Original Article

A1166C Polymorphism of the Angiotensin II Type 1 Receptor Gene and Essential Hypertension in Han, Tibetan and Yi Populations

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Our aim was to clarify whether substitution of cytosine for adenine at position 1166 (A1166C) polymorphism of the angiotensin II type 1 receptor (AT₁R) gene is associated with susceptibility to essential hypertension in Han, Tibetan and Yi populations in China. This study involved 302 normotensive and 446 hypertensive subjects. The polymorphism was detected by polymerase chain reaction of genomic DNA and restriction fragment length polymorphism (PCR-RFLP) in genomic DNA. The data were analyzed by analysis of covariance (ANCOVA), χ^2 test, and multiple logistic regression. In normotensive controls, the A1166 allele frequencies were 0.979, 0.939 and 0.965 in Han, Tibetan and Yi participants, respectively. There was no significant intergroup variation in frequency of the allele in normotensives ($\chi^2 = 4.166$, $p = 0.125$). The frequency of the A1166 allele was significantly higher in Tibetan male hypertensives than that in normotensives ($\chi^2 = 11.46$, $p = 0.001$). There was no significant difference in A1166C genotype distribution and allele frequency between normotensives and hypertensives either in the Han ($p = 0.465$) or Yi ($p = 0.357$) populations. Body mass index in the Han and Yi populations ($p = 0.0001$), age in the Tibetan and Yi populations ($p = 0.0001$), and AA genotype in the Tibetan male population ($p = 0.0034$) all were independent risk factors for hypertension. Diastolic blood pressure levels were significantly higher in Tibetan male subjects with the AA genotype than in those with the AC + CC genotype ($p = 0.0040$). We concluded that the A1166 allele is very common in Han, Tibetan and Yi populations, approximately 1.35-fold more common than in Caucasians. The A1166 allele of the AT₁R gene may be a predisposing factor for essential hypertension in Tibetan males. A1166C polymorphism of the AT₁R gene is probably not involved in the pathogenesis of essential hypertension in Han or Yi populations. (*Hypertens Res* 2002; 25: 515–521)

Key Words: angiotensin II type 1 receptor, A1166C polymorphism, essential hypertension, Han, Tibetan and Yi populations

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Introduction

The renin-angiotensin system plays an important role in regulation of blood pressure and electrolyte homeostasis. Angiotensin II, the major biologically active product of the system, exerts its effects *via* two structurally distinct receptor subtypes: angiotensin II type 1 receptor (AT₁R) and angiotensin II type 2 receptor (1). The human AT₁R gene, which mediates the major cardiovascular effects of angiotensin II, was cloned in 1992 (2) and was present as a single-copy gene on human chromosome 3q 21–25. AT₁R may be an important target for control of angiotensin II-dependent hypertension, as supported by the results of three studies. First, AT₁R antagonists were shown to be effective antihypertensive agents (3). Second, a genome-wide scan suggested that the AT₁R locus is the most significant contributor to hypertension in Finnish populations (4). Third, polymorphisms of the AT₁R gene have been associated with hypertension (5). Although substitution of cytosine for adenine at position 1166 (A1166C) in the AT₁R gene was associated with susceptibility to essential hypertension in French and Finnish populations (5, 6), this finding was not confirmed in other ethnic groups (7, 8), and thus these differences may be due to an ethnic variation. Recently, Paillard *et al.* (9) found that AT₁R sites on platelets are of limited density and that there is no effect of the genotype on receptor number or affinity. No data has been reported thus far on the possible influence of A1166C polymorphism of the AT₁R gene on blood pressure in Chinese.

Essential hypertension, which is frequently associated with low levels of awareness, treatment and control of the disease, is a major public health problem in China (10). Epidemiological studies indicate that the prevalence rates of essential hypertension vary markedly among the various ethnicities in China (11, 12). For example, the Yi ethnic group has the lowest prevalence of hypertension in China (0.22%–3.0%), whereas Tibetans have the highest in China (17.8%) (13). Three genetically distinct Chinese populations “the Han, Tibetan and Yi ethnic groups” were described in 1998 (14). In the present study, we investigated the distribution of the genotypes and alleles of A1166C polymorphism of the AT₁R gene in Han, Tibetan and Yi populations in China, examined the association between the polymorphism and susceptibility to essential hypertension and evaluated independent risk factors for hypertension in Han, Tibetan and Yi populations.

