

Association Study Between the C3123A Polymorphism of the Angiotensin II Type 2 Receptor Gene in the Human X Chromosome and Essential Hypertension in Koreans

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한국인에서 Angiotensin II Type 2 Receptor 유전자에 존재하는 C3123A 다형성과 본태성 고혈압과의 관련성에 관한 연구

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요 약

Renin-angiotensin system (RAS)은 혈압 조절에 중요한 역할을 수행하는 생리적 조절계로써, 이 system을 구성하는 유전자들의 이상은 본태성 고혈압의 발병과 유의하게 관련된 것으로 알려졌다. RAS의 주요한 구성 성분인 angiotensin II는 2 종류의 수용체인 angiotensin II type 1 receptor (AT₁R)와 angiotensin II type 2 receptor (AT₂R)에 의해 그 효과가 매개되기 때문에, 이 수용체를 암호하는 유전자는 본태성 고혈압의 유력한 후보 유전자라고 볼 수 있다. 현재까지의 연구에 의하면, AT₁R 유전자에 존재하는 유전적 변이와 본태성 고혈압과의 관련성에 관해서는 많은 보고들이 있었지만, AT₂R 유전자에 존재하는 유전적 변이가 본태성 고혈압에 유의한 효과를 나타내는 지에 관해서는 이렇다할 연구 성과가 별로 없는 실정이다. 이에 본 연구에서는 한국인 집단을 대상으로 하여, AT₂R 유전자에 존재하는 C3123A 다형성이 한국인 집단에서 본태성 고혈압과 유의한 관련성이 있는지를 분석하였다. 이 유전자는 인간의 X 염색체에 존재하기 때문에, 여성인 경우에는 CC, CA 및 AA로 이루어진 3 유전자형이 존재하지만, 남성인 경우에는 C와 A로 이루어진 2종류의 대립 유전자로 구성되어 있기 때문에, 본 연구에서는 남성과 여성을 개별적으로 나누어서 분석하였다. 연구 결과, AT₂R 유전자에 존재하는 C3123A 다형성은 남녀 모두에서 본태성 고혈압과 유의한 관련성을 나타내지 않았다($P>0.05$). 그렇지만, 이 다형성에 대한 대립 유전자 빈도를 서양인 집단과 비교했을 경우에는, 한국인을 대상으로 한 본 연구에서 A 대립 유전자 빈도가 0.33인 반면에 서양인 집단은 그 빈도가 0.43~0.48로 한국인 집단보다 높은 값을 나타내었다. 따라서, AT₂R 유전자에 존재하는 C3123A 다형성과 본태성 고혈압과의 관련성에 대해서는 한국인과 유전적 배경이 다른서양인 집단을 대상으로 한 추사가 필요할 것으로 사료된다.

Key words : essential hypertension, AT₂R and Korean population

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INTRODUCTION

Essential hypertension is considered as a polygenic and multifactorial disease in which blood pressure is harmfully high without overt cause (Kang *et al.*, 2000). Multiple 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) (Kang *et al.*, 2002).

The RAS has an important role in blood pressure regulation (Tewksbury, 1983; Inagami, 1994), 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 (Zhu *et al.*, 2003).

The component genes of RAS include those encoding for renin, angiotensinogen (AGT), angiotensin I converting enzyme (ACE), angiotensin II type 1 receptor (AT₁R) and angiotensin II type 2 receptor (AT₂R). 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 kidney. The cellular effects of angiotensin II are mediated by two structurally distinct receptor subtypes, AT₁R and AT₂R (Inagami *et al.*, 1994). The AT₁R mediates almost all the known effects of angiotensin II in human (Timmermans *et al.*, 1993), while the role of AT₂R in vascular biology remains to be defined.

The gene coding the AT₂R has been cloned (Tsuzuki *et al.*, 1994) and localized in the human chromosome X (Koike *et al.*, 1994). It spans 5 kb, and is composed of 3 exons and 2 introns (Martin and Elton, 1995). A C3123A polymorphism located in 3'-untranslated region (UTR) of this gene has been described by Katsuya *et al.*, (1997), and known to be associated with the disease conditions such as premature ovarian failure syndrome (Katsuya *et al.*, 1997), cardiac

hypertrophy in women with hypertrophic cardiomyopathy (Deinum *et al.*, 2001) and cardiovascular risk in hypertension (Jones *et al.*, 2003).

