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Linkage and linkage disequilibrium analysis of the lipoprotein lipase gene with lipid profiles in Chinese hypertensive families

Wenjie YANG*†, Jianfeng HUANG*, Cailiang YAO‡, Shaoyong SU*, Donghai LIU*, Dongliang GE* and Dongfeng GU*†

*Division of Population Genetics and Prevention, Cardiovascular Institute, Fu Wai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100037, People's Republic of China, †National Human Genome Center at Beijing, Beijing 100176, People's Republic of China, and ‡School of Public Health, Nanjing Medical University, Nanjing 210008, People's Republic of China

A B S T R A C T

Elevated TG [triacylglycerol (triglyceride)] is a significant independent risk factor for cardiovascular disease. LPL (lipoprotein lipase) is one of the key enzymes in the metabolism of the TG-rich lipoproteins which hydrolyses TG from the chylomicrons and very-LDL (low-density lipoprotein). To investigate the relationship between the *LPL* gene and lipid profiles, especially TG, in 148 hypertensive families, we have chosen seven flanking microsatellite markers and four internal markers of the *LPL* gene and conducted linkage analysis by SOLAR and S.A.G.E. (statistical analysis for genetic epidemiology)/SIBPAL 2 programs, and linkage disequilibrium analysis by QTDT (quantitative transmission/disequilibrium test) and GOLD (graphical overview of linkage disequilibrium). There were statistically significant differences in lipid levels between subjects without and with hypertension within families. A maximum LOD score of 1.3 with TG at the marker D8S261 was observed by SOLAR. Using S.A.G.E./SIBPAL 2, we identified a linkage with TG at the marker 'ATTT' located within intron 6 of the *LPL* gene ($P = 0.0095$). Two SNPs (single nucleotide polymorphisms), *Hin*dIII and *Hin*fI, were found in linkage disequilibrium with LDL-cholesterol levels ($P = 0.0178$ and $P = 0.0088$ respectively). A strong linkage disequilibrium was observed between the *Hin*dIII in intron 8 and *Hin*fI in the exon 9 ($P < 0.00001$, $D' = 0.895$). Linkage disequilibrium was also found between the 'ATTT' polymorphism in intron 6 and two SNPs ($P = 0.0021$ and $D' = 0.611$ for *Hin*dIII; and $P = 0.00004$, $D' = 0.459$ for *Hin*fI). The present study in the Chinese families with hypertension suggested that the *LPL* gene might influence lipid levels, especially TG metabolism. Replication studies both in Chinese and other populations are warranted to confirm these results.

INTRODUCTION

Elevated TG [triacylglycerol (triglyceride)] is an established risk factor for cardiovascular disease and is common among subjects with hypertension. In recent years, more evidence supporting high TG as an independent risk factor for cardiovascular disease has accumulated. By a pooling analysis of 17 studies (46413 men

Key words: hypertension, linkage disequilibrium, lipid, lipoprotein lipase gene, single nucleotide polymorphism, triacylglycerol.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein-cholesterol; IBD, identity-by-descent; LDL, low-density lipoprotein; LDL-C, LDL-cholesterol; LPL, lipoprotein lipase; QTDT, quantitative transmission/disequilibrium test; QTL, quantitative trait locus; SBP, systolic blood pressure; SNP, single nucleotide polymorphism; TC, total cholesterol; TG, triacylglycerol.

Correspondence: Dr Dongfeng Gu, Division of Population Genetics and Prevention, Cardiovascular Institute, Fu Wai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100037, People's Republic of China (email gudf@yahoo.com).

and 10864 women), elevated TG levels were revealed to be associated with an increased cardiovascular risk of 76% in women and 32% in men [1]. Hypertension and abnormalities in lipid metabolism show familial aggregation, and hypertension has been found to occur more often than expected in families with familial hyperlipidaemia [2]. The metabolic syndrome is characterized by clustering of hypertension, hyperlipidaemia, obesity and glucose intolerance in the same individuals. As one of the components of the metabolic syndrome, hyperlipidaemia is not a simple disorder and might form an essential link between lipid metabolism and hypertension, although this interrelation is still not fully understood [3].

