Environmental Mutagens & Carcinogens 22-1 : 30-38 (2002)

Association between Genetic Variation in the Human Antithrombin III Gene and Essential Hypertension in Korean Population

Jung Hee Shin[1], Byung Yong Kang[2], Ki Tae Kim[2*], Kang Oh Lee[3] Jae Hyoun Kim[4], Ae Son Om[5], Sang Duk Oh[6] and Chung Choo Lee[1, 7]

[1]*School of Biological Sciences, Seoul National University, Seoul 151-742, Korea*
[2]*Seoulin Bioscience Institute, Seoulin Bioscience, Co., Ltd, Seoul 134-030, Korea*
[3]*Department of Life Science, Sahmyook University, Seoul 139-742, Korea*
[4]*Dept. of Health Science, College of Natural Science, Dongduk Womens University, Seoul 136-714, Korea*
[5]*College of human Ecology, Hanyang University, Seoul 133-791, Korea*
[6]*Hanyang University ,College of Physical Education, Seoul 133-791, Korea*
[7]*Laboratory of Biology, Gachon Medical School, Inchon 405-760, Korea*

(Received January 28, 2002 / Accepted March 10, 2002)

ABSTRACT : **In view of the effect of antithrombin III on essential hypertension, we investigated the 5' untranslated exon 1 length (I/D) polymorphism and intron 5'-*Dde*I RFLP of the human antithrombin III gene in the Korean patients with essential hypertension. There were no significant differences in the allele, genotype and haplotype frequencies of these polymorphisms between normotensive and essential hypertensive subjects. The significant linkage disequilibrium was however, detected between I/D polymorphism and *Dde* I RFLP. The I/D polymorphism was also significantly associated with BMI, total cholesterol (TC) and HDL-cholesterol levels, while *Dde*I polymorphism with age and BMI. Therefore, our results suggest that the significant association between the genetic polymorphisms in the antithrombin III gene and variable cardiovascular risk factors may reflect the potential role of human antithrombin III gene in cardiovascular function.**

Key words : Antithrombin III, Essential hypertension and RFLP

Introduction

Basically, essential hypertension is defined as high blood pressure in which secondary causes such as renovascular disease, renal failure, pheochromocytoma, aldosteronism, or other causes of secondary hypertension or mendelian forms (monogenic) are not present. Essential hypertension accounts for 95% of all cases of hypertension. Also, it affects approximately 20% of the adult population, and is an insidious disease in which afflicted person from myocardial infranction and stroke. Many factors contribute to the development of essential hypertension, including environments, daily stress and genetics. Essential hypertension is a heterogeneous disorder with different patients having different causal factors that lead to high blood pressure. Thus, the main task in essential hypertension research is to explain the genetic causes of the raised blood pressure. Blood pressure has significant genetic components and behaves as a quantitative trait in all species. Among the different strategies that have been put to trial to identify quantitative trait loci involved in such complex clinical phenotypes, association (case-control) studies using candidate genes represent increasingly preferred methods (Lander *et al.*, 1994; Weeks *et al.*, 1995; Risch *et al.*, 1996; Lander, 1996).

Antithrombin III is a serine inhibitor produced by the liver that can bind to and inactivate most of the clotting enzymes. Antithrombin III inhibits the FVIIa-TF complex, FXa, FIXa, FXIa, FXIIa, Kallikrein and plasmin and is a sensitive marker in activation of coagulation. It functions by providing an exposed reactive site that binds to thrombin very stably, by process greatly enhanced by heparin (Lane *et al.*, 1992; Lee *et al.*, 2000; März *et al.*, 2000). The human antithrombin III gene, which comprises seven exons (Old *et al.*, 1993), is located on the chromosome 1q23-25 and encodes a mature secreted polypeptide of 432

*To whom all correspondence should be addressed

amino acids (Millar *et al.*, 1994). Four DNA sequence polymorphisms have been described recently:(1) a insertion/deletion polymorphism from 5' to exon 1, (2) a GTG to GTA polymorphism in codon 295, (3) a site polymorphism (*Pst*I) of exon 4 and (4) a site polymorphism (*Dde*I) of intron 5 (Prochownik *et al.*, 1983; Old *et al.*, 1992; Bock *et al.*, 1983; Daly *et al.*, 1990).

