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Association of the Apolipoprotein B Gene Polymorphisms With Essential Hypertension in Northern Chinese Han Population[1]

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Objective To study the association of the apolipoprotein B gene polymorphisms with essential hypertension in Northern Chinese Han population. **Methods** *Xba*I and *Eco*RI polymorphisms of the apolipoprotein B (APOB) gene were genotyped by polymerase chain reaction (PCR) and restriction fragment-length polymorphism (RFLP) method in 503 unrelated hypertensive patients and 490 healthy controls recruited from international collaborative study of cardiovascular disease in Asia (InterAsia). **Results** The difference in the genotypic distributions could be neglected across the groups. The prevalence of X+ allele in healthy controls (4.8%) was less frequent in Chinese, and there was no significant difference in the frequency of the X+ allele between cases (5.7%) and controls (P=0.38). The observed E- allele frequencies were closely similar among groups (5.9% in cases *vs* 5.0% in controls, P=0.39). Logitstic regression analyses revealed that the lack of association still persisted after adjustment of other environmental factors. Haplotype analysis showed that X-E+ was most frequent and no haplotype could significantly contribute to essential hypertension. **Conclusion** The APOB gene *Xba*I and *Eco*RI polymorphisms are not associated with essential hypertension in the Northern Chinese Han population. Future studies on single nucleotide polymorphisms in larger samples are needed to further investigate the possible contribution of the APOB gene to essential hypertension.

Key words: Essential hypertension; APOB gene; Polymorphisms; Case-control study

INTRODUCTION

Recent findings indicate that hypertension is the leading risk factor for death among Chinese adults at the age of 40 years or older[1]. As one of the components of metabolic syndrome, dyslipidemia is associated with lipid metabolism and hypertension, although this interrelation is still not fully understood[2]. There is clinical, epidemiological, and genetic evidence that lipid abnormalities are common in hypertensive patients, and connected to the BP level caused by the common obesity and insulin resistance. Some other studies[3-4] have suggested a direct effect of lipids on BP control, as infusion of fat emulsion intralipid with heparin increases BP in healthy subjects. This effect is more pronounced in normotensive subjects with a family history of hypertension. Associations have been found between genes involved in lipid metabolism and hypertension, such as the lipoprotein lipase (LPL) gene[5] and apolipoprotein E (APOE) gene[6].

Apo B is the sole component of low-density lipoprotein (LDL) particles and plays an important role in the homeostasis of LDL cholesterol in plasma. Mutations in the apolipoprotein B gene (APOB) reduce the binding of apo B to LDL receptors and the clearance of plasma LDL, causing a disorder known as familial ligand-defective apo B[7]. The APOB gene variations influence circulation cholesterol concentration and affect susceptibility to coronary artery disease. So far, few studies have been made on the relationship between the APOB gene and hypertension. Frossard and colleagues[8] have demonstrated a statistically significant association between APOB 3' hypervariable region (3' HVR) alleles and essential hypertension in the United Arab

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Emirates (UAEs). They suggested that the APOB gene might constitute a good candidate for determinating genetic susceptibility to essential hypertension. In the study of "Swedish irbesartan left ventricular hypertrophy investigation *versus* atenolol" (SILVHIA), SNP C711T in the APOB gene was associated with the BP response to irbesartan in individuals carrying the C-allele[9].

Two of the extensively studied polymorphisms of the APOB gene are *Xba*I (Pubmed ID: rs693) and *Eco*RI (Pubmed ID: rs1042031). *Xba*I polymorphism is located in the 26th exon of the APOB gene and results in nucleotide substitution (thymidine for cytosine) in the 2488th amino acid (Thr). *Eco*RI is located in the 29th exon (GAA → AAA) and changes the 4154th amino acid (Glu→Lys). In the light of the above associations with cardiovascular diseases, we investigated the relationships between the two polymorphisms of the APOB gene and essential hypertension in the Northern Chinese Han population.

