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Genetic Epidemiology of Renin-Angiotensin System in Korean Population

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ABSTRACT **: Genetic polymorphisms of the renin-angiotensin system (RAS) have been associated with hypertension in various ethnic groups, but no relation between these polymorphisms and hypertension has yet been systematically evaluated. To assess the relationship between allelic variation of RAS genes and hypertension, we performed the case-control studies using genetic markers in Korean normotensives and hypertensives. The allele and genotype frequencies of RAS genes in Korean population were not significantly different between normotensives and hypertensives. To investigate the distribution of allele frequencies among various populations, the data obtained in this study were compared to those in other ethnic groups studied previously. Except for T174M polymorphism of angiotensinogen (AGT) gene, allele frequencies of RAS genes were different among racial groups. The reason for these differences may be due to the difference in various genetic or environmental background or due to the effects by various sample size studied. In addition, it can be emphasized that carefully designed studies are required to minimize the ethnic heterogeneity of the case and control populations.**

Keywords: Genotype, Hypertension and Renin-Angiotensin System

Introduction

Essential hypertension is a heterogeneously multifactorial disease in which blood pressure is harmfully high without overt cause. Both genetic and environmental factors have been implicated in its etiology (Ward, 1990). Candidate genes that determine blood pressure variation include those whose products have a direct role in vascular biology such as components of renin-angiotensin system (RAS).

The RAS has an important role in blood pressure homeostasis (Inagami, 1994; Tewksbury, 1983), and the phenotype abnormalities of the RAS may be associated with the development of essential hypertension. Thus, genes encoding components of the RAS are attractive candidates for the investigation on the genetic basis of essential hypertension.

The component genes of RAS include those encoding for angiotensinogen (AGT), angiotensin-I converting enzyme (ACE), and angiotensin-II type 1 receptor (AT_1R). Angiotensin I is produced from AGT by renin, it subsequently is converted to angiotensin II by ACE. Angiotensin II increases blood pressure by causing vasoconstriction, aldosterone secretion and sodium/water reabsorption in the kidneys. The cellular effects of angiotensin II are mediated by two structurally distinct receptor subtypes, AT_1R and AT_2R (Inagami *et al.*, 1994).

Many studies have identified several molecular variants of genes encoding for components of the RAS. Studies searching for molecular variants of the AGT gene revealed 15 distinct genetic variations, and two of them one with threonine instead of methionine at position 235 (M235T) and one with methionine rather

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than threonine at position 174 (T174M) within exon 2 were found to be significantly associated with plasma AGT level (Inoue *et al.*, 1997; Jeunemaitre *et al.*, 1992), essential hypertension (Caulfield *et al.*, 1994) and coronary heart disease (Katsuya *et al.*, 1995).

An insertion (I)/deletion (D) polymorphism within intron 16 of the ACE gene has been identified (Rigat *et al.*, 1992), and hes been known to be associated with ACE level in plasma (Alvarez *et al.*, 2000; Bloem *et al.*, 1996; Cambien, 1994; Cambien *et al.*, 1988; Foy *et al.*, 1996; Marre *et al.*, 1994; Nakai *et al.*, 1994; Tiret *et al.*, 1992) and several cardiovascular diseases, including hypertension (Ashavaid *et al.*, 2000), left ventricular hypertrophy (Schunkert *et al.*, 1994; Iwai *et al.*, 1994; Lindpaintner *et al.*, 1996), ischemic heart disease (Cambien *et al.*, 1992; Lindpaintner *et al.*, 1995; Nakai *et al.*, 1994), carotid atherosclerosis (Castellano *et al.*, 1995), cerebrovascular disease (Catto *et al.*, 1996), as well as diabetic (Schmidt *et al.*, 1995) and non-diabetic renaldisease (McLaughlin *et al.*, 1996), although some studies have yielded conflicting results.

A·C variant in the 3' untranslated region (A1166C) has been identified through the examination of several poly- morphisms in the AT_1R gene (Tiret *et al.*, 1994). This A1166C polymorphism has been suggested to contribute to hypertension (Bonnardeaux *et al.*, 1994; Dzida *et al.*, 2001) and myocardial infarction (Alvarez *et al.*, 1998; Berge *et al.*, 1997; Tiret *et al.*, 1994) in some population.

The present case-control studies were designed to investigate the relationship between genetic markers of candidate genes in the RAS and hypertension in Korean population.

