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ORIGINAL ARTICLE

Association of lipoprotein lipase gene polymorphisms with obesity and type 2 diabetes in an Asian Indian population

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Aims: Lipoprotein lipase (LPL), a pivotal enzyme in lipoprotein metabolism, catalyzes the hydrolysis of triglycerides of very low-density lipoproteins and chylomicrons. Assuming that the variants in the promoter of the LPL gene may be associated with changes in lipid metabolism leading to obesity and type 2 diabetes, we examined the role of promoter variants (–T93G and –G53C) in the LPL gene in an urban South Indian population.
Methods: The study subjects (619 type 2 diabetic and 731 normal glucose-tolerant (NGT) subjects) were chosen from the Chennai Urban Rural Epidemiology Study, an ongoing population-based study in southern India. The polymorphisms were genotyped using polymerase chain reaction-restriction-fragment length polymorphism (PCR-RFLP). Linkage disequilibrium (LD) was estimated from the estimates of haplotypic frequencies.
Results: The two polymorphisms studied were not in LD. The –T93G was not associated with type 2 diabetes but was associated with obesity. 11.5% of the obese subjects (62/541) had the XG(TG + GG) genotype compared with 6.4% of the nonobese subjects (52/809; $P = 0.001$). The odds ratio for obesity for the XG genotype was 1.766 (95% CI: 1.19–2.63, $P = 0.005$). Subjects with XG genotype also had higher body mass index and waist circumference compared with those with TT genotype. With respect to G53C, subjects with the XC(GC + CC) genotype had 0.527 and 0.531 times lower risk for developing type 2 diabetes and obesity, respectively.
Conclusions: Among Asian Indians, the –T93G SNP of the LPL gene is associated with obesity but not type 2 diabetes, whereas the –G53C SNP appears to be protective against both obesity and type 2 diabetes.
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Introduction

It is well established that Asian Indians have a greater degree of insulin resistance[1–4] and increased susceptibility to diabetes[5–8] compared with Europeans. The 'Asian Indian phenotype' refers to the fact that Indians have increased body fat[4,9,10] and central body obesity despite low rates of obesity as defined by body mass index (BMI).[7–9] It is possible that some of the metabolic abnormalities can be explained by genetic factors. In this respect, the lipoprotein lipase (LPL) gene, which has been associated with important metabolic effects in several populations, is of great interest.[11–12]

LPL is an important target gene for the transcriptional factor peroxisome proliferator activated receptor γ (PPARγ).[13] Transcriptional activation of the LPL gene by fibrates and thiazolidinediones is mediated by PPAR-RXR heterodimers.[14] This activation occurs by the binding of PPARγ and the 9-*cis* retinoic acid receptor (RXR) heterodimers to the PPAR response element (PPRE) sequence (−169 TGCCCTTTCC CCC −157) in the promoter of the LPL gene.[15,16] Important *cis*-acting elements in the promoter of LPL gene have been identified that bind DNA-binding proteins and appear to confer basal and/or ligand-mediated LPL gene transcription.[17] DNA variants in the promoter of the LPL gene have been shown to be associated with changes in lipid metabolism leading to type 2 diabetes and its related traits.[12,18]

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Hence, this study was performed to evaluate the role of sequence variants in the promoter of LPL gene in relation to diabetes, obesity and related traits.

Patients and methods

This is a case-control study of diabetic and normal glucose tolerant (NGT) subjects selected from the Chennai Urban Rural Epidemiology Study (CURES), an ongoing epidemiology study conducted on a representative population (age $\geqslant$20 years) of Chennai (formerly Madras) in southern India. The methodology of CURES is published elsewhere.[19] Initially, 26 001 individuals were recruited based on a systematic random sampling technique. Self-reported diabetic subjects on drug treatment of diabetes were classified as 'known diabetic subjects'. The known diabetic subjects ($n=1529$) were invited to visit the centre for detailed studies. In addition, every 10th individual of the 26 001 individuals ($n=2600$) was invited to undergo oral glucose tolerance tests (OGTT) using 75 g oral glucose load (dissolved in 250 ml of water). Those who were confirmed by OGTT to have 2 h plasma glucose value $\geqslant$11.1 mmol/l (200 mg/dl) based on WHO consulting group criteria were labeled as 'newly detected diabetic subjects' and those with 2 h plasma glucose value $<$7.8 mmol/l (140 mg/dl) as having normal glucose tolerance (NGT).

