Original Article

Lack of Association between Angiotensin II Type 1 Receptor Gene Polymorphism and Hypertension in Japanese

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Angiotensin II type 1 (AT₁) receptor mediates the vasoconstriction and growth-promoting effect of angiotensin II in humans. It has been reported that a polymorphism of the AT₁ receptor gene (an A/C transversion at position 1166; A1166C) may be associated with essential hypertension (HT). However, several conflicting results have also been reported. Therefore, we conducted an association study between A1166C variants of the AT₁ receptor gene and hypertension in the Japanese population. We genotyped this variant in 3,918 subjects (1,492 hypertensive subjects and 2,426 normotensive subjects) recruited from the Suita study. In subjects not receiving antihypertensive medication, the influence of the genotype on blood pressure values adjusted for clinical covariates was analyzed. The genotype distribution did not differ between hypertensive and normotensive subjects in either men (frequency of the C allele: 8.1% vs. 7.8%, $p=0.74$) or women (8.1% vs. 7.7%, $p=0.60$). There were no significant differences in systolic blood pressure, diastolic blood pressure, or pulse pressure among the three genotypes in either men or women who had not received hypertensive medication. Our data suggest that the A1166C polymorphism of AT₁ receptor is unlikely to influence blood pressure status in the Japanese population. (*Hypertens Res* 2003; 26: 131–134)

Key Words: epidemiology, genetics, blood pressure

Introduction

Angiotensin II is an important effector controlling blood pressure and volume in the cardiovascular system. Its importance is reflected by the efficacy of angiotensin-converting enzyme inhibitors in the treatment of hypertension and congestive heart failure. Angiotensin II interacts with two pharmacologically distinct subtypes of cell surface receptors, types 1 and 2. Angiotensin II type 1 (AT₁) receptors seem to play a key role in mediating the vasoconstrictor and growth-promoting effects of angiotensin II (1). It has been reported that a polymorphism of the AT₁ receptor gene (an A/C transversion at position 1166; A1166C) occurs more frequently in hypertensive subjects with a positive family history of hypertension than in control subjects (2). On the other hand, it

has also been reported that this locus is not linked with this disease, and recent studies have demonstrated that the distribution of the genotypes did not differ between normotensive and hypertensive subjects (3, 4). Moreover, a recent population-based survey of Caucasian hypertensives reported lower blood pressure values in CC homozygotes than in heterozygotes and AA homozygotes (5). In response to these controversial results, we performed an association study in a large epidemiological cohort to examine whether A1166C genetic variants influence blood pressure in the Japanese population.

Methods

Subjects

The selection criteria and design of the Suita study have

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Table 1. Characteristics of Men and Women Subjects by AT₁ Receptor Genotype

Characteristics	AT ₁ receptor genotype		
	AA	AC	CC
Men (<i>n</i> = 1,854)			
<i>n</i>	1,575	267	12
Age (years)	60.7 ± 12.1	61.4 ± 12.3	59.4 ± 12.3
Body mass index (kg/m ²)	23.1 ± 2.8	22.7 ± 2.7	22.3 ± 2.60
Waist-to-hip circumference ratio	0.91 ± 0.06	0.90 ± 0.06	0.94 ± 0.04
Alcohol consumption (ml/day)	25.2 ± 26.2	26.4 ± 29.7	23.5 ± 22.5
Smoking habit (%)	38.9	39.7	75
Ischemic heart disease (%)	4.75	6.37	0
Diabetes mellitus (%)	22.2	21.7	33.3
Proteinuria (%)	7.6	6.4	16.7
Use of antihypertensive drugs (%)	18.4	17.2	16.7
Women (<i>n</i> = 2,062)			
<i>n</i>	1,755	290	17
Age (years)	58.8 ± 11.8	59.1 ± 11.6	57.2 ± 13.7
Body mass index (kg/m ²)	22.3 ± 3.2	22.5 ± 2.9	21.1 ± 2.7
Waist-to-hip circumference ratio	0.89 ± 0.08	0.89 ± 0.07	0.86 ± 0.07
Alcohol consumption (ml/day)	5.1 ± 11.2	4.6 ± 10.0	6.1 ± 13.3
Smoking habit (%)	8.1	8.6	11.8
Ischemic heart disease (%)	2.9	4.8	0
Diabetes mellitus (%)	14.2	13.1	5.9
Proteinuria (%)	3.9	4.5	5.9
Use of antihypertensive drugs (%)	16.1	16.2	17.6

Values are the mean ± SEM. AT₁, angiotensin II type 1.

been described previously (6, 7). The present study was approved by the Ethics Committee of the National Cardiovascular Center and by the Committee on Genetic Analysis and Genetic Therapy of the National Cardiovascular Center. Informed consent on genetic analysis was obtained from about 4,000 subjects, and the genotype of A1166C was determined in 3,918 consecutive subjects. Subjects were categorized as hypertensives when they had a systolic blood pressure of 140 mmHg or a diastolic blood pressure of 90 mmHg. Subjects who were taking hypertensive medication were also categorized as hypertensives.