In view of the important role of antithrombin III thrombosis and the extensive range of mutations causing these changes, it is important to investigate the association between essential hypertension and common polymorphisms of the antithrombin gene. The present study examines a possible role of the two polymorphisms, its genetic polymorphisms, i.e. the insertion/deletion polymorphism on 5'-untranslated region and *Dde*I RFLP on intron 5, in the antithrombin III gene in Korean patients with essential hypertension and normal controls.

Materials and Methods

Study subjects

One hundred ninty eight unrelated individuals were chosen from the Seoul Hygiene Hospital, Seoul, Korea. Among them, 99 subjects were patients with essential hypertension. The patients were classified as having essential hypertension if they had systolic blood pressures above 140 mmHg and diastolic blood pressure above 90 mmHg on at least three separate occasions, and had no clinical signs, symptoms and laboratory findings suggestive of secondary hypertension. The others, a randomly selected normal population (99 individuals) was analysed as the control groups (blood pressure value, <140/90 mmHg). Clinical details for these groups were summarized in Table 1. There was statistically significant difference in age distribution, plasma HDL-cholesterol level and plasma apolipoprotein AI (ApoAI) concentration between normotensives and essential hypertensives, respectively.

Determination of plasma lipid levels

Blood samples were obtained in EDTA tubes from individuals who had been fasting for 12-16 hr. Concentration of plasma total cholesterol (TC) and triglyceride were measured by enzymatic colorimetry methods with a commercial kit (Boehringer Mannheim, Germany) and a chemistry analyzer. The day-to-day CVs were 2.5% for TC and 5.1% for triglyceride. HDL-cholesterol was determined by measuring cholesterol in the supernatant after precipitation of the plasma with $MgCl_2$ and dextran sulfate, with a Gilford Impact 400E automated analyzer with reagents and calibrators from Boehringer Mannheim. The day-to-day CV of HDL-cholesterol was 4.0%. Plasma LDL-cholesterol level was calculated by using the formular of Freidwald *et al.*, (1972). Plasma lipoprotein(a) (LP(a)) level was measured by the immunoprecipitation method (SPQ Test System, INCSTAR Corporation, Stillwater, Minnesota, USA) and plasma ApoAI concentration was determied by immunoturbidimetric method (COBAS INTEGRA, ROCHE Diagnostics, USA).

DNA analysis

Blood samples 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

Table 1. Clinical Details of the Study Subjects

Variables	Subjects		P[1]
	Normotensives	Hypertensives	
[a]Age (year)	**56.4 ± 9.5[9]**	**62.9 ± 11.9**	<0.05
BMI (Kg/m^2)[2]	23.4 ± 2.4	24.0 ± 2.5	
TG (mg/dl)[3]	123.0 ± 83.2	134.2 ± 65.5	
TC (mg/dl)[4]	149.5 ± 38.3	152.2 ± 32.1	
LDL-chol (mg/dl)[5]	96.4 ± 38.5	100.4 ± 31.4	
[b]HDL-chol (mg/dl)[6]	**28.3 ± 9.5**	**24.9 ± 9.2**	<0.05
Lp(a) (mg/dl)[7]	14.8 ± 10.9	17.9 ± 12.4	
[c]ApoAI (mg/dl)[8]	**70.8 ± 20.5**	**114.3 ± 31.0**	<0.05

[1]Probability of significant difference between normotensives and essential hypertensive groups.
[2]Body Mass Index, [3]Triglyceride, [4]Total cholesterol, [5]LDL-cholesterol, [6]HDL-cholesterol, [7]Lipoprotein(a) and [8]Apolipoprotein AI. [9]Values are means ± standard deviations.
[a]Statistically significant difference (Student's t-test, P<0.001).
[b]Statistically significant difference (Student's t-test, P=0.029).
[c]Statistically significant difference (Student's t-test, P<0.001).