LPL (lipoprotein lipase) is one of the key enzymes in the metabolism of TG-rich lipoproteins and hydrolyses TG in chylomicrons and very-LDL (low-density lipoprotein). Genetic factors play a significant role in determining lipid levels, and genetic heritabilities of measured fasting lipids, including TG, have been estimated to range from 0.40–0.65 [4]. Therefore the clinical relevance and substantial evidence for a genetic component in TG prompted our present investigation of linkage and linkage disequilibrium analysis in Chinese families with hypertension.

METHODS

Study subjects

Hypertensive families ($n = 148$) were recruited through probands with essential hypertension from suburbs of Beijing (Fangshan and Shijingshan districts), Jiangsu province (Changshu, Taixing and Zhangjiagang districts) and Shanxi province (Hanzhong city). To be eligible for the study, the proband had to meet the following criteria: age > 15 years; either parent with hypertension; two or more siblings with hypertension; resting/sitting SBP (systolic blood pressure) $\geqslant 140$ mmHg and/or DBP (diastolic blood pressure) $\geqslant 90$ mmHg on three different occasions and use of antihypertensive medication. Patients were excluded from the present study if they had taken medication in the past 2 weeks and could not be re-examined without withdrawing treatment for 2 weeks. Trained research staff administered a standard questionnaire. Information on demographic characteristics, including age, gender, personal medical history, family history and lifestyle risk factors, was collected. Anthropometric and blood pressure measurements have been described previously [5]. All study subjects gave informed consent. The study protocol was approved by the Local Research Ethics Committee of the Cardiovascular Institute, Fu Wai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College.

Genotyping

Overnight fasting blood samples were drawn by venipuncture and genomic DNA was obtained from peripheral blood leucocytes by phenol/chloroform extraction. Seven flanking highly polymorphic microsatellite markers (D8S511, D8S1145, D8S261, D8S258, D8S282, D8S560 and NEFL) and four internal markers [two microsatellite and two SNP (single nucleotide polymorphism) markers] of the *LPL* gene were genotyped. Seven microsatellite markers near the *LPL* gene region and two microsatellite markers within the *LPL* gene (one is tetranucleotide 'ATTT' within intron 6 [6] and another is 'CA' repeat in the 3′ flanking region [7]) were genotyped with Genescan™ 3.1 and Genotype 2.1 software™ (Applied Biosystems, Foster City, CA, U.S.A.). Details of Touch-down PCR and genotyping procedure have been published previously [8]. *Hin*dIII (SNP ID: *rs327*) in intron 8 and *Hin*fI (SNP ID: *rs328*) in exon 9 of the *LPL* gene were genotyped by PCR-RFLP (restriction-fragment-length polymorphism). Genotypes of all family members were verified for Mendelian segregation.

Serum lipid measurement

Serum was used for quantifying lipid levels, including TC (total cholesterol), TG and HDL-C (high-density lipoprotein-cholesterol). All the specimens were stored at $-70\,^{\circ}$C at Fu Wai Hospital until laboratory assays were performed. This laboratory participates in the Lipid Standardization Program of the US Centers for Disease Control and Prevention. TC, TG and HDL-C were analysed enzymically on an Hitachi 7060 Clinical Analyser (Hitachi High-Technologies Corporation, Tokyo, Japan) using commercial reagents [9]. Serum control pools were obtained from Solomon Park Research Laboratories (Kirkland, WA, U.S.A.). For the participants who had TG levels < 400 mg/dl, LDL-C (LDL-cholesterol) levels were calculated using the Friedewald equation: LDL-C = (TC − HDL-C − TG)/5 [10].