Although the relationships between the polymorphisms of RAS genes and essential hypertension have been extensively studied (Zhu *et al.*, 2003), there are few reports of the association between the human AT₂R gene and essential hypertension. We therefore conducted a case-control study of AT₂R gene polymorphism in essential hypertension.

MATERIALS AND METHODS

1. Study subjects

A total of 218 individuals (116 males and 92 females) were selected from the outpatients of Dept. of Clinical Pathology, DongIn Clinic, Seoul, Korea. Essential hypertension was diagnosed with systolic blood pressure (SBP) > 140 mmHg or diastolic blood pressure (DBP) > 90 mmHg and/or the need for antihypertensive pharmacotherapy. Subjects with secondary hypertension were excluded from this study. Clinical characteristics of each group are described in Table 1.

2. Determination of clinical phenotypes

Blood samples were obtained in EDTA tubes from individuals who had been fasting for 12~16 hr. The body mass index (BMI) value was calculated by the body weight (kg) divided by the square of the height (m²). Levels of serum triglyceride (TG), total cholesterol (TC) and glucose were measured by enzymatic colorimetry methods with commercial kit (Boehringer Mannheim, Germany) and Hitachi 7150 automatic chemistry analyzer. Serum HDL-cholesterol level was determined by measuring cholesterol in the supernatant after precipitation of the plasma with MgCl₂ and dextran sulfate, with a Gilford Impact 400E automated analyzer with reagents and calibrators from Boehringer Mannheim. Serum lipoprotein (a) (LP (a)) level was measured by the immunoprecipitation method (SPQ Test System, INCSTAR

Table 1. Clinical characteristics of the study subjects

Variables	Mean ± SD ¹ (Number)		Probability
	Normotensives	Hypertensives	
Age (year)	55.6 ± 9.2 (97)	61.9 ± 12.3 (104)	< 0.05
SBP (mmHg) ²	119.1 ± 9.2 (22)	154.3 ± 19.6 (27)	< 0.05
DBP (mmHg) ³	75.9 ± 6.9 (22)	93.7 ± 16.6 (27)	< 0.05
BMI (kg/m ²) ⁴	23.4 ± 2.5 (97)	24.2 ± 2.8 (99)	< 0.05
TG (mg/dL) ⁵	123.5 ± 86.0 (76)	137.6 ± 68.2 (59)	NS ¹¹
TC (mg/dL) ⁶	149.6 ± 38.7 (76)	153.4 ± 33.0 (59)	NS
LDL-chol (mg/dL) ⁷	96.5 ± 38.6 (76)	101.9 ± 31.9 (59)	NS
HDL-chol (mg/dL) ⁸	28.2 ± 10.0 (76)	24.0 ± 10.0 (59)	< 0.05
Lp(a) (mg/dL) ⁹	14.8 ± 11.4 (88)	17.9 ± 11.8 (49)	NS
ApoAI (mg/dL) ¹⁰	71.0 ± 22.2 (12)	109.1 ± 27.4 (23)	< 0.05
Glucose (mg/dL)	94.9 ± 82.7 (60)	77.0 ± 48.3 (24)	NS
Male No./female No.	48/49	68/43	NS

Abbreviations: ¹standard deviation, ²systolic blood pressure, ³diastolic blood pressure, ⁴body mass index, ⁵triglyceride, ⁶total cholesterol, ⁷low density lipoprotein cholesterol, ⁸high density lipoprotein cholesterol, ⁹lipoprotein (a), ¹⁰apolipoproteinAI and ¹¹not significant.