Materials and Methods

Subjects

We obtained 388 blood samples from the outpatients of Seoul Hygiene Hospital, Seoul, Korea. Of these, 116 hypertensive Korean individuals were defined as having a blood pressure above 140/90 mmHg. Subjects with secondary forms of hypertension and those taking antihypertensive drugs were excluded from the study.

Genotyping

Blood samples were obtained after an isolation and determination of lipid profiles were collected in EDTA-containing tubes and were centrifuged at 1,500×g for 10 min. Genomic DNA was isolated from buffy coat by the method of Sambrook *et al.*, (1989) with slight modification. The AGT M235T and T174M polymorphisms were investigated by using PCR-*Tthlll* I (Russ *et al.*, 1993) and *Nco* I digestion (Caulfield *et al.*, 1994), respectively. The I/D polymorphism of the ACE gene was detected by PCR without restriction enzyme digestion (Rigat *et al.*, 1992). The AT_1R A1166C polymorphism was analyzed by using PCR-*Afl* II digestion (Hingorani and Brown, 1995). Primer sequences for PCR reaction were shown in Table 1. Amplified PCR products were digested by each restriction enzymes, and visualized by agarose gel with ethidium bromide staining.

Statistical analysis

Allele frequencies were calculated from genotype frequencies, and the deviation from Hardy-Weinberg equilibrium was analyzed by by χ^2-fitness test. The heterozygosity, polymorphism information content (PIC) and haplotype diversity index (HDI) were measured by the methods of Bostein *et al.* (1980). The odds ratio (OR) and 95% confidence interval (CI) of hypertension associated with allelic variation was calculated by logistic regression analysis. The significance of differences in allele or genotype frequencies between normotensives and hypertensives was also estimated by the χ^2-independence test. The estimated frequencies of haplotypes were

Table 1. Polymorphiç sites and primer sequences for the PCR amplification of 3 candidate genes

Gene	Polymorphism	Primer sequence	Reference
Angiotensinogen	M235T	5'-CAGGGTGCTGTCCACACTGGACCCC-3' 5'-CCCTTTGTGCAGGGCCTGGCTCTCT-3'	Russ *et al.*, 1993
	T174M	5'-GATGCGCACAAGGTCCTG-3' 5'-CAGGGTGCTGTCCACACTGGCTCGC-3'	Caulfield *et al.*, 1994
Angiotensin I converting enzyme	I/D	5'-CTGGAGACCACTCCCATCCTTTCT-3' 5'-GATGTGGCCATCACATTCGTCAGAT-3'	Rigat *et al.*, 1992
Angiotensin II Type 1 receptor	A1166C	5'-ATAATGTAAGCTCATCCACCAAGAAG-3' 5'-TCTCCTTCAATTCTGAAAAGTACTTAA-3'	Hingorani and Brown, 1995

calculated by the method of Morgan *et al.* (1990). The degree of linkage disequilibrium, Δ and D' were measured by the methods of Hill and Robertson (1968) and Lewontin (1964). The level of statistical significance was assumed to be $p<0.05$. All statistical analysis was performed by the SAS (version 6.12) computer program.

Results

AGT gene

Figure 1A showed that the M235T and T174M polymorphic sites are located in the exon 2 of AGT gene. Figure 1B showed the M235T polymorphic patterns of the AGT gene. By PCR, M allele revealed a band of 164 bp, and T allele yielded bands of 141 bp and 24 bp. The genotype frequencies of the M235T polymorphism in the AGT gene were shown in Table 2. The genotype frequencies of MM, MT and TT were 4, 31 and 65% in normotensives, and 3, 41 and 56% in hypertensives, respectively. The heterozygosity and PIC values of M235T polymorphism represented the values of 0.31 and 0.26 for normotensives, and 0.36 and 0.30 for hypertensives, respectively. According to the heterozygosity and PIC values, the M235T polymorphism of the AGT gene showed the reasonably high degree of polymorphism in the both groups. The observed genotype distributions were not significantly deviated from those expected for Hardy-Weinberg equilibrium. There were no significant differences in allele and genotype frequencies between normotensives and hypertensives by case-control comparison.

Figure 1C showed the T174M polymorphic patterns of the AGT gene. By PCR, T allele revealed a band of 303 bp, and T allele yielded bands of a 211 bp and 92 bp.

