Original Article

Polymorphism of the Angiotensin Converting Enzyme Gene and Blood Pressure in a Japanese General Population (the Shigaraki Study)

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Recent studies have described a linkage between angiotensin converting enzyme (ACE) gene polymorphism and hypertension in a large number of hypertensive sibs. Moreover, among Japanese, the DD genotype of the ACE gene has been reported to be a genetically predisposing factor for hypertension in a large general population. However, there is some controversy regarding the association between the ACE insertion/deletion (I/D) polymorphism and systemic hypertension. Therefore, we examined the influence of the ACE I/D polymorphism in a random Japanese population. The participants were 2,892 subjects (1,110 males: mean age, 58.4 ± 15.5 ; 1,782 females: mean age, 56.2 ± 15.7) who underwent medical examinations in 1999 in Shigaraki, a suburban town located in an urban area in Shiga prefecture. Among them, 2,395 subjects (917 males: mean age, 58.0 ± 15.7 ; 1,478 females: mean age, 56.3 ± 15.6) who gave their informed consent for genetic analysis were enrolled in the present study. Every year since 1991, we have repeatedly performed medical examinations in Shigaraki. ACE genotypes were determined by polymerase chain reaction (PCR) methods. Logistic analysis revealed that age ($p < 0.001$; odds ratio = 1.091), body mass index (BMI) ($p < 0.001$; odds ratio = 1.211), and family history of hypertension ($p < 0.001$; odds ratio = 0.371) were associated with hypertension (systolic blood pressure greater than 140 mmHg and diastolic greater than 90 mmHg). There was no association between ACE polymorphism and blood pressure. There were also no significant differences in blood pressure among males or females across the three genotypes. Moreover, in an analysis of 1,484 subjects (549 males: mean age, 62.8 ± 12.1 ; 935 females: mean age, 61.2 ± 12.6) who were followed-up since 1991, there was no association between ACE polymorphism and changes in blood pressure ($p = 0.3905$). We conclude that ACE polymorphism does not appear to have any significant association with blood pressure, changes in blood pressure or sex in Japanese subjects, who have a more homogeneous genetic background than any other group reported to date. (Hypertens Res 2002; 25: 843–848)

Key Words: angiotensin converting enzyme insertion/deletion polymorphism, blood pressure, general population, cohort study, subsequent blood pressure

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Introduction

Among whites, a large, population-based study has shown evidence of an association and genetic linkage of the angiotensin converting enzyme (ACE) locus with hypertension and with diastolic blood pressure in men but not women (1). Moreover, in sex-specific analyses of young white males, genetic variation in the region of the ACE gene significantly influenced inter-individual blood pressure variation in males (2, 3). In an analysis of the elderly it was reported that the DD genotypes at the ACE locus were less common in elderly hypertensive Caucasian patients than in age-matched controls (4). However, it has also been reported that the insertion/deletion (I/D) polymorphism of the ACE gene was not a marker for isolated systolic hypertension and systolic-diastolic hypertension in a large elderly sample (5). Among Japanese, the ACE gene polymorphism has been reported to be associated with an increased risk of hypertension, suggesting that this polymorphism is a mild but definite genetic risk factor for essential hypertension in men (6). Another cohort population study reported that there is a tendency for higher diastolic blood pressure in men with DD genotypes; however, there is no convincing evidence that ACE genotypes are associated with hypertension among Japanese (7).

In a meta-analysis of 23 case-control studies, neither hypertension nor a family history of hypertension was significantly associated with the D allele (8). Clearly, the association between ACE I/D polymorphism and systemic hypertension remains controversial.

The purpose of this study was to elucidate the relationship between ACE genotypes and blood pressure, including changes in blood pressure and a sex-specific association, in Japanese residents in a rural community.

Methods

Study Population

Of 2,892 local residents who underwent a mass medical examination in 1999 at Shigaraki, a mountain farming community with a population of about 15,000, a total of 2,395 subjects consisting of 917 men (mean age, 58.0 ± 15.7) and 1,478 women (mean age, 56.3 ± 15.6) were enrolled in this genetic study after providing their full informed consent (9). Of these, 227 were excluded because their genotype was not determined (80 men, 147 women). After this exclusion, 2,168 subjects (819 men: mean age, 58.4 ± 15.5 ; 1,349 women: mean age, 56.2 ± 15.7) in whom genotyping of the ACE I/D polymorphism was successful were eligible for the present study. The participants ranged in age from 30 to 79 years, and represented approximately 19.6% of the population of Shigaraki. A disproportionately small number of young men were enrolled in the study, probably because most young men in the community received routine health

examinations at their place of work. However, half of the subjects over 50 years old participated in this study. The basic sampling of the population started in 1991 with a cohort-based study, and 100 questions about lifestyle were also surveyed. The study protocol was approved by the Institutional Review Board of the Shiga University of Medical Science (No.11-15, 1999).