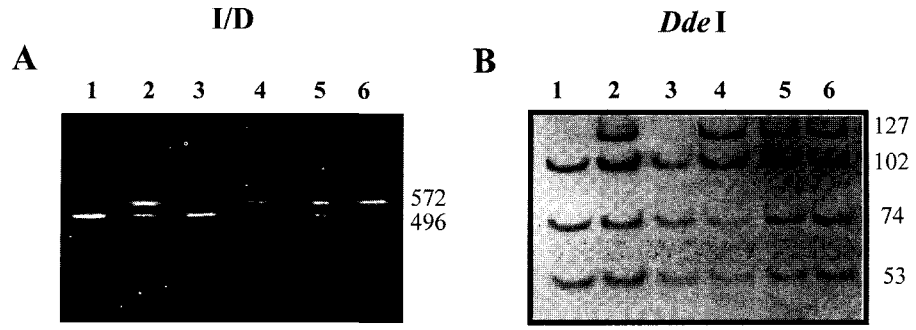

Fig. 1. Two polymorphic patterns of ATIII gene. A) I/D polymorphic patterns. Lane 1 and 3, DD genotypes; lane 2, 4 and 5, ID genotypes; lane 6, II genotypes. B) *Dde* I RFLP patterns. Lane 1 and 3, D2/D2 genotypes; lane 2, 4~6, D1/D2 genotypes.

of Sambrook *et al.*, (1989) with slight modification. Polymerase Chain Reaction (PCR) techniques were used for insertion/deletion (I/D) polymorphism of 5'-untranslated exon 1 and *Dde* I PFLP of antithrombin III gene (Liu *et al.*, 1995). Briefly, total 50 μl of the reaction mixture contained 200-400 ng of genomic DNA, 100 ng of each primer, 200 μl of each dNTP, and buffers recommended by the manufacturer. The sequences of the primers for two polymorphisms studied were:

(a) I/D polymorphism; sense, 5'-CCACAGGTGTAA CATTGTGT-3', and nonsense, 5'-GAGATAGTGTGAT CTGAGGC-3'; (b) *Dde*I RFLP; sense 5'-GAATTCCC ATCT GATTGAAGCCA-3', and nonsense, 5'-TGCAT GCCTTAACACTGGAAACAGGC-3'. Amplification was carried out with automated thermocycler: one cycle at 94°C for 7 min, 30 cycles at 55°C for 30s, at 72°C for 30s and at 94°C for 90s, and a final polymerization at 72°C for 10 min. Ten μl of PCR product of the intron 5 polymorphism was restriction-digested overnight with 5 units of *Dde*I at 37°C. Amplified product for 5'-untranslated exon 1 and digested product for intron 5 locus were size-fractionated after 2% agarose gel electrophoresis in TBE buffer for 40 min along with molecular markers. Ethidium bromide was incorporated into the gel. The gels were directly photographed on an UV transilluminator and genotyped. The 76 bp dimorphism of the I/D polymorphism produces a short (allele D) and a long (allele I) fragment (Fig. 1A). The amplified intron 5 product is 229 bp, from which a fragment of 127 bp is produced in the absence of *Dde*I site (allele D1) while fragments of 53 and 74 bp are produced in the presence of the *Dde*I site (allele D2) (Fig. 1B).