Statistical analysis

Descriptive statistics (means ± S.D.) were used to evaluate the characteristics of all participants using SPSS 11.0. Student's t test was used to compare subjects by age, BMI (body mass index) and lipid profiles. A P value < 0.05 was considered statistically significant. Complex diseases represent a new level of genetic intricacy for which analytical tools have proved inadequate [11]. A plethora of approaches for analysis of complex diseases has emerged to deal with these disorders. SOLAR and S.A.G.E. [statistical analysis for genetic epidemiology; release 4.0. (2001) Department of Epidemiology and Biostatistics, Rammelkamp Center for Education and Research, MetroHealth Campus, Case Western Reserve University, Cleveland, OH, U.S.A.] programs have proved flexible and extensive for genetic linkage analysis, for which different algorithms have been implemented.

Table 1 Characteristics and lipid levels of family members

Values are means ± S.D.

	Parents			Offspring		
	With hypertension	Without hypertension	*P* value	With hypertension	Without hypertension	*P* value
n	154	111		346	188	
Gender (male/female)	57/97	75/36		225/121	109/79	
Age (years)	72.14 ± 8.95	71.73 ± 10.29	0.201	47.89 ± 8.39	43.13 ± 8.05	0.565
BMI (kg/m^2)	24.29 ± 4.69	22.46 ± 2.99	0.043	26.25 ± 3.40	24.72 ± 3.24	0.030
TC (mg/dl)	197.59 ± 46.23	189.14 ± 39.46	0.457	188.45 ± 35.66	182.10 ± 31.93	0.020
TG (mg/dl)	151.98 ± 94.85	131.48 ± 136.27	0.041	159.31 ± 111.56	124.72 ± 68.19	0.000
HDL-C (mg/dl)	48.24 ± 12.90	53.62 ± 13.07	0.035	46.75 ± 12.44	47.74 ± 12.94	0.401
LDL-C (mg/dl)	114.85 ± 44.31	109.23 ± 34.76	0.326	106.44 ± 37.62	109.42 ± 30.27	0.562

The pedigree-based variance component method has been implemented in the SOLAR package. This approach is based on specifying the expected genetic covariances between arbitrary relatives as a function of the IBD (identity-by-descent) among all relative pairs at a QTL (quantitative trait locus). Multipoint linkage analysis increases the power to detect true linkage and decreases the false positive rate. In addition, it incorporates a number of more complex genetic models by allowing for additional sources of genetic and non-genetic variance [12]. S.A.G.E. is a sib-pair-based program for detecting linkage between the quantitative trait and polymorphic markers. Based on the Haseman–Elston algorithm, the S.A.G.E./SIBPAL 2 program uses regression of the mean-corrected cross product of sibling pairs and the mean proportion of alleles sharing IBD. The underlying basis for this approach is to compare the quantitative variation in a trait between siblings as a function of the alleles sharing IBD by utilizing the SIBPAL 2 subroutine program of S.A.G.E. The covariate effects evaluated were age, sex and BMI. The QTDT (quantitative transmission/disequilibrium test) procedure was used for lipid profile analysis with the SNPs *Hin*dIII and *Hin*fI. It is a general linkage disequilibrium test that is applicable to the analysis of quantitative traits. The model also makes use of the powerful and flexible variance component framework to construct tests of linkage or linkage disequilibrium [14]. Allele frequencies were calculated by the genotyping data. The heterozygosity (H) was calculated as $H = [1 - (p^2 + q^2)]$, where p is the frequency of one allele and q the frequency of the other allele. Haplotype frequencies for *Hin*dIII, *Hin*fI and ATTT were estimated using the GOLD (graphical overview of linkage disequilibrium) program, and the extent of disequilibrium was expressed in terms of D'.