Blood Pressure Measurement

In 1991 and eight years later in 1999, systolic (sBP) and diastolic blood pressure (dBP) were measured twice on the right arm using a standard sphygmomanometer with the subjects seated following a rest of at least 5 min. Korotkov's first and fourth points were regarded as the sBP and dBP, respectively, and the blood pressure was measured by a well-trained nurse. The mean of the two measurements of each pressure was used for data analysis. In this study, subjects with a sBP ≥ 140 mmHg or a dBP ≥ 90 mmHg or who were using antihypertensive agents were defined as hypertensives. Δ blood pressure was defined as the difference between the blood pressures in 1991 and 1999, and %blood pressure as the Δ blood pressure divided by the blood pressure in 1999.

DNA Studies

DNA was isolated from peripheral leukocytes and the ACE genotype was determined as in our previous report (10). The sense primer was 5'-CTGGAGACCACTCCCATCCTTTCT-3', and the antisense primer was 5'-GATGTGGCCATCACA TTCGTCAGAT-3'. These primers allowed the detection of a 490-base pair (bp) genomic DNA segment corresponding to the insertion allele (I) as well as a 190-bp segment corresponding to the deletion allele (D). The amplification profile consisted of an initial denaturation at 94°C for 60 s, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 58°C for 60 s, and extension at 74°C for 120 s. The polymerase chain reaction (PCR) products were resolved in 1.5% agarose gels and visualized with ethidium bromide staining. The DD genotype of the ACE gene was reconfirmed by a second PCR using Taq extender (Stratagene, La Jolla, USA).

Statistical Analyses

Statistical analyses were performed using Stat View 4 software (Abacus Concepts Inc., Berkeley, USA). The frequency was compared by a contingency table analysis. Numerical data were analyzed by unpaired Student's *t*-test or one-way analysis of variance (ANOVA). Multiple regression analysis was performed to investigate the possible influence of the ACE genotype on blood pressure, with gender, age, body mass index (BMI), hypertension (negative = 0, positive = 1), and the genotype of the ACE gene (II = 0, ID + DD = 1) included as independent variables. Backward selection was used and a *p* value of 0.10 or greater was required for a vari-

Table 1. Characteristics of the Study Population

	ACE polymorphism			<i>p</i> value
	II	ID	DD	
<i>N</i> (2,168)	821	941	406	
Male/female (819/1,349)	311/510	362/579	146/260	0.6819
Age (years)	56.4 ± 15.7	57.7 ± 15.6	56.7 ± 15.4	0.1628
BMI (kg/m ²)	22.5 ± 3.1	22.6 ± 3.2	22.7 ± 3.0	0.3796
sBP (mmHg)	128.3 ± 19.2	129.3 ± 18.8	128.7 ± 20.1	0.5682
dBP (mmHg)	75.8 ± 11.8	76.3 ± 11.8	76.5 ± 11.7	0.5247
PP (mmHg)	52.6 ± 13.4	53.0 ± 13.3	52.2 ± 14.1	0.5739
CHO (mg/dl)	192.6 ± 35.9	192.3 ± 34.2	192.3 ± 34.4	0.9797
TG (mg/dl)	130.9 ± 88.1	131.2 ± 87.6	127.7 ± 82.1	0.7734
Smoking (P/N) (%)	259/562 (31.5)	310/631 (32.9)	133/273 (32.8)	0.8092
FH (P/N) (%)	248/573 (30.2)	298/643 (31.7)	142/264 (34.9)	0.2400
Hypertension (P/N) (%)	253/568 (30.8)	306/635 (32.5)	119/287 (29.3)	0.4755
DM (%)	64/757 (7.8)	75/866 (8.0)	31/375 (7.6)	0.9764

Values are means ± SD and numbers (%); BMI, body mass index; sBP, systolic blood pressure; dBP, diastolic blood pressure; PP, pulse pressure; CHO, total cholesterol; TG, triglycerides; smoking, habits of smoking; (P/N), positive/negative; FH, family history of hypertension; DM, diabetes mellitus.

