Apolipoprotein(a) Gene Polymorphism in the Korean Population: Is There Any Relevance to Essential Hypertension?

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Key Words

Apolipoprotein(a) · Essential hypertension · Polymorphism

Abstract

Objective: To investigate the role of the apolipoprotein(a) [*Apo(a)*] gene in predicting essential hypertension in the Korean population, we undertook a case-control study using the TTTTA repeat and the Met/Thr polymorphisms of the *Apo(a)* gene. **Methods:** The study subjects consisted of 74 essential hypertensives and 211 normotensive individuals. For polymorphism analysis, DNA was amplified by polymerase chain reaction (PCR). In the case of the Met/Thr polymorphism, amplified PCR products were digested with the restriction enzyme *KspI*. Genotyping was performed by a 10% nondenaturing polyacrylamide gel. **Results:** There were no significant differences in the allele and genotype frequencies of these polymorphisms between normotensive and essential hypertensive subjects ($p > 0.05$). Furthermore, levels of lipoprotein(a) in the plasma were not significantly associated with the different genotypes of the

Apo(a) gene in either group ($p > 0.05$). **Conclusion:** The polymorphisms of these two loci on the *Apo(a)* gene are unlikely to contribute to the etiology of essential hypertension in the Korean population.

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Introduction

Lipoprotein(a) [Lp(a)] is a plasma lipoprotein that includes a low-density lipoprotein component and a large glycoprotein, apolipoprotein(a) [apo(a)] [1]. Lp(a) was reported for the first time in 1963 by Berg [2], and the physiological role of this lipoprotein is still unclear. According to many epidemiological studies, high levels of plasma Lp(a) correlate with diverse cardiovascular diseases [3–7]. Therefore, this lipoprotein has been considered an independent risk factor for cardiovascular diseases. Furthermore, plasma Lp(a) levels are largely genetically determined, and over 90% of interindividual variation in this lipoprotein is due to sequence variation in the *Apo(a)* gene [8]. Among the remaining 10%, it is estimated that variation of the kringle-4 repeat number in the

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Apo(a) gene may account for about 40–70% of interindividual differences in plasma Lp(a) levels [9]. In addition, the (TTTTA)_n repeat polymorphism of the *Apo(a)* gene is likely to be one of the factors associated with the plasma Lp(a) level, and may account for 10–14% of interindividual differences in plasma Lp(a) levels in the Caucasian population [10]. In the case of the Asian population, Park et al. [11] reported that the (TTTTA)_n repeat polymorphism has an effect on the serum Lp(a) level in the general Japanese population. However, these are only limited reports regarding the role of the *Apo(a)* gene in essential hypertension. Therefore, we investigated the relationship between the genetic polymorphisms of the *Apo(a)* gene and essential hypertension in the Korean population.

Subjects and Methods

Subjects

We collected 285 blood samples from outpatients of the Seoul Hygiene Hospital, Seoul, Korea. Seventy-four Korean individuals with essential hypertension were identified in this group, with blood pressure above 140/90 mm Hg, and the remaining 211 were normotensives. Of these, there was an equal number of males and females (91 each). The gender information for the remaining 29 persons was unavailable. The age and body mass indices were recorded.

Biochemical Assays

Blood samples were obtained in EDTA tubes from individuals who had been fasting for 12–16 h. The plasma samples were separated within 30 min of collection by centrifugation at 2,000 *g* for 15 min at 4 °C, and aliquots of plasma were stored at –70 °C in deep-freeze. Plasma Lp(a) levels were measured by immunonephelometry within 2 weeks. Unfortunately, owing to a shortage of plasma samples, we could not measure the plasma Lp(a) levels for all samples studied.

