Original Article

Lipoprotein Lipase Gene Polymorphisms and Blood Pressure Levels in the Northern Chinese Han Population

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The lipoprotein lipase (LPL) gene has been investigated extensively in linkage studies and in studies of its association with lipid profiles and coronary artery disease (CAD), and this gene has also been reported to have an association with hypertension. In our previous linkage study on 148 Chinese hypertensive families, the regions at or near the LPL gene were found to be associated with systolic blood pressure (SBP) and diastolic blood pressure (DBP). Thus the LPL gene is a logical candidate gene for involvement in the underlying cause of essential hypertension (EH). In the present study, we identified 22 sequence variants by directly sequencing 10 exons and flanking regions of the LPL gene, and investigated the occurrence of 3 of these variants, IVS4-214C>T, 7754C>A and S447X, in a case-control study including 501 normotensive (NT) subjects and 497 EH subjects. In males, the frequencies of the genotypes of each of the 3 variants did not differ significantly between the NT and EH groups. Among the EH group in females, ANCOVA revealed no significant difference in blood pressure levels according to the 7754C>A genotype. However, in female, the distribution of the 7754C>A genotype and the frequency of the A allele of 7754C>A differed significantly between the NT and EH groups (p=0.032 and p=0.027, respectively) with 0.78 (95% confidence interval (CI): 0.56 to 1.07; p=0.12) of odds ratio for the A allele. Moreover, haplotype analysis revealed that T-A-C and T-C-G haplotypes (in the order of IVS4-214C>T, 7754C>A and S447X) were statistically more frequent in the NT group than in the EH group in females and males, respectively. Our indivisual single nucleotide polymorphism (SNP) analysis did not provide substantial evidence of an association between polymorphisms in the LPL gene and hypertension status and/or blood pressure levels in this cohort, but the more powerful haplotypes analysis suggested an association between the LPL gene and hypertension.
(*Hypertens Res* 2004; 27: 373−378)

***Key Words*: hypertension, lipoprotein lipase, polymorphism, haplotype**

Introduction

Hypertension is believed to be a complex disorder influenced by multiple genetic factors and environmental factors. Since hypertension is found to occur more often than expected in families with familial combined hyperlipidemia and other types of familial lipid syndromes (*1*, *2*), genes involved in triglycerides metabolism, such as lipoprotein lipase (LPL), may be involved in the genetic component of the develop-

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This work was supported by Grants-in-Aid No. H020220030130 of the Biomedical Project of the Council of Science and Technology, Beijing, KSCX2-2-07 and KJCX1-08 of the Knowledge Innovation Program of the Chinese Academy of Sciences, and 2002BA711A05 of the Tenth National Five-Year Plan Key Programs from the Ministry of Science and Technology of the People's Republic of China.

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Received October 24, 2003; Accepted in revised form February 25, 2004.

ment of hypertension. LPL is involved in the transformation of dietary lipids into sources of energy for peripheral tissues (*3*) and plays an important role in triglycerides metabolism, since it is crucial for the hydrolysis of triglycerides and chylomicrons. Thus the LPL gene is a logical gene to test for a possible association with hypertension. Several linkage studies have been conducted, but the results have been inconsistent. Wu and his colleagues provided significant evidence for linkage of systolic blood pressure (SBP) to a genetic region at or near the LPL gene in their study on Taiwanese families (*4*). In addition, a study in a population of Mexican-Americans suggested a linkage of a five-marker haplotype to SBP (*5*). On the other hand, Hunt and colleagues failed to replicate these positive findings in Caucasians (*6*). Because the genomic sequence of the LPL gene is highly polymorphic and a number of variants have been screened (*7, 8*), associations of markers near the LPL gene locus and polymorphisms within this locus with hypertension have been tested in different populations. Ma and colleagues found that the D8S282 marker near the LPL gene locus contributed to the variance of SBP in healthy Chinese subjects from Hong Kong, especially in females (*9*). Polymorphism S447X, which truncates 2 amino acids, has been studied extensively in relation to plasma triglycerides, coronary artery disease (CAD) and blood pressure (BP) levels (*10, 11*). Sass and colleagues showed an association between the G447 allele and both lower SBP and pulse pressure (PP) levels in females in the Stanislas cohort (*11*), and Clee *et al*. found that both male and female carriers of the S447X variant had decreased diastolic blood pressure (DBP) and SBP (*12*). Moreover, our previous linkage study suggested that the LPL gene and adjacent genomic region might contribute to individual BP variation in the Chinese population (*13, 14*). We therefore performed the present association study by screening variants in the LPL gene and assessing their association, both individually and as haplotypes, with essential hypertension (EH) in a northern Chinese Han population.

