Association Study of *Apolipoprotein L-I* Lys166Glu and Ile244Met Gene Variants with Obesity in Chinese Subjects

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Aims: ApoL-I is a newly found component of high-density lipoprotein, and has a potential role in lipid metabolism. This study was mainly to examine the possible association of the *ApoL-I* gene polymorphism with obesity and plasma lipid levels in the Chinese population. **Methods:** Three hundred nineteen Han Chinese (109 obese and 210 nonobese control subjects) in Chengdu area were studied using polymerase chain reaction-restriction fragment length polymorphism analysis. **Results:** The Lys and Glu allele frequencies of the *apoL-I* gene at the Lys166Glu site in obese and nonobese control groups were 0.830, 0.170 and 0.814, 0.186, respectively. The Ile and Met allele frequencies of the gene at Ile244Met site in the obese and the control groups were 0.844, 0.156 and 0.831, 0.169, respectively. The 166Glu and 244Met allele frequencies of the two polymorphisms in obese subjects were not different from those in the controls, respectively ($p > 0.05$). In the nonobese group, subjects with genotype Lys/Lys at the Lys166Glu site or Ile/Ile at the Ile244Met site had a higher serum mean concentration of triglyceride (TG) as compared with those of corresponding Glu or Met allele carriers ($p < 0.05$). In addition, serum apoC-III and apoE levels were affected by genotypes of Lys166Glu and Ile244Met polymorphisms, respectively, in the control group (all $p < 0.05$). **Conclusion:** The current study, for the first time, provides evidence that Lys166Glu and Ile244Met polymorphisms in *apoL-I* gene are associated with TG, apoC-III, and apoE levels in nonobese Chinese. However, these polymorphisms are not associated with obesity in the population.

Introduction

OBESITY, A HIGHLY prevalent and chronic disorder, is strongly associated with an adverse dyslipidemic profile and increased risks including diabetes, elevated blood pressure, and coronary artery disease (Kissebah and Krakower, 1994; Lemieux and Després, 1994). Although this relationship has been described in detail, its pathophysiological basis remains unclear. Several studies have suggested a biologic cascade in which enhanced plasma flux of free fatty acid from visceral adipocytes in the basal condition, often accompanied by its poor suppressibility by insulin in the nonfasting state, could result in overproduction of triglyceride (TG) and apolipoprotein B (apoB), thus giving rise to formation of the small, dense subfractions of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) (Kissebah and Peiris, 1989; Björntorp, 1990; Kissebah and Krakower, 1994; Bergman *et al.*, 2001). Furthermore, insulin resistance associated with compensatory hyperinsulinemia may directly influence hepatic

production of these lipid-lipoprotein subcomponents to augment this process. Prospective twin studies, familial segregation and intercorrelation analyses, have supported the existence of genetic factors, which influence expression of an adverse lipid profile (Després *et al.*, 1992; Mitchell *et al.*, 1996).

Many of the environmental factors that influence the risk of obesity are known, but genetic factors predisposing to obesity are poorly understood. Numerous gene variants have been reported to be associated with obesity or obesity-related traits (Perusse *et al.*, 2005), but only a few of the associations have been replicated in other populations (Verdich *et al.*, 2004; Perusse *et al.*, 2005).

ApoL-I, a member of a multigene family (apoL-I to apoL-IV) on human chromosome 22 (Duchateau *et al.*, 2001; Page *et al.*, 2001), is a 42 kDa protein that is found in the plasma mainly associated with the high-density lipoprotein (HDL) component (Duchateau *et al.*, 1997). The functions of apoL-I are currently not clear. Duchateau *et al.* (2000) reported that ApoL-I protein levels were positively associated with TG and

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cholesterol levels in normolipidemic, hyperlipidemic, and diabetic subjects. Like other members in the *apoL* gene family, the *apoL-I* gene also has putative sterol response elements (sterol regulatory element) in its promoter region, and conserved amphipathic helices in its sequence (Duchateau *et al.*, 2001; Page *et al.*, 2001). Therefore, it is suggested that *apoL-I* is closely related to lipid metabolism (Duchateau *et al.*, 2001; Page *et al.*, 2001). In addition, Vanhamme *et al.* recently found that *apoL-I* was a trypanosome lytic factor in human serum, suggesting that it may also play an important role in biology (Vanhamme *et al.*, 2003).