Table 2. Characteristics of the Study Population (Excluding 438 Hypertensive Therapy Patients)

	ACE polymorphism			<i>p</i> value
	II	ID	DD	
<i>N</i> (1,730)	671	734	325	
Male/female (656/1,074)	247/424	289/445	120/205	0.5637
Age (years)	53.8 ± 15.8	54.5 ± 15.9	53.8 ± 15.3	0.6385
BMI (kg/m ²)	22.2 ± 3.0	22.2 ± 3.0	22.5 ± 3.0	0.1959
sBP (mmHg)	127.3 ± 20.6	128.0 ± 19.2	127.1 ± 20.0	0.7316
dBP (mmHg)	76.0 ± 12.7	76.3 ± 12.2	76.3 ± 11.8	0.8743
PP (mmHg)	50.5 ± 12.3	50.6 ± 12.3	49.5 ± 12.2	0.4122
CHO (mg/dl)	191.4 ± 35.8	190.3 ± 34.0	191.2 ± 35.6	0.8129
TG (mg/dl)	127.1 ± 85.9	124.1 ± 84.2	126.1 ± 84.7	0.7983
Smoking (P/N) (%)	239/432 (35.6)	263/471 (35.8)	123/202 (37.9)	0.7713
FH (P/N) (%)	178/493 (26.5)	202/532 (27.5)	103/222 (31.7)	0.2228
DM (P/N) (%)	37/634 (5.5)	50/684 (6.8)	17/308 (5.2)	0.4779

Abbreviations are listed in Table1.

able to be removed. To examine the independent contribution of ACE gene polymorphisms on hypertension, while adjusting for the effects of other clinical characteristics, we used a logistic analysis. For hypertension, a baseline logistic model was developed by applying a backward stepwise selection with the following factors as covariates: gender (female = 0, male = 1), BMI, family history of hypertension (negative = 0, positive = 1), and polymorphism of the ACE gene (II = 0, ID + DD = 1). Backward selection was used, and a *p* value of 0.10 or greater was required for a variable to be removed from the model.

Results

Table 1 shows the characteristics of the study population.

The frequency of the ACE I/D polymorphism was not significantly different from that in our previous report (10). No significant differences were observed among the II, ID, and DD groups with respect to male/female ratio, age, BMI, blood pressure, pulse pressure, hyperlipidemia, frequency of smoking habits, family history of hypertension, presence of hypertension, or diabetes mellitus (DM). Logistic analysis revealed that age (*p* < 0.001; odds ratio = 1.091), BMI (*p* < 0.001; odds ratio = 1.211), and family history of hypertension (*p* < 0.001; odds ratio = 0.371) were associated with hypertension. Table 2 shows the characteristics of the subjects not receiving antihypertensive therapy. We again analyzed these 1,730 subjects. The results for these subjects were similar to those reported in our previous study. No significant differences in blood pressure were observed among

Table 3. Characteristics of the Male Study Population (Excluding 438 Hypertensive Therapy Patients)

	ACE polymorphism			<i>p</i> value
	II	ID	DD	
<i>N</i> (656)	247	289	120	
Age (years)	54.9 ± 16.1	55.9 ± 15.9	57.0 ± 14.3	0.4530
BMI (kg/m ²)	22.4 ± 2.9	22.5 ± 2.9	22.7 ± 2.9	0.6550
sBP (mmHg)	130.1 ± 20.0	130.3 ± 16.9	132.7 ± 19.4	0.4094
dBp (mmHg)	78.0 ± 13.5	78.3 ± 11.4	79.5 ± 12.1	0.5608
PP (mmHg)	52.0 ± 13.4	52.0 ± 11.8	53.2 ± 13.2	0.6520
CHO (mg/dl)	184.1 ± 32.4	184.7 ± 35.5	182.0 ± 31.8	0.7660
TG (mg/dl)	155.0 ± 104.0	142.9 ± 103.0	146.7 ± 103.4	0.3948
Smoking (P/N) (%)	186/61 (75.3)	231/58 (79.9)	104/16 (86.7)	0.0395
FH (P/N) (%)	56/191 (22.7)	69/220 (23.9)	36/84 (30.0)	0.2914
DM (P/N) (%)	20/227 (8.1)	25/264 (8.7)	10/110 (8.3)	0.9736

Abbreviations are listed in Table 1.