Methods

Subjects

The present study was performed as part of the International Collaborative Study of Cardiovascular Disease in Asia (InterASIA), from which all DNA samples and clinical data were collected (*15*). The local bioethical committee approved the study protocol and informed consent for participation was obtained from all subjects. The samples involved in this investigation were collected from 501 normotensive (NT) control subjects and 497 EH patients of northern Han Chinese origin residing in Beijing and in the provinces of Shandong and Jilin. All measurements and interviews were taken in the standard manner as described previously (*15*). All EH patients had a mean SBP $\geq$ 160 mmHg and/or mean DBP $\geq$ 100 mmHg on three consecutive occasions, and control subjects had an SBP$<$140 mmHg and a DBP$<$90 mmHg. BP values of EH patients undergoing treatment were adjusted according to an algorithm described in analyses of the Framingham data (*16*). None of the subjects had secondary forms of hypertension, CAD or diabetes. In all subjects, 474 EH patients were matched to NT subjects for the region of residence, gender and age.

Sequencing and Genotyping

DNA of all subjects was extracted from the peripheral blood leukocytes by using the standard protocols. Genomic segments consisting of about 250 bp both of the 5′flanking region and the 3′flanking region of all 10 exons of the LPL gene in 29 randomly selected subjects were amplified by polymerase chain reaction (PCR). Sequence variants were determined by direct sequencing of purified PCR products using an autosequencer (ABI 3700; Perkin-Elmer Biosystems, Norwalk, USA). PCR and restriction fragment length polymorphism (RFLP) methods were used to determine the genotypes of IVS4-214C>T with *Tsep*I and 7754C>A with *Hae*III in all subjects. The following primers were used: for IVS4-214C>T, 5′-AAGCCTGTTTCCTCCCACT-3′ (forward) and 5′-TAGCTCCTATTCTACAGTCATG-3′(reverse); and for 7754C>A, 5′-TGAAGTTTCCACAAATAAGGC-3′(forward) and 5′-TCTCCTGCTTTTACTCTG-3′(reverse). The primers and enzyme used for determination of S447X were described previously (*17*).

Statistical Analysis

The data were analyzed using SAS statistical software (SAS Institute Inc., Cary, USA), the program HWE (*18*), the program 2LD (http://www.iop.kcl.ac.uk/IoP/Departments/PsychMed/GepiBst/software.shtml) and the program Haplotyper (*19*). Quantitative data are presented as the mean$\pm$SD. Hardy-Weinberg equilibrium was assessed by Fisher's exact test using the program HWE. The differences in clinical characteristics between the EH and NT groups were assessed by two-sample *t*-test for quantitative variables and χ^2 test for categorical ones. Comparisons of the frequencies of the alleles, genotypes, and haplotypes between the EH and NT groups were made using the χ^2 test. Multivariate logistic regression analysis was employed to assess the relationship between each of the polymorphisms and the probability of having hypertension while considering the effects of other predictor variables. Odds ratios (OR) and 95% confidence intervals (CIs) were computed from the above regression parameters. Blood pressure levels according to the genotypes of each variant in both the EH group and NT group were compared by ANCOVA. The program Haplotyper was used to estimate the haplotypes of each subject based on the genotyping data. Pairwise linkage disequilibrium coefficients were calculated from estimated haplotype frequencies using the program 2LD, and D' denoted the extent of disequilibrium. Values of