Genotyping

Genomic DNA was prepared from the buffy coats of whole blood (5 ml) after lysis of the red blood cells [12]. The polymerase chain reaction (PCR) primer sequences used to detect the (TTTTA)_n repeat polymorphism of the *Apo(a)* gene are 5'-ATTTGCGGAAAGATTGATACTATGC-3' for the forward primer and 5'-CCAAAATCACGTCAGTGCACCTTCAA-3' for the reverse primer [13]. PCR was performed in a final volume of 50 µl (100 ng of genomic DNA, 20 pmol of each primer, 200 µM each of the four dNTPs, 1.5 mM MgCl₂, 50 mM KCl and 10 mM Tris-HCl, pH 8.4, and 2.5 units of *Taq* DNA polymerase). The reactions were denatured at 96 °C for 1 min, annealed at 55 °C for 1 min and extended at 70 °C for 2 min over 30 cycles with a final extension time of 10 min at 72 °C. After PCR amplification, an aliquot (5 µl) of each PCR product was loaded onto a 10% nondenaturing polyacrylamide gel. The band patterns were detected by silver staining.

The PCR primer sequences used to detect the Met/Thr polymorphism of the *Apo(a)* gene are 5'-CGATACAGGCCCTTGGTGT-TACCG-3' for the forward primer and 5'-GCCCTAGGCTTG-

Table 1. Genotype and allele frequencies for the (TTTTA)_n repeat polymorphism of the *Apo(a)* gene in normotensive and essential hypertensive subjects

a Genotype frequencies

Genotype	Normotensive subjects		Hypertensive subjects	
	n	%	n	%
8/5	10	5.2	4	5.9
8/6	1	0.5	0	0.0
8/8	107	56.0	44	64.7
9/5	1	0.5	0	0.0
9/8	58	30.4	16	23.5
9/9	9	4.7	3	4.4
10/8	9	2.1	0	0.0
10/9	1	0.5	1	1.5
Total	191	100.0	68	100.0

b Allele frequencies

Allele	Normotensive subjects n (frequency)	Hypertensive subjects n (frequency)
5	11 (0.0288)	4 (0.0294)
6	1 (0.0026)	0 (0.0000)
8	287 (0.7513)	108 (0.7941)
9	78 (0.2042)	23 (0.1691)
10	5 (0.0131)	1 (0.0074)
Total	382 (1.0000)	136 (1.0000)

There were no statistically significant differences in genotype and allele frequencies between normotensive and hypertensive subjects.

GAACCTGGATG-3' for the reverse primer [14]. PCR was performed in a final volume of 50 µl (100 ng of genomic DNA, 20 pmol of each primer, 200 µM each of the four dNTPs, 1.5 mM MgCl₂, 50 mM KCl and 10 mM Tris-HCl, pH 8.4, and 2.5 units of *Taq* DNA polymerase). The reactions were denatured at 94 °C for 1 min, annealed at 64 °C for 1 min and extended at 72 °C for 30 s over 50 cycles with a final extension time of 10 min at 72 °C. Amplified PCR products (5 µl) were digested with the restriction enzyme *Ksp*I, then separated by electrophoresis on a 10% polyacrylamide gel and detected by silver staining.

Statistical Analysis

Allele frequencies were calculated from genotype frequencies, and the correlation between the genetic marker and essential hypertension was analyzed by a χ^2 test. For the (TTTTA)_n repeat polymorphism with more than two alleles, the statistical significance of the association between the genetic marker and essential hypertension was estimated with a Monte Carlo simulation using the Clump (version 1.6) program [15]. The polymorphism information content was

Table 2. Comparison of plasma Lp(a) levels in the indicated genotypes of the (TTTTA)_n repeat polymorphism in normotensive and hypertensive subjects

Genotype	Plasma Lp(a), mg/dl	
	normotensive subjects	hypertensive subjects
8/5	24.9 ± 12.3 (4)	24.8 ± 5.5 (4)
8/6	16.4 ± 0.0 (1)	0.0 ± 0.0 (0)
8/8	16.3 ± 12.8 (60)	20.2 ± 14.6 (34)
9/8	17.7 ± 17.3 (33)	13.9 ± 6.6 (15)
9/9	12.3 ± 7.7 (8)	9.6 ± 6.3 (3)
10/8	8.0 ± 0.0 (1)	0.0 ± 0.0 (0)
10/9	0.0 ± 0.0 (0)	18.4 ± 0.0 (1)

Values are means ± SD. Figures in parentheses show the number of subjects. There were no statistically significant differences in plasma Lp(a) levels across the genotypes in either group (Kruskal-Wallis test: $p = 0.5170$ for normotensive subjects, $p = 0.1090$ for subjects with essential hypertension).

estimated using the method of Botstein et al. [16]. The relative risk of essential hypertension associated with allelic variation was expressed in terms of an odds ratio with a 95% confidence interval. Comparison of the variables across genotypes was performed using a nonparametric Mann-Whitney U test or a Kruskal-Wallis test. All statistical analyses were performed using the SPSSWIN (version 8.0) software package.

