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Association of lipoprotein lipase (*LPL*) single nucleotide polymorphisms with type 2 diabetes mellitus

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Abbreviations: AUCGLU, glucose area under the curve; AUCINS, insulin area under the curve; BMI, body mass index; DBP, diastolic blood pressure; FDR, false discovery rate; HbA1C, glycosylated hemoglobin; HDLc, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; LDLc, low density lipoprotein cholesterol; LPL, lipoprotein lipase; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; TCHOL, total cholesterol; TG, triglyceride; UTR, untranslated region; WHR, waist hip ratio

Abstract

The etiology and pathogenesis of type 2 diabetes mellitus (T2DM) are not completely understood although it is often associated with other conditions such as obesity, hypertension, and dyslipidemia. Lipoprotein lipase (LPL) is a key enzyme in human lipid metabolism that facilitates the removal of triglyceride-rich lipoproteins from the bloodstream. LPL hydrolyzes the core of triglyceride-rich lipoproteins (chylomicrons and very low density lipoprotein) into free fatty acids and monoacylglycerol. To gain insight into the possible role of LPL in T2DM, nine single nucleotide polymorphisms (SNPs) of *LPL* were analyzed for the association with T2DM using 944 unrelated Koreans, including 474 T2DM subjects and 470 normal healthy controls. Of the nine *LPL* SNPs we analyzed, a significant association with multiple tests by the false discovery rate (FDR) was observed between T2DM and SNP rs343 (+13836C > A in intron 3). SNP rs343 was also marginally associated with some of T2DM-related phenotypes including total cholesterol, high density lipoprotein cholesterol (HDLc), and log transformed glycosylated hemoglobin in 470 normal controls, although no significant association was detected by multiple tests. In total, our results suggest that the control of lipid level by LPL in the bloodstream might be an important factor in T2DM pathogenesis in the Korean population.

Keywords: Asian continental ancestry group; diabetes mellitus, type 2; haplotypes; lipoprotein lipase; polymorphism, single nucleotide

Introduction

The normal glucose level in the blood is coordinately maintained by a complex interplay between the insulin responsiveness of key insulin target tissues (skeletal muscle, liver, adipose tissue, kidney, and brain) and glucose-stimulated insulin secretion by pancreatic β cells (Lowell and Shulman, 2005). In type 2 diabetes mellitus (T2DM), a failure in this coordination causes insulin resistance or insufficient insulin secretion by the pancreas, ultimately leading to high blood glucose levels (Alberti and Zimmet, 1998). Although genetic and environmental factors are believed to be closely related to T2DM, the exact mechanisms that underlie dysfunction of insulin uptake or secretion in T2DM are not clear.

Deviations from normal levels of fats in the blood (dyslipidemia) are consistently associated with T2DM. Dyslipidemia associated with T2DM is characterized by high levels of both plasma triglycerides (TGs) and very low density lipoprotein (VLDL) and low levels of high density lipoprotein (HDL) (Taskinen, 2003). Several lines of evidence

indicate that high TGs play a role in insulin resistance and T2DM (Mingrone *et al*., 1999; Yang *et al*., 2003a). TGs are present in circulating chylomicrons and VLDL particles and hydrolyzed by lipoprotein lipase (LPL) into nonesterified fatty acids and 2-monoacylglycerol for tissue utilization and HDL formation (Mead *et al*., 2002; de Vries *et al*., 2003; Kalmar *et al*., 2005). Thus, LPL has a central role in both VLDL and HDL metabolism. Deficiency or dysfunction of LPL has been associated with various diseases including atherosclerosis, chylomicronemia, obesity, Alzheimer's disease, and the dyslipidemia related to diabetes and insulin resistance (Mead *et al*., 2002). In T2DM, LPL activity is commonly deficient and contributes to the increased level of serum TGs and the decreased level of HDL (Taskinen, 1987; Klannemark *et al*., 2000; Kalmar *et al*., 2005).

Several studies have shown an association between genetic variations in *LPL* and lipid metabolism in T2DM patients (Marcais *et al*., 2000; Ma *et al*., 2003; Socquard *et al*., 2006). For example, an association between higher plasma TGs and lower HDL cholesterol levels and the H+ allele of *LPL Hin*dIII polymorphism has been reported in Chinese patients with early-onset T2DM (Ma *et al*., 2003). Several studies have also shown associations between polymorphisms in *LPL* and T2DM-related complications (Ukkola *et al*., 1995; Mattu *et al*., 2002; Solini *et al*., 2004; Ng *et al*., 2006; Javorsky *et al*., 2006). For instance, the LPL S447X polymorphism is associated with nephropathy in T2DM patients (Ng *et al*., 2006). However, to our knowledge, few studies have identified genetic variations in *LPL* that are directly associated with T2DM, although a recent study reported that a polymorphism in the promoter region (-G53C) of the LPL gene is protective against T2DM (Radha *et al*., 2007).

