Int J Clin Exp Pathol 2016;9(2):2181-2191
www.ijcep.com /ISSN:1936-2625/IJCEP0018534

Original Article

Endothelial lipase gene polymorphisms are associated with serum HDL-C levels but not coronary heart disease

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Received October 26, 2015; Accepted December 24, 2015; Epub February 1, 2016; Published February 15, 2016

Abstract: Background: The purpose of this study was to confirm the association of endothelial lipase gene (*LIPG*) variants with coronary heart disease (CHD) and plasma lipid levels. The study also investigated whether *LIPG* interacts with environmental factors to influence the risk of CHD in Chinese Han population. Methods: 1,514 Chinese Han subjects were recruited. Four tag single nucleotide polymorphisms (SNPs) (rs2000813 C>T, rs3786248 A>G, rs9958734 T>C, and rs3786247 A>C) in *LIPG* were selected from the HapMap website and genotyped using MALDI-TOF mass spectrometry. Data analysis was performed using SPSS 16.0 and SNPStats. Results: SNP rs2000813 was significantly associated with plasma levels of HDL-C (P = 0.035), and carriers of rs2000813 T allele had significantly higher levels of HDL-C than those with rs2000813 CC genotype. No significant differences were observed in allele frequencies and genotype distributions of the four *LIPG* SNPs between CHD patients and controls. Even after adjusting for age, sex, smoking, drinking, and lipid profiles, no significant association was found between *LIPG* SNPs and CHD in inheritance models and allele frequency comparisons. Furthermore, no significant interaction was observed between *LIPG* polymorphisms and hypertension, smoking, or alcohol drinking on the risk of CHD. Conclusions: Variants in *LIPG* might influence serum HDL-C levels, but they were not found to increase the risk of CHD in Chinese Han population. Our study did not demonstrate that *LIPG* variants interact with environmental factors to affect the susceptibility of individuals to CHD.

Keywords: *LIPG*, polymorphisms, coronary heart disease, HDL-C

Introduction

The endothelial lipase (EL) gene (*LIPG*), located on chromosome 18q21.1 and discovered in 1999, is a newly identified member of the triglyceride lipase gene family, which also includes lipoprotein lipase (LPL), hepatic lipase (HL), and pancreatic lipase (PL) [1-3]. EL is mainly synthesized by endothelial cells. It is also expressed in the liver, lung, macrophage, testis, ovary, and placenta [4]. EL has 45% cDNA identity to LPL and 40% to HL [5]. Unlike LPL and HL, EL has a greater phospholipase activity than triglyceride lipase [4, 6]. A research on EL lipolytic activity showed that the high-density lipoprotein cholesterol (HDL-C) is the preferred substrate for EL [6]. It has been reported that overexpression of EL in mice by recombinant EL adenovirus remarkably reduced plasma levels of HDL-C and apolipoproteins A-I (ApoA-I) [7], while plasma levels of HDL cholesterol and apoA-I and E were elevated in EL-deficient mice compared with wild-type littermates [8]. Some studies demonstrated that EL overexpression led to an increased uptake of HDL-apolipoproteins in the liver and kidney [9] and cholesterol in the liver through scavenger receptor class B type I (SR-BI) [10]. Thus, the catabolism and remodeling of HDL particles are promoted by EL.

Studies have shown that HDL-C is associated with the risk of cardiovascular diseases and atherosclerosis [8, 11]. However, the specific role of EL in the development of atherosclerosis is complicated and has not yet been firmly established [12-14]. Ishida et al. reported that EL might influence the development of atherosclerosis due to its effect on HDL-C and contrib-

ute to monocyte recruitment and cholesterol uptake to produce an atherogenic effect [12]. Yasuda et al. explored the role and expression of endothelial lipase in macrophages and found that EL was promoted by inflammation and thus increased macrophage uptake of LDL. The results suggest that EL may promote the development of atherosclerosis [13]. In contrast, a study found that EL did not play a role in the progression of atherosclerosis in apolipoprotein E- or low-density lipoprotein receptor-deficient mice [14].

