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Association between Angiotensin I-Converting Enzyme Gene Polymorphism and Hypertension in Selected Individuals of the Bangladeshi Population

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The genetic factors that contribute to the development of coronary artery disease (CAD) are poorly understood. It is likely that multiple genes that act independently or synergistically contribute to the development of CAD and the outcome. Recently, an insertion/deletion (I/D) polymorphism of the human angiotensin I-converting enzyme (ACE) gene, a major component of the renin-angiotensin system (RAS), was identified. The association of the ACE gene D allele with essential hypertension and CAD has been reported in the African-American, Chinese, and Japanese populations. However, other studies have failed to detect such an association. It has been suggested that these inconsistencies may be due to the difference in backgrounds of the population characteristics. In the present study, we investigated the I/D polymorphism of the ACE gene in 103 subjects of both sexes, consisting of 59 normal controls and 44 patients with hypertension. The allele and genotype frequency were significantly different between the hypertensive and control groups ($p < 0.01$). Among the three ACE I/D variants, the DD genotype was associated with the highest value of the mean systolic blood pressure [SBP] and mean diastolic blood pressure [DBP] ($p = < 0.05$) in men, but not in women. In the overall population, the mean SBP and DBP was highest in DD subjects, intermediate in I/D subjects, and the least in II subjects.

Keywords: Angiotensin, Angiotensin converting enzyme, Genotype, Hypertension, Polymorphism

Introduction

Hypertension or pathological elevation of blood pressure is regulated by a number of physiological system and biochemical processes. Each of the systems and processes are influenced by a variety of genetic and environmental factors (Jeunemaitre *et al*., 1992). As one of the genetic factor polymorphisms of the angiotensin I-converting enzyme (ACE) gene, the encoding component of the renin-angiotensin system (RAS) has been proposed as an independent factor for hypertension and other cardiovascular diseases (Raynolds *et al*., 1993; Higaki *et al*., 2000). The major function of ACE is the conversion of angiotensin I to vasoactive, natriuretic octapeptide angiotensin II. Angiotensin II binds to plasma membrane receptors. It also produces arterioler constriction and a rise in systolic and diastolic blood pressure. The ACE is encoded by a 21 Kb gene that consists of 26 exons, and is located on chromosome 17. A polymorphism of the ACE gene involves the insertion (I) or deletion (D) of a 287 bp Alu repeat sequence near the 3' end of intron 16 (Rigat *et al.*, 1990). Since its identification, several studies have shown that the DD genotype of the I/D polymorphism in the ACE gene is associated with hypertension and other cardiovascular risk factors. Significant association of hypertension with D allele of the ACE gene has been documented in the African-American (Duru *et al*., 1994), Chinese (Chiang *et al*., 1996), and Japanese populations (Morise *et al*., 1994; Nakano *et al*., 1998). However, many other studies have failed to detect any such association (Jeunematre *et al*., 1992; Harrap *et al*., 1993; Kamdar *et al*., 1994). Genetic and environmental heterogeneity among different ethnic groups may account for this inconsistent result (Barley *et al*., 1994; Staessen *et al*., 1997). In this study, we focused on the I/D polymorphism of the ACE gene in patients with essential hypertension and normotensive control subjects.

Materials and Methods

Subjects The study included a total of 103 subjects of both sexes, consisting of 59 age-matched controls and 44 patients with hypertension. The hypertensive individuals were recruited from the internal medicine ward of the Dhaka

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Table 1. Clinical characteristics of hypertensive and control subjects

	Control (n = 59)	Hypertensive (n = 44)	*p*
Age years	43.5 ± 12.6	47.3 ± 11.5	0.126
Sex: male/female	40(67.8)/19(32.2)	30(68.2)/14(31.8)	0.967
systolic blood pressure (mmHg)	118 ± 8.73	149.4 ± 6.21	<0.001
diastolic blood pressure (mmHg)	82.1 ± 5.73	111.2 ± 9.53	<0.001
Family history of hypertension	23(39.0)	25(56.8)	0.074

Medical College Hospital (DMCH). These individuals were selected from the outpatients that were treated in the hospital, as well as ones in periodic follow-ups thereafter. Blood pressure was measured using a mercury sphygmomanometer, as recommended by the American Heart Association (Kirkendal *et al*., 1980). Special care was taken to control and avoid stimuli that may influence blood pressure. Hypertension was defined as a sustained diastolic blood pressure > 90 mm Hg that is accompanied by an elevated systolic blood pressure > 140 mm Hg. Secondary hypertension was excluded by clinical evaluation, and none had evidence of cardiac or renal failure. The normotensive control subjects were recruited from Red Crescent Blood Centre. They had no history of hypertension and were not on anti-hypertensive drug therapy. All of the participants were Bangladeshi, and belonged mainly to the urban population of Dhaka City. They were between the ages of 20 to 65 years.