Table 1. Characteristics of Study Participants

Parameter	Total		Males		Females	
	NT (501)	EH (497)	NT (261)	EH (259)	NT (240)	EH (238)
Age (years)	53.7±9.1	53.5±9.3	52.3±9.0	52.1±9.3	55.2±9.1	55.1±9.2
BMI (kg/m^2)	24.3±3.6	26.3±3.8**	24.1±3.6	25.9±3.7**	24.5±3.6	26.8±4.0**
SBP (mmHg)	117.6±11.7	176.9±28.0**	118.8±11.5	169.7±26.1**	116.2±11.7	184.9±27.9**
DBP (mmHg)	75.0±8.0	104.3±12.2**	77.0±7.5	105.5±11.5**	73.0±8.0	103.0±12.8**
Cr (μmol/l)	68.9±12.1	70.7±14.9*	73.7±10.0	76.1±13.5*	63.7±12.0	64.8±14.1
TG (mmol/l)	1.42±0.86	1.69±1.06**	1.41±0.87	1.60±1.03*	1.43±0.84	1.79±1.08**
HDL-C (mmol/l)	1.32±0.34	1.25±0.30**	1.28±0.34	1.27±0.32	1.37±0.33	1.24±0.28**
Glu (mmol/l)	5.57±1.67	5.93±1.80**	5.58±1.76	5.75±1.10	5.56±1.55	6.14±2.32**
Cho (mmol/l)	5.06±1.04	5.22±0.98*	4.92±1.02	5.13±0.95*	5.22±1.06	5.31±1.01
LDL-C (mmol/l)	3.09±0.86	3.19±0.85	2.99±0.81	3.14±0.81*	3.20±0.90	3.24±089
Drinking (%)	33.3	34.6	57.5	61.0	7.1	5.9
Smoking (%)	42.9	40.6	69.3	66.4	14.2	12.6

NT, normotensive; EH, essential hypertension; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Cr, creatinine; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; Glu, glucose; Cho, cholesterol; LDL-C, low density lipoprotein cholesterol. * $p \leq 0.05$, ** $p \leq 0.01$.

Table 2. Genotype Distribution in NT Subjects and EH Patients in Males and Females

Variants	Genotypes	Males			Females		
		NT (n=261)	EH (n=259)	p	NT (n=240)	EH (n=238)	p
S447X	CC	215	226	0.095	204	203	0.999
	CG	43	33		34	33	
	GG	3	0		2	2	
IVS4-214C>T	CC	107	115	0.559	101	113	0.456
	CT	113	111		115	101	
	TT	41	33		24	24	
7754C>A	CC	203	204	0.379	185	200	0.032
	CA	57	51		51	38	
	AA	1	4		4	0	

NT, normotensive; EH, essential hypertension.

$p<0.05$ were considered to indicate statistical significance.

Results

Table 1 shows the general clinical characteristics of the patients with EH and the NT subjects. The SBP, DBP, body mass index (BMI), triglycerides level (TG) and glucose level (Glu) were significantly higher in the EH group than in the NT group. No significant differences in age or serum concentrations of creatinine (Cr) were observed between the two groups.

Results of Sequencing

A total of 20 novel variants and 2 previously reported variants (7754C>A, S447X) were identified by means of direct sequencing. All novel variants were located in introns, and the two other polymorphisms, 7754C>A (*7*) and S447X, were located in coding regions. More than half (12) of the variants appeared only once in the 29 samples, and the quality of sequencing for 3 of the variants located at the 5′end of 7754C>A was too low to be reliable. To cover a greater genomic region by means of linkage disequilibrium, we chose the variant 7754C>A for genotyping and analyzing. Polymorphism 7754C>A is in exon 8 and causes no amino acid change, while S447X is located in exon 9 and results in a Ser-to-stop codon change. All genotype distributions of IVS4-214C>T, 7754C>A and S447X were in accordance with Hardy-Weinberg equilibrium.