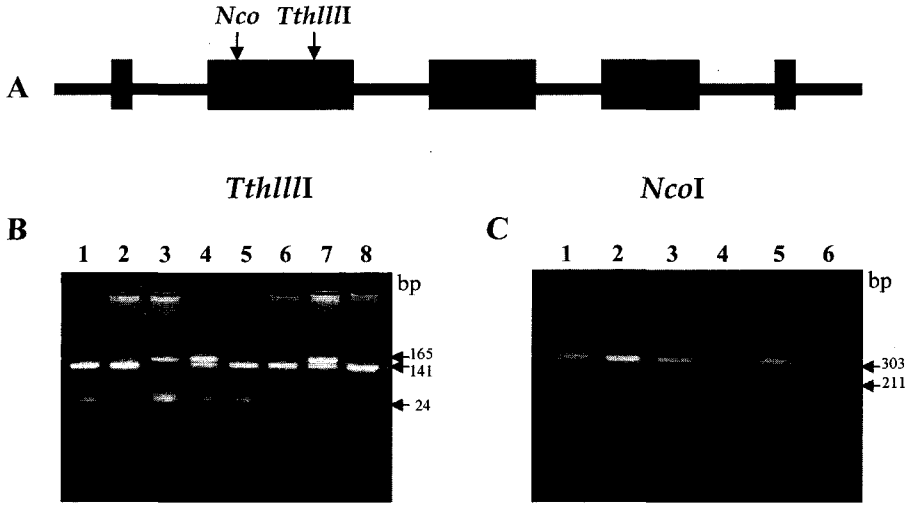

Fig. 1. Schematic diagram of the angiotensiongen gene to show the polymorphisms within the gene. A) Restriction map of the angiotensinogen gene. B) Patterns of M235T polymorphism at the angiotensinogen gene. Lane 3, MM genotypes; Lane 4 and 7, MT genotype; Lane 1, 2, 5, 6 and 8, TT genotypes. C) Patterns of T174M polymorphism at the angiotensinogen. Lane 1~3, 5 and 6, TT genotypes; Lane 4, TM genotypes.

Table 2. Genotype and allele frequencies of the M235T polymorphism in the AGT gene between normotensives and hypertensives

	Genotype No. (%)			Allele No. (%)		H^1	PIC^2
	MM	MT	TT	M	T		
Normotensives	6(4)	48(31)	102(65)	60(19)	252(81)	0.31	0.26
Hypertensives	4(3)	47(41)	65(56)	55(24)	177(76)	0.36	0.30
Chi-square		2.786		1.599			
Probability		0.248		0.206			
Odds ratio(CI)[3]				0.76(0.51-1.16)			

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Percentages are given in parentheses.
Both groups were in Hardy-weinberg equilibrium ($\chi^2 = 0.011$, df = 1, $p>0.05$ in normotensives; $\chi^2 = 1.650$, df = 1, $p>0.05$ in hypertensives).

Table 3. Genotype and allele frequencies of the T174M polymorphism in the AGT gene between normotensives and hypertensives

	Genotype No. (%)			Allele No. (%)		H^1	PIC^2
	TT	TM	MM	T	M		
Normotensives	72(84)	14(16)	0(0)	158(92)	14(8)	0.15	0.14
Hypertensives	58(72)	22(28)	0(0)	138(86)	22(14)	0.24	0.21
Chi-square		3.037		2.699			
Probability		0.080		0.100			
Odds ratio(CI)[3]			1.80(0.85-3.82)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Percentages are given in parentheses.
Both groups were in Hardy-weinberg equilibrium (χ^2 = 0.718, df = 1, p>0.05 in normotensives; χ^2 = 2.012, df = 1, p>0.05 in hypertensives).

The genotype frequencies of the T174M polymorphism in the AGT gene were shown in Table 3. The genotype frequencies of TT, TM and MM were 84, 16 and 0% in normotensives, and 72, 28 and 0% in hypertensives, respectively. MM genotype was not observed in our subjects. The heterozygosity and PIC values of T174M polymorphism represented the values of 0.15 and 0.14 for normotensives, and 0.24 and 0.21 for hypertensives, respectively. According to the heterozygosity and PIC values, the T174M polymorphism of the AGT gene showed the relatively low degree of polymorphism in the both groups. The observed genotype distributions were not significantly deviated from those expected for Hardy-Weinberg equilibrium. There were no significant differences in allele and genotype frequencies between two groups by case-control comparison.