Statistical Anaysis

Allelic frequencies were estimated by the gene counting method. Deviation in genotype distribution from that expected for Hardy-Weinberg equilibrium was estimated by χ^2-fitness test. The heterozygosity and polymorphism information content (PIC) was estimated by the methods of Bostein *et al.*, (1980). The significance of differences in allele frequencies between populations was also estimated by χ^2-independence test. The relative risk of essential hypertension associated with allelic variation was expressed in terms of an odds ratio (OR) with 95% confidence interval (CI). The Student's t-test or one-way ANOVA test was performed to compare the mean levels of biochemical parameters among different genotypes. Maximum likelihood estimates (MLE) of haplotypes were obtained by iterative two-steps algorithm called expectation-maximatization (EM). A Monte-Carlo simulation using the Clump (version 1.6) program was performed to test the statistical significance of the association between the haplotype distribution and essential hypertension (Sham and Curtis, 1995). The degree of nonrandom association was determined by calculation of the delta (Δ) (Hill and Robertson, 1968) and D' (Lewontin, 1964) between the polymorphic sites in the antithrombin III gene. To test the significance of linkage disequilibrium, $n\Delta^2$ value was used as the χ^2 distribution with 1 df (degree of freedom). Statistical significance was accepted at the $P<0.05$. All statistical analyses were performed by the computer program of SPSSWIN (version 8.0).

Results

Genotype distribution

In the present study, we attempted to clarify the distribution of two polymorphisms in the antithrombin III gene in Korean population. The data in Table 2 and 3 present the gene frequencies, the values of heterozygosity

Table 2. Genotype and allele frequencies of the insertion/deletion polymorphism in the antithrombin III gene between normotensives and essential hypertensives

	Genotype No. (%)			Allele No. (%)		H[1]	PIC[2]
	I I	I D	DD	I	D		
Normotensives	17(21)	47(57)	19(23)	81(49)	85(51)	0.4997	0.3749
Hypertensives	29(30)	53(55)	15(16)	111(57)	83(43)	0.4896	0.3697
Chi-square		2.890		2.5490			
Probability		0.2360		0.1100			
Odds ratio(CI)[3]			1.40(0.93-2.13)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.
There were no statistically significant differences in genotype and allele frewuencies between normotensives and essential hypertensive subjects.

Table 3. Genotype and allele frequencies of the *Dde* I RFLP in the Intron 5 of the Antithrombin III gene between normotensives and essential hypertensives

	Genotype No. (%)			Allele No. (%)		**H[1]**	**PIC[2]**
	D1/D1	D1/D2	D2/D2	D1	D2		
Normotensives[4]	0(0)	42(42)	57(58)	42(21)	156(79)	0.3343	0.2784
Hypertensives[4]	0(0)	52(53)	47(48)	52(26)	146(74)	0.3873	0.3123
Chi-square		2.0250		1.3950			
Probability		0.1550		0.2380			
Odds ratio(CI)[3]			1.32(0.83-2.11)				

[1]Heterozygosity, [2]Polymorphism Information Content, [3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.
There were no statistically significant differences in genotype and allele frewuencies between normotensives and essential hypertensive subjects.
[4]The observed genotype distribution was significantly deviated from Hardy-weinberg equilibrium ($P<0.05$).

and PIC for 5' untranslated exon 1 I/D and intron 5'-*Dde*I polymorphisms of the antithrombin III gene in Korean normotensive and essential hypertensive groups, respectively. In the case of I/D polymorphism, the genotype and allele frequencies were not significantly different between normotensives and essential hypertensives. The observed genotype distributions of this polymorphism were not significantly different from those expected for Hardy-Weinberg equilibrium. The frequencies of II, ID and DD genotypes were 21, 55, and 24%, respectively, in normotensives, and 30, 55, and 16%, respectively, in essential hypertensives. The heterozygosity and PIC values of I/D polymorphism represented values of 0.4993, and 0.3747, respectively, for normotensives, and 0.4896, and 0.3697, respectively, for essential hypertensives. According to the heterozygosity and PIC values, I/D polymorphism showed a reasonably high degree of polymorphism in the both groups.

For *Dde*I RFLP, there were no significant differences in allele and genotype frequencies between the two groups. The observed genotype distribution of this RFLP was, however, significantly deviated from Hardy-Weinberg equilibrium (χ^2-fitness test, $P<0.05$). The frequencies of D1/D1, D1/D2, and D2/D2 genotypes were 0, 42, and 58%, respectively, in normotensives, and 0, 54, and 47%, respectively, in essential hypertensives, respectively. The heterozygosity and PIC values of I/D polymorphism represented values of 0.3343 and 0.2784 for normotensives, and 0.3937 and 0.3150 for essential hypertensives, respectively. According to the heterozygosity and PIC values, *Dde*I RFLP showed a relatively low degree of polymorphism in the both groups compared with the I/D polymorphism.