7754C>A Variant

The frequency we observed for the minor allele of 7754C>A (0.11) was similar to that reported by Morabia *et al*. (*8*) (0.12). A significant association of the C allele with EH was observed (Table 2), but was only confined to females (p=

Table 3. Blood Pressure Levels in EH Group According to 7754C>A Genotypes

	Genotype		p^*
	CC	CA+AA	
Males			
n	204	55	
SBP (mmHg)	170.8±26.6	165.5±23.8	0.397
DBP (mmHg)	105.5±12.0	105.2±9.8	0.583
Females			
n	200	38	
SBP (mmHg)	184.2±27.3	188.1±31.2	0.301
DBP (mmHg)	102.6±12.8	104.9±12.9	0.486

Data are presented as means±SD. *The mean values are adjusted and compared by ANCOVA. SBP, systolic blood pressure; DBP, diastolic blood pressure.

0.027 for alleles, $p=0.0315$ for genotypes). In the female subgroup the odds ratio for hypertension associated with A-allele carrier was 0.78 (95% CI: 0.56 to 1.07; $p=0.12$) after adjusting for other factors such as BMI, age and smoking. In the comparison of BP levels according to 7754C>A genotypes in the NT group and, separately, in the EH group after adjustment, no association of genotypes with BP levels was found either in the male or the female subgroup (data for the EH group are shown in Table 3).

IVS4-214C>T and S447X Variants

The frequency of the T allele of IVS4-214C>T was 0.36 in the NT group and 0.33 in the EH group ($p=0.168$), and no association with either hypertension status or BP levels was found. The genotype frequencies of S447X were almost the same between the NT group and HT group. In addition, there were no associations of S447X with either EH or BP variation in either males or females.

Haplotype Analysis

The estimated haplotype frequencies derived from IVS4-214C>T, 7754C>A and S447X in males and females are shown in Table 4. A total of 6 haplotypes (in the order of IVS4-214C>T, 7754C>A and S447X from left to right) were observed in all subjects. A comparison of overall frequency differences across each haplotype between the EH group and the NT group showed no significant difference. However, in females the haplotype T-A-C was more frequent in the NT group than in the EH group (47 *vs*. 29, $p=0.034$), and in males the haplotype T-C-G occurred more frequently in the NT group than in the EH group (46 *vs*. 27, $p=0.023$). These 3 variants are found to be in strong linkage disequilibrium to each other. The D' value was 0.67 between IVS4-214C>T and 7754C>A, 0.85 between IVS4-214C>T and S447X, and approximately 1 between 7754C>A and S447X.

Discussion

By individual single nucleotide polymorphisms (SNPs) analysis, the present study provided no substantial evidence to support a role of the LPL gene in hypertension in the Han of northern China, although an association of 7754C>A with hypertension was observed before adjusting for other clinical factors. For the purpose of gene identification, one of usual attempts was to bring the earlier linkage results by family based genome-wide linkage studies and to establish an association between BP and alleles of the genetic markers in question by the case control studies afterwards (*20*). In our recent linkage study involving 148 hypertensive families, a linkage of SBP and DBP with the marker D8S261 in the LPL gene region was found by quantitative trait linkage analysis ($p=0.002$ for SBP, and $p=0.04$ for DBP). This result indicated that the LPL gene and adjacent region might contribute to individual BP variation in the Chinese population (*13*, *14*). In the present association study, we followed up this finding by investigating whether variants of the LPL gene were responsible for BP variability in the Chinese population.

Although the S447X variant of the LPL gene has been shown to result in premature termination of the LPL gene translation (*21*) and several studies had revealed a positive association between this variant and BP levels, we failed to replicate these findings in the present study. The allele fre-

Table 4. Haplotypes Distribution in NT Subjects and EH Patients by Gender

Haplotype	Males			Females		
	NT ($n=522$)	EH ($n=518$)	*p*	NT ($n=480$)	EH ($n=476$)	*p*
C-C-C	313	321	0.507	303	314	0.359
T-C-C	101	105	0.709	80	87	0.512
C-A-C	11	14	0.531	12	9	0.520
T-A-C	48	45	0.774	47	29	0.0345
T-C-G	46	27	0.023	36	33	0.735
C-C-G	3	6	0.310	2	4	0.450