Methods

Subjects

The study was approved by the ethical committee of our institution, and the recruited subjects gave their informed consent for the genetic analysis. All subjects were recruited ran-

domly from outpatient clinics in Fangshan County of Beijing, in Lhasa of the Tibet Autonomous Region and in the Liangshan Yi Autonomous Prefecture of Sichuan Province. A structured questionnaire was used for data collection on demographic variables, medical history of hypertension and lifestyles. The study group comprised 302 normotensive controls and 446 hypertensive patients. Blood pressure was measured in the right upper arm of subjects after they had sat for at least 30 min using a calibrated mercury sphygmomanometer, and all blood pressure values were taken as the average of three readings. Hypertension subjects were defined as those having a systolic blood pressure (SBP) of 140 mmHg or greater or a diastolic blood pressure (DBP) of 90 mmHg or greater. Few of the subjects had previously received antihypertensive agents. All the hypertensive patients were required to be free of secondary hypertension or diabetes mellitus, and to have been diagnosed with hypertension before the age of 60 years. Normotensive subjects were selected from the same population as the hypertensive participants, and had an SBP of less than 140 mmHg and a DBP of less than 90 mmHg, and no history of essential hypertension in their first-degree relatives. The sex, age, SBP, DBP and body mass index (BMI) of the subjects are summarized in Table 1.

Analysis of Genomic DNA

Peripheral blood leukocytes were digested with proteinase K and then genomic DNA was extracted using phenol and chloroform, followed by ethanol precipitation.

A1166C polymorphism in the 3 untranslated region of the AT₁R gene was detected by polymerase chain reaction of genomic DNA and restriction fragment length polymorphism (PCR-RFLP) as described by Wang *et al.* with some modifications (15). The sequence of the sense primer was 5'-ATA ATG TAA GCT CAT CCA CCA AGA AG-3', and that of the antisense primer was 5'-TCT CCT TCA ATT CTG AAA AGT ACT TAA-3'. About 100 ng of genomic DNA were amplified in a total volume of 25 μ l containing 25 mmol/l Tris-HCl (pH = 8.3 at 25°C), 25 mmol/l KCl, 3 mmol/l MgCl₂, 5% formamide, 4 pmol for each primer, 80 μ mol/l for each deoxynucleotide triphosphate, and 0.50 units of DNA polymerase (Biotechnology Center FORWARD GROUP Ltd., Shanghai, P.R. China). PCR amplification was carried out in a PTC-200 MJ Research Peltier Thermal Cycler (Perkin-Elmer Corp., Foster City, USA) and followed by an initial denaturation at 94°C for 3 min. This was followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 45 s, and extension at 72°C for 45 s with a final extension at 72°C for 5 min. Ten microliters of unpurified PCR product was diluted to 20 μ l in a recommended buffer containing 10 units of Bfr I enzyme (Boehringer Mannheim GmbH, Mannheim, Germany) and incubated at 37°C for 5 h. The digestion products were separated on 3% agarose gel and visualized directly under UV lighting with ethidium bro-

Table 1. Main Characteristics of Participants by Population

Group	n	Male/Female	Age (years)	BMI (kg/m ²)	SBP (mmHg)	DBP (mmHg)
Han	239					
NT	72	45 / 27	50.0 ± 10.6	22.8 ± 2.3	118.3 ± 9.0	76.5 ± 6.7
HT	167	85 / 82	48.4 ± 8.7	26.1 ± 3.1	161.1 ± 18.1*	102.7 ± 9.7*
Tibetan	330					
NT	115	59 / 56	44.1 ± 11.2	24.9 ± 3.0	114.3 ± 11.6	75.8 ± 7.5
HT	215	120 / 95	53.6 ± 12.2	23.6 ± 3.5	160.6 ± 31.0*	104.9 ± 13.7*
Yi	179					
NT	115	115 / 0	44.3 ± 5.8	21.1 ± 2.7	112.8 ± 8.2	73.3 ± 6.3
HT	64	64 / 0	51.2 ± 11.9	24.8 ± 3.5	158.8 ± 28.9*	103.4 ± 15.3*

Values are the mean ± SD unless otherwise indicated. NT, normotensive; HT, hypertensive; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. * $p = 0.001$, for hypertensive vs. normotensive subjects by ANCOVA in each ethnic group.

Fig. 1. A1166C polymorphism of the AT₁R gene was detected. The photo shows three genotypes (AA, CC, and AC) seen on an ethidium bromide-stained gel after running Bfr I digestion of PCR-amplified samples. The homozygous (AA) has a band of 166 bp, the homozygous (CC) has a band of 139 bp, and the heterozygous (AC) has two bands. The first lane shows DNA marker (pBR322–Msp I digest).

mid-staining (Fig. 1). The validity of genotyping for the AA, AC and CC genotypes was confirmed by sequencing of the amplified DNA from several subjects using the dideoxy-mediated chain-termination method.