Association with biochemical parameters

Table 4 presents the comparision of anthropometric data and intermediate phenotypes across I/D polymorphism. The I/D polymorphism was significantly associated with BMI, TC and HDL-cholesterol level (one-way ANOVA test, $P<0.05$). The comparison of the anthropometric data and intermediate phenotypes across *Dde*I RFLP is shown in Table 5. *Dde*I RFLP was significantly associated with

Table 4. The comparison of the anthropometric data and intermediate phenotypes according to antithrombin III insertion/deletion genotypes

Variables	Genotypes		
	II (No.)[8]	ID (No.)	DD (No.)
Age(year)	61.0 ± 10.0(45)	59.5 ± 12.3(98)	58.4 ± 10.5(34)
*BMI(kg/m^2)[1]	**23.3 ± 2.7(42)**	**24.0 ± 2.4(87)**	**22.8 ± 3.4(32)**
Tg(mg/dl)[2]	144.3 ± 80.0(36)	125.2 ± 80.7(75)	109.8 ± 69.4(26)
*TC(mg/dl)[3]	**160.9 ± 33.4(36)**	**142.1 ± 36.5(75)**	**151.5 ± 32.9(26)**
LDL-chol(mg/dl)[4]	104.1 ± 35.0(36)	92.51 ± 36.6(75)	100.0 ± 34.1(26)
*HDL-chol(mg/dl)[5]	**27.9 ± 9.6(36)**	**24.9 ± 10.0(75)**	**29.5 ± 9.1(26)**
Lp(a)[6]	16.2 ± 11.0(38)	15.8 ± 11.1(75)	15.3 ± 13.5(28)
APOA1(mg/dl)[7]	101.6 ± 39.4(10)	101.1 ± 32.7(25)	118.0 ± 35.3 (8)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol, [6]Lipoprotein(a), [7]Apolipoprotein AI and [8]Number. Value are mean ± SD (standard deviation).

*There were the significant differences in BMI, plasma TC and HDL-cholesterol levels between genotypes (For BMI, one-way ANOVA test, P=0.031; for TC, one-way ANOVA test, P=0.029; for HDL-cholesterol level, P=0.044).

Table 5. The comparison of the anthropometric data and intermediate phenotypes according to antithrombin III /*Dde* I genotypes

Variables	Genotypes		
	D1/D1 (No.)[8]	D1/D2 (No.)	D2/D2 (No.)
*Age (year)	**0 ± 0(0)**	**61.3 ± 11.4(92)**	**58.1 ± 10.9(103)**
*BMI (kg/m^2)[1]	**0 ± 0(0)**	**24.1 ± 2.3(84)**	**23.3 ± 2.5(95)**
Tg (mg/dl)[2]	0 ± 0(0)	129.0 ± 63.9(70)	126.8 ± 85.4(82)
TC (mg/dl)[3]	0 ± 0(0)	152.4 ± 38.5(70)	149.2 ± 33.2(82)
LDL-chol (mg/dl)[4]	0 ± 0(0)	98.9 ± 36.8(70)	97.5 ± 34.6(82)
HDL-chol (mg/dl)[5]	0 ± 0(0)	27.0 ± 10.3(70)	26.7 ± 8.8(82)
Lp (a)[6]	0 ± 0(0)	15.4 ± 11.5(73)	16.4 ± 11.7(84)
APOA1 (mg/dl)[7]	0 ± 0(0)	100.7 ± 31.9(24)	103.7 ± 38.6(22)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol, [6]Lipoprotein(a), [7]Apolipoprotein AI and [8]Number. Value are mean ± SD (standard deviation).