Haplotypes observed in overall subjects are not listed in the table. NT, normotensive; EH, essential hypertension.

quencies in our population (G, 0.08; C, 0.92) differed from the previously published frequencies found in the Stanislas cohort (G, 0.12; C, 0.88) (*11*). This discrepancy may have been due to differences between ethnic groups in the genomic region around the LPL gene and/or differences in the methods used to select subjects, since our subjects were not only selected from general populations but also cases were matched to NT subjects with respect to the region of residence, gender and age, whereas Sass *et al*. (*11*) selected subjects from a general population without a similar process of match.

Polymorphism 7754C>A has not previously been investigated for a possible association with hypertension. Although we observed a statistically significant difference in the distribution of the 7754C>A genotypes between the NT and EH groups, after adjustment for other clinical factors such as BMI and age in the multivariate logistic regression analysis this SNP showed no contribution to the development of hypertension. This negative result was enhanced by ANCOVA analysis of the BP levels according to the genotypes of 7754C>A. The above disparity may have been caused by interference from other contributive factors, since we observed the different results before and after adjustment of other clinical factors.

Haplotypes combined by adjacent SNPs are considered to have more information content than individual SNP and thus have more power to explore the association between candidate genes and complex diseases (*22*−*26*). Several association studies based on the distribution of haplotypes derived from genotypes have been performed (*27*, *28*). The Haplotyper program is based on the Monte Carlo method, and can infer haplotypes for a large number of linked SNPs accurately and rapidly. It is robust to the violation of Hardy-Weinberg equilibrium, to the presence of missing data, and to the occurrences of recombination hotspots (*19*). We thus applied this program to estimate haplotypes by using genotypes of IVS4-214C>T, 7754C>A and S447X in the present study. The most prevalent haplotype was C-C-C (in order of IVS4-214C>T-7754C>A-S447X) with the frequency of 0.63. The T-A-C and T-C-G haplotypes were statistically associated with EH in females and males, respectively. Because of the very strong LD across these 3 variants, these findings are not surprising. Nevertheless, the number of subjects having positive haplotypes was too small (for T-C-G in males: 46 in the NT group and 27 in the EH group; for T-A-C in females: 47 in the NT group and 29 in the EH group) to detect potential associations at a statistically more significant level. Moreover, the low reliability should be taken into account when interpreting the findings between positive haplotypes and hypertension status.

A certain kind of discrepancy exists between the findings of our previous quantitative trait linkage analysis and the present association study by individual SNP analysis, and this difference might be related to any of the following factors. First, with respect to methodology, it is difficult to identify the genetic factors of complex diseases using only the present standard molecular techniques of linkage analysis. Inconsistency between linkage studies and the subsequent association analyses has been found even for major candidate genes, such as angiotensinogen (*29*, *30*) and angiotensin-converting enzyme (ACE) (*31*−*33*). Second, the sample size in the present study may not have been large enough to identify or confirm the results derived from linkage studies. Additionally, only three SNPs were involved in the present study, and they were not likely to be causal or in linkage disequilibrium with a disease-causing variant. Those variants that affect the function of a protein or its expression would be studied for the reasons of clinical significance and practical or statistically feasible, therefore it might be valuable to investigate the SNPs which altered an amino acid or resulted in a premature stop codon, or to investigate the SNPs which located at the promoter region and had effects on the transcription of the LPL gene (*34*).

In conclusion, our previous linkage study suggested that the genomic region near the LPL gene might contribute to individual BP variation. In the present study, single SNP analysis did not provide substantial evidence to support an association between polymorphisms in the LPL gene and hypertension status and/or BP levels; however, the more powerful haplotypes analysis did suggest an association between the LPL gene and hypertension. A further study employing a greater number of subjects and SNPs would be appropriate to determine whether the LPL gene contributes to BP variation in the Northern Chinese Han population.

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