The haplotype distriburions between the two polymorphic sites were shown in Table 4. There were no significant differences in haplotype distributions between normotensive and essential hypertensives. However, AGT M235T and T174M polymorphisms indicated the significant linkage disequilibrium (Δ = –0.19, D' = –0.94, χ^2 = 10.57, df = 1, P = 0.0011).

Table 4. Haplotype analysis of the AGT gene in the normotensives and hypertensives

Haplotypes		Normotensives	Hypertensives
M235T	T174M		
M	M	0.0007	0.0032
M	T	0.2162	0.2273
T	M	0.0539	0.1143
T	T	0.7292	0.6552
X^2 = 4.4698, df = 2, P = 0.1070			
HDI^1		0.4186	0.5060
PIC^2		0.3655	0.4490

[1]Haplotype Diversity Index, [2]Polymorphism Information Content.
There were no significant differences in haplotype frequencies between normotensives and hypertensives (χ^2 = 4.4698, df = 2, p> 0.05).

ACE gene

The I/D polymorphism of the ACE gene was located in intron 16 of this gene, and the genotype patterns of this polymorphism were displayed in Fig. 2A. This polymorphism was detected without digestion by restriction enzyme. After PCR amplification. I allele yielded a 490 bp band, and D allele gave a 190 bp band. The genotype frequencies of the I/D polymorphism in the ACE gene were shown in Table 5. The genotype frequencies of II, ID and DD were 30, 51 and 19% in normotensives, and 34, 43 and 23% in hypertensives, respectively. The heterozygosity and PIC values of *Bam* HI RFLP represented the values of 0.49 and 0.337 for normotensives, and 0.49 and 0.37 for hypertensives, respectively. According to the heterozygosity and PIC values, I/D polymorphism showed a reasonably high degree of polymorphism in the both groups. The observed genotype distribution of this polymorphism was in Hardy-Weinberg equilibrium. There were no significant differences in allele or genotype frequencies between two groups.

AT_1R gene

The A1166C polymorphism of the AT_1R gene was located in 3'-untranslated region of this gene, and the genotype patterns of this polymorphism were displayed in Fig. 2B. This polymorphism was detected by digestion with restriction enzyme, *Afl* II. After PCR amplification and *Afl* II digestion. A allele yielded a 166 bp band, and C allele gave a 139 bp and 27 bp bands. The genotype frequencies of the A1166C polymorphism in the AT_1R gene were shown in Table 6. The genotype frequencies of AA, AC and CC were 90, 10 and 0% in normotensives, and 94, 6 and 0% in hypertensives, respectively. CC

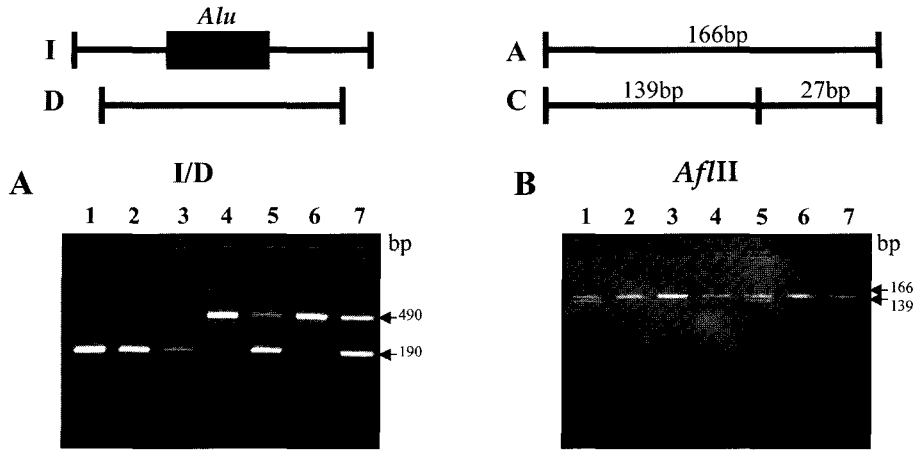

Fig. 2. Schematic diagram of the ACE and AT_1R genes to show the polymorphisms within the gene. A) I/D polymorphism patterns of the ACE gene. Lane 4 and 6, II genotypes; Lane 5 and 7, ID genotypes; Lane 1~3, DD genotypes. B) A1166C polymorphic patterns of the AT_1Rgene. Lane 2~4, 6 and 7, AA genotypes; Lane 1 and 5, AC genotypes.