To identify *LPL* polymorphisms, we sequenced the *LPL* genomic region from 24 unrelated Koreans and detected 31 genetic variants. In an attempt to reveal the genetic variations in the *LPL* gene associated with T2DM, we analyzed nine selected single nucleotide polymorphisms (SNPs) in 944 unrelated Koreans (474 T2DM subjects and 470

Table 1. Clinical profile of study subjects.

Phenotype group	Phenotypes	Normal controls (470)	T2DM cases (474)	*P*-value
General	Age (years)	64.0 ± 2.9 (470)	64.6 ± 2.8 (474)	0.001
	Sex (male/female)	208/262 (470)	204/270 (474)	0.743
Obesity	BMI (kg/m^2)	23.3 ± 3.1 (470)	25.1 ± 3.1 (474)	< 0.0001
	WHR	0.908 ± 0.066 (470)	0.931 ± 0.067 (473)	< 0.0001
	Body fat (%)	26.3 ± 7.5 (321)	29.9 ± 7.1 (343)	< 0.0001
Hypertension	Systolic BP (mmHg)	121.0 ± 17.3 (470)	129.2 ± 18.4 (474)	< 0.0001
	Diastolic BP (mmHg)	75.4 ± 9.9 (470)	77.7 ± 10.3 (474)	0.01
Dyslipidemia	Triacylglyceride (mg/dl)	149.2 ± 71 (470)	200.3 ± 141.0 (474)	< 0.0001
	Total cholesterol (mg/dl)	180.5 ± 31.7 (470)	195.0 ± 42.5 (474)	< 0.0001
	HDL cholesterol (mg/dl)	44.3 ± 9.9 (470)	42.7 ± 9.8 (474)	0.011
	LDL cholesterol (mg/dl)	106.6 ± 27.7 (466)	115.7 ± 38.6 (443)	< 0.0001
Blood glucose	Glucose 0 (mg/dl)	74.5 ± 3.5 (470)	118.1 ± 35.3 (325)	< 0.0001
	Glucose 60 (mg/dl)	124.2 ± 36.0 (470)	249.5 ± 52.9 (268)	< 0.0001
	Glucose 120 (mg/dl)	98.7 ± 22.3 (470)	247.7 ± 62.0 (268)	< 0.0001
	AUCGLU (mg · hr/dl)	210.8 ± 40.2 (470)	430.2 ± 90.5 (268)	< 0.0001
	HbA1c (%)	5.5 ± 0.2 (470)	7.3 ± 1.5 (474)	< 0.0001
Blood insulin	Insulin 0 (μU/ml)	6.7 ± 6.6 (470)	8.8 ± 6.9 (325)	< 0.0001
	Insulin 60 (μU/ml)	30.6 ± 29.5 (470)	26.2 ± 28.2 (267)	0.053
	Insulin 120 (μU/ml)	22.6 ± 23.1 (470)	33.4 ± 40.7 (267)	< 0.0001
	AUCINS (μU · hr/ml)	45.2 ± 37.0 (470)	47.4 ± 44.1 (267)	0.499
	HOMA-IR	1.2 ± 1.2 (470)	2.6 ± 2.0 (325)	< 0.0001

Values are mean ± standard deviation (SD). Values in parentheses are the number of subjects assessed for each clinical profile. *P* values: T2DM cases versus normal controls. (BMI, body mass index; WHR, waist hip ratio; BP, blood pressure; Glucose 0, fasting plasma glucose level; Glucose 60 and 120, plasma glucose level 60 and 120 min after glucose ingestion, respectively; Insulin 0, fasting plasma insulin level; Insulin 60 and insulin 120, plasma insulin level 60 and 120 min after glucose ingestion, respectively; AUCGLU, glucose area under the curve; AUCINS, insulin area under the curve; HbA1C, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment-insulin resistance; HDL, high density lipoprotein; LDL, low density lipoprotein).

normal controls). Using the SNP genotyping data from these nine SNPs, we also analyzed the association of *LPL* SNPs with T2DM-related phenotypes such as obesity, blood pressure, dyslipidemia, blood glucose, and blood insulin in normal healthy controls. Our data demonstrated a significant association between one SNP in *LPL* intron 3 (+13836C > A) and T2DM. In addition, this polymorphism was also marginally associated with total cholesterol (TCHOL), high density lipoprotein cholesterol (HDLc), and log transformed glycosylated hemoglobin (log-HbA1C), when the false discovery rate (FDR) estimation to account for the multiple comparisons was not considered in the analysis.