Recent studies reported that *LIPG* polymorphisms were linked to the risk of coronary heart disease (CHD) and serum lipid levels [15-17]. Huang et al. demonstrated that allelic frequencies and genotypic distributions of *LIPG* SNP rs3813082 were significantly different between acute coronary syndrome (ACS) subjects and healthy controls, and subjects with the minor allele (AC+CC) of SNP rs3813082 had higher plasma levels of lipid in ACS patients, suggesting a significant association between *LIPG* rs3813082 and ACS as well as lipid levels in elderly Uygur individuals in Xinjiang, China [15]. Besides, Tang et al. assessed the effect of *LIPG* polymorphisms on coronary artery disease (CAD) in 530 Chinese participants living in Jiangsu Province, China, and their results suggested that SNP rs2000813 of *LIPG* might have a protective effect on CAD and be associated with HDL-C levels [16]. The association of *LIPG* SNP rs200813 with acute myocardial infarction was also observed in a case-control study including 214 Japanese subjects [17].

However, some studies reported opposite results, showing that *LIPG* polymorphisms were not associated with the risk of CHD or plasma levels of high-density lipoprotein [18-20]. Chen et al. investigated the association of 42 candidate genes involving in synthesis, maturation, or catabolism of HDL with plasma levels of HDL-C and severity of coronary atherosclerosis in 784 unrelated Caucasians, and they found that *LIPG* variants rs2000813 and rs4939585 were not associated with the severity and development of coronary atherosclerosis and HDL levels [18]. The study by Jensen et al. did not support that *LIPG* 584C/T variant (rs2000813) was associated with lipid levels or CHD in healthy Caucasian subjects [19]. Cai and colleagues reported no significant association between *LIPG* 584C/T polymorphism and unstable angina pectoris or plasma lipid levels in middle-aged and elderly populations [20].

Although studies of the association between *LIPG* SNPs and CHD as well as lipid levels have been conducted in Caucasian, Japanese, Chinese Han and Uygur populations [15-20], the results are conflicting and controversial. In addition, the interaction of *LIPG* variants and environmental factors on CHD has not been investigated in Chinese Han population. Therefore, we intended to analyze the association of *LIPG* polymorphisms with CHD and plasma lipid levels, and understand whether *LIPG* variants interact with environmental factors to influence the risk of CHD in Chinese Han population.

Materials and methods

Study subjects

This study included two case-control populations. The participants of the first group were recruited from the General Hospital of Jilin Chemical Group Corporation, Jilin City, Northeast of China, and the subjects of the second group were from the First Hospital of Jilin University. 404 male and 350 female patients, aged 40 to 85 years (mean age: 63±8 years), were recruited from inpatients admitted to the Cardiology Departments of the above two hospitals. The diagnosis of CHD was based on the criteria of the American College of Cardiology Foundation and the American Heart Association [21] and confirmed by the evidence of coronary angiography analyzed by experienced cardiologists.

Control subjects were recruited *via* a routine health examination in the same hospitals during the same period of time when patients were recruited. They had no biological relationships with the patients and were not affected by CHD. We recruited subjects who did not have symptoms and signs of atherosclerotic vascular diseases, history of coronary heart disease, or abnormal electrocardiogram as controls. There were 760 control subjects, including 378 males and 382 females aged 40 to 85 years (mean age: 63±8 years).

All subjects were unrelated Chinese Hans living in Jilin City, Northeast of China, and received a standard questionnaire that included sex, age,

smoking and drinking habits, history of other cardiovascular diseases, hypertension, and diabetes mellitus. In addition, blood pressure and plasma lipid and glucose levels were obtained from all subjects. Patients with malignant tumor, infection, immune system diseases, or severe renal or hepatic diseases were excluded from the study. Diabetes mellitus was defined according to 1999 World Health Organization (WHO) criteria of definition, diagnosis and classification of diabetes mellitus and its complications [22]: fasting blood glucose ≥ 7.0 mmol/L, 2-hour post-meal blood glucose ≥ 11.1 mmol/L, or/and under treatment of diabetes mellitus. Diagnosis of hypertension was according to the 2010 Chinese guidelines for the management of hypertension [23]: blood pressure ≥ 140/90 mmHg or/and taking antihypertensive drugs currently.

This study was approved by the ethics committee of Jilin University School of Public Health, and informed consents were provided by all subjects.