Table 5. Genotype and allele frequencies of the I/D polymorphism in the ACE gene between normotensives and hypertensives

	Genotype No. (%)			Allele No. (%)		H^1	PIC^2
	II	ID	DD	I	D		
Normotensives	82(30)	138(51)	52(19)	302(56)	240(44)	0.49	0.37
Hypertensives	36(34)	46(43)	25(23)	118(55)	96(45)	0.49	0.37
Chi-square		2.062		0.021			
Probability		0.357		0.885			
Odds ratio(CI)[3]			1.02(0.74-1.41)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Percentages are given in parentheses.
Both groups were in Hardy-weinberg equilibrium (χ^2 = 0.282, df = 1, p>0.05 in normotensives; χ^2 = 1.812, df = 1, p>0.05 in hypertensives).

Table 6. Genotype and allele frequencies of the A1166C polymorphism in the AT_1R gene between normotensives and hypertensives

	Genotype No. (%)			Allele No. (%)		H^1	PIC^2
	AA	AC	CC	A	C		
Normotensives	139(90)	16(10)	0(0)	294(95)	16(5)	0.10	0.09
Hypertensives	96(94)	6(6)	0(0)	198(97)	6(3)	0.06	0.06
Chi-square		1.550		1.480			
Probability		0.213		0.224			
Odds ratio(CI)[3]			0.56(0.21-1.45)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Percentages are given in parentheses.
Both groups were in Hardy-weinberg equilibrium (χ^2 = 0.443, df = 1, p>0.05 in normotensives; χ^2 = 0.107, df = 1, p>0.05 in hypertensives).

genotype was not detected in our subjects. The heterozygosity and PIC values of A1166C polymorphism represented the values of 0.10 and 0.09 for normotensives, and 0.06 and 0.06 for hypertensives, respectively. According to the heterozygosity and PIC values, I/D polymorphism showed very low degree of polymorphism in the both groups. The observed genotype distribution of this polymorphism was in Hardy-Weinberg equilibrium. There were no significant differences in allele or genotype frequencies between two groups.

Discussion

Essential hypertension is a multifactorial disease in which the genetic and environmental factors play an important role. These factors may differ in each racial

or ethnic groups. Thus, the prevalence of essential hypertension differs widely among various populations. The renin-angiotensin system is one of candidates for the implication of the genetic basis of essential hypertension.

AGT is a crucial rate determinant of this pressor system, and regions within or near the AGT gene on chromosome 1q42-43 have been linked to essential hypertension (Jeunemaitre *et al.,* 1992). Additionally, a point mutation of the AGT gene, resulting in an amino acid substitution of threonine for methionine at position 235 (M235T), has been associated with essential hypertension (Caulfield *et al.*, 1994; Hata *et al.*, 1994; Jeunemaiter *et al.*, 1992), although some studies have indicated negative results (Cheung *et al.*, 1998; Rotimi *et al.*, 1994). The present study was unable to detect a significant association of the M235T polymorphism of the AGT gene with hypertension. Although our result could be taken to imply that there is essentially no relation between AGT gene and risk of hypertension in Korean population, some limitations to the present study must be taken into account. First, like the other studies, our study is retrospective and thus provides only an imperfect insight into causal processes. Second, given the high frequency (about 80%) of the T allele in Korean population, attempts to find an association between the putative risk factor and hypertension are inherently constrained by low statistical power. Overall, a full understanding of the genetic contribution to the pathophysiology of essential hypertension requires the evaluation of several different ethnic groups (Cooper and Rotimi, 1994), because the same molecular variant may exhibit very different risk associations in genetically different populations. The 235T allele frequency of Koreans (0.81) was higher than that of Caucasians (0.38~0.40) (Bennett *et al.*, 1993; Fornage *et al.*, 1995), but similar to that of other Asians and Africans (0.76~0.91) (Jeunemaitre *et al.*, 1997; Rotimi *et al.*, 1996).