Table 4. Characteristics of the Female Study Population (Excluding 438 Hypertensive Therapy Patients)

	ACE polymorphism			<i>p</i> value
	II	ID	DD	
<i>N</i> (1,074)	424	445	205	
Age (years)	53.1 ± 15.6	53.6 ± 15.8	52.0 ± 15.6	0.4501
BMI (kg/m ²)	22.1 ± 3.1	22.0 ± 3.1	22.4 ± 3.1	0.2275
sBP (mmHg)	125.7 ± 20.9	126.4 ± 20.5	123.7 ± 19.6	0.3083
dBp (mmHg)	74.8 ± 12.1	74.9 ± 12.5	74.5 ± 11.3	0.9164
PP (mmHg)	50.9 ± 13.3	51.5 ± 13.7	49.2 ± 13.4	0.1489
CHO (mg/dl)	195.7 ± 37.0	193.9 ± 32.6	196.6 ± 36.6	0.6004
TG (mg/dl)	110.9 ± 68.5	111.9 ± 66.6	114.0 ± 69.1	0.8642
Smoking (P/N) (%)	53/371 (12.5)	32/413 (7.2)	19/186 (9.3)	0.0294
FH (P/N) (%)	122/302 (28.8)	133/312 (29.9)	67/138 (32.7)	0.6037
DM (P/N) (%)	17/407 (4.0)	25/420 (5.6)	7/198 (3.4)	0.3576

Abbreviations are listed in Table 1.

the II, ID, and DD groups. Tables 3 and 4 show the characteristics of the subjects by gender. Male subjects with ACE DD genotypes and female subjects with ACE II genotypes were more likely to be smokers than the male or female subjects with other genotypes. However, no significant differences in blood pressure were observed among the II, ID, and DD groups. Moreover, we analyzed subjects not receiving hypertensive treatment as well as subjects younger than 60 years. No significant differences were observed among the II, ID, and DD groups with respect to male/female ratio, age, BMI, blood pressure, pulse pressure, hyperlipidemia, frequency of smoking habit, family history of hypertension, or presence of DM (data not shown). Similarly, when subjects were divided by gender there were also no significant differences between males or females across the three genotypes with regard to age, BMI, blood pressure, pulse pressure, hyperlipidemia, frequency of smoking habits, family history of hypertension or presence of DM (data not shown). Table 5 shows the characteristics of the subjects followed up since 1991. No significant differences were observed among the II,

ID, and DD groups with respect to male/female ratio, age, difference or change in blood pressure, or BMI. Table 6 shows the characteristics of the subjects followed up since 1991 according to gender. There were no differences or changes in blood pressure or BMI by ACE polymorphism. We also analyzed subjects younger than 60 years, and found no differences or changes in blood pressure or BMI by ACE polymorphism (data not shown). Only age ($p < 0.0001$) and %BMI ($p < 0.0001$) were strongly associated with change in blood pressure over 8 years, there were no associations between ACE polymorphism and either blood pressure or change in blood pressure.

Discussion

There were no differences or changes in blood pressure in relation to ACE I/D polymorphism in our study. However, recent reports have suggested that an ACE I/D polymorphism is associated with age-related essential hypertension (2, 5, 9, 11). Moreover, some studies have recently suggested that

Table 5. Characteristics of the Subjects Followed-Up Since 1991

	ACE polymorphism			<i>p</i> value
	II	ID	DD	
<i>N</i> (1,484)	541	655	288	
Male/female (549/935)	201/340	247/408	101/187	0.7380
Age (years)	61.2 ± 12.6	62.7 ± 12.0	60.8 ± 12.9	0.0422
ΔsBP (mmHg)	2.75 ± 17.8	1.16 ± 16.7	2.01 ± 17.1	0.2800
ΔdBP (mmHg)	- 0.21 ± 11.9	- 0.17 ± 11.2	0.79 ± 10.9	0.4226
ΔBMI (kg/m ²)	0.07 ± 1.2	0.09 ± 1.4	0.13 ± 1.29	0.7804
%sBP (%)	1.13 ± 13.0	0.15 ± 12.3	0.72 ± 12.3	0.4018
%dBP (%)	- 1.55 ± 15.7	- 1.21 ± 14.8	0.18 ± 13.9	0.2696
%BMI (%)	0.10 ± 5.3	0.21 ± 6.1	0.35 ± 5.70	0.8331

ΔBP (BMI) was calculated as: BP (BMI) in 1999 - BP (BMI) in 1991. % BP (BMI) was calculated as: ΔBP (BMI) divided by BP (BMI) in 1999. Other abbreviations are listed in Table 1.