Results

Subjects

The age distribution of normotensives (56.4 ± 9.6 years) and essential hypertensives (61.6 ± 9.2 years) differed significantly. The body mass index was not significantly different between normotensives (23.4 ± 2.3) and essential hypertensives (24.1 ± 2.6). The ratio of males to females was statistically similar (for normotensives, the ratio of males to females was 1:1; for essential hypertensives, it was 45/29).

Plasma Lp(a) Levels

The mean value of Lp(a) levels in plasma was 16.1 ± 13.4 mg/dl in 121 normotensive subjects and 17.8 ± 12.3 mg/dl in 60 subjects with essential hypertension. Although plasma Lp(a) levels were slightly higher in essential hypertensive subjects than in normotensive subjects, this difference was not statistically significant.

Fig. 1. Electrophoretic patterns of (TTTTA)_n polymorphisms of the *Apo(a)* gene. Lane 1: 8/5 repeat; lane 2: 8/6 repeat; lane 3: 8/8 repeat; lane 4: 9/9 repeat; lanes 5 and 6: 10/9 repeat; M: size marker (phi X-174 *Hinf*I).

(TTTTA)_n Repeat Polymorphism

Figure 1 shows the patterns of the (TTTTA)_n repeat polymorphism of the *Apo(a)* gene revealed by PCR. Overall, five different alleles were detected, with 5, 6, 8, 9 or 10 repeats corresponding to the PCR product of 93–118 bp. The genotype and allele frequencies of the (TTTTA)_n repeat polymorphism are given in table 1. Homozygote 8/8 subjects were most frequently identified among both the normotensive subjects and subjects with essential hypertension. The second most frequent genotype was the 9/8 heterozygote. Other genotypes occurred at frequencies of less than 6%. The allele with 6 repeats was observed only among the normotensive subjects. Heterozygosity was 0.39 and 0.34 for the normotensive and essential hypertensive subjects, respectively, and the polymorphism information content was 0.34 and 0.30, respectively. There were no significant differences in allele and genotype frequencies between the two groups (Monte Carlo simulation, $p > 0.05$, $n = 10,000$). Plasma Lp(a) levels for the various genotypes of the *Apo(a)* (TTTTA)_n repeat polymorphism are given in table 2. For this polymorphism, there were no significant differences in plasma Lp(a) levels across the genotypes in either group (Kruskal-Wallis test, $p > 0.05$).

Fig. 2. The electrophoretic patterns of the Met/Thr polymorphism of the *Apo(a)* gene. Lanes 1 and 3: Thr/Thr homozygotes; lanes 2 and 6: Met/Met homozygotes; lanes 4 and 5: Met/Thr heterozygotes; M: size marker.

Met/Thr Polymorphism

The patterns of the Met/Thr polymorphism produced by this technique are displayed in figure 2. In the Thr-coding allele containing the ACG codon, the PCR product is cleaved into 105- and 26-bp fragments by *KspI*. The Met-coding allele containing the ATG codon remains uncleaved (131 bp). Table 3 shows the genotype and allele distributions of the Met/Thr polymorphism of the *Apo(a)* gene. Heterozygosity was 0.48 and 0.47 for the normotensive subjects and subjects with essential hypertension, respectively, and the polymorphism information content was 0.38 and 0.37, respectively. There were no significant differences in allele and genotype frequencies between the two groups (χ^2 test, $p > 0.05$). Plasma Lp(a) levels for the various genotypes of the *Apo(a)* Met/Thr polymorphism are given in table 4. Like the (TTTTA)_n repeat polymorphism, this polymorphism also did not show any significant differences in the plasma Lp(a) levels across the genotypes in either group (Kruskal-Wallis test, $p > 0.05$).