In one study Albert *et al.* detected the relationship between Arg166Glu polymorphism of *apoL-I* gene and coronary heart disease (CHD) with low HDL levels, and the result indicated that the variation of Arg166Glu was associated with increased *apoL-I* level accompanied by elevated fasting glucose (FG) in these patients, but not with changes of serum TG, total cholesterol (TC), and HDL-cholesterol (HDL-C) levels (Albert *et al.*, 2005). Our recent study showed that Lys166Glu and Ile244Met polymorphisms in the *apoL-I* gene are associated with TG levels in subjects with endogenous hypertriglyceridemia in Chinese (Li *et al.*, 2009).

However, the association between obesity and *apoL-I* polymorphisms has not been evaluated. Due to the patients with obesity being characterized with dyslipidemia including high TGs and low HDL-C levels accompanied by abnormal apolipoprotein levels, it is a matter of significance to investigate the influence of *apoL-I* polymorphisms on the lipid and apolipoprotein concentrations in the obese population. Therefore, in Chinese, we determined the genotypes of Arg166Glu and Ile244Met polymorphisms in the *apoL-I* gene in obese patients and nonobese controls, and examined whether the *apoL-I* genetic variations confer the association with obesity or the lipid levels in the populations.

Materials and Methods

Subjects

For this study, blood samples were taken from 319 volunteers (200 men, 119 women, aged 55.09 ± 10.72 years) who were taking part in a routine health examination at three hospitals of the Sichuan University and Sichuan Normal University in Chengdu, China. All these subjects were current or retired staff members of the Universities, and apparently healthy and unrelated individuals. After a 12- to 14-h overnight fast, the blood from each individual was collected and analyzed for serum concentrations of lipids, lipoproteins, and apolipoproteins. Body mass index (BMI) was calculated from height and weight measurements using the formula: $BMI = \text{body weight}/(\text{height})^2$ in kg/m^2 . According to the World Health Organization guidelines for Asians, individuals with $BMI \geq 25 \text{ kg}/\text{m}^2$ are classified as obese (WHO Expert Consultation, 2004). The obese group ($n=109$) presented with obese characteristics ($BMI \geq 25 \text{ kg}/\text{m}^2$) and comprised 74 men and 35 women. The control group ($n=210$) of subjects with $BMI < 25$ comprised 126 men and 84 women. All of the subjects were Han Chinese. Subjects with internal complications, such as CHD, diabetes mellitus, and hypertension, were excluded. All study participants gave their informed consent and the study was approved by our institutional review board.

Quantitative analysis

Serum TC and TGs were measured by an enzymatic method (kits; Zhong Sen Co.). HDL-C was determined after sodium phosphotungstate/magnesium chloride precipitation of LDL by polyvinyl sulfate. Serum apoA-I, apoA-II, apoB-100, apoC-II, apoC-III, and apoE were quantified by radial immunodiffusion kit developed by our laboratory (Bingwen, 1995).

DNA extraction and genotyping

Genomic DNA was isolated from 500 μL peripheral blood according to the method of Erlich (1989). The polymerase chain reactions (PCRs) were performed in a final volume of 25 μL containing 10% $10 \times$ PCR buffer, 2 mM MgCl_2 , 0.2 mM dNTPs, 0.6 U Taq DNA polymerase (MBI Fermentas), and sense and antisense primers, 0.5 μM of each. We used 100 ng DNA per PCR. Genotypes for the polymorphic *ApoL-I* markers Lys166Glu and Ile244Met were determined as previously described (Duchateau *et al.*, 2001).

Statistical analysis

Allele frequencies of *apoL-I* polymorphism were estimated by gene counting. Hardy-Weinberg equilibrium was tested in cases and controls by a chi-square test. Allele and genotype frequencies were compared between cases and controls by chi-square analysis. To evaluate the effect of *apoL-I* polymorphism on the variation of quantitative variables of lipid and apolipoprotein, an ANOVA was carried out. Adjustment for age and sex was performed by an analysis of covariance. Statistical analyses were performed using SPSS statistical software.

Results

The lipid and lipoprotein profiles for both obese and control groups are shown in Table 1. Serum TC, HDL-C, and apoA-I were significantly lower in the obese group compared with those in the control group ($p < 0.05$ or $p < 0.01$).