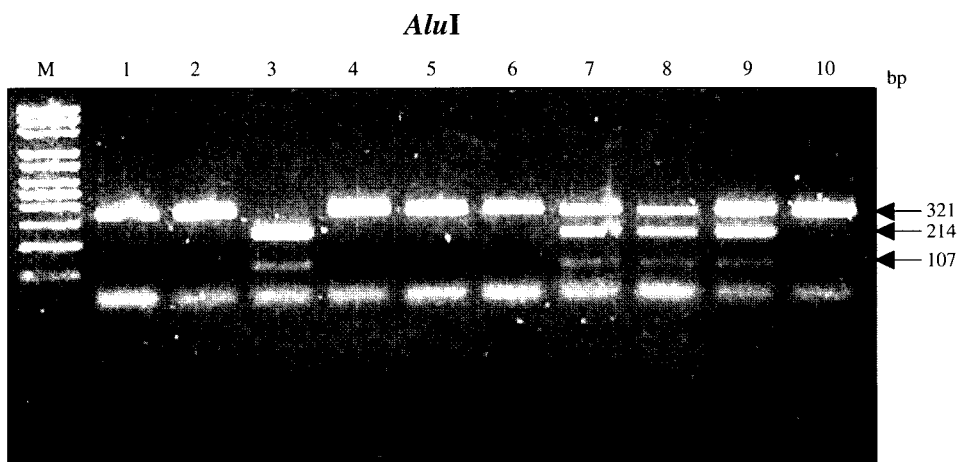


Fig. 1. An *AluI* RFLP of the AT₂R gene in the female subjects. Lane M, molecular size marker; lane 1, 2, 4~6 and 10, CC genotypes; lane 7~9, CA genotypes; lane 3, AA genotype.

Corporation, Stillwater, Minnesota, USA), and serum apoAI concentration determined by immunoturbidimetric method (COBAS INTEGRA, ROCHE Diagnostics, USA). Serum LDL-cholesterol level was calculated by using the formular of Friedewald *et al.*, (1972).

3. Genotyping

Genomic DNA was extracted from whole blood using Wizard Genomic DNA Isolation kit (Promega,

Co. Ltd., USA). The C3123A polymorphism of AT₂R gene was detected by PCR followed by *AluI* (New England Biolabs, Inc., USA) restriction enzyme digestion. Briefly, total 50 µL of the reaction mixture contained 200 ng of genomic DNA, 10 pmol of each primer, 200 µM of each dNTP, and buffers recommended by the manufacturer. The sequences of the primer for the C3123A polymorphism studied were: 5'-GGATTCAGATTTCTCTTTGAA-3' and 5'-GCATAGGAGTATGATTTAATC-3' (Katsuya

et al., 1997). Amplification was carried out with Perkin-Elmer 9700 automated thermal cycler (Perkin-Elmer, ABI, USA); one cycle at 94°C for 3 min, 40 cycles at 94°C for 45 sec, at 53°C for 1 min and at 72°C for 2 min with a final cycle at 72°C for 10 min. Final PCR product was digested with the restriction enzyme, *AluI*, electrophoresed in 2% agarose gel (SeaKem LE agarose, FMC BioProducts, USA) and visualized by ethidium bromide staining. The size of PCR products after *AluI* digestion was 321 bp for the C allele, and a set of 214 bp and 107 bp for the A allele, which were clearly resolved on 2% agarose gel (Fig. 1).

4. Statistical analysis

Allele frequencies were calculated by the gene counting method. The heterozygosity and polymorphism information content (PIC) values were calculated by the methods of Bostein *et al.* (1980). The significance of differences in genotype and allele frequencies between populations was estimated by χ^2 -independence test. Using a student's *t*-test or one-way ANOVA test performed the comparisons of the variables across the clinical phenotypes. Statistical significance was set at the $P=0.05$ level. All statistical analysis was performed using the computer pro-

gram of SPSS for windows (version 11).

RESULTS AND DISCUSSION

The objective of present study was to estimate the genotype and allele distributions of the AT₂R/C3123A polymorphism in Korean normotensives and essential hypertensives, respectively. Table 2 shows the genotype and allele distributions for the AT₂R/C3123A polymorphism in the both groups, respectively. Because AT₂R gene is located within the human X chromosome (Koike *et al.*, 1994), Hardy-Weinberg equilibrium and genotype distribution could be tested only in female group. In female group, observed genotype distribution was in Hardy-Weinberg equilibrium, and the genotype frequencies of CC, CA and AA were 42.8, 38.8 and 18.4% in normotensives, and 50.0, 41.2 and 8.8% in essential hypertensives, respectively. There were no significant differences in genotype distribution between two groups in female group ($P>0.05$). In male group, Frequencies of the A allele were about 0.27 for normotensives and about 0.26 for essential hypertensives, respectively. Like female group, there was no statistically significant difference between normotensives and essential hypertensives in allele frequencies