Statistical Analysis

All statistical analysis was performed using a statistical software package, SAS version 6.12 (SAS Inst., Inc., Cary, USA). Differences in blood pressure measurements between normotensive and hypertensive subjects in each group were analyzed by analysis of covariance (ANCOVA) adjusted for confounding variables. A genotype was assigned to each subject and allele frequencies were estimated based on the genotype distributions. Because of the very low frequency of the 1166C allele, CC homozygous subjects were combined with AC heterozygous subjects in analysis to increase the

statistical power. A χ^2 test was used to assess the goodness-of-fit between the observed allele frequencies and the expected ones by Hardy-Weinberg equilibrium and to evaluate the difference in genotype distribution between normotensive and hypertensive subjects. Multiple logistic regression analysis was used to assess the relationship between hypertension and A1166C genotypes, with adjustment for the effects of other predicted variables. The odds ratio and its 95% confidence interval (CI) were calculated for each risk of hypertension. Differences in blood pressure levels among the subjects with different genotypes were analyzed by ANCOVA, and adjusted for sex, age and BMI. Values of $p < 0.05$ were considered to indicate statistical significance.

Results

Characteristics of the Study Subjects

The distributions of sex, age, BMI, SBP and DBP for the Han, Tibetan and Yi populations are summarized in Table 1. Within each population, there were significant differences in both SBP and DBP between normotensive and hypertensive subjects after adjustment for sex, age, and BMI ($p = 0.001$). No other significant intragroup differences were found.

Genotype Distributions and Allele Frequencies

The genotype distributions and allele frequencies of the A1166C polymorphism in normotensive and hypertensive subjects of the Han, Tibetan and Yi populations are shown in Table 2.

The distributions of genotype frequencies in normotensive and hypertensive subjects showed that their alleles frequencies were coincident with Hardy-Weinberg equilibrium for all three populations. The homozygous CC genotype was not observed in the Tibetan or Yi populations or in Han normotensives. For normotensive controls, the A1166 allele frequencies of the AT₁R gene were 0.979, 0.939 and 0.965 in

Table 2. Genotype Distribution and Allele Frequencies between Normotensive and Hypertensive Subjects

	<i>n</i>	Genotypes (%)		Alleles		Total alleles on chromosomes	
		AA	AC + CC	A	C	A	C
Han	239						
NT	72	69 (95.8)	3 (4.2)	0.979	0.021	141	3
HT	167	156 (93.4)	11 (6.6)	0.967	0.033	323	11
χ^2			0.534				0.518
<i>p</i>			0.465				0.472
Tibetan	330						
NT	115	101 (87.8)	14 (12.2)	0.939	0.061	216	14
HT	215	207 (96.3)	8 (3.7)	0.981	0.019	422	8
χ^2			8.604				8.307
<i>p</i>			0.003				0.004
Yi	179						
NT	115	107 (93.0)	8 (7.0)	0.965	0.035	222	8
HT	64	57 (89.1)	7 (10.9)	0.945	0.055	121	7
χ^2			0.849				0.812
<i>p</i>			0.357				0.368

n, number.**Table 3. Genotype Distribution and Allele Frequencies between Normotensive and Hypertensive Tibetan Subjects in Tibetans by Sex**

	<i>n</i>	Genotypes (%)		Alleles		Total alleles on a chromosomes	
		AA	AC + CC	A	C	A	C
Male	179						
NT	59	49 (83.1)	10 (16.9)	0.915	0.085	108	10
HT	120	117 (97.5)	3 (2.5)	0.988	0.012	237	3
χ^2			11.46				15.39
<i>p</i>			0.001				0.001
Female	151						
NT	56	52 (92.9)	4 (7.1)	0.964	0.036	108	4
HT	95	90 (94.7)	5 (5.3)	0.974	0.026	185	5
χ^2			0.217				0.211
<i>p</i>			0.641				0.646

n, number.

the Han, Tibetan and Yi populations, respectively. There was no significant intergroup variation in frequency of the allele in normotensives ($\chi^2 = 4.166$, $p = 0.125$).