TABLE 1. CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF CONTROL AND OBESE GROUPS

	BMI (<25.00) n = 210	BMI (≥ 25.00) n = 109
Age (years)	55.77 ± 9.70	55.86 ± 9.95
Sex (M/F)	126/84	74/35
TG (mM) ^a	2.01 ± 1.73	2.24 ± 1.38
TC (mM)	5.61 ± 1.07	5.36 ± 0.89^b
HDL-C (mM)	1.33 ± 0.36	1.12 ± 0.31^c
LDL-C (mM)	3.34 ± 1.06	3.15 ± 0.83
apoA-I (mg/dL)	130.09 ± 29.73	120.48 ± 21.15^c
apoA-II (mg/dL)	28.4 ± 4.61	28.06 ± 4.89
apoB-100 (mg/dL)	88.52 ± 20.72	87.80 ± 15.73
apoC-11 (mg/dL)	6.26 ± 4.27	6.62 ± 3.09
apoC-111 (mg/dL)	14.30 ± 6.43	15.28 ± 6.79
apoE (mg/dL)	5.22 ± 2.36	5.21 ± 1.69

^aLog-transformed TG values were used in the analysis.

Compared with control group. ^b $p < 0.05$, ^c $p < 0.01$.

TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; BMI, body mass index.

TABLE 2. GENOTYPE AND ALLELE FREQUENCY FOR THE LYS166GLU AND ILE244MET SITES OF *apoL-I* GENE IN CONTROL AND OBESE GROUPS

	Frequency		
	BMI < 25 (n=210)	BMI ≥ 25 (n=109)	Total (n=319)
Lys166Glu			
Genotype			
Lys/Lys	0.667 (140)	0.679 (74)	0.671 (214)
Lys/Glu	0.295 (62)	0.303 (33)	0.298 (95)
Glu/Glu	0.038 (8)	0.018 (2)	0.031 (10)
Allele			
Lys	0.814 (342)	0.830 (181)	0.820 (523)
Glu	0.186 (78)	0.170 (37)	0.180 (115)
Ile244Met			
Genotype			
Ile/Ile	0.700 (147)	0.707 (77)	0.702 (224)
Ile/Met	0.262 (55)	0.275 (30)	0.267 (85)
Met/Met	0.038 (8)	0.018 (2)	0.031 (10)
Allele			
Ile	0.831 (349)	0.844 (184)	0.835 (533)
Met	0.169 (71)	0.156 (34)	0.165 (105)

The distribution of the Lys166Glu and Ile244Met polymorphisms agrees with Hardy–Weinberg equilibrium in both control and obese groups.

The PCR-amplified fragment (containing Lys166Glu and Ile244Met polymorphic sites) from each sample was digested with restriction enzyme *HindIII* for Lys166Glu site and *NcoI* for Ile244Met site, and analyzed by agarose gel electrophoresis (Li *et al.*, 2009).

Genotypes of Lys166Glu and Ile244Met polymorphisms were found to be in Hardy–Weinberg equilibrium in both the obese and control groups, respectively. The frequency data are presented in Table 2. The mutations underlying Lys166→Glu (AAG→GAG) and Ile244→Met (ATA→ATG) were analyzed by PCR-restriction fragment length polymorphism analysis. The Lys and Glu allele frequencies of the *apoL-I* gene at codon 166 in obese and control groups were 0.830, 0.170

and 0.814, 0.186, respectively; the Ile and Met allele frequencies of the gene at codon 244 in obese and the control groups were 0.844, 0.156 and 0.831, 0.169, respectively. The 166Glu and 244Met allele frequencies of the two polymorphisms in obese subjects were not different from those in the controls, respectively ($p > 0.05$).

To assess the possible impact of polymorphic sites in the *apoL-I* gene on lipid metabolism, we analyzed the serum lipids and lipoprotein levels in different genotypes of codon 166 and codon 244 polymorphisms in both control and obese groups (Tables 3 and 4). Because of the relatively small number of subjects with Glu/Glu (codon 166) or Met/Met (codon 244) genotype, heterozygotes and homozygotes for the Glu allele or Met allele were pooled in these analyses, respectively.