MATERIAL AND METHODS

Subjects

A total of 993 (503 unrelated hypertensive cases and 490 unrelated normotensive) Northern Chinese Han from Jilin and Shandong Provinces and Beijing were included in this study. All DNA samples and clinical data were collected from InterASIA[10]. The measurements and interviews were taken under standard conditions as described previously[11]. This project was approved by local bioethical committee and informed consent was obtained from each participant before data collection. Hypertension was defined as an average systolic blood pressure (SBP) ⩾ 140 mmHg, an average diastolic blood pressure (DBP) ⩾ 90 mmHg on three successive blood pressure measurements, and/or current treatment for hypertension with anti-hypertensive medication. Stage-2 hypertension was defined as an average SBP ⩾ 160 mmHg and/or DBP ⩾ 100 mmHg according to the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7). The control subjects had SBP < 140 mmHg and DBP < 90 mmHg, and were age- (within 2 years), gender-, and area-matched with patients. All participates had no clinical signs, symptoms, and laboratory findings suggestive of secondary hypertension, coronary heart disease or diabetes mellitus.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by using standard phenol-chloroform method[12]. The polymorphisms were determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis. The polymorphic site was classified as (+) or (-) according to the presence or absence at the cutting site of corresponding restriction enzyme. The PCR amplification conditions for two polymorphisms involved 40 cycles at 94℃ for 15 s, at 56℃ for 15 s and at 72℃ for 30 s, a final extension at 72℃ for 7 min. A 253 bp DNA fragment containing *Xba*I polymorphism site was amplified using the following primers: 5′ -TTG ATA CAT TCG GTC TCG TG-3′ (forward) and 5′ - CAA AAT CCG TGA GGT GAC-3′ (reverse). The primers used for EcoRI polymorphisms were 5′ - CAA AGT TCC TCC CTA GTG T-3′ (forward) and 5′ - CCA GGG ACT CAA GGA TAA C -3′ (reverse). PCR products were digested and then subjected to electrophoresis in 3% agarose gels. Two bands of 223 bp and 30 bp were produced for individuals with X+ allele, and fragments of 108 bp and 51 bp represented E+ allele.

Statistical Analysis

Quantitative variables were expressed as $\bar{x} \pm s$. The data were analyzed using SAS program (SAS Institute Inc., Cary, NC, USA). Hardy-Weinberg equilibrium was evaluated by Fisher's exact test using the program HWE[13]. The differences in clinical characteristics between cases and controls were assessed by Student's *t*-test for quantitative variables and χ^2-test for categorical ones. Since the homozygotes were too few to analyze separately, subjects with heterozygous or homozygous minor alleles were taken into one group. The frequencies of the genotypes and alleles between cases and controls were compared by the χ^2-test. Multivariate analysis was performed to investigate the independent effect of the two polymorphisms. All statistical tests were two-tailed, and $P<0.05$ was considered statistically significant.

We used the EM algorithm-based function *Haplo.em* to estimate each individual's haplotypes in the entire sample. Then the *Haplo.score* approach was used to test the association of statistically inferred haplotypes with essential hypertension[14]. *Haplo.em* and *Haplo.score* were implemented in the *Haplo.stats* package developed using the *R* language (http://www.r-project.org).

RESULTS

The demographic and clinical data of all individuals are summarized in Table 1. Besides

systolic blood pressure (SBP), diastolic blood pressure (DBP), and body mass index (BMI), levels of serum total cholesterol (TC), triglycerides (TG) and serum glucose (GLU) were significantly higher in cases than in controls. The patients also had higher levels of low density lipoprotein cholesterol (LDLC) and lower levels of high density lipoprotein cholesterol (HDLC) than controls. There were no significant differences in age, gender, drinking, and smoking status between cases and controls.

TABLE 1

Comparison of Clinical Characteristics Between Cases and Controls ($\overline{x} \pm s$)

	Cases (503)	Controls (490)	*P* Value
Age (Years)	53.57±9.34	53.51±9.22	0.9212
Gender (Male/Female)	262/241	257/233	0.9092
Systolic Blood Pressure (mmHg)	177.07±28.05	117.47±11.64	<0.0001
Diastolic Blood Pressure (mmHg)	104.34±12.28	75.05±8.01	<0.0001
Body Mass Index (kg/m^2)	26.33±3.85	24.29±3.58	<0.0001
Glucose (mmol/L)	5.93±1.79	5.60±1.68	0.0026
Total Cholesterol (mmol/L)	5.23±0.99	5.06±1.05	0.0122
Triglyceride (mmol/L)	1.70±1.06	1.43±0.86	<0.0001
High-density Lipoprotein Cholesterol (mmol/L)	1.25±0.30	1.32±0.34	0.0012
Low-density Lipoprotein Cholesterol (mmol/L)	3.19±0.86	3.09±0.87	0.0544
Creatinine, μmol/L	70.83±15.21	68.92±12.12	0.0283
Smokers	204	211	0.4237
Drinkers	173	164	0.7584

Note. Smokers, the number of cigarette consumers who smoked not less than 100 cigarettes; Drinkers, the number of alcohol consumers who drank not less than 12 times during the year ahead of the interview.

The genotypic and allelic frequencies of *Xba*I and *Eco*RI polymorphisms are shown in Table 2. Genotype distributions in cases and controls did not differ from those expected for Hardy-Weinberg proportions at both polymorphic sites. Univariate analysis indicated that *Xba*I and *Eco*RI polymorphisms were not associated with essential hypertension. Compared with wild-type individuals, the detected carriers of X+ allele (X-X+/X+X+) were not at an increased risk for essential hypertension (*P*=0.36). The observed X+ allele frequencies were closely similar among groups (5.7% for cases, 4.8% for controls, *P*=0.38). The presence of E-allele (E-E+/E-E-) did not alter the risk of hypertension (*P*=0.55), and there was no significant difference in E-allele frequency between cases and controls (5.9% *vs*. 5.0%, *P*=0.39). Further studies stratified by gender revealed no significant difference between the groups. Moreover, the lack of association persisted even after controlling other environmental risk factors by multiple logistic regression analysis (X-X+/X+X+ *vs*. X-X-, OR=1.26, 95%CI: 0.82 to 1.94; E-E+/E-E- *vs*. E+E+, OR=1.13, 95%CI: 0.74 to 1.73).