* There were the significant differences in age and BMI between genotypes (For age, Students test, P=0.043; for BMI, Students test, P=0.043)

age and BMI (Students t-test, P<0.05).

Haplotype analysis

The haplotype distribution and the linkage disequilibrium statistics reflecting the extent or significance of pair-wise nonrandom associations between the two polymorphic sites are shown in Table 6. There was no statistically significant difference in haplotype frequency between the two groups (Monte-Carlo simulation, $T_2 = 2.1431$, df = 2, P = 0.3426, simulation number = 10,000). However, the significant pair-wise linkage disequilibrium between I/D polymorphism and *Dde*I RFLP in the antithrombin gene was detected in the both groups by χ^2-test.

Table 6. Haplotype frequencies and linkage disequilibrium statistic (D', Δ) between pairs of two DNA polymorphisms in the antithrombin III gene

Haplotypes		Normotensives	Hypertensives
Ins/Del	*Dde* I		
Ins	D1	0.197532	0.265609
Ins	D2	0.296370	0.307308
Del	D1	0.009785	0.000016
Del	D2	0.496313	0.427067
Total chromosomes		164	192
D'		**0.4695**	**–0.5192**
Δ		**0.9069**	**–0.9999**
χ^2		**36.151**	**51.757**
P		**<0.001**	**<0.001**

There was no significant difference in haplotype frequency between normotensives and essential hypertensives (Monte-Carlo simulation, $T_2 = 2.1431$, df = 2, P = 0.3426, simulation number = 10,000).

Discussion

Hypertension is one of the most common disease in civilized contries. It is currently seen as a "complex" genetic trait caused by multiple susceptibility genes which are modulated by gene-environment and gene-gene interactions. Also, their etiology is complex with substantial

environmental components. There is a strong indication that multiple genes are implicated. Specific candidate genes have been tested for linkage and association with a blood pressure or the diagnosis of hypertension. Nevertheless, the genetic alterations responsible for inherited "essential" hypertention remain largely unknown, and the success to date in identifying susceptibility genes has been very limited. Depending on the genetic factors of human essential hypertension, it appears that DNA polymorphisms at the candidate genes may play a significant role as useful genetic markers in the association study.

The defect of antithrombin III gene may occur so that quantitative decrease or qualitative abnormality of plasma antithrombin III is accompanied with frequent thrombosis. The critical antithrombin III level to maintain the fluidity of blood is thought to be more than 50% of normal. Thus, the defect of antithrombin III gene may occur to elevate the blood pressure, and elevated blood pressure can predispose to the essential hypertension. Aiming at deciphering the genetic architecture of blood pressure regulation and essential hypertension (Emmerich *et al.*, 1994; Oquma *et al.*, 1992), we determined the distribution of the polymorphisms of antithrombin III gene in Korean normotensive and hypertensive groups.

The present study revealed that there were no significant differences between Korean normotensive subjects and subjects with essential hypertension in allele or genotype frequencies of the I/D polymorphism and *Dde*I RFLP in the antithrombin III gene. Therefore, it is unlikely that these polymorphisms are significantly associated with the etiology of essential hypertension among Koreans.

For the I/D polymorphism, there were statistically significant association in BMI, plasma TC and HDL-cholesterol levels across the genotypes in Korean population. II genotypes represented significantly higher BMI value and plasma TC-level and lower HDL-cholesterol level than DD genotypes. BMI and plasma TC level are considered the cardiovascular risk factors (Schulte *et al.*, 2001; Froom *et al.*, 2000), while plasma HDL-cholesterol level is known to a protective factor for various cardiovascular diseases (Fuh *et al.*, 1987; Jayakumari *et al.*, 1993). Thus, II genotype of I/D polymorphism in the antithrombin III gene may be one of the genetic components in the pathogenesis of various cardiovascular diseases, although this polymorphism did not show the significant association with essential hypertension in Korean population.

