for the LVMI, ECGLVH and CTR measurements are also shown in Table 3. In patients with the Gly/Gly genotype of the β_1ADR Ser49Gly polymorphism, CTR was significantly higher compared to the CTRs of patients with other genotypes. In patients with the Glu27Gln genotype of the β_2ADR polymorphism, both ECGLVH and LVMI were significantly higher compared to these measures in patients with the Gln27Gln genotype.

To evaluate genetic cooperation between β_1ADR Ser49Gly polymorphism and β_2ADR Glu27Gln polymorphism, we analyzed the combined influences of these two polymorphisms on arterial and cardiac phenotypes (Table 4). Although the numbers of patients with Ser49Gly/Glu27Gln and Gly49Gly/Gln27Gln were very small ($n=7$), patients with Gly49Gly/Gln27Gln showed higher PWV and CTR, but lower LVMI compared with the other combined genotypes. Patients with Ser49Ser/Glu27Gln ($n=33$) showed a hyperreactive hyperemia ratio, which meant improved vasodilator response.

Moreover, we performed haplotype-analysis for the β_1ADR and β_2ADR polymorphisms (Table 5). Although the number of patients with Gly49Gly/Arg389Arg in the β_1ADR was

Table 4. Combination Analysis of β-Adrenoceptor Genotypes and Arterial Functional Changes/Cardiac Remodeling

	n	PWV (m/s)	Hyperemia		ECGLVH (mm)	LVMI (g/m^2)	CTR (%)
			Reactive	NTG			
Ser49Ser/Gln27Gln	180	9.1±0.1	1.54±0.05	1.01±0.05	3.0±0.1	133±3.3†	49.8±0.5$^{\#}$
Ser49Ser/Glu27Gln	33	9.2±0.3†	1.88±0.19*	0.84±0.05	3.1±0.2	130±4.9†	50.0±1.4$^{\#}$
Ser49Gly/Gln27Gln	73	9.1±0.2$^{\#}$	1.55±0.08	1.00±0.09	2.8±0.1	126±4.3†	48.8±0.7$^{\#}$
Ser49Gly/Glu27Gln	7	7.8±0.5*,$^{\#}$	1.51±0.17	1.02±0.13	3.3±0.4	169±20.7	50.2±0.9
Gly49Gly/Gln27Gln	7	10.5±0.5*	1.51±0.29	0.79±0.10	2.6±0.3	119±8.1†	56.9±2.9

PWV, pulse wave velocity; reactive, reactive hyperemia; NTG, nitroglycerin-induced hyperemia; ECGLVH, electrocardiographic left ventricular hypertrophy; LVMI, left ventricular mass index; CTR, cardio-thoracic-ratio. *$p<0.05$ and **$p<0.01$ *vs*. Ser49Ser/Gln27Gln; $^{\#}p<0.05$ *vs*. Gly49Gly/Gln27Gln; $^{\dagger}p<0.05$ *vs*. Ser49Gly/Glu27Gln.

Table 5. The Haplotype Analysis of β_1- and β_2-Adrenoceptor Polymorphism

		n	PWV (m/s)	Hyperemia		ECGLVH (mm)	LVMI (g/m^2)	CTR (%)
				Reactive	NTG			
β_1	Ser-Ser/Arg-Arg	150	9.1±0.2*	1.66±0.07	1.04±0.06	3.0±0.1	134±4.1	49.3±0.7**
	Ser-Gly/Arg-Arg	56	9.0±0.2*	1.50±0.08	1.02±0.11	2.9±0.1	129±5.0	48.7±0.9**
	Gly-Gly/Arg-Arg	7	10.5±0.5	1.51±0.29	0.79±0.10	2.6±0.3	119±8.1	56.9±2.9
	Ser-Ser/Arg-Gly	71	9.3±0.2*	1.52±0.08	0.91±0.03	2.9±0.1	132±4.3	50.7±0.7**
	Ser-Gly/Arg-Gly	18	9.1±0.4*	1.66±0.22	0.95±0.07	2.8±0.3	128±10.1	49.5±1.0**
	Gly-Gly/Arg-Gly	10	9.3±0.4	1.32±0.12	0.74±0.06$^{\#}$	3.2±0.3	129±14.7	49.8±0.8*
β_2	Gly-Gly/Gln-Gln	59	9.1±0.2	1.66±0.10	1.02±0.10	3.1±0.1†	137±6.7	50.2±1.1
	Gly-Arg/Gln-Gln	145	9.3±0.1	1.53±0.06†	1.01±0.05	2.9±0.1	130±3.2†	49.8±0.5
	Gly-Arg/Glu-Gln	21	8.9±0.4	1.68±0.16	0.84±0.05	2.9±0.3	125±6.9†	50.6±1.4
	Arg-Arg/Gln-Gln	55	9.0±0.2	1.44±0.07†	0.93±0.05	2.8±0.1†	126±5.3†	49.0±0.8
	Arg-Arg/Glu-Gln	19	9.0±0.4	1.98±0.29	0.89±0.07	3.4±0.2	153±8.9	49.3±2.0