Tag SNP selection

LIPG gene is located in chromosomal region 18q12.1.Tag SNPs for *LIPG* were chosen from the HapMap databases (HapMap Genome Brower release # 24). In the public HapMap database (phase II Nov08, on NCBI B36 assembly, dbSNP b126) tag SNPs were selected under the following options: Han Chinese in Beijing population (CHB), r^2 cut-off of 0.8, tagger pairwise as pairwise methods, and MAF cut-off of 5%. We also consider the potential role of the tag SNPs which were selected from HapMap database in the progress of coronary heart disease and their influence to the plasma level of HDL-C based on the prior studies. Four tag SNPs (rs2000813 C>T, rs3786248 A>G, rs9958734 T>C, and rs3786247 A>C) of *LIPG* were chosen. The rs2000813 is located in the exon 3 of LIPG gene, which results in amino acid substitution. The rs3786248, rs9958734, and rs3786247 are in the three prime untranslated region.

DNA extraction, purification, and SNP genotyping

Genomic DNA was extracted from peripheral blood samples using standard protocol (phenol/chloroform extraction), and the quality and quantity were determined by spectrophotometry.

Genotyping was conducted by polymerase chain reaction (PCR) and MALDI-TOF mass spectrometry [24] using the MassARRAY iPLEX System (Sequenom Inc., San Diego, CA)

Data analysis

Data analyses were performed using SPSS 16.0 for Windows and the program SNPStats [25]. Clinical and biochemical data were expressed as mean ± SD or direct counts (percentage). Continuous variables were compared using the independent-sample t-test, and categorical variables were compared using the Chi-square test. The association between four *LIPG* SNPs and quantitative clinical traits was assessed by general linear model. The Chi-square test was used to check if genotype distributions were in Hardy-Weinberg equilibrium (HWE) and if allele frequencies and genotype distributions were significantly different between cases and controls. Logistic regression analysis was used to detect associations between SNPs and CHD after adjustment for age, sex, smoking, drinking, and plasma lipid levels. The strength of association was expressed as odds ratios (ORs) and 95% confidence intervals (95% CI). The gene-environment interaction in case-only analysis was analyzed by unconditional logistic regression with alleles or genotypes as independent variables and hypertension exposure as a dependent variable. Interactions of four SNPs and smoking as well as drinking on CHD were analyzed by SNPStats with adjustment for age and sex. Unadjusted odds ratios with 95% CI were obtained from 2×4 table comparisons by Chi-square test *P*-value <0.05 was considered statistically significant.

Results

Clinical and biochemical characteristics of CHD patients and healthy controls are presented in **Table 1**. The distributions of age and sex in cases were not significantly different from that of controls (age: $P = 0.963$; sex: $P = 0.135$), indicating that cases and controls were matched by age and sex. Compared with controls, CHD patients had a higher prevalence of hypertension, diabetes mellitus, smoking, and alcohol consumption (all *P*-values were less

Table 1. Characteristics of study subjects

Characteristic	CHD (n = 754)	Control (n = 760)	*P*
Age (years)	63±8	63±8	0.963
Male, n (%)	404 (53.6)	378 (49.7)	0.135
Hypertension, n (%)	444 (58.9)	0 (0.0)	**<0.001**
Diabetes mellitus, n (%)	110 (14.6)	0 (0.0)	**<0.001**
Smoking, n (%)	378 (50.1)	88 (11.6)	**<0.001**
Alcohol drinking, n (%)	107 (14.2)	55 (7.2)	**<0.001**
TC (mmol/L)	1.72±1.12	1.25±0.66	**<0.001**
TG (mmol/L)	4.35±1.06	4.11±0.80	**<0.001**
LDL-C (mmol/L)	2.78±0.90	2.62±0.71	**<0.001**
HDL-C (mmol/L)	1.15±0.33	1.31±0.25	**<0.001**
GLU (mmol/L)	6.25±2.28	5.05±0.70	**<0.001**
SBP (mmHg)	158.21±34.45	124.27±12.48	**<0.001**
DBP (mmHg)	92.60±18.61	81.44±38.87	**<0.001**

Data are presented as mean ± SD or number (percentage). Bold *P* values are statistically significant.