Materials and Methods

Sequencing analysis for *LPL* SNP identification

Genetic variants of the LPL gene were identified by sequencing analysis of DNA from 24 unrelated Koreans. We sequenced the entire exons, 5' untranslated region (UTR), 3' UTR, up to several hundred base pairs of exon-intron boundary regions (including the splice junctions), and the promoter region (approximately 1.0 kb upstream from the transcriptional start site). The information for the gene and genomic DNA sequence of *LPL* was obtained from GenBank (http://www.ncbi/nlm.nih.gov/). Genomic regions targeted for sequencing were amplified by PCR from genomic DNA of 24 immortalized cell lines generated from unrelated Koreans. Primers for PCR were designed using the Primer3 program (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) (Rozen and Skaletsky, 2000) (Supplemental Table 1). PCR-amplified fragments were sequenced on both strands using an ABI Prism 3730 sequencer (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The PolyPhred program (http://www.droog.gs. wasington.edu/PolyPhred.html) was used to assemble the sequences and identify SNPs (Nickerson *et al*., 1997).

Subjects

Two community cohorts (Ansung and Ansan) in South Korea were initiated from 2001 as major projects for the Korean Health and Genome Study (KHGS). The Ansan cohort primarily represents an urban community, whereas the Ansung cohort represents a rural community. All participants in either cohort were 40–69 years old, had resided within the boarders of the survey area for at least 6 months, and were mentally and physically healthy to participate. Cohort examinations with participants' consent were accomplished biennially for the prospective studies. To date, 2,239 men and 2,779 women in Ansung, and 2,523 men and 2,497 women in Ansan have participated in the cohorts.

In this study, 474 unrelated individuals were recruited from 10,038 individuals in the Ansung-Ansan prospective community cohort as T2DM subjects according to World Health Organization criteria. A total of 470 unrelated individuals who had no history of diabetes, no first-degree relatives with diabetes, fasting plasma glucose level less than 126 mg/dl, plasma glucose level 120 min after glucose ingestion (glucose 120) less than 140 mg/dl, and HbA1C level less than 5.8% were recruited as normal control subjects from the cohort. To be eligible, normal control subjects also had to be free of medications for diabetes, hypertension, and dyslipidemia. Both T2DM and normal control subjects were $\geq$ 60 years of age. The clinical profiles of the study subjects are summarized in Table 1. The study was approved by the institutional review board of the Korean National Institute of Health. All subjects gave written informed consent.

Genotyping

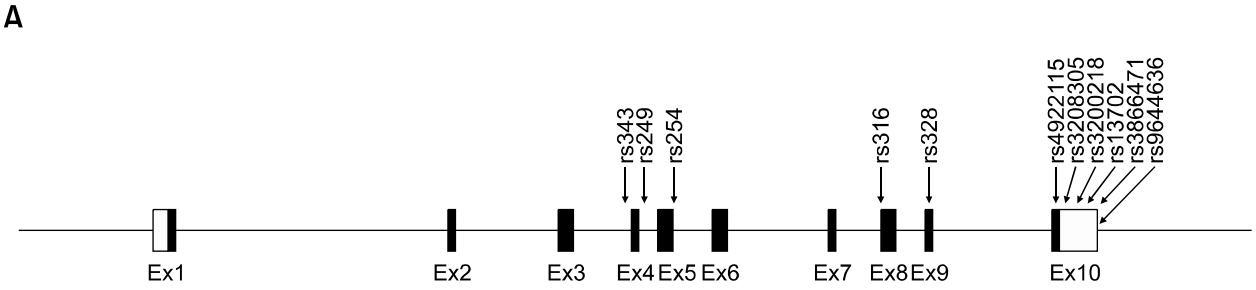
Among 31 *LPL* SNPs identified from 24 unrelated Korean subjects, 11 SNPs (rs343, +13836C>A; rs249, +14055T>C; rs254, +14946C>G; rs316, +21485C>A; rs328, +22773C>G; rs4922115, +25879G>A; rs3208305, +26697A>T; rs3200218, +27120A>G; rs13702, +27541T>C; rs3866471, +27718C>A; rs9644636, +27945T>G) were selected for subsequent large scale genotyping. Selection was based on their tagging status. Minor allele frequencies of 31 *LPL* SNPs obtained from sequencing results of 24 unrelated Korean subjects were used to calculate the pair-wise r^2 coefficient (Hao *et al*., 2007). SNPs were chosen as tagging SNPs if they were common SNPs (minor allele frequency $\geq$0.02) and were predicted by r^2 value $\geq$ 0.8. SNPs in exons or promoter regions were preferred in SNP selection (Supplemental Figure 1 and Supplemental Table 1) (Stram *et al*., 2003).