Table 2. Genotype and allele frequencies of the C3123A polymorphism in the 3'-UTR of the AT₂R gene between normotensives and hypertensives

	Genotype No. (%)			Allele No. (%)		H ¹	PIC ²
	CC	CT	TT	C	T		
Female							
Normotensive	21 (42.8)	19 (38.8)	9 (18.4)	61 (62.2)	37 (37.8)	0.4700	0.3596
Hypertensive	34 (50.0)	28 (41.2)	6 (8.8)	96 (70.6)	40 (29.4)	0.4152	0.3290
Chi-square		2.3732			1.4378		
Probability		0.3053			0.2305		
Male							
Normotensive	-	-	-	35 (72.9)	23 (27.1)	-	-
Hypertensive	-	-	-	32 (74.4)	11 (25.6)	-	-
Chi-square		-			1.6053		
Probability		-			0.2052		

¹Heterozygosity was calculated as $H = 1 - \sum p_i^2$ (p_i : allele frequency). ²Polymorphism Information Content was calculated as $PIC = 1 - \sum p_i^2 - \sum \sum 2p_i^2 p_j^2$ (p_i : allele frequency). Frequency is given as a percentage in parenthesis. Observed genotype distributions of female group were in Hardy-Weinberg equilibrium (For normotensives, $\chi^2 = 1.5010$, $df = 1$, $P = 0.2205$; For essential hypertensives, $\chi^2 = 0.0050$, $df = 1$, $P = 0.9452$).

Table 3. The comparison of the clinical characteristics according to the genotypes in the C3123A polymorphism of the AT₂R gene in female subjects

Variables	Mean \pm SD ¹ (Number)		
	CC (No.)	CA (No.)	AA (No.)
Age (year)	61.6 \pm 11.4 (52)	62.8 \pm 10.2 (47)	60.7 \pm 10.5 (15)
SBP (mmHg) ²	139.9 \pm 32.0 (13)	128.2 \pm 20.0 (10)	120.0 \pm 0.0 (1)
DBP (mmHg) ³	86.2 \pm 24.5 (13)	79.3 \pm 11.0 (10)	75.0 \pm 0.0 (1)
BMI (kg/m ²) ⁴	23.7 \pm 3.1 (48)	23.7 \pm 3.1 (45)	22.7 \pm 2.0 (13)
TG (mg/dL) ⁵	134.8 \pm 67.3 (32)	135.8 \pm 72.1 (33)	106.6 \pm 32.9 (11)
TC (mg/dL) ⁶	157.3 \pm 29.4 (32)	150.2 \pm 29.6 (33)	170.8 \pm 30.5 (11)
LDL-chol (mg/dL) ⁷	104.6 \pm 31.5 (32)	98.7 \pm 26.5 (33)	120.1 \pm 27.4 (11)
HDL-chol (mg/dL) ⁸	25.7 \pm 7.4 (32)	24.3 \pm 8.6 (33)	29.4 \pm 11.2 (11)
Lp(a) (mg/dL) ⁹	18.4 \pm 12.7 (32)	15.7 \pm 12.4 (34)	18.2 \pm 9.0 (11)
ApoAI (mg/dL) ¹⁰	103.6 \pm 35.1 (13)	110.8 \pm 26.4 (7)	77.5 \pm 14.8 (2)
Glucose (mg/dL)	129.3 \pm 103.0 (15)	98.1 \pm 56.9 (16)	72.4 \pm 55.7 (7)

Abbreviations: ¹standard deviation, ²systolic blood pressure, ³diastolic blood pressure, ⁴body mass index, ⁵triglyceride, ⁶total cholesterol, ⁷low density lipoprotein cholesterol, ⁸high density lipoprotein cholesterol, ⁹lipoprotein (a) and ¹⁰apolipoproteinAI.

of male group ($P > 0.05$). In the light of the results from association studies, it is likely that AT₂R/C3123A polymorphism is useful as a genetic marker to explain the development or sexual dimorphism of essential hypertension in Koreans.

Table 3 and 4 display the comparison of clinical phenotypes across the AT₂R/C3123A polymorphism in female and male groups, respectively. This polymorphism was not significantly associated with any cardiovascular risk factors in the both groups, respectively ($P > 0.05$). Therefore, the results from our one-way ANOVA tests suggest that this polymorphism is not one of the genetic component for cardiovascular risk.