PWV, pulse wave velocity; reactive, reactive hyperemia; NTG, nitroglycerin-induced hyperemia; ECGLVH, electrocardiographic left ventricular hypertrophy; LVMI, left ventricular mass index; CTR, cardio-thoracic-ratio. *$p<0.05$ and **$p<0.01$ *vs*. Gly-Gly/Arg-Arg; $^{\#}p<0.05$ *vs*. Ser-Ser/Arg-Gly; $^{\dagger}p<0.05$ *vs*. Arg-Arg/Glu-Gln.

very small ($n=7$), patients with this haplotype showed higher PWV and CTR compared with patients having the other haplotypes. Patients with the Arg16Arg/Glu27Gln haplotype in the β_2ADR showed higher reactive hyperemia and LVMI.

Discussion

In this hospital-based study, the frequencies of five SNPs of three βADRs in a patient population were determined. With respect to the two SNPs of β_1ADR SNPs, the data for the Ser49Gly SNPs were not significantly different from those in a report from Sweden (*27*), and Arg389Gly also exhibited the same percentage of SNPs as reported by a Japanese group (*14*). In addition, the previously reported frequencies of two β_2ADR SNPs, Gly16Arg and Glu27Gln, in a Japanese (*28*) and a Taiwanese (*29*) cohort were identical to the respective frequencies determined here. Finally, the frequency of Trp64Arg for the β_3ADR reflected the findings of another Japanese group (*30*). Based on the accordance of our findings with these previous studies, our results would seem to be an accurate evaluation of the frequencies of these five βADR SNPs.

The β_1ADR are positioned at the cell membrane of cardiomyocytes. Subjects with the Gly allele in the Ser49Gly polymorphism have been reported to have a lower heart rate (*20*), but in another study, the Ser49Gly β_1ADR polymorphisms did not seem to exert a major effect on the changes in heart rate and blood pressure of patients with essential hypertension during 12 weeks of treatment with atenolol (*31*). The C allele of β_1AR 1165C>G encodes arginine at amino acid 389, whereas the G allele encodes glycine. *In vitro* studies of isoproterenol stimulation showed that the Arg-389 receptors produce higher levels of adenylyl cyclase activity than the Gly-389 receptors (*32*), resulting in enhanced cardiac sensitivity to catecholamines. In another study, the Gly16 and Glu27 polymorphisms in the β_2ADR were associated with sympathetic overactivity, as reflected by high plasma norepinephrine levels (*33*). In a study by Eisenach *et al*., dietary sodium restriction blunted the increase in nitric oxide-mediated, β_2ADR responsiveness in Gly16 homozygotes of the forearm following administration of normal dietary sodium, while baseline CO decreased and peripheral resistance were

increased by the sodium restriction (*34*). In male twins with highly similar genetic and environmental backgrounds, the Arg64 variant of the β_3ADR polymorphism was found to be associated with insulin resistance and higher post-prandial hyperglycemia (*35*).

In the previous studies, PWV was shown to be effective as an index of stiffened vessels and decreased compliance, which was strongly correlated with increased pulse pressure and aging, and NTG-induced hyperemia was considered to be a marker of endothelium-independent vasodilatation. This type of dilatation was considered to be medial smooth-muscle-cell–related vasodilatation. Previous reports have suggested that βADR mediates smooth muscle relaxation in the small resistance arteries and large conduit arteries (*36*). In the present study, we only found an association between a genetic polymorphism in Ser49Gly of the β_1ADR and the aortic stiffness as measured by PWV, and the Gly49Gly genotype showed a genetic association with NTG-induced hyperemia. Although functional analysis is required, Ser49Gly polymorphism of the β_1ADR might influence arterial functional changes.

We also performed a combined analysis of the various polymorphism and haplotype results. This analysis revealed several statistically significant parameters in regard to the intermediate phenotype; however, we were unable to reach a definitive conclusion about these findings due to the small number of subjects studied. Although the present study had several limitations, it nonetheless underscored the possibility of a relationship between βADR SNPs and intermediate phenotypes, such as functional arterial changes and cardiac remodeling. Clinically, 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, cross-sectional research that included patients with essential hypertension and had several limitations. First, a large number of clinical studies will be required to analyze the relationship between polymorphisms and intermediate phenotypes, such as LVH and arterial stiffness. Moreover, for the haplotype and combined analyses, the number of subjects in this study was too small to provide definitive results, and the statistical power was low. Second, subjects showed heterogeneity of clinical background, which influences cardiovascular events, with some patients having taken medicines and some patients having been sick for a longer or shorter period of time. Third, to strictly evaluate the genetic influences of βADR genes, a cohort study would be much better. Fourth, as our study was a hospital-based study, there may have been a selection-bias. In general, it is better to use a population-based study for analyzing genetic influences, in order to avoid such a bias. Nonetheless, although there were several important limitations and although a larger, population-based cohort study might be required, the present study showed an association between βADR gene polymorphisms and arterial or cardiac remodeling in hypertensive patients.

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