Association analysis of a polymorphism of the angiotensin I-converting enzyme gene and angiotensin II type 1 receptor gene in Korean population

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Previously, we made a study report on the genotype distribution and the gene frequency of angiotensin I-converting enzyme (ACE) in Korean population, and on the association between hypertension and genetic variance of ACE. This time, we have investigated a rapid mismatch-PCR/RFLP assays for the variant of the angiotensin II type 1 receptor (AT_1R) gene (an A→C transversion at position 1166 of AT_1R gene), a mutation which may interact with the ACE polymorphism in the determining of risk of myocardial infarction. The genotype distributions of Koreans' angiotensin II type I receptor gene were AA (66.3%):AC (28.1%):CC (5.6%), thus the AA genotype was most numerous, and the allele frequency was A:C = 0.803:0.197. Genotype distributions were shown as AA (76.8%):AC (20.9%):CC (2.3%), the allele frequency was A:C = 0.872:0.128 in the male group, and AA (47.4%):AC (41.0%):CC (11.6%), A:C = 0.679:0.321 in the female group. Differences were highly significant between the male and female groups ($p<0.0001$). Genotype distributions between angiotensin II type I receptor gene and angiotensin converting enzyme gene showed that there is no significance between AT_1R genotypes and ACE genotypes in total subjects ($p>0.05$).

Keywords: Angiotensin I-converting enzyme, Angiotensin II type 1 receptor, Genotype, Genetic polymorphism

INTRODUCTION

Essential hypertension is a common human disease believed to result from the interplay of multiple genetic and environmental determinants. The functions of the dicarboxypeptidase angiotensin converting enzyme (ACE) include the metabolism of bradykinin and the conversion of angiotensin I to angiotensin II. Angiotensin II, which is a biologically active peptide in the renin-angiotensin-aldosteron system, plays an important role in the homeostasis of blood pressure, electrolyte balance and cardiovascular hypertrophy (Hall *et al.*, 1990). Most of the known actions of angiotensin II are exerted through the angiotensin II type I receptor (AT_1R), which is present in particular in vascular smooth muscle cells and in the myocardium. Two subtypes of cell surface receptors have been identified (angiotensin II type I; AT_1, angiotensin II type II; AT_2) in man and the AT_1 receptor , a G-protein-coupled receptor, has been cloned and sequenced (Furuta *et al.*, 1992). This receptor is thought to mediate the major pressor and trophic actions of angiotensin II (Timmermans *et al.*, 1993).

Recently, a polymorphism ($A^{1166}\rightarrow C$) in the 3' untranslated region of the gene encoding AT_1 receptor was associated with essential hypertension (Bonnardeaux *et al.*, 1994). In the present study we investigated the genotype distribution and the gene frequency for C^{1166} variant of the AT_1R gene in Korean population.

MATERIALS AND METHODS

Subjects

We studied 267 Korean adults (172 men and 95 women).

Isolation of Genomic DNA

Genomic DNA was extracted from human blood using Blin and Stafford's (1976) method. Briefly, 600 μl of blood was diluted in 600 μl of PBS buffer, vortexed, and centrifuged. The pellets were resuspended in 600 μl of lysis buffer [10

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mM Tris-HCl ; pH 8.0, 100 mM EDTA; pH 8.0, 0.5% SDS, 20 μg/ml RNase] were added. Overnight digestion with proteinase K (100 μg/ml) at 55°C was followed by centrifugation and precipitation of the supernatant in ethanol. The solution of the TE (10 mM Tris-HCl, 1 mM EDTA; pH8.0) buffer was added to the DNA pellet.

Identification and Detection of Polymorphisms of the AT_1 Receptor Gene

The sequence of the upstream primer is 5'-ATA ATG TAA GCT CAT CCA CCA AGA AG-3'. The downstream primer is 5'-TCT CCT TCA ATT CTG AAA AGT ACT TAA-3'. PCR was performed in a final volume of 20 μl which contained 100ng of genomic DNA, 10 pmol of each primers, 250 μM each of the four dNTP, 1.5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl, pH 8.4 and 0.4 unit of *Taq* polymerase. Amplication was carried out in an Perkin-Elmer PCR (Norwalk, CT, USA) for 30 cycles with steps of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 2.5 min. The PCR products were digested by *Afl*II restriction enzyme, which recognize CTTAAG sequence, with an incubation at 37°C overnight. Digested products were electrophoresed on 2.5% agarose gel, and DNA was visualized directly with ethidium bromide staining. Genotypes were assigned as AA, AC, CC.

Statistical Analysis

Statistical analysis was performed with the Statistical Analysis System (version 6.04; SAS Institute, Inc). Allele frequencies in different groups were compared by the use of gene counting and χ^2 analysis (SAS, 1989).

RESULTS

267 DNA samples from unrelated adult Korean subjects were assayed, and genotypes were easily assigned on all occasions. *Afl*II digests of PCR amplicon showed 3 genotype patterns, AA, AC, and CC. *Afl*II digestion of the PCR products showed 139 bp and 27 bp bands for the C^{1166} allele and 166 bp fragment for the A^{1166} allele (Fig. 1).