Table 2. Lipid levels in control subjects with different genotypes

SNP	Genotype	TC	TG	LDL-C	HDL-C
rs2000813	CC (n = 395)	4.09±0.80	1.24±0.69	2.62±0.71	1.30±0.25
	CT+TT (n = 365)	4.13±0.81	1.26±0.63	2.62±0.72	1.33±0.25
	P	0.478	0.717	0.971	**0.035**
rs3786248	AA (n = 474)	4.14±0.82	1.26±0.69	2.65±0.72	1.32±0.24
	AG+GG (n = 286)	4.06±0.78	1.22±0.61	2.58±0.71	1.29±0.26
	P	0.095	0.344	0.136	0.077
rs9958734	TT (n = 292)	4.14±0.82	1.25±0.72	2.66±0.72	1.31±0.24
	CT+CC (n = 468)	4.09±0.79	1.24±0.62	2.60±0.71	1.31±0.26
	P	0.372	0.899	0.234	0.664
rs3786247	AA (n = 262)	4.16±0.81	1.28±0.76	2.67±0.72	1.32±0.24
	AC+CC (n = 498)	4.09±0.80	1.23±0.61	2.59±0.71	1.31±0.26
	P	0.171	0.323	0.128	0.442

Data was represented as mean ± SD, and *P* value was obtained with the general linear model adjusted for age, sex, smoking, and drinking. Bold *P* value is statistically significant.

than 0.001). The levels of TC, TG, LDL-C, GLU, SBP, and DBP were significantly higher in CHD patients compared to controls, but the HDL-C level was significantly higher in controls compared to CHD patients (all *P*-values were less than 0.001).

We explored the association of four LIPG SNPs with serum lipid levels in the control group using general linear model. SNP rs2000813 was significantly associated with plasma levels of HDL-C ($P = 0.035$), and the carriers of T allele had significantly higher levels of HDL-C than those with the CC genotype (1.33±0.25 *vs.* 1.30±0.25 mmol/L). However, no significant association between the four *LIPG* SNPs and three other serum lipid parameters (TC, TG, and LDL-C) was observed in controls in our study (**Table 2**).

The genotype distributions of the four *LIPG* SNPs in the control group were confirmed to be in Hardy-Weinberg equilibrium (HWE) (data not sh own). Allele frequencies and genotype distributions of the four SNPs in *LIPG* are displayed in **Table 3**. No significant differences were observed in allele frequencies and genotype distributions of the four *LIPG* SNPs between cases and controls. After adjustment for age, sex, smoking, drinking and lipid profiles, we did not find a significant association between *LIPG* SNPs and CHD in inheritance models and allele comparisons (**Table 4**).

Because control subjects were not affected with hypertension, interactions of *LIPG* polymorphisms and hypertension were analyzed only in cases. *LIPG* polymorphisms showed no interaction with hypertension on the risk of CHD ($P>0.05$) (**Table 5**). Likewise, there were no significant interactive effects of *LIPG* polymorphisms and hypertension on CHD after adjusting for age and sex.

Since cigarette smoking and alcohol assumption are known risk factors for CHD, we explored the interaction of the four *LIPG* SNPs and these two risk factors on CHD (**Tables 6** and **7**). We found no interactions between *LIPG* polymorphisms and smoking (rs2000813: $P = 0.384$; rs3786248: $P = 0.491$; rs9958734: $P = 0.834$; and rs3786247: $P = 0.920$). Smoking increased

Table 3. Genotype distribution and allele frequency differences between coronary heart disease patients and controls

SNPs	Genotype/Allele	CHD	Control	*P*
rs2000813	CC	390 (52.9)	395 (52.0)	0.921
	CT	295 (40.0)	312 (41.1)	
	TT	52 (7.1)	53 (7.0)	
	C	1075 (72.9)	1102 (72.5)	0.791
	T	399 (27.1)	418 (27.5)	
rs3786248	AA	437 (59.3)	474 (62.4)	0.274
	AG	252 (34.2)	249 (32.8)	
	GG	48 (6.5)	37 (4.9)	
	A	1126 (76.4)	1197 (78.8)	0.120
	G	348 (23.6)	323 (21.2)	
rs9958734	TT	264 (35.8)	292 (38.4)	0.534
	CT	342 (46.4)	344 (45.3)	
	CC	131 (17.8)	124 (16.3)	
	T	870 (59.0)	928 (61.1)	0.260
	C	604 (41.0)	592 (38.9)	
rs3786247	AA	265 (35.8)	262 (34.5)	0.772
	AC	346 (46.8)	356 (46.8)	
	CC	129 (17.4)	142 (18.7)	
	A	876 (59.2)	880 (57.9)	0.470
	C	604 (40.8)	640 (42.1)	