As a pilot study, we screened 150 type 2 diabetic and 150 age- and sex-matched NGT subjects for mutations in the region comprising the PPRE sequence of the LPL gene by direct sequencing. The identified variants were studied further in a large number of study subjects (619 type 2 diabetic and 731 NGT subjects) chosen randomly from CURES. All study subjects were genotyped for the presence of the identified variants and these were correlated with clinical and metabolic parameters. Informed consent was obtained from all study participants, and the study was approved by the Institutional Ethics Committee.

The BMI was calculated using the formula, weight (kg)/height (m^2). The subjects chosen for the study were categorized as nonobese and obese according to the WHO (World Health Organization) Asia Pacific Guidelines (nonobese, BMI $<25\,kg/m^2$; obese, BMI$\geqslant 25\,kg/m^2$).[20] Biochemical analyses were done on Hitachi-912 Autoanalyser (Hitachi, Germany) using kits supplied by Roche Diagnostics (Mannheim, Germany). Fasting plasma glucose (GOD-POD method), serum cholesterol (CHOD-PAP method), serum triglycerides (GPO-PAP method) and high-density lipoprotein (HDL) cholesterol (Direct method-polyethylene glycol-pretreated enzymes) were measured by enzymatic methods. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald's formula. Glycated hemoglobin (HbA1C) was estimated by high-performance liquid chromatography using the Variant machine (Bio-Rad, Hercules, CA, USA). Serum insulin concentration was estimated using enzyme-linked immunosorbent assay (Dako, Glostrup, Denmark). Insulin resistance was calculated using the homeostasis assessment (HOMA-IR) model using the following formula:

$$\text{fasting insulin (mIU/ml)} \times \text{fasting glucose (mmol/l/22.5)}$$

Genomic DNA was isolated from the whole blood by the phenol-chloroform method of DNA extraction.[21] The polymerase chain reaction (PCR) fragment comprising the SNPs –T93G and –G53C were amplified using the following primers: forward, 5′-GCTGATCCATCTTGCCAATGTTA-3′; and reverse, 5′-CCGCGGTTTGGCGC TGAGCAAGT-3′. SNP –T93G was detected using the enzyme *Hae*III and –G53C was detected using *Bcl*I. The RFLP products were resolved by 3% agarose gel electrophoresis.

Statistical Package for Social Sciences (SPSS, Windows, version 10.0) was used for statistical analysis. The effects of the two polymorphisms on quantitative and categorical variables were analyzed. Allele frequencies were estimated by gene counting. Agreement with Hardy–Weinberg expectations was tested using a χ^2 goodness-of-fit test. One-way ANOVA or Student's *t*-test as appropriate was used to compare groups for continuous variables. χ^2 test was used to compare the proportions of genotypes or alleles. Risk variables were adjusted for age for comparison between different genotypes. Regression analysis was carried out using diabetes as dependent variable and the genotypes as independent variables. Haplotype frequencies were estimated using an Expectation-Maximization (EM) algorithm, which equates each genotypic frequency to the sum of the probabilities of all possible haplotypic configurations resulting in that genotype. Linkage disequilibrium (LD) was estimated from the estimates of haplotypic frequencies.

Results

Compared to NGT subjects, the diabetic subjects had significantly higher BMI (diabetes: 26.1 ± 4.2 vs NGT: $24.0\pm4.7\,kg/m^2$, $P=0.003$) and waist circumference (diabetes: 92.3 ± 9.4 vs NGT: 87.2 ± 11.4 cm, $P=<0.0001$). Systolic and diastolic blood pressure ($P<0.0001$), serum cholesterol ($P<0.0001$) and triglycerides ($P<0.0001$) were also significantly higher among subjects with type 2 diabetes compared with NGT subjects (Table 1).