Collection of blood samples Approximately 5 ml of peripheral blood samples were collected in a screw cap tube that contained 20% EDTA. The specimen was capped and transported to the laboratory on dry ice. It was then stored at −20ºC if not assayed immediately.

Determination of genotype Genomic DNA was isolated from whole blood according to the published protocols for extracting DNA (Gustincich *et al*., 1991). To determine the ACE genotype, genomic DNA was amplified by a polymerase chain reaction (PCR) initially using a flanking primer pair, and subsequently with a primer pair that specifically recognized the insertion specific sequence, when necessary, in order to confirm the specificity of the amplification reactions (Yoshida *et al*., 1996). The sense oligonucleotide primer was 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3', and the antisense primer was 5'-GAT GTG GCC ATC ACA TTC GTC AGA TTT -3'. The PCR mixture contained 50 ng genomic DNA, 10 pmol of each primer, 250 µmol/l dNTP, 1.5 mmol/l MgCl2, 10 mmol/l Tris-HCl. pH 8.3, and 1.0 unit Taq DNA polymerase (Sigma) in a final volume of 25 µl. The amplification cycle was performed on a GeneAmp 9700 thermal cycler (Perkin-Elmer). After initial denaturation at 94ºC for 5 min, the DNA was amplified by 35 cycles: denaturation for 1 min, annealing at 58ºC for 1 min, and extension at 72ºC for 3 min, followed by a final elongation at 72ºC for 5 min. Amplification products were separated by electrophoresis on a 2% agarose gel, and visualized under ultraviolet light after ethidium bromide staining. The PCR product is a 190 bp fragment in the presence of a deletion (D) allele, and a 490 bp fragment in the absence of a deletion (I) allele. Thus, each DNA sample revealed one of three possible patterns after electrophoresis: a 490 bp band (II genotype), a 190 bp band (DD genotype), or both 490 and 190 bp bands (I/D genotype) (Rigat *et al*., 1992).

Statistical analysis Statistical analysis was performed using SPSS version 10.0. Allele frequencies were deduced from genotype frequencies. The differences in allele and genotype frequencies between the groups were tested by the Chi-square test. The same test was also used to examine if the observed genotype frequencies were in the Hardy-Weinberg equilibrium. The association between ACE I/D polymorphism and clinical variables was examined by one-way ANOVA.

Results

The clinical details of the hypertensive and control subjects are presented in Table 1. The two study groups were well-matched for gender and age. The mean systolic blood pressure [SBP] and diastolic blood pressure [DBP] were significantly higher in the hypertensive subjects than in the control subjects ($P < 0.001$). There was, however, no significant difference in families with a history of hypertension between the control and hypertensive subjects ($P = 0.07$). The ACE genotype distribution in total study subjects and controls were in the Hardy-Weinbergs equilibrium ($x^2 = 5.37$, $df = 2$, $P = 0.068$ and $x^2 = 3.69$, $df = 2$, $P = 0.158$), but significantly deviated in hypertensives ($x^2 = 10.4$, $df = 2$, $P = 0.005$).

The respective frequency of the DD, I/D, and II genotype among hypertensive individuals were 50% (n = 22), 38.6% (17), and 11.4% (n = 5), respectively. Among the control

Table 2. Distribution of ACE genotype and allele frequency in the control and hypertensive subjects

Variable	Control	Hypertensive	*p*
Genotype, Number (%)			
II	19 (33.2)	5 (11.4)	
I/D	26 (44.1)	17 (38.6)	
DD	14 (23.7)	22 (50.0)	<0.01
Alleles			
I	64 (54.2)	27 (30.6)	
D	54 (45.7)	61 (69.3)	<0.001