Table 4. Association of *LIPG* SNPs with coronary heart disease

SNPs	Comparisons	OR (95% CI)	*P*
rs2000813	Homozygote comparison (TT vs. CC)	1.06 (0.66-1.73)	0.799
	Heterozygote comparison (CT vs. CC)	1.03 (0.80-1.32)	0.816
	Dominant model (CT/TT vs. CC)	1.03 (0.82-1.31)	0.778
	Recessive model (TT vs. CT/CC)	1.05 (0.66-1.68)	0.835
	Allele contrast (T vs. C)	0.98 (0.83-1.15)	0.791
rs3786248	Homozygote comparison (GG vs. AA)	1.59 (0.95-2.66)	0.077
	Heterozygote comparison (AG vs. AA)	1.17 (0.91-1.51)	0.232
	Dominant model (AG/GG vs. AA)	1.22 (0.96-1.56)	0.104
	Recessive model (GG vs. AG/AA)	1.50 (0.91-2.49)	0.114
	Allele contrast (G vs. A)	1.14 (0.96-1.36)	0.120
rs9958734	Homozygote comparison (CC vs. TT)	1.26 (0.89-1.79)	0.186
	Heterozygote comparison (CT vs. TT)	1.19 (0.92-1.55)	0.192
	Dominant model (CT/CC vs. TT)	1.21 (0.95-1.55)	0.128
	Recessive model (CC vs. CT/TT)	1.15 (0.84-1.57)	0.388
	Allele contrast (C vs. T)	1.09 (0.94-1.26)	0.260
rs3786247	Homozygote comparison (CC vs. AA)	0.98 (0.69-1.38)	0.898
	Heterozygote comparison (AC vs. AA)	1.06 (0.81-1.38)	0.669
	Dominant model (AC/CC vs. AA)	1.04 (0.81-1.33)	0.781
	Recessive model (CC vs. AC/AA)	0.95 (0.70-1.29)	0.727
	Allele contrast (C vs. A)	0.95 (0.82-1.10)	0.470

Except for allele contrast, ORs and 95% CI were adjusted for age, sex, smoking, drinking, TC, TG, LDL, and HDL. OR, odds ratio; CI, confidence interval.

the risk of CHD (OR = 8.65, 7.21, 7.54, and 7.63 respectively for SNPs rs2000813, rs3786248, rs9958734, and rs3786247)), while *LIPG* SNPs were not associated with CHD even after stratifying the data by smoking habits to explore the association of the four SNPs with CHD. In addition, smoking subjects with variant alleles of the four *LIPG* SNPs had an increased risk of CHD compared to wild-type homozygous subjects who were not exposed to smoking (OR = 7.27, 9.71, 9.49, and 7.92, respectively, for rs2000813, rs3786248, rs9958734, and rs3786247). Similarly, no interaction was observed between *LIPG* polymorphisms and alcohol drinking on CHD (*P* = 0.902, 0.076, 0.555, and 0.386, respectively, for SNPs rs2000813, rs3786248, rs9958734, and rs3786247). We found that drinking was also associated with an increased risk of CHD (OR = 2.13, 1.66, 1.87, and 1.74, respectively, for SNPs rs2000813, rs3786248, rs9958734, and rs3786247). Among the drinking subjects, carriers with AG and GG genotypes of SNP rs3786248 had a higher risk of CHD compared to those with AA genotypes (OR = 2.02, 95% CI = 1.02-3.98).

Discussion

The present study demonstrated that variation in *LIPG* might influence the serum level of HDL-C. Nevertheless, *LIPG* variants were not found to be associated with the risk of CHD in Chinese