Direct sequencing of the promoter region comprising the PPRE element of LPL gene revealed two variants, namely, a T$\rightarrow$G substitution at the nucleotide position -93 and a G$\rightarrow$C substitution at the nucleotide position -53. The frequencies of the –93G and -53C variant alleles were 4.3 and 4.2%, respectively, in the pilot study. The genotype–phenotype analysis in this pilot screening revealed that $-$T93G was associated with the various measures of obesity and $-$G53C was associated with type 2 diabetes and obesity.

The two identified promoter variants, $-$T93G and $-$G53C, were studied further in a large number of study subjects ($n=1350$) to examine the role of these variants in relation

to type 2 diabetes, obesity and its related traits. The two polymorphisms were not in linkage disequilibrium. The pairwise LD between the two loci (−93T/G and −53G/C) in the LPL gene was not significant at level 0.05, indicating that the loci are unlikely to be in LD. The pairwise LD values (D′) between −93 and −53 loci were 0.0159 ($P=0.7894$) in type 2 diabetic subjects and 0.1024 ($P=0.1282$) in NGT subjects. Nevertheless, two-sample *t*-tests were performed to evaluate the differences in haplotypic frequencies between type 2 diabetic and NGT subjects but no significant differences were found.

The genotype and allele frequencies of the sequence substitution in the promoter region at −93 bp were not statistically significant between the NGT and type 2 diabetic subjects (Table 2). Stratification of the study subjects based on obesity according to the Asia Pacific guidelines revealed that the frequency of the pooled genotype class TG and GG (hereafter denoted as 'XG') genotype of −93 T→G SNP was significantly higher among obese subjects (62/541, 11.5%) compared with nonobese subjects (52/809, 6.4%, $P=0.001$). Statistically significant differences were observed in the distribution of genotypes ($P=0.001$) and alleles ($P=0.002$) between obese and nonobese subjects. Regression analysis revealed the odds ratio for obesity for the individuals carrying the XG genotype to be 1.892 (95% confidence intervals (CI): 1.29–2.78, $P=0.001$). This remained significant even after adjustment for age, gender and type 2 diabetes (OR: 1.766 (95% CI: 1.19–2.63, $P=0.005$).

The association of this variant with obesity was further supported by obesity-related traits. The NGT subjects with XG genotype of −93 T→G SNP had higher BMI (TT, $23.4\pm4.6\,kg/m^2$ vs XG, $25.4\pm6.7\,kg/m^2$, $P=0.003$) and waist circumference (TT, 83.6 ± 11.7 cm vs XG, 87.3 ± 14.3 cm, $P=0.03$) values compared with those with TT genotype. Similarly, type 2 diabetic genotypes of −93 T→G SNP in NGT and type 2 diabetic subjects (data not shown).

The genotype ($P=0.008$) and allele ($P=0.044$) frequencies of −53 G→C promoter polymorphism of the LPL gene were statistically significant between NGT and type 2 diabetic subjects and the XC appeared to be protective against both obesity and diabetes (Table 3). Regression analysis revealed the odds ratio (adjusted for age, sex and BMI) for diabetes for XC (GC + CC) genotype to be 0.527 (95% CI: 0.29–0.96, $P=0.036$). To study the role of this variant in obesity-related traits, the study subjects were stratified based on BMI. The genotype and allele frequencies of −53 G→C variant were statistically significant between the obese and nonobese subjects (Table 3). The odds ratio for obesity for the XC genotype was 0.531 (95% CI: 0.30–0.94, $P=0.031$) and this remained significant even after adjustment for age, sex and diabetes (OR: 0.561 (95% CI: 0.03–0.99, $P=0.05$)). None of

Table 1 Clinical and biochemical characteristics of the study subjects

	NGT subjects (n = 731)	*Type 2 diabetic subjects* (n = 619)	P-*value*
Age (years)	49 ± 12	43 ± 13	<0.0001
BMI (kg/m^2)	24.0 ± 4.7	26.1 ± 4.2	<0.0001
Waist (cm)	87.2 ± 11.4	92.3 ± 9.4	<0.0001
Systolic blood pressure (mm Hg)	120 ± 17	128 ± 18	<0.0001
Diastolic blood pressure (mm Hg)	75 ± 10	77 ± 11	<0.0001
Fasting plasma glucose (mmol/l)	4.8 ± 0.5	9.1 ± 3.8	<0.0001
HbA1c (%)	5.7 ± 0.5	8.9 ± 2.0	<0.0001
Fasting serum insulin (μIU/ml)	9.0 ± 6.1	11.4 ± 7.4	<0.0001
HOMA-IR	1.8 ± 1.3	4.3 ± 3.3	<0.0001
Serum cholesterol (mg/dl)	176 ± 37	201 ± 42	<0.0001
Serum triglycerides (mg/dl)	112 ± 65	180 ± 130	<0.0001
HDL cholesterol (mg/dl)	43 ± 10	42 ± 9	<0.0001
LDL cholesterol (mg/dl)	110 ± 32	122 ± 38	<0.0001