RESULTS

Our samples included 148 hypertensive families in a total of 799 subjects, which included 328 affected sib-pairs with hypertension. Table 1 shows the characteristics and lipids levels of all family members according to hypertension status in parents and offspring. TC and LDL-C were normally distributed. TG ($P = 0.216$) and HDL-C ($P = 0.348$) were also normally distributed after log transformation. We used transformed data in the analysis (Table 1 lists the non-transformed data) and age, sex and BMI as covariates were adjusted. Between affected and unaffected subjects, there were statistically significant differences in BMI, TG and HDL-C among the parents and in BMI, TC and TG among the offspring.

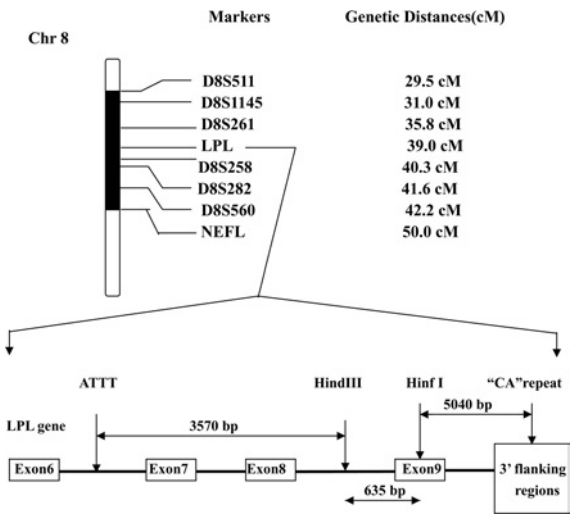

Figure 1 Position of the macrosatellite and SNP markers in the *LPL* gene region

Seven microsatellite markers were selected from the flanking region of the *LPL* gene and two microsatellite and two SNP markers were selected in the *LPL* gene. The positions of these markers are shown in Figure 1. Table 2 lists the heterozygosity and genetic distance of these markers. By SOLAR, two-point and multipoint linkage analyses were conducted for nine microsatellite markers with lipid phenotypes (the data for the linkage analyses of the lipids are available in Tables 1–6 at

Table 2 Heterozygosity and genetic distance of markers

Markers	Heterozygozity	Genetic distance (cM or Mbp)
D8S511	0.67	29.5 cM
D8S1145	0.73	31.0 cM
D8S261	0.78	35.8 cM
D8S258	0.70	40.3 cM
D8S282	0.71	41.6 cM
D8S560	0.75	42.2 cM
NEFL	0.81	50.0 cM
ATTT	0.72	19.825 Mbp
CA repeat	0.84	19.835 Mbp
*Hin*dIII	0.35	19.829 Mbp
*Hin*fI	0.15	19.830 Mbp

http://www.clinsci.org/cs/108/cs1080137add.htm). The largest LOD score (1.3) was identified with TG at the marker D8S261, which resides approx. 3.0 cM from the *LPL* gene, and a maximum multipoint LOD score of 0.04 was found at 36 cM, which is near the marker D8S261 (35.8 cM). No LOD scores larger than 1.0 were obtained from any other microsatellite markers with lipid profiles in either the two-point or multipoint linkage analyses.

Quantitative linkage analyses of the nine microsatellite markers with lipid profiles were conducted using the S.A.G.E./SIBPAL 2 program (the complete data for the analysis of the lipid profiles is available in Tables 7–10 at http://www.clinsci.org/cs/108/cs1080137add.htm). No significant evidence was found for linkage of the marker D8S261 with TG ($P = 0.1971$) or other lipid profiles. Only the marker 'ATTT' in intron 6 of the *LPL* gene was found to be linked with TG ($P = 0.0095$). Linkage disequilibrium was observed between LDL-C and *Hin*dIII ($P = 0.0178$) and *Hin*f I ($P = 0.0088$) respectively, by the QTDT procedure (Table 3). Allele frequencies of *Hin*dIII and *Hin*fI were 0.7769 and 0.2231 and 0.9213 and 0.0787 respectively. A strong linkage disequilibrium was observed between *Hin*dIII in intron 8 and *Hin*fI in exon 9 ($P < 0.00001$, $D' = 0.895$). Linkage disequilibrium was also found between the 'ATTT' polymorphism in intron 6 and two SNPs ($P = 0.0021$, $D' = 0.611$ for *Hin*dIII; $P = 0.00004$, $D' = 0.459$ for *Hin*fI).