Table 5. Case-only analysis of *LIPG* SNP-hypertension interactions on CHD

Genotype	Hypertension		OR (95% CI)	*P*	OR[a] (95% CI)	*P*
	Yes (n = 433)	No (n = 304)				
rs2000813						
CC	231 (53.3)	159 (52.3)	1		1	
CT	174 (40.2)	121 (39.8)	0.99 (0.73-1.35)	0.948	0.98 (0.72-1.34)	0.924
TT	28 (6.5)	24 (7.9)	0.80 (0.45-1.44)	0.460	0.81 (0.45-1.45)	0.482
CT+TT	202 (46.7)	145 (47.7)	0.96 (0.71-1.29)	0.779	0.96 (0.71-1.28)	0.765
C	636 (62.8)	439 (62.3)	1			
T	376 (37.2)	266 (37.7)	0.98 (0.80-1.19)	0.808		
rs3786248						
AA	251 (58.0)	186 (61.2)	1		1	
AG	155 (35.8)	97 (31.9)	1.18 (0.86-1.62)	0.296	1.19 (0.87-1.63)	0.285
GG	27 (6.2)	21 (6.9)	0.95 (0.52-1.74)	0.875	0.95 (0.52-1.74)	0.877
AG+GG	182 (42.0)	118 (38.8)	1.14 (0.85-1.54)	0.382	1.15 (0.85-1.55)	0.370
A	657 (66.1)	469 (68.6)	1			
G	337 (33.9)	215 (31.4)	1.12 (0.91-1.38)	0.290		
rs9958734						
TT	158 (36.5)	106 (34.9)	1		1	
CT	199 (46.0)	143 (47.0)	0.93 (0.67-1.29)	0.680	0.93 (0.67-1.29)	0.654
CC	76 (17.6)	55 (18.1)	0.93 (0.61-1.42)	0.727	0.92 (0.60-1.41)	0.703
CT+CC	275 (63.5)	198 (65.1)	0.93 (0.69-1.27)	0.651	0.93 (0.68-1.26)	0.623
T	515 (59.5)	355 (58.4)	1			
C	351 (40.5)	253 (41.6)	0.96 (0.77-1.18)	0.678		
rs3786247						
AA	159 (36.6)	106 (34.8)	1		1	
AC	201 (46.2)	145 (47.5)	0.92 (0.67-1.28)	0.635	0.92 (0.66-1.27)	0.606
CC	75 (17.2)	54 (17.7)	0.92 (0.60-1.42)	0.724	0.92 (0.60-1.41)	0.702
AC+CC	276 (63.4)	199 (65.2)	0.92 (0.68-1.26)	0.616	0.92 (0.68-1.25)	0.586
A	519 (59.7)	357 (58.5)	1			
C	351 (40.3)	253 (41.5)	0.95 (0.77-1.18)	0.663		

[a]: ORs and 95% CI were adjusted for age and sex.

Han population. We also failed to find significant interactions of *LIPG* polymorphisms with environmental factors on CHD susceptibility.

In 2002, Delemos and his colleagues [5] identified 17 polymorphic sites in *LIPG*, and six of them were potentially functional variants. They reported a significant difference of allele frequencies of *LIPG* SNP Asn396Ser between high and normal HDL-C subjects from the Caucasian population; however, allele frequencies of *LIPG* SNP rs2000813 were not significantly different between high and normal HDL-C subjects [5].

A growing body of studies have focused on the association of *LIPG* polymorphisms with plasma lipid levels [2, 26-29], but the results are inconsistent and inconclusive. Durlach et al. demonstrated that *LIPG* SNP Thr111Ile (rs2000813) was associated with HDL-C levels in 396 type 2 diabetes mellitus (T2DM) patients including 225 men and 171 women. They found that the T allele frequency was 0.331 and subjects with the TT genotype had increased levels of HDL-C [26]. Paradis et al. investigated the impact of SNP rs2000813 on HDL profiles of 497 subjects enrolled from the Quebec Family Study [27]. Women who were homozygous for the minor allele (TT) of SNP rs2000813 had significantly higher levels of HDL compared to those women with the wild-type allele (CC+CT). However, they observed no significant differences in plasma HDL levels in men with different genotypes of SNP rs2000813 [27]. Mank-

Table 6. Interaction of *LIPG* polymorphisms and smoking on coronary heart disease

Smoking	Genotype	Case/Control	OR[a] (95% CI)	OR[a] (95% CI)
	rs2000813			
No	CC	185/350	1	1
No	CT+TT	181/322	1.06 (0.82-1.37)	1.06 (0.82-1.37)
Yes	CC	205/45	8.65 (5.98-12.51)	1
Yes	CT+TT	166/43	7.27 (4.97-10.64)	0.84 (0.53-1.34)
	rs3786248			
No	AA	218/416	1	1
No	AG+GG	148/256	1.11 (0.85-1.44)	1.11 (0.85-1.44)
Yes	AA	219/58	7.21 (5.17-10.06)	1
Yes	AG+GG	152/30	9.71 (6.35-14.85)	1.35 (0.83-2.19)
	rs9958734			
No	TT	124/254	1	1
No	CT+CC	242/418	1.19 (0.91-1.55)	1.19 (0.91-1.55)
Yes	TT	140/38	7.54 (4.96-11.45)	1
Yes	CT+CC	231/50	9.49 (6.53-13.80)	1.26 (0.79-2.02)
	rs3786247			
No	AA	124/228	1	1
No	AC+CC	243/444	1.01 (0.77-1.32)	1.01 (0.77-1.32)
Yes	AA	141/34	7.63 (4.94-11.78)	1
Yes	AC+CC	232/54	7.92 (5.48-11.45)	1.04 (0.64-1.68)

[a]: ORs and 95% CI were adjusted for age and sex.