In the nonobese group, subjects with genotype Lys/Lys at Lys166Glu site had a higher serum mean concentration of TG as compared with those of Glu allele carriers (2.24 ± 2.00 mM vs. 1.54 ± 0.80 mM, $p < 0.05$), and subjects with genotype Ile/Ile at the Ile244Met site had a higher serum mean concentration of TG as compared with those of Met allele carriers (2.23 ± 1.96 mM vs. 1.50 ± 0.80 mM, $p < 0.05$). In addition, in this group, subjects with Lys/Lys or Ile/Ile genotype had a higher serum mean concentration of apoC-III and apoE, as compared with those of corresponding Glu or Met allele carriers. The above differences were still significant after adjustment for sex and BMI concomitant. There were no significant changes in lipid and apolipoprotein levels in obese subjects (Tables 3 and 4).

Discussion

The human *apoL* gene polymorphisms were first reported by Duchateau *et al.* in Caucasian population (Duchateau *et al.*, 2001), and recently we also demonstrated the presence of the polymorphisms in the Han Chinese, the largest population group in the world (Li *et al.*, 2009). The genotype and allele frequencies of *apoL-I* Lys166Glu and Ile244Met polymorphisms were not different between the two populations, suggesting that the difference in levels of diversity in the gene variants might be small among some populations.

TABLE 3. MEAN VALUES ($\bar{x} \pm SD$) OF SERUM LIPID AND APOLIPOPROTEIN LEVELS FOR LYS166GLU SITE OF *apoL-I* GENE IN CONTROL AND OBESE GROUPS

	BMI (<25.00)		BMI (≥25.00)	
	Lys/Lys (n=140)	Glu allele (n=70)	Lys/Lys (n=74)	Glu allele (n=35)
TG (mM) ^a	2.24±2.00	1.54±0.80 ^b	2.34±1.43	2.02±1.25 ^b
TC (mM)	5.65±1.09	5.51±1.04	5.36±0.85	5.37±0.99
HDL-C (mM)	1.33±0.38	1.34±0.33	1.12±0.28	1.13±0.38
LDL-C (mM)	3.29±1.11	3.46±0.95	3.13±0.78	3.19±0.94
apoA-I (mg/dL)	129.75±21.38	130.76±41.89	119.35±19.59	122.63±24.29
apoA-II (mg/dL)	28.27±4.30	28.77±5.21	28.26±5.00	27.64±4.72
apoB-100 (mg/dL)	90.08±20.73	85.37±20.48	89.49±13.80	84.24±18.91
apoC-11 (mg/dL)	6.50±3.54	5.77±5.45	6.77±3.17	6.30±2.95
apoC-111 (mg/dL)	15.19±6.89	12.53±4.68 ^b	15.40±6.89	15.04±6.65
apoE (mg/dL)	5.49±2.71	4.69±1.25 ^b	5.26±1.70	5.08±1.70

The relationships between the genotype and plasma TG, apoC-11, and apoC-111 levels remained significant even with adjustment for age and sex, whereas other values were similar after these adjustments.

^aLog-transformed TG values were used in the analysis.

Compared with Lys/Lys genotype carriers in the same group, ^b $p < 0.05$.

TABLE 4. MEAN VALUES ($\bar{x} \pm SD$) OF SERUM LIPID AND APOLIPOPROTEIN LEVELS FOR Ile244Met SITE OF *apoL-I* GENE IN CONTROL AND OBESE GROUPS

	BMI (<25.00)		BMI (≥ 25.00)	
	Ile/Ile (n=147)	Met allele (n=63)	Ile/Ile (n=77)	Met allele (n=32)
TG (mM) ^a	2.23 $\pm$ 1.96	1.50 $\pm$ 0.80 ^b	2.33 $\pm$ 1.44	2.01 $\pm$ 1.21
TC (mM)	5.68 $\pm$ 1.07	5.43 $\pm$ 1.05	5.39 $\pm$ 0.88	5.29 $\pm$ 0.94
HDL-C (mM)	1.32 $\pm$ 0.38	1.35 $\pm$ 0.31	1.13 $\pm$ 0.28	1.12 $\pm$ 0.39
LDL-C (mM)	3.33 $\pm$ 1.10	3.37 $\pm$ 0.96	3.15 $\pm$ 0.79	3.14 $\pm$ 0.94
apoA-I (mg/dL)	130.92 $\pm$ 31.92	128.15 $\pm$ 23.97	119.54 $\pm$ 19.94	122.47 $\pm$ 24.03
apoA-II (mg/dL)	28.17 $\pm$ 4.25	28.05 $\pm$ 5.36	28.30 $\pm$ 4.91	27.48 $\pm$ 4.89
apoB-100 (mg/dL)	90.31 $\pm$ 20.48	84.31 $\pm$ 20.84	89.73 $\pm$ 14.21	83.16 $\pm$ 18.32
apoC-III (mg/dL)	6.43 $\pm$ 3.47	5.85 $\pm$ 5.73	6.76 $\pm$ 3.18	6.28 $\pm$ 2.89
apoC-III (mg/dL)	15.06 $\pm$ 6.90	12.51 $\pm$ 4.74 ^b	15.42 $\pm$ 7.08	14.94 $\pm$ 6.12
apoE (mg/dL)	5.47 $\pm$ 2.65	4.63 $\pm$ 1.30 ^b	5.28 $\pm$ 1.76	5.03 $\pm$ 1.54