DNA Studies

DNA was isolated from peripheral leukocytes according to standard procedures. Polymorphisms were determined by the TaqMan system. The primers and probes for genotype determination were as follows: Sense 5'-CATTCCTCTGCAGCACTTCACT-3', Antisense 5'-CGGTTCAGTCCACATAATGCAT-3', Probe for A(1166) 5'-Fam-AAATGAGCATTAGCTACT-MGB-3', Probe for C(1166) 5'-Fam-AAATGAGCCTTAGCTACT-MGB-3'. Each reaction included 20 ng of genomic DNA, 30 pmol of each primer, 12.5 pmol of each TaqMan probe and 1X TaqMan Universal Master Mix (PE Biosystems, Foster City, USA) in a volume of 50 µl. Polymerase chain reaction (PCR) cycling conditions were 1 cycle at 50°C for 2 min, 1 cycle at 95°C for 10 min, and 40 cycles

at 95°C for 15 s and 60°C for 1 min. The results were analyzed using an ABI PRISM 7700 Sequence Detection System (PE Biosystems) using allelic discrimination software supplied by the manufacture.

Statistical Analyses

Values are expressed as the means ± SEM. All statistical analyses were performed with the JMP statistical package (SAS Institute Inc., Cary, USA). Multiple regression and multiple logistic analyses were performed with other covariates. Differences in numerical data among the groups were calculated by one-way analysis of variance (ANOVA) and unpaired Student's *t*-test. Values of *p* < 0.05 were considered to indicate statistical significance. Differences in frequencies were tested by contingency table analysis.

Results

AT₁ Receptor Genotype and Allele Frequencies and Clinical Characteristics

The overall frequencies of the genotypes AA, AC, and CC were as follows: in men, 84.9%, 14.5%, and 0.6%, respectively; in women, 85.1%, 14.1%, and 0.8%; and overall, 85.0%, 14.1%, 0.8%. The allele frequencies for A and C were 92.1% and 7.9%, 92.0% and 8.0%, and 92.0% and 8.0%, respectively, in men, in women, and

Table 2. Genotype and Allele Distribution of AT₁ Receptor A1166C Polymorphism in Hypertensive Subjects and Normotensive Subjects

	Men (<i>n</i> (%))		Women (<i>n</i> (%))	
	Hypertensive	Normotensive	Hypertensive	Normotensive
Genotype				
AA	627 (84.4)	948 (85.2)	632 (84.4)	1,123 (85.5)
AC	112 (15.1)	157 (14.1)	112 (15.0)	178 (13.6)
CC	4 (0.5)	8 (0.7)	5 (0.6)	12 (0.9)
	$\chi^2 = 0.55, p = 0.76$		$\chi^2 = 1.09, p = 0.58$	
Allele				
A	1,366 (91.9)	2,053 (92.2)	1,376 (91.9)	2,424 (92.3)
C	120 (8.1)	173 (7.8)	122 (8.1)	202 (7.7)
	$\chi^2 = 0.11, p = 0.74$		$\chi^2 = 0.27, p = 0.60$	

Table 3. Blood Pressure of Subjects Not Receiving Antihypertensive Medication by AT₁ Receptor Genotype