DISCUSSION

Hypertension and lipid metabolism disorders are major risk factors of cardiovascular diseases, such as coronary heart disease and stroke. They often occur concomitantly in the same individuals and have an evident familial aggregation. This suggests that common genetic and environmental effects on these factors are present and genes conferring susceptibility to the development of hypertension might be closely linked with the genes leading to hyperlipidaemia.

The *LPL* gene is an important candidate and has been found to be linked and associated with blood pressure or lipid phenotypes in some studies [15–17]. In a previous study, we reported linkage and association of the *LPL* gene with hypertension and blood pressure phenotypes [5,8]. The marker D8S261, which resides approx. 3.0 cM from the *LPL* gene, was identified to be linked with SBP, with a maximum two-point LOD score of 2.52 and a maximum multipoint LOD score of 2.03. In addition, allele 3 of the marker D8S261 was found to be associated with hypertension. In present study, we also observed a maximum LOD score of 1.3 with TG at the marker D8S261. This is consistent with similar results from Taiwanese [16] and Caucasian [18] populations. These findings suggest that variations in the *LPL* gene may influence blood pressure and lipid metabolism (especially TG metabolism) and be potentially linked with TG, although the LOD score was not very large in the present study. No similar findings have been reported previously, and these results are the first demonstration of a relationship between any serum lipid concentration and the *LPL* gene in Chinese hypertensive families.

Support for the linkage of the *LPL* gene or the marker D8S261 with TG can be found in a Finnish family study [19]. A maximum LOD score of 1.5 with TG was reported

Table 3 Linkage disequilibrium tests of *Hin*dIII and *Hin*fI SNPs in the *LPL* gene with lipid phenotypes by QTDT

LnLk, log (likelihood); V, variances; df, degrees of freedom.

Phenotypes	SNPs	df(0)	LnLk(0)	df(V)	LnLk(V)	χ^2	*P* value
TC	*Hin*dIII	449	2190.74	448	2190.74	0	0.9870
	*Hin*fI	449	2190.74	448	2190.73	0	1.1500
TG	*Hin*dIII	448	−30.19	447	−30.21	0	0.8542
	*Hin*fI	448	−30.19	447	−30.19	0	0.8770
HDL-C	*Hin*dIII	450	−388.23	449	−389.66	2.85	0.0912
	*Hin*fI	450	−388.23	449	−389.75	3.03	0.0820
LDL-C	*Hin*dIII	385	1982.78	384	1979.97	5.62	0.0178
	*Hin*fI	385	1982.78	384	1979.35	6.87	0.0088

at 50 cM from the p-telomere in the *LPL* gene region on chromosome 8. We also examined a marker, NEFL, located 50 cM from the p-telomere. Unfortunately, no significant linkage results were found with TG or other lipid profiles. In another healthy monozygotic and dizygotic twin-pairs study, Knoblauch et al. [18] found a linkage relationship with TG at the marker D8S261 ($P = 0.05$). It has been confirmed that variants of the *LPL* gene are associated with the level of TG in case-control studies, and results supporting this are reported predominantly from patients with coronary heart disease [20] or hyperlipidaemia [21] and a small proportion from studies in healthy subjects [19,22], although few reports have been in hypertensive patients.