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment; LDL, low-density lipoprotein; NGT, normal glucose-tolerant.

Table 2 Association of −93 T→G promoter polymorphism of the LPL gene with type 2 diabetes and obesity

Study subjects classified based on diabetes status			
	NGT subjects (n = *731*)	*Type 2 diabetic subjects* (n = *619*)	P-*value*
TT	677 (92.6%)	559 (90.3%)	
XG (TG+GG)	54 (7.4%)	60 (9.7%)	0.15
Minor allele frequency (G)	0.04	0.05	0.16
Study subjects classified based on BMI			
	Non-obese subjects (BMI <25) (*n* = 809)	Obese subjects (BMI ⩾ 25) (*n* = 541)	
TT	757 (93.6%)	479 (88.5%)	0.001
XG (TG+GG)	52 (6.4%)	62 (11.5%)	
Minor allele frequency (G)	0.05	0.06	0.002
Unadjusted odds ratio			
TT vs XG	1.892 (95% CI: 1.29–2.78)		0.001
Odds ratio adjusted for age, sex and diabetes			
TT vs XG	1.766 (95% CI: 1.19–2.63)		0.005

Abbreviations: BMI, body mass index; CI, confidence interval; LPL, low-density lipoprotein; NGT, normal glucose-tolerant.

the clinical and biochemical parameters were statistically significant between the genotypes of this promoter SNP in NGT and type 2 diabetic subjects.

Discussion

The important finding of this study is that the −93 T→G SNP of the LPL gene is associated with the increased risk of developing obesity, whereas the −53 G→C SNP is associated with the reduced risk of developing obesity and type 2 diabetes in this South Indian population (Figure 1). This is one of the first reports investigating the association of these polymorphisms with type 2 diabetes and obesity in Asian Indians.

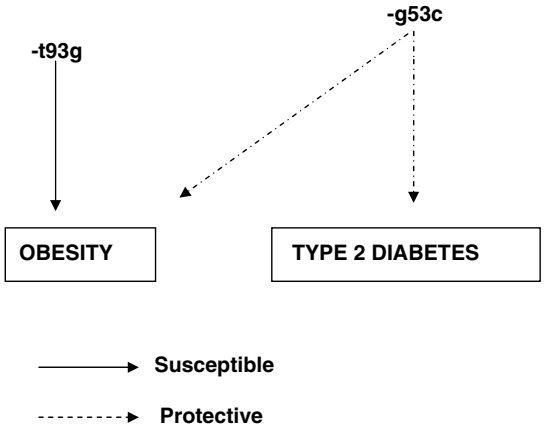

Figure 1 Schematic representation of association of the studied polymorphism.

Subjects with the XG genotype of −93 T→G SNP were at 1.766 times higher risk of developing obesity compared with those with TT genotype. Furthermore, subjects with XG genotype had significantly higher BMI and waist circumference values compared with those with TT genotype. The T→G substitution at nucleotide −93 lies 2 bases 5′ to a totally conserved inverted GA box (5′-CCTCCCCCC-3′, nt −91 to −83) in the LPL promoter.[22] This motif binds the transcription factors Sp1 and Sp3 and is essential for LPL promoter activity. The basic recognition unit of the Sp family transcription factors is a motif with a consensus sequence 5′-(G/T)GGGCGGPu-Pu-Py-3′, known as a GC box. Furthermore, GA and GT boxes (or their inverted forms, CT and CA elements) can also bind Sp proteins with similar specificity in the promoters of LPL gene. It is known that the nucleotides flanking this conserved element may influence binding affinity of Sp1 and Sp3.[22] Furthermore, this variant lies within the region of LSE-2, identified by Tanuma *et al*.[23] as a potential LSE-2, and hence it is possible that this T-to-G change at −93 results specifically in the disruption of LSE-2, leading to increased expression of LPL.