Genotyping of total 944 subjects was performed for 11 SNPs selected in *LPL* by an allelic discrimination assay using the TaqMan (Aplied Bio systems) reaction as described (Hurd *et al*., 2000). Duplicate genotyping for about 10% of samples was performed as a quality control. Only SNP genotyping results that satisfied the concordance rate in duplicates > 99% and the genotype

success rate > 97% were allowed for subsequent association analyses.

Statistics

Deviation from the expected Hardy-Weinberg frequency was examined with the chi-square (χ^2) test. Minor allele frequencies and heterozygosities in terms of functional location were calculated from each SNP genotyping result of study subjects. A haplotype block was generated by a linkage disequilibrium (D') plot of *LPL* in study subjects using HaploView v3.2 (http://www.broad.mit.edu/mpg/haploview/)(Barrett *et al*., 2005). Haplotypes were also inferred by HaploView analysis for given SNPs of the LPL gene. The associations of SNPs or haplotypes with T2DM between controls and T2DM patients were determined by logistic regression analysis while controlling for age, sex, and body mass index (BMI). The associations between SNPs or haplotypes and T2DM-related subphenotypes were determined by linear regression analysis while controlling for age, sex, and BMI. Only 470 normal control subjects were used for linear regression analyses of T2DM-related subphenotypes in order to exclude possible influences of T2DM treatment on the phenotypic values in the T2DM patients. Among several phenotypes (Table 1), the levels of TG and all blood glucose and insulin related phenotypes were transformed with logarithm to normalize their skewed distribution. Several clinical parameters of T2DM related phenotypes were compared between T2DM cases and control subjects, showing a statistically significant difference between the two groups except with respect to parameters such as age, insulin level 60 min after glucose ingestion (Insulin 60), and insulin area under the curve (AUCINS) (Table 1). The SAS statistical software package (SAS Institute Inc., Cary, NC) was used to perform



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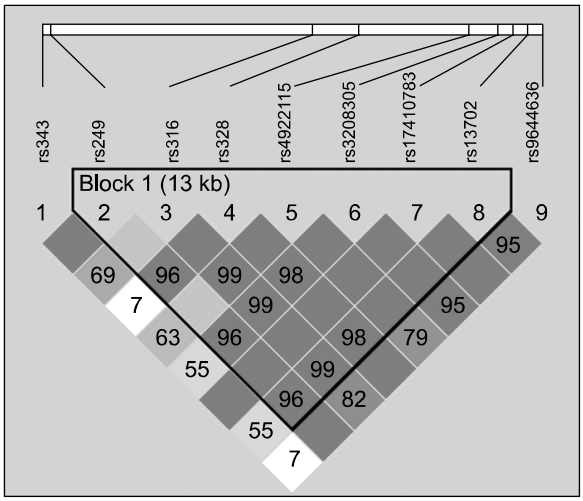

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Hap ID	Haplotype	Frequency
H1	TCCGAAA	0.65201
H2	TCCGAGA	0.12712
H3	TACATAG	0.08157
H4	CCGGTAG	0.07362
H5	TCGGTAG	0.05456

Figure 1. Gene map and haplotypes in *LPL*. (A) Gene map and SNPs in the LPL gene on chromosome 8p22. Black boxes denote coding exons, and white boxes denote 5' and 3' UTRs. The locations of the SNPs within the gene are shown by arrows. (B) Haplotype block generated by a linkage disequilibrium (D') plot of the LPL gene in subjects from the Ansung-Ansan cohort in Korea. All numbers in squares represent the D' value expressed as a percentile. All squares without numbers represent D' = 1. Plots were generated using HaploView v3.2. (C) Haplotypes of the LPL gene. Haplotypes with frequency > 0.05 are presented.

general statistical analyses. Statistical significance was determined at a two-tailed value of $P < 0.05$. For the multiple comparisons, the FDR estimation method was applied to the association analyses (Pounds and Cheng, 2006; Forner *et al*., 2008).