TABLE 2

Genotype Frequencies of Two Polymorphisms in the APOB Gene

Polymorphisms	Genotype	Total (Male/Female)		*P* Value
		Cases	Controls	
*Xba*I	X-X-	443 (232/211),	441 (232/209)	0.36 (0.59/0.37)
	X-X+/X+X+	55+1 (27/29)	45+1 (23/23)	
	X+ %	5.7 (5.4/6.0)	4.8 (4.5/5.2)	0.38 (0.51/0.56)
*Eco*RI	E+E+	445 (233/212)	440 (231/209)	0.55 (0.72/0.63)
	E-E+/E-E-	53+3 (29/27)	49+0 (26/23)	
	E- %	5.9 (5.9/5.9)	5.0 (5.0/5.0)	0.39 (0.54/0.54)

Note. Genotype distributions did not differ significantly from those expected under Hardy-Weinberg equilibrium.

Three haplotypes involved in *Xba*I and *Eco*RI polymorphisms were observed in all subjects. The X-E+ haplotype was most frequently observed in both cases (0.884) and controls (0.901). Comparison of the overall frequencies between groups across each haplotype revealed no significantly different haplotype in cases and controls (data not shown).

DISCUSSION

Since Hegele[15] reported the association of the APOB gene *Xba*I polymorphism with myocardial

infarction, a series of studies have demonstrated correlations between the APOB gene and atherosclerotic coronary artery disease and dyslipidemia[16-18]. However, controversial data existed in these studies. Since blood lipid levels are closely associated with hypertension, the APOB gene might be involved in the development of hypertension. Higashimori *et al.*[19] genotyped APOB 3' HVR in 187 Japanese and found the APOB 3' HVR was not associated with essential hypertension. Frossard and colleagues[8] then designed an association study in 437 UAEs and found that the APOB variant was significantly associated with essential hypertension. In the present study, we explored the APOB *Xba*I and *Eco*RI polymorphisms in essential hypertensive cases and controls.

There is general agreement that genetic heterogeneity across races and ethnicities exists in the APOB gene polymorphisms. Previous studies reported that the allele frequencies were 33%-61%[16, 20-22] and 15%-22%[16, 20-22] in Caucasoids, and 2%-9%[23-26] and 4%-7%[23-26] in Mongoloids for X+ allele and E- allele, respectively. Our results are very similar to those published for the Chinese and Korean populations of Mongolian origin. As a possible explanation for the differences in allele frequency of the APOB polymorphisms among populations studied, the differences in the genetic background might be a more important factor than environmental variations, such as diet or life-style[27]. Another possibility is the differences in linkage disequilibrium between *Xba*I/*Eco*RI and other polymorphic sites of the APOB gene among populations. Different levels of linkage disequilibrium, which may be due to genetic drift by a founder effect or selective mechanism, could be caused by different sample sizes and the bias of sample selection for the studied population[28]. Thus, an ideal association study should be performed on samples from homogenous population. Population admixture may cause a false positive genetic association. In this respect, our samples selected from the InterASIA study, were all the Han ethnic of northern rural area of China and were appropriate for association study. In addition, strict training processes and rigorous quality assurance programs were used to ensure the quality of data collection. Additional strengths of the study included a high response rate, three blood pressure measurements, detailed information on history of hypertension and standard laboratory methods[29].

Some limitations should be taken into consideration when interpreting our results. First, our study design was confident to detect odds radio of 1.7 in our population (80% power, 5% significance level, two-sided test). In fact, the pathophysiological mechanism by which polymorphisms of the APOB gene could influence susceptibility to essential hypertension is yet unknown. So each of genetic polymorphisms of the APOB gene may confer a modestly increased or reduced risk that may be added to, amplified, or even overcome by other acquired environmental and/or additional genetic factor[30] in the development of essential hypertension. Second, the APOB gene is about 43 kb in length, and consists of 29 exons and codes for a protein containing 4536 amino acids[23]. A number of polymorphisms are required to extensively explore the possible effect of the APOB gene polymorphisms on essential hypertension[31]. Third, sine the study subjects were not recruited prospectively, a survival bias could not be excluded. Taken these reasons into consideration, the lack of association between *Xba*I/*Eco*RI variant and essential hypertension in our study should not be interpreted as data negating the potential role of the APOB gene in essential hypertension.

In conclusion, there is no evidence that the APOB gene *Xba*I and *Eco*RI polymorphisms are associated with human essential hypertension in the Northern Chinese Han population. Future studies are needed on tagging SNPs with the Hapmap data and relationship between the APOB gene polymorphisms and essential hypertension.

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