Frequencies of the AA and AC + CC genotypes were 87.8% and 12.2% in Tibetan normotensives, and 96.3% and 3.7% in hypertensives, respectively (Table 2). Derived frequencies for the A and C alleles were 0.939 and 0.061 in Tibetan normotensives, and 0.981 and 0.019 in Tibetan hypertensives, respectively. The frequency of the AA genotype was significantly higher in Tibetan hypertensives than in normotensives ($\chi^2 = 8.604$, $p = 0.003$). No significant difference in the A1166C genotype distribution or allele frequencies between normotensives and hypertensives was found in either the Han ($\chi^2 = 0.534$, $p = 0.465$) or Yi populations ($\chi^2 = 0.849$, $p = 0.357$).

When the Tibetan subjects were divided into male and fe-

male subgroups, the distribution of the A1166C genotype differed significantly between normotensive and hypertensive groups in males ($\chi^2 = 11.46$, $p = 0.001$), but there was no difference in females ($\chi^2 = 0.217$, $p = 0.641$) (Table 3).

For comparison, an overview of data reported from other major population groups is provided (Table 4). In Han normotensives, the frequency of the A1166 and C1166 alleles were 0.979 and 0.021, respectively, similar to the values observed in Japanese (8, 17) and African (18), and approximately 1.14 to 1.50-fold those observed in Caucasians (5, 19–22).

Multiple Logistic Regression Analysis of Various Risk Factors for Hypertension

The results of the logistic regression analysis showed that

Table 4. Ethnic/Country Allele Frequencies of AT₁R A1166C Polymorphism

Ethnic groups	Countries	n	Genotypes (%)			Alleles		Ref.
			AA	AC	CC	A	C	
African	Ghana	47	45 (95.7)	2 (4.3)	0 (0.0)	0.979	0.021	18
Caucasian	Italy	143	63 (44.1)	61 (42.7)	19 (13.3)	0.654	0.346	19
	Canada	100	56 (56.0)	37 (37.0)	7 (7.0)	0.745	0.255	20
	France	723	355 (49.1)	319 (44.1)	49 (6.8)	0.712	0.288	21
		298	221 (74.2)	72 (24.2)	5 (1.7)	0.862	0.138	5
	Germany	147	75 (51.0)	61 (41.5)	11 (7.5)	0.718	0.282	22
		172	81 (47.1)	76 (44.2)	15 (8.7)	0.692	0.308	7
	Australia	84	39 (46.4)	42 (50.0)	3 (3.6)	0.714	0.286	15
Japanese	Japan	631	525 (83.2)	103 (16.3)	3 (0.5)	0.913	0.087	17
	Japan	215	172 (80.0)	40 (18.6)	3 (1.4)	0.893	0.107	8
Chinese	Hong Kong, China	174	152 (87.4)	21 (12.1)	1 (0.5)	0.934	0.066	16
	Beijing, China	72	69 (95.8)	3 (4.2)	0 (0.0)	0.979	0.021	present study
	Tibetan, China	115	101 (87.8)	14 (12.2)	0 (0.0)	0.939	0.061	present study
	Yi, China	115	107 (93.0)	8 (7.0)	0 (0.0)	0.965	0.035	present study

n, number.

Table 5. Adjusted Odds Ratio of Essential Hypertension by Multivariate Logistic Regression

Variables	β	Odds ratio	95% CI	p
Han (329)				
BMI	0.52	1.67	1.42–1.97	0.0001
Tibetan (male, 179)				
AA genotype	2.06	7.87	1.98–31.35	0.0034
Age	0.07	1.07	1.05–1.09	0.0001
Yi (male, 179)				
Age	0.09	1.10	1.05–1.15	0.0001
BMI	0.36	1.44	1.27–1.63	0.0001

CI, confidence interval.

BMI in the Han and Yi populations ($p = 0.0001$), age in the Tibetan and Yi populations ($p = 0.0001$), and AA genotype in Tibetan male subjects ($p = 0.0034$) all were independent risk factors for hypertension (Table 5).

Further analysis indicated that the difference in the distribution of genotypes between Tibetan hypertensives and normotensives could only be found in males with an odds ratio of 7.87 and 95% CI of 1.98 to 31.35 for AA genotype vs. AC + CC genotype $p = 0.0034$, while the difference in females was not significant (data not shown). These findings suggested that the increased risk of hypertension in the Tibetan population associated with A1166 allele was specific in males.

Relationship between Genotypes and Blood Pressure

Blood pressure levels in the three populations, adjusted for age, BMI and sex, are presented in Table 6 for each genotype. No significant association between the A1166C geno-

type distribution and SBP or DBP was found in either the Han or Yi populations. However, DBP levels were significantly higher in Tibetan male subjects with the AA than in those with the AC + CC genotype ($p = 0.0040$).