Allayee et al. [17] observed a linkage of DBP (LOD score = 1.8) to the *LPL* gene in dyslipidaemic families. Inversely, we found some suggestive evidence for linkage of TG with the *LPL* gene in hypertensive families. To our knowledge, there are some common metabolic pathways in the metabolism of hypertension and hyperlipidaemia. A possible pathophysiological mechanism could involve the ability of elevated TG to induce hyperinsulinaemia, which could lead to hypertension through its effects on renal haemodynamics, sodium reabsorption and retention and vascular hypertrophy [23]. Of course, we cannot be certain that the *LPL* gene alone is responsible for these findings, since there may be another unknown locus influencing lipid metabolism and blood pressure.

In the *LPL* gene, we selected two common LPL variants which have an obvious effect on lipid metabolism. The LPL *Hin*dIII and *Hin*fI SNPs were observed to be associated with variations in lipid levels, coronary artery disease, ischaemic cerebrovascular disease and diabetes in a Canadian Quebec family and Japanese and Australian populations [24–27]. In our present study, *Hin*dIII and *Hin*fI SNPs were observed in linkage disequilibrium with LDL-C by QTDT. However, this result did not allow us to conclude the presence of a relationship between LDL-C and the *LPL* gene or the two SNPs at the present time. These data suggest a potential effect of the *LPL* gene on lipid metabolism, but these analyses should be considered exploratory and require further investigation in larger studies.

Razzaghi et al. [28] observed an association of *Hin*fI with levels of TG and HDL-C in an Hispanic population. Both *Hin*dIII and *Hin*fI SNPs were discovered in linkage disequilibrium with lipids or blood pressure levels in some studies, because *Hin*dIII was found to have a very strong linkage disequilibrium with the *Hin*fI variant [29]. Similarly, strong linkage disequilibrium was also identified between the *Hin*dIII in intron 8 and *Hin*fI in exon 9 ($P < 0.00001$, $D' = 0.895$) in our present study. *Hin*fI locates downstream of *Hin*dIII by 647 bp, so it is not surprising that linkage disequilibrium is observed between the two SNPs. Any phenotype's association with the intron variants of a certain gene, such as *Hin*dIII, is likely to be as a result of linkage disequilibrium, with the actual aetiological mutations in the exon, promoter or exon–intron juncture region of a gene.

In addition, the 'ATTT' marker in the *LPL* gene was found to be linked with TG by S.A.G.E./SIBPAL 2. The discrepancies in the algorithm, model and information abstracted from the original data may have affected the result of the different positions at the putative TG marker. Linkage disequilibrium was found between 'ATTT' and the two SNPs ($P = 0.0021$, $D' = 0.611$ for *Hin*dIII; $P = 0.00004$, $D' = 0.459$ for *Hin*fI). Therefore we cannot rule out the possibility that linkage disequilibrium exists between 'ATTT' and other markers or candidates lying within this region that cause disease. Further studies should help to clarify this scenario. Perhaps the methods are not yet optimal, but the future holds much promise.

Our results in favour of the *LPL* gene influencing lipid levels, especially TG metabolism in Chinese populations, are partially in agreement with, or confirmed by, reports of some studies [19,20,28], although discrepancies in the findings do exist between the present study and others. We cannot exclude that the use of medication for hyperlipidaemia may have adversely affected blood lipid levels and slightly underestimated the prevalence of hyperlipidaemia. However, our large number of subjects were selected from rural areas in China, where awareness, treatment and control of cardiovascular disease are very low and few people know about their own serum lipid levels and medication taken. This enabled us to examine a completely natural distribution of inter-individual variation in quantitative lipid phenotypes. In addition, differences in ethical background, study samples and marker sets may contribute to the identification of different genes related to certain kinds of phenotypes and to what appears to be a lack of replication across studies.

In summary, we examined eleven genetic polymorphisms of the *LPL* gene in terms of linkage and linkage disequilibrium with lipid profiles in 148 Chinese hypertensive families. D8S261, ATTT, *Hin*dIII and *Hin*fI markers might be in linkage and/or associated with and exert potential effects on lipid metabolism. Further investigations are warranted to elucidate the relationship between the *LPL* gene polymorphisms and lipid profiles in a variety of ethnic groups.

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