The allele frequencies for AT₂R/C3123A polymorphism in different populations are displayed in Table 5. As seen in Table 5, Korean population has much lower an allele frequency (0.33) than Caucasian populations ranging from 0.43~0.48 (Katsuya *et al.*, 1997; Deinum *et al.*, 2001; Jones *et al.*, 2003). This discrepancy in the allele frequency may be explained by differences in genetic background. That is, it might be due to genetic drift by a founder effect or a selective mechanism. Because the racial differences was observed in the allele frequencies of this polymorphism, studies in other racial or ethnic group includ-

Table 4. The comparison of the clinical characteristics according to the alleles in the C3123A polymorphism of the AT₂R gene in male subjects

Variables	Mean \pm SD ¹ (Number)	
	C (No.)	A (No.)
Age (year)	54.4 \pm 11.3 (65)	56.1 \pm 9.8 (22)
SBP (mmHg) ²	138.1 \pm 12.8 (15)	149.5 \pm 25.7 (10)
DBP (mmHg) ³	87.3 \pm 11.8 (15)	90.2 \pm 10.4 (10)
BMI (kg/m ²) ⁴	24.0 \pm 2.2 (66)	24.5 \pm 2.3 (24)
TG (mg/dL) ⁵	126.9 \pm 94.9 (46)	130.7 \pm 91.1 (13)
TC (mg/dL) ⁶	147.3 \pm 42.9 (46)	137.2 \pm 41.6 (13)
LDL-chol (mg/dL) ⁷	94.8 \pm 41.2 (46)	81.6 \pm 44.8 (13)
HDL-chol (mg/dL) ⁸	27.7 \pm 9.8 (46)	25.6 \pm 10.7 (13)
Lp(a) (mg/dL) ⁹	13.1 \pm 9.9 (45)	17.9 \pm 13.5 (15)
ApoAI (mg/dL) ¹⁰	91.2 \pm 25.9 (10)	57.6 \pm 16.1 (3)
Glucose (mg/dL)	72.2 \pm 65.5 (33)	87.7 \pm 77.9 (13)

Abbreviations: ¹standard deviation, ²systolic blood pressure, ³diastolic blood pressure, ⁴body mass index, ⁵triglyceride, ⁶total cholesterol, ⁷low density lipoprotein cholesterol, ⁸high density lipoprotein cholesterol, ⁹lipoprotein (a) and ¹⁰apolipoproteinAI.

ing Caucasians will be great interest in the context of association with essential hypertension.

There are several limitations of our study that must be emphasized. An important consideration is the statistical power when small differences in allele frequencies do not achieve statistical significance. Essential hypertension is a polygenic disorder, and

Table 5. Allele frequencies of AT₂R/C3123A polymorphism in different populations

Population	Chromosome number	Frequency		Reference
		C allele	A allele	
Caucasian				
USA	40	0.52	0.48	Katsuya <i>et al.</i> , 1997
Dutch	143	0.57	0.43	Deinum <i>et al.</i> , 2001
UK	2382	0.55	0.45	Jones <i>et al.</i> , 2003
Mongolian				
Korean	234	0.67	0.33	Present study

many genes with minor effect are likely to be involved in the development of it (Doris, 2002). Thus large samples are required to achieve a strong association. Since the present study employed modest sample size of 117 normotensives and 101 essential hypertensives, the result has shown a relatively low statistical power in achieving statistical significance. Secondly, we did not measure any intermediate phenotypes such as plasma renin and aldosterone level as well as salt sensitivity in this study. Although our data do not suppose a probable role of AT₂R/C3123A polymorphism in the development of essential hypertension, it could not exclude the possibility that there are the significant association between this genetic polymorphism and some subgroup of hypertensive patients such as salt-sensitive or salt-resistant hypertensives.

In conclusion, we could not find any significant association between the AT₂R/C3123A polymorphism and essential hypertension in a group of Korean origin. However since this polymorphisms indicted racial difference in allele frequencies, and our sample size was modest, further studies using larger sample size and other ethnic groups are necessary to elucidate the genetic basis of essential hypertension.

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