	AT ₁ receptor genotype			ANOVA
	AA	AC	CC	<i>p</i>
Men (<i>n</i> = 1,518)				
<i>n</i>	1,285	223	10	
SBP (mmHg)				
Unadjusted	125.8 ± 0.5	126.4 ± 1.2	126.2 ± 5.8	0.88
Age-adjusted	125.8 ± 0.5	126.5 ± 1.1	128.0 ± 5.4	0.93
Age and BMI-adjusted	125.7 ± 0.5	127.1 ± 1.1	128.6 ± 5.3	0.73
DBP (mmHg)				
Unadjusted	80.3 ± 0.3	79.5 ± 0.7	77.1 ± 3.3	0.40
Age-adjusted	80.4 ± 0.3	78.8 ± 0.7	74.3 ± 3.3	0.38
Age and BMI-adjusted	80.4 ± 0.3	79.2 ± 0.7	74.7 ± 3.2	0.69
PP (mmHg)				
Unadjusted	45.5 ± 0.4	46.9 ± 0.9	49.1 ± 4.1	0.22
Age-adjusted	45.3 ± 0.3	47.7 ± 0.8	53.8 ± 3.6	0.20
Age and BMI-adjusted	45.3 ± 0.3	47.8 ± 0.8	53.9 ± 3.6	0.17
Women (<i>n</i> = 1,730)				
<i>n</i>	1,473	243	14	
SBP (mmHg)				
Unadjusted	125.0 ± 0.5	126.1 ± 1.2	123.6 ± 5.2	0.67
Age-adjusted	125.0 ± 0.5	126.9 ± 1.1	124.1 ± 4.7	0.73
Age and BMI-adjusted	125.0 ± 0.5	126.6 ± 1.1	125.5 ± 4.7	0.80
DBP (mmHg)				
Unadjusted	77.5 ± 0.3	79.0 ± 0.7	78.4 ± 2.8	0.12
Age-adjusted	77.3 ± 0.3	80.2 ± 0.7	79.5 ± 2.8	0.12
Age and BMI-adjusted	77.3 ± 0.3	80.0 ± 0.7	80.7 ± 2.7	0.17
PP (mmHg)				
Unadjusted	47.5 ± 0.4	47.2 ± 0.9	45.3 ± 3.6	0.77
Age-adjusted	47.6 ± 0.3	46.8 ± 0.8	44.7 ± 3.2	0.82
Age and BMI-adjusted	47.6 ± 0.3	46.7 ± 0.8	44.9 ± 3.2	0.80

Values are the mean ± SEM. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; BMI, body mass index.

overall. The observed genotype frequencies were in agreement with those predicted by Hardy-Weinberg equilibrium.

The clinical characteristics of male and female subjects are summarized in Table 1. There were no significant differences among the three genotypes in any characteristics in either men or women.

Relationship between the AT₁ Receptor Genotype and Blood Pressure

The genotype distribution and the allele distribution of the A1166C variants of AT₁ receptor gene polymorphisms in hypertensive subjects were compared to those in normotensive

subjects for both men and women (Table 2). No significant differences in the genotype distribution or the allele distribution were observed between hypertensive subjects and normotensive subjects in either men or women.

The influence of the AT₁ receptor genotype on residuals of blood pressure values after adjusting for age and body mass index (BMI) is shown in Table 3. No significant influence of the genotype on blood pressure was observed.

Discussion

Bonnardeaux *et al.* identified a positive association between A1166C variants of AT₁ receptor gene polymorphisms and hypertension in 206 Caucasian patients with essential hypertension (2). Wang *et al.* did a case-control study of the A1166C variant in 108 Caucasian hypertensive subjects and found that the A1166C variant was associated with hypertension (8). On the other hand, Bonnardeaux *et al.* failed to detect such an association using affected sibling pair analysis, and recent studies have found no difference in the distribution of the genotypes between normotensive and hypertensive subjects (3, 4). Two other studies conducted in Japan also failed to show a significant association between this polymorphism and hypertension (9, 10). Moreover, a recent population based survey of Caucasian hypertensives reported lower blood pressure values in CC homozygotes than in heterozygotes and AA homozygotes (5).

In the original report with Caucasian subjects, the frequencies of the 1166C allele were 0.36 in hypertensives and 0.28 in normotensives (2). However, in the present study, the frequencies of the 1166C allele were 0.09 in hypertensives and 0.08 in controls. Since similar results have also been reported in other studies in Japan, it would appear that the C allele is less frequent in Japanese than in whites. This different allele frequency may have affected the different results between Japanese and whites.

Since a population of only several hundred subjects may be too small to draw statistically certain conclusions, and because independent confirmation is of critical value, we genotyped this variant in a large cohort representing the general Japanese population (the Suita study) consisting of 3,918 subjects.

Our results indicate that the genotype distribution did not differ between hypertensive and normotensive subjects, and that the genotype had no significant effects on blood pressure values in either men or women. However, the present observations do not necessarily exclude the possibility that the AT₁ receptor gene is involved in hypertension in Japanese. It is still possible that some other polymorphisms in this gene may influence blood pressure. Takahashi *et al.* identified seven polymorphisms in the 5'-flanking region of the AT₁ receptor gene and found a significantly higher frequency of the (-535)T allele in hypertensive subjects (11). However, the sample size was small and further confirmation may still be required.

In future studies, it will be necessary to identify a large number of polymorphisms throughout the AT₁ receptor gene in Japanese and to perform association studies between these polymorphisms and blood pressure.

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