Discussion

Identification of genes involved in the development of essential hypertension is still a major challenge (23–26). The prevalence of hypertension in China increased from 7.7% in 1980 to more than 11.8% in 1991 (10). In the present report, we included a sample of the majority Han population, which accounts for approximately 92% of the population of China, as well as samples of two minority populations, the Tibetan and Yi. These three genetically distinct populations with different prevalence rates of essential hypertension provided an excellent opportunity to study genetic factors that contribute to the pathogenesis of essential hypertension.

In our study, we found that although the frequencies of the AA genotype and A allele of the AT₁R gene tended to be lower in Tibetan normotensives than in Han normotensives, the distribution of the genotype and allele did not differ significantly between Tibetan and Han. The frequency of the A1166 allele in Han normotensive was 0.979, significantly higher than that in Caucasian populations (5, 19–22). Furthermore, we believe that the A1166 allele may be a predisposing factor for essential hypertension in Tibetan males but not in Han or Yi populations.

The differences in allele frequency among different ethnic groups were larger than those between hypertensive and normotensive subjects within the same ethnic group. This observation, coupled with the high allele frequency of 0.913 reported in the Japanese population (17) and that of 0.979 reported in Africans (18), led to the hypothesis that large

Table 6. Comparison of Blood Pressure Measurements According to A1166C Genotypes

		<i>n</i>	Systolic blood pressure			Diastolic blood pressure		
		748	AA	AC + CC	<i>p</i> [*]	AA	AC + CC	<i>p</i> [*]
Han	Male	130	142.6 ± 2.0	151.8 ± 8.4	0.2900	94.3 ± 1.2	96.6 ± 5.0	0.5290
	Female	109	153.0 ± 2.3	158.5 ± 8.8	0.5409	95.9 ± 1.6	95.5 ± 6.0	0.9591
Tibetan	Male	176	142.3 ± 2.2	127.1 ± 7.7	0.0570	94.6 ± 1.2	81.6 ± 4.3	0.0040
	Female	154	148.3 ± 2.7	146.1 ± 10.7	0.8395	96.1 ± 1.4	94.1 ± 5.8	0.7455
Yi	Male	179	129.3 ± 1.7	128.6 ± 5.8	0.9067	84.2 ± 1.2	82.1 ± 3.8	0.5950

n, number. * *p* values were estimated by ANCOVA adjusted for age, sex and BMI.

differences in allele frequencies among ethnic groups may be important for essential hypertension.

No evidence for an association between A1166C polymorphism and essential hypertension was found in either the Han or Yi populations. The high frequency of the A1166 allele makes it impossible that this variant will be useful as a risk marker for hypertension either within the Han or Yi populations. In Tibetan males, however, the A1166 allele was significantly more frequent in hypertensives than in normotensives. In this case, our estimate differed from previously reported results (5, 7, 8, 15). The discrepancy among these studies may be due to ethnic differences. Our study showed that Tibetan male subjects with the AA genotype had higher DBP than those without it, which is consistent with the result in hypertensive subjects by Castellano *et al.* (19). Our results can be explained in either of two ways. First, it is possible that androgens could increase sodium reabsorption *via* angiotensin II-mediated increases in aldosterone (27, 28). Second, A1166C polymorphism itself does not seem to be functional, but it may be in linkage disequilibrium with an unknown genetic variant in an important functional region of the AT₁R gene in the Tibetan male population. We have recently described significant association of essential hypertension with microsatellite alleles located in the 3' end of the AT₁R gene, insertion/deletion polymorphism in the angiotensin converting enzyme gene and M235T polymorphism in the angiotensinogen gene in the Han population (29, 30). An association was recently reported between A - 6G variant of the angiotensinogen gene and essential hypertension in the Han, Tibetan and Yi Populations (31). Williams *et al.* (18) suggested that, in terms of causing hypertension, combinations of alleles at a variety of candidate loci might be more important than the variants found at any single locus. We are currently engaged in investigating gene-gene interactions among the renin-angiotensin system gene polymorphisms.

In conclusion, the A1166 allele of the AT₁R gene is very common in the Han, Tibetan and Yi populations in China, with a frequency approximately 1.35-fold higher than that in Caucasians. The A1166 allele of the AT₁R gene may be a predisposing factor in the development of essential hypertension in Tibetan males, but A1166C polymorphism does not appear to be involved in the pathogenesis of essential hyper-

tension in either the Han or Yi populations.

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