Korean SNP database

The information for most SNPs described in this study is available in the Korean SNP database (http://www.ksnp.ngri.re.kr/SNP/index.jsp) that was constructed at the Center for Genome Sciences (Korean National Institute of Health).

Results

We identified a total of 31 genetic variants of the LPL gene from 24 unrelated Korean subjects by sequencing (Supplemental Figure 1 and http://www.ngri.re.kr/SNP/index.jsp). Among them, 11 SNPs selected as tagging SNPs based on the criterion of pair-wise correlation coefficient, $r^2 \geq 0.8$ were genotyped from a total of 474 patient and 470 control subjects to discover genetic polymorphisms implicated in T2DM. The average genotyping success rate was 99.3% and a minimum was 97.1% for SNP rs328. Duplicate genotyping for 10% of the samples as a quality control showed no mismatches among duplicates, indicating high genotyping reproducibility.

Eleven SNPs of the LPL gene analyzed in this study were localized in introns (rs343, rs249, and rs254), the coding region (rs316 and rs328), 3'UTR (rs4922115, rs3208305, rs3200218, rs13702, and rs3866471) and 3' downstream region (rs9644636) on chromosome 8p22 (Figure 1A). In the normal control subjects, the genotype distributions of nine SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$) (Table 2) but two (rs254 and rs3866471) were not. Thus, in subsequent association studies we further analyzed the nine SNPs that were in Hardy-Weinberg equilibrium. The minor allele frequencies of these nine SNPs were 0.137, 0.074,

Table 2. Genotype distribution of *LPL* polymorphisms in type 2 diabetes mellitus (T2DM) cases (n = 474) and normal control (NC) subjects (*n* = 470) in the Korean population.

Locus[a]	Location	rs#	Group	Genotype			Frequency	Heterozygosity	HWE[b]
				CC	AC	AA			
+13836C>A	Intron 3	rs343	T2DM	373 (78.9%)	94 (19.9%)	6 (1.3%)	0.112	0.2391	0.4565
			NC	327 (69.9%)	131 (28.0%)	10 (2.1%)	0.161		
				TT	CT	CC			
+14055T>C	Intron 4	rs249	T2DM	402 (85.0%)	70 (14.8%)	1 (0.2%)	0.076	0.1351	0.0711
			NC	405 (86.7%)	57 (12.2%)	5 (1.1%)	0.072		
				CC	AC	AA			
+21485C>A	Exon 8 (T388T)	rs316	T2DM	403 (85.0%)	68 (14.3%)	3 (0.6%)	0.078	0.1529	0.7321
			NC	389 (83.1%)	76 (16.2%)	3 (0.6%)	0.088		
				CC	CG	GG			
+22773C>G	Exon 9 (S474S)	rs328	T2DM	347 (74.9%)	108 (23.3%)	8 (1.7%)	0.134	0.2279	0.6204
			NC	344 (75.8%)	101 (22.2%)	9 (2.0%)	0.131		
				GG	AG	AA			
+25879G>A	3' UTR	rs4922115	T2DM	396 (84.3%)	71 (15.1%)	3 (0.6%)	0.082	0.1622	0.5644
			NC	383 (82.0%)	81 (17.3%)	3 (0.6%)	0.093		
				AA	AT	TT			
+26697A>T	3' UTR	rs3208305	T2DM	288 (61.1%)	166 (35.2%)	17 (3.6%)	0.212	0.3419	0.5888
			NC	289 (61.8%)	155 (33.1%)	24 (5.1%)	0.217		
				AA	AG	GG			
+27120A>G	3' UTR	rs3200218	T2DM	345 (73.4%)	121 (25.7%)	4 (0.9%)	0.137	0.2396	0.2384
			NC	361 (77.0%)	104 (22.2%)	4 (0.9%)	0.119		
				TT	TC	CC			
+27541T>C	3' UTR	rs13702	T2DM	289 (61.1%)	167 (35.3%)	17 (3.6%)	0.212	0.3426	0.5978
			NC	288 (61.7%)	155 (33.2%)	24 (5.1%)	0.217		
				TT	GT	GG			
+27945T>G	3'down-stream	rs9644636	T2DM	339 (72.0%)	121 (25.7%)	11 (2.3%)	0.152	0.2708	0.6925
			NC	322 (69.0%)	133 (28.5%)	12 (2.6%)	0.168		

[a]The number and + sign in the locus indicate the nucleotide number downstream from translational start site; [b]*P* values of deviation from HWE among all subjects

0.083, 0.132, 0.088, 0.215, 0.128, 0.215, and 0.16 at positions +13836C>A (rs343), +14055T>C (rs249), +21485C>A (rs316), +22773C>G (rs328), +25879G>A (rs4922115), +26697A>T (rs3208305), +27120A>G (rs3200218), +27541T>C (rs13702), and +27945T>G (rs9644636), respectively. The observed heterozygosity of each polymorphic locus in the population is indicated in Table 2.