Table 6. Characteristics of the Subjects Followed-Up Since 1991 by Gender

	ACE polymorphism			<i>p</i> value
	II	ID	DD	
Male (549)	201	247	101	
Age (years)	62.8 ± 12.6	63.3 ± 11.8	61.6 ± 11.5	0.5168
ΔsBP (mmHg)	3.27 ± 16.9	1.00 ± 16.3	3.78 ± 18.3	0.2364
ΔdBP (mmHg)	- 0.63 ± 11.8	- 1.45 ± 10.7	0.76 ± 10.3	0.2347
ΔBMI (kg/m ²)	0.17 ± 1.1	0.08 ± 1.2	0.20 ± 1.2	0.5791
%sBP (%)	1.61 ± 12.1	0.10 ± 11.9	1.76 ± 12.8	0.3225
%dBP (%)	- 2.15 ± 15.3	- 2.84 ± 14.5	0.01 ± 13.2	0.2542
%BMI (%)	0.55 ± 5.0	0.10 ± 5.5	0.72 ± 5.1	0.5106
Female (935)	340	408	187	
Age (years)	60.3 ± 12.5	62.3 ± 12.1	60.3 ± 13.6	0.0553
ΔsBP (mmHg)	2.44 ± 18.3	1.26 ± 17.0	1.06 ± 16.4	0.5678
ΔdBP (mmHg)	0.03 ± 11.9	0.61 ± 11.4	0.81 ± 11.2	0.7013
ΔBMI (kg/m ²)	0.01 ± 1.2	0.10 ± 1.5	0.10 ± 1.3	0.6123
%sBP (%)	0.84 ± 13.5	0.18 ± 12.5	0.16 ± 12.0	0.7470
%dBP (%)	- 1.20 ± 15.9	- 0.23 ± 14.9	0.28 ± 14.3	0.5132
%BMI (%)	- 0.17 ± 5.4	0.29 ± 6.5	0.15 ± 6.0	0.5874

Abbreviations are listed in Tables 1 and 5.

there is a sex-specific association between this gene and hypertension. That is, a male-specific association was seen between the ACE I/D polymorphism and hypertension (1-3, 6, 12). However, another case-control study found no significant association between ACE I/D polymorphism and hypertension irrespective of whether men and women were analyzed separately or together (13). Further, these results did not change appreciably when the gender groups were subdivided according to more stringent criteria. These discrepancies are difficult to explain. Because hypertension is a multifactorial disease, one possible explanation is that its expression is mediated by ACE, as well as angiotensinogen and other factors related to the renin-angiotensin system. There is some controversy regarding the association of the

ACE locus with blood pressure and hypertension. It was reported in one study that approximately 10 gene loci are responsible for hypertension (14). It is possible that the polymorphism of the ACE gene has only a small effect on hypertension, and that this effect is masked by differences in genetic background or environmental factors, including lifestyle, which would affect the various gene loci. Moreover, in our analysis of elderly hypertensives, we found no significant association between ACE I/D polymorphism and hypertension. However, other reports have shown that genetic variation at the ACE locus may be associated with some determinants for blood pressure in the elderly (5, 11). These discrepant results may suggest that there is actually very little or no association between the ACE I/D genotypes and hy-

pertension, especially since, by way of contrast, the association between hypertension and both age and BMI was strong in the present analysis and a previous report (6). However, in the same study population analysis, there was at best a minimum association between ACE I/D genotypes and hypertension. Iso *et al.* conducted a study to examine the relation between left ventricular mass and subsequent blood pressure changes over a 6–8 year follow-up period in adult Japanese men from rural and urban populations (15). Prior to their study, however, there had been no examination of the associations between ACE gene polymorphism and change in blood pressure. Our study is the first to analyze the relation between ACE I/D polymorphism and change in blood pressure in a cohort representative of the general population in Japan. During the 8-year follow-up, we found no significant associations between the ACE I/D polymorphism and changes in blood pressure. One hypothesis is that homeostasis compensations, such as those of the kidney in blood pressure regulation, successfully normalize the blood pressure, even in subjects who have quantitative changes in ACE gene function. This hypothesis was reported in a study using mice having functional copies of the ACE gene at its normal chromosomal location (16). Importantly, our study evaluated a large number of subjects in a rural community. Furthermore, the potential selection bias was minimized by using a study population based on medical examination.

In our study, the D allele frequency (40.4%) was higher than those in previous reports in Japan (34.3–39.0%) (6, 7). In fact, the ACE genotype distribution in this study did not conform to Hardy-Weinberg's law. One possible reason for the excess of the DD genotype is that we used a small number of subjects. Another possible reason is that our subjects were highly genetically uniform. The ACE DD genotype has been associated with longevity (17). Although our participants included more than half of the Shigaraki residents who were older than 50 years. Finally, it is highly unlikely that ACE I/D genotyping errors occurred in this study. We confirmed the DD genotype of the ACE gene by a second PCR using Taq extender. Moreover, in the case of equivocal genotypes such as ID and DD, we performed a third PCR and verified the genotype of ACE gene.

In conclusion, ACE polymorphism did not appear to have any significant effect on blood pressure or change in blood pressure, and did not appear to be sex-specific in this rural Japanese general population. Further investigations will need to be performed in large-scale populations.

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