Table 3. Genotype and allele frequencies for the Met/Thr polymorphism of the *Apo(a)* gene in normotensive and hypertensive subjects

	Genotype			Allele		H	PIC
	Met/Met	Met/Thr	Thr/Thr	Met	Thr		
Normotensive subjects	54 (26)	140 (67)	14 (7)	248 (60)	168 (40)	0.4815	0.3656
Hypertensive subjects	20 (28)	47 (66)	4 (6)	87 (61)	55 (39)	0.4746	0.3620
χ^2		0.2057			0.1204		
p		0.9023			0.7286		
Odds ratio (CI)			1.07 (0.73–1.58)				

Frequency is given as a percentage in parentheses. There were no statistically significant differences in genotype and allele frequencies between normotensive and hypertensive subjects. H = Heterozygosity; PIC = polymorphism information content; CI = 95% confidence interval.

Table 4. Correlation of plasma Lp(a) levels with the Met/Thr polymorphism

	Plasma Lp(a), mg/dl		
	Met/Met	Met/Thr	Thr/Thr
Normotensive subjects	18.5 ± 14.9 (34)	15.8 ± 13.1 (76)	11.0 ± 9.1 (11)
Hypertensive subjects	16.2 ± 13.5 (16)	18.8 ± 11.9 (41)	13.5 ± 14.2 (3)

Values are given as means ± SD. Figures in parentheses show the number of subjects. There were no statistically significant differences in plasma Lp(a) levels across the genotypes in either group (Kruskal-Wallis test: $p = 0.2070$ for normotensive subjects, $p = 0.4620$ for subjects with essential hypertension).

Discussion

The exact pathogenesis of essential hypertension is unknown. A number of risk factors, including genetic defects, are known to predispose subjects to essential hypertension. Therefore, the identification of genetic risk factors should enhance our understanding of the molecular basis of essential hypertension. Allelic association studies are commonly used to identify the susceptibility genes for complex traits such as essential hypertension [17].

Until now, few data have been reported regarding plasma Lp(a) levels among people with essential hypertension. Some authors have found normal plasma Lp(a) levels in these subjects [18, 19], whereas others have reported increased plasma Lp(a) levels [20–22]. In the present study, the mean plasma Lp(a) level of subjects with essential hypertension was not significantly different from that of normotensive subjects. This result implies that high plasma Lp(a) levels may not be an independent risk factor in the etiology of essential hypertension among Koreans. There were no significant differences between normotensive Korean subjects and subjects with essential hypertension in allele or genotype frequencies of the (TTTTA)_n repeat and Met/Thr polymorphisms of the *Apo(a)* gene. Furthermore, these two polymorphisms were not significantly associated with plasma Lp(a) levels in either group of subjects. Therefore, it is unlikely that these polymorphisms are associated with the etiology of essential hypertension among Koreans. Hong et al. [23] reported that the (TTTTA)_n repeat polymorphism of the *Apo(a)* gene was significantly associated with the plasma Lp(a) level in a coronary artery disease group, while the Met/Thr poly-

morphism was linked with the plasma Lp(a) level in the control group. Differences in the selection of study subjects may influence the difference in the association between the genetic variations of *Apo(a)* gene and the plasma Lp(a) level. However, we cannot exclude the possibility that these polymorphisms play a small role in the pathogenesis of this disease, because minor gene effects may be masked in a disease as complex as essential hypertension. Furthermore, the statistical power of retrospective case-control studies can be low when assessing correlations between genetic markers and complex diseases. Therefore, further studies using larger sample sizes are required to understand the influence of the *Apo(a)* gene on the pathogenesis of essential hypertension.

Conclusion

To our knowledge, this study is the first report of an investigation of a possible association between the genetic polymorphisms of the *Apo(a)* gene and essential hypertension in the Korean population. Our results suggest that the (TTTTA)_n repeat and Met/Thr polymorphisms of the *Apo(a)* gene are not significantly associated with essential hypertension and plasma Lp(a) levels in Koreans.

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