One haplotype block comprising seven SNPs (rs249, rs316, rs328, rs4922115, rs3208305, rs3200218, and rs13702) could be constructed due to very strong linkage disequilibrium (LD) among nine SNPs in the LPL gene (Figure 1B). We used HaploView v3.2 to generate the haplotype block (Barrett *et al*., 2005). Among all possible haplotypes from seven SNPs, five common haplotypes with frequencies greater than 5% were used for further analyses (Figure 1C).

To identify genetic variants of the *LPL* gene that are associated with T2DM, we performed logistic regression analysis for nine SNPs and five haplotypes. Logistic analyses controlling for age, sex, and BMI indicated a significant association (protective effect) of one SNP (rs343, +13826C>A) with T2DM ($P = 0.002$, OR = 0.64, co-dominant model; $P = 0.003$, OR = 0.62, dominant model). The association of SNP rs314 was still retained even after the multiple comparisons with FDR estimation ($P = 0.038$ in the codominant model). The other eight SNPs and all five haplotypes were not associated with T2DM (Table 3). It is noteworthy that rs343 was not in a haplotype block generated in this study due to weak LD with other SNPs (Figure 1B). The frequency of the +13836A genotype was higher in normal control subjects (30.1%) than in T2DM subjects (21.2%) (Table 2).

To gain insight into the genetic relevance of *LPL* polymorphisms to T2DM risk factors (such phenotypes related to obesity, hypertension, dyslipidemia, blood glucose, and blood insulin), linear regression analyses between *LPL* polymorphisms (including nine SNPs and five haplotypes) and risk factors

Table 3. Logistic analysis of *LPL* SNPs and haplotypes in T2DM and normal control subjects while controlling for age, sex, and BMI as covariates.

SNP ID or haplotype ID	Co-dominant		Dominant		Recessive	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
rs343	0.64 (0.48-0.85)	0.002	0.62 (0.46-0.85)	0.003	0.46 (0.15-1.35)	0.156
rs249	1.04 (0.73-1.50)	0.824	1.12 (0.76-1.64)	0.577	0.22 (0.02-2.08)	0.187
rs316	0.82 (0.58-1.16)	0.260	0.81 (0.56-1.17)	0.264	0.73 (0.13-3.96)	0.713
rs328	1.08 (0.82-1.44)	0.583	1.10 (0.80-1.52)	0.541	1.01 (0.37-2.73)	0.990
rs4922115	0.82 (0.58-1.16)	0.259	0.81 (0.56-1.17)	0.264	0.73 (0.13-3.97)	0.711
rs3208305	0.99 (0.79-1.26)	0.949	1.07 (0.81-1.41)	0.633	0.63 (0.32-1.25)	0.187
rs3200218	1.24 (0.92-1.67)	0.152	1.27 (0.93-1.74)	0.140	1.13 (0.27-4.78)	0.866
rs13702	0.99 (0.78-1.25)	0.937	1.07 (0.81-1.41)	0.638	0.63 (0.32-1.24)	0.177
rs9644636	0.91 (0.70-1.19)	0.492	0.89 (0.66-1.19)	0.420	1.03 (0.44-2.45)	0.939
H1	0.94 (0.77-1.15)	0.530	0.80 (0.52-1.23)	0.305	0.98 (0.74-1.28)	0.854
H2	1.24 (0.92-1.67)	0.152	1.27 (0.93-1.74)	0.139	1.13 (0.27-4.75)	0.872
H3	0.82 (0.58-1.17)	0.268	0.81 (0.56-1.18)	0.272	0.73 (0.13-3.98)	0.717
H4	1.04 (0.73-1.49)	0.821	1.14 (0.77-1.68)	0.515	0.19 (0.02-1.67)	0.133
H5	1.21 (0.80-1.83)	0.368	1.17 (0.76-1.81)	0.481	3.81 (0.36-40.12)	0.265

Table 4. Regression analyses of rs343 with total cholesterol (TCHOL), high density lipoprotein cholesterol (HDLc), and natural log transformed glycosylated hemoglobin (ln-HbA1C) while controlling for age, sex, and BMI as covariates in 470 normal controls.