As shown in Table 1, genotype distributions and allele frequencies of angiotensin II type I receptor gene were comparable to those previously obtained by Caucasian group (Hingorani and Brown, 1995). Genotype distributions of Koreans angiotensin II type I receptor gene were AA (66.3%):AC (28.1%):CC (5.6%), thus AA genotype was most numerous, the allele frequency was A:C = 0.803:0.197. Table 2 shows genotype distributions and allele frequencies of angiotensin II type I receptor gene between Korean male and female. Genotype distributions showed AA (76.8%):AC

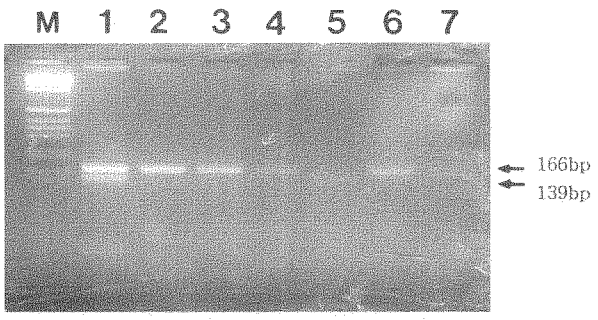

Fig 1. *Afl*II digests of PCR amplicons from 7 unrelated individuals resolved on a 2.5% agarose gel with ethidium bromide staining. AA homozygotes (2, 3, 4, 6 and 7), AC heterozygote (1) and CC homozygote (5) is shown. M; marker (1 kb Ladder).

Table 1. Genotype distributions and allele frequencies of angiotensin II type I receptor gene between Korean and Caucasian.

	Korean (n = 267)	Caucasian (n = 221)
Genotypes, n (%)		
AA	177 (66.3)	114 (51.6)
AC	75 (28.1)	89 (40.3)
CC	15 (5.6)	18 (8.1)
χ^2 (2 df) = 10.868, p = 0.004		
Alleles, n (frequency)		
A	429 (0.803)	317 (0.717)
C	105 (0.197)	125 (0.283)
χ^2 (1 df) = 9.971, p = 0.002		

Table 2. Genotype distributions and allele frequencies of angiotensin II type I receptor gene in Korean classified by sex

	Total (n = 267)	Male (n = 172)	Female (n = 95)
Genotypes, n (%)			
AA	177 (66.3)	132 (76.8)	45 (47.4)
AC	75 (28.1)	36 (20.9)	39 (41.0)
CC	15 (5.6)	4 (2.3)	11 (11.6)
χ^2 (2 df) = 26.115, p < 0.0001			
Alleles, n (frequency)			
A	429 (0.803)	300 (0.872)	129 (0.679)
C	105 (0.197)	44 (0.128)	61 (0.321)
χ^2 (1 df) = 28.905, p < 0.0001			

(20.9%):CC (2.3%), the allele frequency was A:C = 0.872: 0.128 in the male group, and AA (47.4%):AC (41.0%):CC (11.6%), A:C = 0.679:0.321 in the female group. Differences were highly significant between the male and female groups ($p<0.0001$). Table 3 shows genotype distributions between angiotensin II type I receptor gene (AT_1R) and angiotensin converting enzyme gene (ACE). The analysis showed that there is no significance between AT_1R genotypes and ACE genotypes in total subjects ($p>0.05$).

DISCUSSION

Polymorphisms of the genes encoding components of the renin-angiotensin system have been used to implicate this system in the genetic predisposition to essential hypertension (Zee *et al.*, 1992), and cardiovascular disease (Rigat *et al.*, 1990, 1992). Angiotensin II also has hypertrophic, and possibly hyperplastic, effects on vascular smooth muscle cells and cardiomyocytes, and increases extracellular collagen matrix synthesis (Geisterfer *et al.*, 1988 and Daemen *et al.*, 1991). The cellular effects of angiotensin II are mediated by two structurally distinct receptor subtypes, AT_1 and AT_2.

We also investigated genotype distributions and allele frequencies of angiotensin II type I receptor gene in Korean population. Genotype distributions and allele frequencies of angiotensin II type I receptor gene were quite different between Koreans and Caucasians (Table 1). In comparison with Caucasian distributions (Hingorani and Brown, 1995), there were significant differences ($p<0.01$). Compared between Koreans and Caucasians, we found that Koreans have more genotype AA, and Caucasions have more genotype AC.

The important fact was that genotype distributions and allele frequencies of angiotensin II type I receptor gene between male and female were quite different in Korean population (Table 2). It showed that the Korean male group had more AA genotype and A allele than the Korean female.

Table 3. Genotype distributions between angiotensin II type I receptor gene (AT_1R) and angiotensin converting enzyme (ACE) gene

AT1R genotype	AA (n=177)	AC (n=75)	CC (n=15)
ACE genotype (n)			
I I (91)	61 (22.85%)	25 (9.36%)	5 (1.87%)
ID (126)	76 (28.46%)	41 (15.36%)	9 (3.37%)
DD (50)	40 (14.98%)	9 (3.37%)	1 (0.37%)
$\chi 2$ (4 df)=6.505,	p=0.164		

Further analysis showed that 76.8% of male had genotype AA. The Korean female group had more AC genotype, CC genotype and C allele than the Korean male group. However, ACE genotypes of Koreans (Yang *et al.*, 1997) were similar distribution in male and female ($p>0.05$). Distributions of ACE genotype of the subjects (n=267) showed II:ID:DD = 18.6%:48.3%:33.1% in the male group and II:ID:DD = 18.9%: 45.3%:35.8% in the female, respectively. Taken together, the ratio of ACE genotype was II (18.7%):ID (47.2%):DD (34.1%). In Table 3, the analysis showed that there is no significance between AT_1R genotypes and ACE genotypes in total subjects. However, there is significance in the male group ($p<0.01$).

ACKNOWLEDGEMENT

This paper was supported by Joongwon Research Institute of Kon-Kuk University. 1997

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