With respect to T174M polymorphism in the AGT gene, positive (Jeunemaitre *et al.*, 1992) as well as negative associations (Jeunemaitre *et al.*, 1997; Morise *et al.*, 1995; Rotimi *et al.*, 1994) with hypertension have been documented. In our study, there were no significant differences in allele and genotype frequencies between normotensives and hypertensives in Korean subjects. Thus, it is considered that the T174M polymorphism of the AGT gene may not contribute to the ethiology of hypertension in Korean population. The allele distribution of T17M polymorphism was relatively uniform among the populations studied including our result. M allele frequency (0.04~0.12) of this polymorphism was very low in all ethnic groups reported (Chiang *et al.*, 1997a; Jeunemaitre *et al.*, 1997; Rotimi *et al.*, 1996).

By haplotype analysis, the significant linkage disequilibrium between M235T and T174M polymorphic sites was observed. This observation implies that the haplotype constructed by these two polymorphisms decreases the haplotype diversity index (HDI) for family-based linkage analysis, while it did not require the large sample size to perform the association study. Therefore, the association study may be better than linkage analysis to detect the disease susceptibility gene in the case of two polymorphisms in the AGT gene.

The ACE polymorphism may have important clinical relavance. A number of associations of the ACE I/D polymorphism with cardiovascular disease have been recognized (Bohn *et al.,* 1993; Cambien *et al.*, 1992; Cambien *et al.,* 1994; Evans *et al.*, 1994; Friedl *et al.*, 1995; Lindpaintner *et al.*, 1995; Mattu *et al.*, 1995; Morris *et al.*, 1994; Schunkert *et al.*, 1994; Tiret *et al.*, 1993). Also, the presence of D allele may be related to higher level of ACE in the plasma (Alvarez *et al.*, 2000; Bloem *et al.*, 1996; Cambien, 1994; Cambien *et al.*, 1988; Foy *et al.*, 1996; Marre *et al.,* 1994; Nakai *et al.*, 1994; Tiret *et al.*, 1992). On the other hand, the association of the I/D polymorphism with hypertension has been mostly inconclusive (Ashavaid *et al.*, 2000; Dzida *et al.*, 2001; Harrap *et al.*, 1993; Higashimori *et al.*, 1993; Morise *et al.*, 1994; Zee *et al.*, 1992). The allele and genotype frequencies in Korean hypertensives were not significantly different than those of controls. There are some differences among the various ethnic groups in the ACE I/D polymorphism. In general, the frequency of the D allele in Caucasian population is higher than that of the I allele. The I allele frequency has a frequency of approximately 0.47 in Caucasian populations (Cambien *et al.*, 1994; Dzida *et al.*, 2001). In contrast, Barley *et al.*, (1994) reported a considerably higher I allele frequency in Polynesian (Samoans) and South African native (Yanomami) populations. In normotensive subjects, the I allele frequency in Korean population was very similar to that in Chinese and Japanese populations (Chiang *et al.*, 1997b; Higashimori *et al.*, 1993). The I allele frequencies of Korean (0.56) and Japanese (0.59) populations had the intermediate

values among Caucasian (0.47), Samoan (0.91) and Yanomami (0.85) populations. Thus, D allele frequency of Caucasian populations were higher than that of Asian population.

Angiotensin II receptors, which mediate the vasoconstrictive and salt-conserving actions of the renin-angiotensin system, also represent interesting candidate genes for essential hypertension. Two subtypes of cell surface receptors have been identified (AT_1 and AT_2) by ligand binding studies (Chiu *et al.*, 1989). Examination of several polymorphisms in the AT_1R gene has detect the Afl II RFLP by A·C transversion in the 3'-untranslated region at nucleotide 1166 (Bonnardeaux *et al.*, 1994). In the present study, we assessed whether the AT_1R gene might be involved in human hypertension by directly studying A1166C genotypes in hypertensive subjects. The differences in allele and genotype frequencies between normotensives and hypertensives were not significant. However, the allele distribution of AT_1R A1166C polymorphism showed the significant differences between Koreans and Caucasians. The C allele frequencies of Caucasians (0.20~0.29) (Bonnardeaux *et al.*, 1994; Dzida *et al.*, 2001; Wang *et al.*, 1997) were considerably higher than that of Koreans (0.05). A possible explanation for this difference might be due to genetic drift by founder effect or natural selection.

In conclusion, except for T174M polymorphism in the AGT gene, the allele frequencies of three RAS genes investigated were considerably different among ethnic groups. Therefore, these data suggest that it is of great importance to investigate the association of the polymorphisms in the candidate gene with hypertension in homogeneous ethnic group. Series of our studies are contributing to clarification of genetic basis of multifactorial disease such as essential hypertension.

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