Table 7. Interaction of *LIPG* polymorphisms and drinking on coronary heart disease

Drinking	Genotype	Case/Control	OR[a] (95% CI)	OR[a] (95% CI)
	rs2000813			
No	CC	328/363	1	1
No	CT+TT	302/342	0.97 (0.78-1.21)	0.97 (0.78-1.21)
Yes	CC	62/32	2.13 (1.35-3.34)	1
Yes	CT+TT	45/23	2.16 (1.28-3.65)	1.02 (0.53-1.97)
	rs3786248			
No	AA	383/437	1	1
No	AG+GG	247/268	1.06 (0.85-1.32)	1.06 (0.85-1.32)
Yes	AA	54/37	1.66 (1.07-2.58)	1
Yes	AG+GG	53/18	3.35 (1.93-5.82)	2.02 (1.02-3.98)
	rs9958734			
No	TT	232/272	1	1
No	CT+CC	398/433	1.08 (0.87-1.35)	1.08 (0.87-1.35)
Yes	TT	32/20	1.87 (1.04-3.36)	1
Yes	CT+CC	75/35	2.51 (1.62-3.89)	1.34 (0.67-2.68)
	rs3786247			
No	AA	233/243	1	1
No	AC+CC	400/462	0.90 (0.72-1.13)	0.90 (0.72-1.13)
Yes	AA	32/19	1.74 (0.96-3.16)	1
Yes	AC+CC	75/36	2.17 (1.40-3.36)	1.25 (0.62-2.50)

[a]: ORs and 95% CI were adjusted for age and sex.

Seymour et al. provided evidence of an association between *LIPG* variation and HDL-C levels, especially for intronic variants C+42T/In5 and T+2864C/In8 [2]. Yamakawa-Kobayashi et al. observed significant associations between two genetic variants (-384A/C and 2237G/A) in *LIPG* and plasma HDL cholesterol levels in 340 unrelated healthy Japanese children, but they did not observe a significant association between other *LIPG* SNPs (584C/T, 2037T/C, 2842T/A, and 3082T/C) and serum HDL-C levels [28]. Similarly, Mank-Seymour et al. confirmed that there was a lack of association of SNP rs2000813 with HDL levels in an intermediate HDL-C population (between 35 and 60 mg/dl HDL-C) [2]. Besides, data from Cai et al. suggested that there was no significant association between *LIPG* 584C/T polymorphisms (rs2000813) and lipid levels in either CAD patients or healthy controls [29]. In the present study, our data showed that SNP rs2000813 was significantly associated with plasma levels of HDL-C (P = 0.035), and carriers of the T allele had significantly higher levels of HDL-C than those with the CC genotype (1.33±0.25 *vs*. 1.30±0.25 mmol/L). In addition, we did not observe a significant association between the four *LIPG* SNPs and three other serum lipid parameters (TC, TG, and LDL-C). The conflicting results of association of *LIPG* polymorphisms and HDL, especially for the common variant rs2000813, may be attributable to the effect of

differences in allele frequencies in different ethnic groups, life styles, or sample sizes. Delemos et al. demonstrated that the minor allele frequency of SNP rs2000813 was 0.103 in African Americans, 0.312 in White American controls, and 0.326 in high HDL-C White Americans [5]. Also, the frequency of the T allele was 0.304 and 0.351, respectively, in French women and men with T2DM (26), and it was 0.241 in Japanese school-aged children [28] and 0.218 in Chinese Han population living in the south of Jiangsu Province, China [29]. In our study, the frequency of the T allele in the total study subjects was 0.273, which was higher than that of the subjects from Japan or Chinese Han population in Jiangsu Province, China, and it was lower than that of Caucasians. Smith et al. found a significant interaction of *LIPG* polymorphisms with physical activity on regulation of plasma levels of HDL cholesterol [30]. Paradis et al. reported that the impact of dietary fat on plasma HDL levels in women was influenced by mutation in *LIPG* [29], indicating that different life styles and habits may modulate the effect of *LIPG* polymorphisms on HDL profiles.