The relationships between the genotype and plasma TG levels remained significant even with adjustment for age and sex, whereas other values were similar after these adjustments.

^aLog-transformed TG values were used in the analysis.

Compared with Ile/Ile genotype carriers in the same group, ^b $p < 0.05$.

This study first detected the relationship of *apoL-I* polymorphisms with obesity and related traits in a population. Our work does not provide evidence in favor of *apoL-I* Lys166Glu and Ile244Met being a candidate gene for conferring genetic susceptibility to obesity in a South West Chinese population.

Previously, Albert *et al.* (2005) showed that apoL-I is positively associated with TGs in CAD patients with low HDL, and there was a tendency toward higher apoL-I levels, accompanied by elevated FG, in the 166 Glu allele homozygotes, but no genotype-dependent effect of Lys166Glu site on TG levels. Our nonobese (control) subjects showed a significantly higher TG levels in 166 Lys allele homozygotes, as well as 244 Ile allele homozygotes, suggesting an association of these polymorphisms with TG levels in our control population. The possible mechanism of the effect of *apoL-I* genotypes on serum TG concentrations was not clear. It might be by the indirect role of apoL-I, since apoL-I is not the component of apoB-containing lipoprotein. It can be speculated that apoL-I might, like other HDL-containing apolipoproteins as ApoA-I and AIV, modulate the activity of lipoprotein lipase resulting in impaired TG metabolism in TG-containing lipoproteins (mainly VLDL). The variants of apoL-I might lead to a different rate of TG transversion within the lipoproteins and affect the serum TG levels. This warrants further investigation.

We did not find a similar genotype effect on TG or any other lipid and apolipoprotein levels in the obese group. A possible explanation for this is that in obesity the genotype effects on the lipid levels might be masked by the obesity-associated metabolic abnormalities. We genotyped 278 additional patients with type 2 diabetes mellitus and did not find any association between the genotypes and the lipid and apolipoprotein levels either in total or in obese and nonobese separated subgroups (data not shown). It supports the previous findings that the disturbed lipid levels might mask the role of the *apoL* gene variants on the lipid variations.

Interestingly, this study also found that in nonobese subjects the *apoL-I* Lys166Glu and Ile244Met polymorphisms were significantly associated with apoC-III and apoE (Tables 3 and 4), two apolipoprotein components relating to TG/TG-

associated lipoprotein metabolism. This may suggest the complexity of the polymorphisms affecting lipid metabolism.

Although up to now the exact physiological function of apoL-I was not well defined, the data currently available have indicated that apoL-I might play some role in the metabolism of lipid (TG), which is linked to the risk of disease (CHD). The current study has provided evidence that genetic variations of polymorphic sites in *apoL-I* gene might affect plasma TG variability in nonobese Chinese subjects.

Conclusion

The current study, for the first time, provides evidence that Lys166Glu and Ile244Met polymorphisms in *apoL-I* gene are associated with TG and apoC-III and apoE levels in nonobese Chinese. However, these polymorphisms are not associated with obesity in the population. Data presented in this study need to be confirmed in a larger population.

Acknowledgments

This work was supported by a grant from the National Natural Sciences Foundation of China (No. 39770322), Program for Changjiang Scholars and Innovative Research Team in University (IRT0935), and Research Seed Fund from West China Second Hospital of Sichuan University. The authors thank overweight/obese and control subjects who donated blood samples for this study.

Disclosure Statement

No competing financial interests exist.

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