In the case of *Dde*I RFLP, the observed genotype distribution was not in Hardy-Weinberg equlibrium. Founder effect might be operating in *Dde*I RFLP of the antithrombin III gene in Korean population. D1D2 heterozygote is significanltly higher in age and BMI values than D2D2 homozygote. Because age is closely related with obesity index (Luhrmann *et al.*, 2001), this finding may imply that older subjects with D1D2 heterozygote rather than D2D2 homozygote, are included in the present study.

By pair-wise haplotype analysis, the significant linkage

Table 7. Comparison of allele frequencies of I/D polymorphism at the antithrombin III gene from various ethnic groups

Populations	Sample number	Allele frequencies		P[1]	Reference
		I	D		
Caucasian	41	0.25	0.75	P<0.05[2]	Liu *et al.*, 1995
Indian	221	0.35	0.65	P<0.05	Liu *et al.*, 1995
Chinese	251	0.63	0.37	P<0.05	Liu *et al.*, 1995
Korean	83	0.49	0.51		Present study

[1]Probability value by χ^2-fitness test.
[2]The significant difference in allele frequencies among ethnic groups studied.

Table 8. Comparison of allele frequencies of *Dde* I RFLP at the antithrombin III gene from various ethnic groups

Populations	Sample number	Allele frequencies		P[1]	Reference
		D1	D2		
Caucasian	41	0.17	0.83	NS[2]	Daly *et al.*, 1990
Indian	221	0.16	0.84	NS	Daly *et al.*, 1990
Chinese	251	0.23	0.77	NS	Daly *et al.*, 1990
Korean	99	0.21	0.79		Present study

[1]Probability value by χ^2-fitness test.
[2]The significant difference in allele frequencies among ethnic groups studied.

disequilibrium between two polymorphic sites was detected. This finding suggests that the haplotype occurred by two polymorphisms decreases the information content for linkage analysis, while it did not require the large sample size to perform the association study. Therefore, association study may be better than linkage analysis to discover the disease susceptibility gene in the case of two polymorphisms in the antithrombin III gene.

The allele distribution of I/D polymorphism in the antithrombin III gene was different among various ethnic groups (Table 7). The reason for this phenomenon may be explained by different genetic background of populations or various sample sizes. It seems to be important for carefully designed studies to minimize the ethnic heterogeneity of the case and control populations. In contrast, the allele distribution of *Dde*I RFLP in the antithrombin III gene was relatively uniform among the ethnic groups studied (Table 8). It is likely that this RFLP arose before the divergence of man into different racial groups. Because of the possible absence of selective forces at this locus, neither allele may have progressed to fixation.

Within the limitations of the present study of 83 normotensive and 97 essential hypertensive subjects, we were unable to demonstrate the association between the alleles of I/D polymorphism nor those of *Dde*I RFLP in the antithrombin gene, and the occurrence of essential hypertension. It could be however, argued that the relatively small number of subjects may give a low probability of detecting a small effect of the polymorphism (a small gene effect is expected in the case of a disease as complex as essential hypertension). Moreover, these types of study design are prone to type II error (that is, failing to reject the null hypothesis that there is no differences in allelic distributions between the two groups when it is false). The lack of association indicates that the particular DNA changes causing the polymorphisms are not responsible for essential hypertension, and that polymorphisms are not in linkage disequilibrium with other loci that are responsible. Negative finding generated by retrospective case-control studies can in no way be advocated to rule out the gene effects in clinical phenotype under investigation. This is why we cannot exclude the possibility that antithrombin III gene is somehow involved in the pathogenesis of essential hypertension. It is of interest to examine the possible association of these two linked loci of the antithrombin III gene with the expression of the gene product or with structural changes/deficiencies in the antithrombin III. Also, as expected, taking into account the complexity of the genetic component of essential hypertension, association studies using more candidate genes is required to establish the genetic basis of essential hypertension in Korean population.

Acknowledgement

The human blood samples were kindly supplied by Dr. Seung Hee Cho, Clinical Pathology, Seoul Hygiene Hospital, Seoul, Korea.

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