Results from the present study showed no significant differences in allele frequencies and genotype distributions of the four *LIPG* SNPs between patients and controls. After adjustment for age, sex, smoking, drinking, and lipid profiles, we did not find significant associations between *LIPG* SNPs and CHD in inheritance models and allele frequency comparisons. Data from Vergeer et al. showed a lack of association of *LIPG* SNP rs2000813 and other variants in *LIPG* with the risk of CAD and deep venous thrombosis [31]. Results from Jensen et al. also did not support that SNP rs2000813 was associated the risk of CHD in healthy Caucasians [19]. Besides, a recent study found that allele frequencies and genotype distributions of rs2000813 were not significantly different between early-onset CHD patients and healthy controls, and that no association between SNP rs2000813 and CHD or the severity of CHD was shown in Chinese Han population [29]. However, several studies reported the opposite results, demonstrating that *LIPG* polymorphisms were associated with the risk of CHD and atherosclerosis, especially for SNP rs2000813 [16, 17, 32]. The inconsistent results suggest that *LIPG* variants may not always be implicated in the development of CHD or atherosclerosis. In addition, no *in vivo* experiment has been conducted to investigate the influence of *LIPG* polymorphisms on the expression of endothelial lipase. Hence, the relationship between *LIPG* polymorphisms and CHD are unclear and needed to be explored in further studies.

The development in Medical Genetics has helped us in understanding the influence of genes on diseases, and many diseases discovered are not simply affected by a single gene. A number of common diseases or complex phenotypes may be the results of interactions between genes and environmental factors [33], and the interaction may result in varying associations between genes and diseases, depending on the extent of exposure to environmental factors [34]. It is well-known that the development of CHD is contributed by multiple genetic and environmental factors, including age, sex, obesity, smoking, diabetes mellitus, hypertension, plasma levels of lipid, and gene polymorphisms [35, 36]. Some studies have investigated the interaction between *LIPG* polymorphisms and environmental factors on serum lipid and blood pressure levels [37-41]. Previous studies showed that SNP rs2000813 interacts with obesity to modulate serum levels of apolipoprotein-B (Apo-B) in subjects of Bai Ku Yao minority in China [37, 38], and the levels of pulse pressure were different among smokers with different genotypes of *LIPG* SNP rs2000813 [39]. The interaction of LIPG variants with cigarette smoking on blood pressure was not observed in the study by Yin et al [39]. In addition, Yin et al. demonstrated that *LIPG* rs2000813 did not interact with obesity to influence blood pressure [40]. Up to date, the interaction between *LIPG* variants and environmental factors on CHD is scarcely explored and has not been investigated in Chinese Han population. In our study, we found that smoking and alcohol drinking were both associated with an increased risk of CHD, and among study subjects who were alcohol drinkers, carriers of AG and GG genotypes of rs3786248 had a higher risk of CHD in comparison to those with AA genotypes (OR = 2.02, 95% CI = 1.02-3.98), but no significant interaction of *LIPG* variants with environmental factors on susceptibility to CHD was observed.

Our study has several limitations. First, the inclusion criteria for controls (i.e., subjects who did not have symptoms and signs of atheroscle-

rotic vascular diseases, history of CHD, and abnormal electrocardiograms) instead of using coronary angiography as exclusion criterion for CHD may result in recruiting control subject with asymptomatic CHD. Second, because our study subjects were recruited from two separate hospitals, selection biases, such as admission rate and prevalence-incidence biases, might occur. Third, our subjects were selected from Chinese Han population living in Northeast of China, and their life styles, eating habits, and living conditions may be different from other ethnic groups, suggesting that the research about the association of *LIPG* polymorphisms with CHD should be investigated in other regions and race groups in China.

In conclusion, our study found that variants in *LIPG* may influence the serum level of HDL-C, but they were not associated with the risk of CHD disease in Chinese Han population. Furthermore, we found no interactions of *LIPG* variants with environmental factors on susceptibility to CHD.

Acknowledgements

Our study was supported by National Natural Science Foundation of China (grant number: 30870952 and 81573230), Scientific Research Foundation of the Health Bureau of Jilin Province, China (grant number: 2011R009), Bethune plan B foundation (grant number: 2013102009), grants from Science and Technology Department of Jilin Province, China (grant number: 20080735), and grants from International Cooperation of Science and Technology Department of Jilin Province (grant number: 20140413016GH).

Disclosure of conflict of interest

None.

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