Table 3. Feature of men and women by ACE genotype

Subjects	ACE genotype			*p*
	DD	ID	II	
Male (n = 70)	23	31	16	
Age	49.0 ± 13.5	46.9 ± 12.0	45.9 ± 12.2	0.725
Systolic blood pressure	139.7 ± 15.9	130.4 ± 15.5	127.1 ± 17.1	<0.05
Diastolic blood pressure	100.6 ± 15.3	95.4 ± 16.1	87.1 ± 15.7	<0.05
Female (n = 33)	13	12	8	
Age	35.7 ± 8.6	42.7 ± 11.3	44.8 ± 9.8	0.097
Systolic blood pressure	137.3 ± 20.6	127.9 ± 16.5	130.0 ± 15.3	0.421
Diastolic blood pressure	97.6 ± 16.0	90.8 ± 17.0	88.7 ± 16.6	0.412

Variables are mean ± SD

subjects, they were 23.7% (n = 14), 44.1% (n = 26), and 32.2% (n = 19), respectively. The frequencies of the D and I allele among the patients with hypertension were 69.3% and 30.6%, and among the controls they were 45.7% and 54.2%, respectively. Statistical analyses showed that the ACE DD genotype was significantly higher in hypertensive subjects ($P < 0.01$). The frequency of the D allele was also more frequent in the hypertensive subjects than in the control groups ($P < 0.001$). To examine the sex-specific association with hypertension, we compared genotypes separately among men and women (Table 3). The results showed that the mean systolic blood pressure [SBP] and diastolic blood pressure [DBP] was significantly associated with the ACE DD genotype in men ($P < 0.05$), but not in women. In the overall population, the mean SBP and mean SBP were highest in the DD subjects, intermediate in the I/D subjects, and lowest in the II subjects.

Discussion

The Angiotensin-I converting enzyme (ACE) is one of the components of the renin-angiotensin system, and it has attracted attention in the development of cardiovascular disease. An ACE inhibitor is known to significantly reduce mortality or the incidence of myocardial infarction in patients who have hypertension or ischemic heart disease (Pfeffer *et al*., 1992; Cambein *et al*., 1995; Wenzel *et al*., 1997). Recently, a polymorphic marker that is found on intron 16 of the ACE gene was correlated with circulating concentrations of ACE. The marker has a strong linkage disequilibrium with the true variant. It consists of the presence (I) or absence (D) of a 287 bp Alu repeat sequence. Individuals with the II genotype have the lowest circulating ACE concentrations, whereas those with the DD genotype have the highest concentrations (Samani *et al*., 1994). A significant association of the ACE gene D allele with essential hypertension was documented in the African-American (Duru *et al*., 1994), Chinese (Chiang *et al*., 1996), and Japanese populations (Morise *et al*., 1994; Nakano *et al*., 1998). On the other hand, the I allele was associated with high blood pressure in an Australian population with strong evidence of familial hypertension (Zee *et al*., 1992). It has been suggested that the population heterogeneity in the association of ACE I/D polymorphism with essential hypertension may be due to significant variations of population backgrounds (Barley *et al*., 1994).

In this study we had the opportunity to investigate the association of the ACE gene insertion/deletion (I/D) polymorphism with hypertension in selected individuals from the Bangladeshi population. We determined the ACE genotype of 103 subjects that consisted of 50 healthy controls and 44 hypertensive individuals. The two groups were well matched for age and sex, but significantly different with respect to SBP and DPB and family history of hypertension (Table 1). This study demonstrated a positive association between I/D polymorphism and hypertension in the Bangladeshi population. The frequency of the ACE DD genotype was 50% in the hypertensive group. This was significantly higher than the control groups ($P < 0.01$) (Table 2). This is in accordance with studies that were conducted in selected groups from the Turkish and in Japanese populations (Turgay *et al*., 1999; Higaki *et al*., 2000). The frequency of D allele (69.3%) in the hypertensive group was significantly higher than in the control groups ($P < 0.001$). Table 2 also shows that DD, but not I/D, was associated with hypertension compared with the II genotype. This suggests that the hypertensive effect appears with recessive inheritance. This study also found a positive association of the ACE DD genotype with hypertension in men ($P < 0.05$), which is consistent with the Suita study that was conducted in Japan (Higaki *et al*., 2000). The mechanism of the sex-specific association of hypertension with the DD genotype is unclear. One reason is that estrogen might play a protective effect against hypertension, and it has also been found that the sex specificity decreases in elderly subjects and increases in younger subjects.

In this study of Bangladeshi people, a significant association of ACE I/D polymorphism and hypertension was observed. The result of this study supports the hypothesis that the DD genotype is in linkage disequilibrium with a

functional variant of the ACE gene. The sample size in this study was relatively small, which may raise some questions, but a clearer interrelation between this allele and hypertension is established, and should be taken into account by physicians.

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