Functional studies using luciferase gene as a reporter have shown that the −93G promoter had 24% higher activity than the −93T in a rat smooth muscle cell line.[24] Using a human adrenal cell line, NCIH295, which has been shown to secrete LPL,[25] a similar (18%) increase in luciferase activity was seen with the G allele.[24] Although this variant was not associated with altered lipid profile in this study population, the association of this promoter variant with different measures

Table 3 Association of -53 G→C promoter polymorphism of the LPL gene with type 2 diabetes and obesity

Study subjects classified based on diabetes status			
	NGT subjects (n = 731)	*Type 2 diabetic subjects (n = 619)*	P-*value*
GG	680 (93%)	597 (96%)	0.008
XC	51 (7%)	22 (4%)	
Minor allele frequency (G)	4%	3%	0.044
Unadjusted odds ratio			
GG vs XC	0.486 (95% CI: 0.28–0.84)		0.009
Odds ratio adjusted for age, sex and BMI			
GG vs XC	0.527 (95% CI: 0.29–0.96)		0.036
Study subjects classified based on obesity			
	Non-obese subjects (BMI <25) (*n* = 809)	Obese subjects (BMI ⩾25) (*n* = 541)	0.039
GG	752 (93%)	519 (96%)	
XC	57 (7%)	22 (4%)	
Minor allele frequency (G)	5%	3%	0.024
Unadjusted odds ratio			
GG vs XC	0.531 (95% CI: 0.30–0.94)		0.031
Odds ratio adjusted for age, sex and diabetes			
GG vs XC	0.561 (95% CI: 0.03–0.99)		0.050

Abbreviations: BMI, body mass index; CI, confidence interval; LPL, low-density lipoprotein.

of obesity suggests it could be an important contributor to obesity and type 2 diabetes in this ethnic group.

The −53 G→C promoter polymorphism of the LPL gene was found to be a rare variant in Dutch, Black and Chinese populations.[12] However, in this study population, this variant was significantly associated with the reduced risk of developing obesity and type 2 diabetes. Subjects with the XC genotype had 0.527 times and 0.531 times lower risk for developing type 2 diabetes and obesity. None of the clinical and biochemical parameters were statistically significant between the genotypes of this promoter SNP in NGT and type 2 diabetic subjects. This may be because of the lower frequency of the heterozygous variants in this study.

The −G53C substitution is located between the CCAAT (nt −65 to −61) and Oct-1 (nt −46 to −39) motifs and within a putative Ca^{2+}-responsive element, 5′-TGAGGTTT-3′ (nt. −54 to −47), similar to the (TGACGTTT) of the c-fos gene promoter.[26] DNase I protection assays have revealed a footprint in the region extending approximately from nt −52 to −35.[27] The −53 substitution may affect the binding of Oct-1 to the octamer site or some of other transcription factors to the putative Ca^{2+}-responsive element. Hence, the location of this variant and its association with type 2 diabetes and obesity seems to reveal that this SNP could have some functional role in this study population.

It has been shown that there is significant diversity in allele frequencies at many autosomal loci within different castes in South India.[28] To address the issue of population stratification, a cross-validation using genomic controls was done.[29] A case-control study at six unlinked marker loci believed to be unrelated to the disease under study but known to have allelic diversity among different populations was carried out. The allele frequency difference between cases and controls was not statistically significant at any of the six loci studied. This indicates that the findings in this study are not likely to be an artifact of population sub structuring.

In a recent study by Radha *et al*.,[30] the *Hind*III and Ser447 Ter polymorphisms of the LPL gene were shown to be associated with low HDL cholesterol and hypertriglyceridemia in Asian Indians. This study shows further evidence of the importance of the LPL gene in relation to type 2 diabetes and obesity in this ethnic group. Taken together, the findings in the present study and our earlier study[30] thus confirm that the LPL gene polymorphism is associated with several components of the metabolic syndrome in Asian Indians. However, the findings will need to be confirmed in replication studies and, most importantly, will need to be supported by mechanistic studies.

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