Phenotype	C/C	C/R[a]	R/R	*P*-value		
				Co-dominant	Dominant	Recessive
TCHOL	327 (180.44 ± 31.57)[b]	131 (178.96 ± 31.90)	10 (202.30 ± 32.77)	0.606	0.939	0.038
HDLc	327 (44.14 ± 9.85)	131 (44.40 ± 10.04)	10 (50.10 ± 10.62)	0.179	0.403	0.036
log-HbA1C	327 (0.74 ± 0.02)	131 (0.74 ± 0.02)	10 (0.72 ± 0.03)	0.090	0.254	0.018

[a]C and R indicate common allele and rare allele, respectively; [b]Numbers in parentheses indicate mean ± standard deviation. Unit for both TCHOL and HDLc is mg/dl, and unit for log-HbA1C is %.

were performed in normal control subjects (Supplemental Tables 2 and 3). For these linear regression analyses, we only used 470 normal control subjects since treatment for T2DM may affect on the phenotypic values. Without considering multiple tests, significant associations were detected between rs343 (+13826C>A) and T2DM-related subphenotypes such as total cholesterol (TCHOL; $P = 0.038$, recessive model), high density lipoprotein cholesterol (HDLc; $P = 0.036$, recessive model), and log-transformed glycosylated hemoglobin (log-HbA1C; $P = 0.018$, recessive model) (Table 4). The relevance of HDLc and HbA1C to rs343 agrees with T2DM association of this polymorphism, whereas TCHOL is not consistent (see Discussion).

Three *LPL* SNPs, rs3208305, rs13702, and rs9644636, were not associated with any T2DM-related risk factors, whereas other *LPL* SNPs primarily had significant associations with blood glucose-related phenotypes (rs316 with log-glucose 0, and log-HbA1C; rs328 with log-glucose 60, and log-AUCGLU; rs4922115 with log-glucose 0, and log-HbA1C). In addition, rs316 was associated with BODY_FAT (obesity-related phenotype), and rs3200218 was associated with WHR (obesity-related phenotype) and TCHOL and LDLc (dyslipidemia-related phenotype) (Supplemental Table 2). Five haplotypes also showed significant associations with at least one T2DM-related risk factor (Supplemental Table 3).

When *LPL* SNPs were examined with the FDR estimation for the association with T2DM-related phenotypes, most *P* values corrected for the multiple comparisons were not in the statistically significant levels. However, it is noteworthy that our results still suggest the possible involvement of *LPL* SNPs in the pathogenesis of T2DM via T2DM-related phenotypes.

Discussion

LPL is one of the candidate genes of dyslipidemia (Hoffer *et al*., 1998; Julien *et al*., 1998; Pillarisetti and Saxena, 2003; Pruneta-Deloche *et al*., 2005) and hypertension (Williams *et al*., 1994; Yang *et al*., 2003b; Li *et al*., 2004; Chen *et al*., 2005) because its gene product is a major regulator of triglyceride clearance in the blood. LPL catalyzes the hydrolysis of triglycerides of circulating chylomicrons and VLDL, excesses of which are the potent causes of both disorders. T2DM is a common metabolic disorder for which the etiology is not yet clearly understood. About one-third of T2DM patients have hypertension and/or dyslipidemia, indicating the relationship between these disorders and T2DM (Tkac, 2005). High triglyceride levels in the blood (hypertriglyceridemia) are also seen in 20～60% of T2DM patients, and these levels are two to three times higher than in non-diabetic subjects (Taskinen, 1992; Klannemark *et al*., 2000). Furthermore, insulin resistance is associated with increased cholesterol synthesis and decreased cholesterol absorption in normoglycemic men (McPherson and Jones, 2003). Thus, we hypothesized that there may be a genetic link between the LPL gene and T2DM. Interestingly, *LPL* has been predicted to be one of nine potent T2DM genes by the application of seven independent computational disease gene prioritization methods to the analysis of 9,556 positional candidate genes for T2DM and the related trait, obesity (Tiffin *et al*., 2006).

As one way to address our hypothesis, we studied possible associations between T2DM and *LPL* polymorphisms. Our findings in this study demonstrate that individuals who have the A allele at *LPL* +13836 (rs343 on chromosome 8p22) appear to be protected against T2DM (Tables 2 and 3). Allele and genotype frequencies of *LPL* +13836C>A in the Korean population observed in this study were compared with those in other populations obtained from HapMap data (Table 5). Similar patterns of both frequencies were detected between Korean and Japanese populations rather than other populations, suggesting that *LPL* +13836C>A might be a useful marker for a T2DM association study in the Japanese population as well. The SNP rs343 (+13836C>A) is located in intron 3 on chromosome 8p22. Thus, it is likely that T2DM-associated *LPL* +13836C>A serves as a disease marker rather than a disease-causing SNP, although the possibility that this SNP may be a regulatory variant in modulating splicing (Baralle and Baralle, 2005) or

Table 5. Allele frequencies and genotype frequencies of *LPL* +13836C>A (rs343) among different population groups.

Population	Genotype frequency			Allele frequency	
	CC	AC	AA	C	A
European	0.814	0.186		0.907	0.093
African	0.967	0.033		0.983	0.017
Chinese	0.689	0.244	0.067	0.811	0.189
Japanese	0.756	0.200	0.044	0.856	0.144
Korean	0.744	0.239	0.017	0.863	0.137

Allele frequencies and genotype frequencies of all populations except Korean were obtained from HapMap data (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=343).

transcription factor binding (Liao *et al*., 2004) cannot be excluded.

Power analysis was performed using the Quanto program (Gauderman, 2002) to compute sample size or power for the case-control association study of the LPL gene. Assuming $\alpha = 0.05$, we had 89% power for *LPL* rs343 which had a minor allele frequency of 0.137 to detect an odds ratio of 0.64 in the protective component of the study in the co-dominant model. We also had 83% and 27% power to detect odds ratio of 0.62 and 0.46 in dominant and recessive models, respectively. The sample sizes of T2DM case for 80% power were 388, 405, and 1954 for the case-control association study of *LPL* rs343 in the co-dominant model, dominant model, and recessive model, respectively, indicating that the sample size was adequate for our study using both co-dominant and dominant models, but not sufficient using recessive model.

Not only genetic factors (such as family history) but also environmental factors (such as obesity, hypertension, dyslipidemia, blood glucose, and blood insulin) are considered to be crucial risk factors for T2DM. Therefore, understanding the genetic relationship between *LPL* and those T2DM-related risk factors might help unravel the etiology of T2DM. We analyzed nine *LPL* SNPs as well as five haplotypes from 470 normal control subjects to detect their association with several T2DM-related risk factors including phenotypes related to obesity, hypertension, dyslipidemia, blood glucose, and blood insulin. Without considering multiple tests, overall results of linear regression analyses controlling for age, sex, and BMI as covariates are summarized in Supplemental Tables 2 and 3.

Without considering multiple tests, the SNP rs343 was also marginally associated with dyslipidemia-related phenotypes (TCHOL and HDLc) as well as the blood gluose-related phenotype (log-HbA1C) (Table 4). Because glucose binds to hemoglobin to produce HbA1C, the level of HbA1C in circulating blood can be used as an indicator of the blood glucose level. Increased levels of blood glucose due to insulin resistance of tissues or the defect of pancreatic β-cell in insulin secretion are responsible for the high levels of HbA1C in the blood. Regression analyses of log-HbA1C with rs343 (+13836C>A) indicated that individuals who have a homozygous A allele have a lower level of HbA1C compared to individuals harboring the C allele (P = 0.018, recessive model; Table 4). This result is consistent with the protective effect of the A allele for T2DM (Table 3). The homozygous A allele also contributes to the high level of HDLc (P = 0.036, recessive model; Table 4). Considering the central role of LPL in HDL formation (Mead *et al*., 2002), this observation agrees with the beneficial genetic predisposition of the A allele with respect to T2DM (Table 3). The significant relevance of rs343 to TCHOL was also detected from regression analyses, in which a higher TCHOL level was evident in individuals homozygous for the A allele (P = 0.038, recessive model; Table 4). Considering the protective effect of the A allele- containing genotype for T2DM, this result does not appear to be consistent with the association result of rs343 with T2DM (Table 3). However, it is possible that this observed association might be affected by other various genetic and environmental factors or the complicated interactions of both factors.

In our study, the association of SNP rs343 with T2DM (P = 0.002 in the co-dominant model; P = 0.003 in the dominant model) was still significant at the $\alpha = 0.05$ level even after multiple tests by the FDR estimation, while the association with T2DM-related phenotypes was not retained after multiple tests possibly due to the insufficient sample size (n = 470). Thus, the replication study in the large population would be necessary to prove the association between rs343 and T2DM-related phenotypes. In conclusion, we report one *LPL* SNP (rs343) that has a significant association in the Korean population with T2DM. This finding supports our hypothesis that the *LPL* gene product, which controls lipid levels in the blood, might be one of the important genetic factors in determining the onset of T2DM in the Korean population.

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