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Distribution and Effect of *apo E* Genotype on Plasma Lipid and Apolipoprotein Profiles in Overweight/Obese and Nonobese Chinese Subjects

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Background: Apolipoprotein E (apo E) polymorphism has been reported to influence some lipid profile abnormalities in some ethnic groups. This study was conducted mainly to examine the possible association of *apo E* polymorphism with overweight/obesity in a South West Chinese population. *Methods:* Four hundred and fifty-four Han Chinese (282 overweight/obese and 172 normal weight control subjects) in Chengdu area were studied using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. *Results:* The genotype and allele frequencies of *apo E* polymorphism in cases of overweight/obesity showed no significant statistical difference compared to those of controls. In the control group, both apo E4 and E3 allele carriers had the higher serum LDL-C and lower triglycerides (TGs) and apo E concentrations than those with apo E2 carriers, while apo E2 allele carriers had higher serum apo C-II levels than apo E3 carriers ($P < 0.05$). In overweight/obese group, genotype-related low density Lipoprotein-Cholesterol (LDL-C) variations were also evident, with the changes being in a same direction as the effect in the controls, and apo B100 levels were decreased and apo E increased in apo E2 allele carriers when compared with respective apo E4 and apo E4/apo E3 allele carriers (all $P < 0.05$). *Conclusion:* Polymorphism of the *apo E* gene is associated with altered plasma LDL-C and TG, as well as apo B, apo C-II, and apo E concentrations. The effects on TG, apo B, and apo C-II levels are BMI dependent in Chinese population of Chengdu area. J. Clin. Lab. Anal. 26:200–205, 2012.

Key words: overweight/obese; apo E; gene polymorphism; PCR-RFLP; genotype

INTRODUCTION

Cardiovascular diseases (CVD) are the leading causes of morbidity, mortality, and disability in industrialized countries, and they are poised to also become the major causes of mortality in developing nations within the next decade. In most cases, the progress of CVD is influenced by multifactorial inheritance and environmental factors (1–4).

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Apolipoproteins that form the protein part of the lipoproteins are important in lipid metabolism. It regulates the transport and redistribution of lipoproteins in blood as a cofactor. Apolipoprotein E (Apo E), a 35 kDa glycoprotein consisting of 299 amino acids (5), plays a fundamental role in the lipid metabolism. Apo E participates in the clearance of chylomicron remnants and very low-density lipoprotein (VLDL) by serving as a ligand for LDL receptors (5). It is also important for intestinal cholesterol absorption (6) and plasma lipid maintenance (7). The *apo E* gene, located at chromosome 19q 13.2 and consisted of four exons and three introns spanning 3,597 nucleotides (8), is composed by three alleles (E2, E3, and E4) that give rise to six different genotypes (E2E2, E2E3, E2E4, E3E3, E3E4, and E4E4) (5). The E3 allele differs from the E2 allele by an amino acid substitution of arginine for cysteine at codon 158, while the E4 differs from E3 by a substitution of arginine for cysteine at residue 112 (9–11). Many studies assessing the role of apo E polymorphism on plasma lipids have shown that the presence of the E4 allele is associated with elevations in LDL-C, while the presence of E2 is associated with decreased levels of LDL-C (12). The associations of *apo E* genes with lipid levels and hyperlipidemia suggest that *apo E* alleles contribute to the genetic risk of developing atherosclerotic vascular disease (13). In addition, an association between *apo E* alleles and various chronic conditions, such as Alzheimer's disease (14), cognitive impairment (15), osteoporosis (16), breast cancer (17), end-stage renal disease (18), diabetes (19), multiple sclerosis (20), and longevity (21), has been documented.

To date, there are no data available for South West Chinese subjects with overweight/obese in the genetic polymorphism of apo E and its relation with increased cardiovascular risk factors in the population group. In this study, we aimed at evaluating the distribution of apo E alleles that can influence cardiovascular risk of the Chinese overweight/obese and control subjects.

MATERIALS AND METHODS

Subjects

For this study, blood samples were taken from 454 volunteers (264 men, 190 women, aged 55.62 ± 10.21 years) who were taking part in a routine health examination at three hospitals of 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 12–14 hr 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 = body weight/(height)^2 in kg/m^2. According to the World Health Organization guidelines for Asians, individuals with BMI $\geq$ 23 kg/m^2 are classified as overweight and those with BMI $\geq$ 25 kg/m^2 as obese (22). The overweight/obese group (n = 282) presented with overweight and obese characteristics (BMI $\geq$ 23 kg/m^2) and comprised 169 men and 113 women. The control group (n = 172) of subjects with normal weight (BMI < 23 kg/m^2) comprised 95 men and 77 women. All of the subjects were Han Chinese. Subjects with internal implications, such as coronary heart disease (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

Total serum cholesterol (TC) and triglycerides (TGs) were measured by enzymatic method (kits, Zhong Sen Co., Beijing, China). High-density lipoprotein-cholesterol (HDL-C) was determined after sodium phosphotungstate/magnesium chloride precipitation of low-density lipoprotein by polyvinyl sulfate. Serum apo A-I, apo A-II, apo B100, apo C-II, apo C-III, and apo E were quantified by radial immunodiffusion kit developed by our laboratory (23). Serum LDL-C concentration was calculated according to the Friedewald equation.

DNA Extraction and Genotyping

Genomic DNA was isolated from 500 μl peripheral blood according to the method of Erlich (24). The investigated DNA sequences were amplified by the following primers: 5′- AACAACTGAC CCCGGTGGCG -3′ as a sense primer (P1) and 5′- ATGGCGCTG AGGCCGCGCTC -3′ as an antisense primer (P2) (25). The PCRs were performed in a final volume of 25 μl containing 10% 10 × PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 U Taq DNA polymerase (MBI Fermentas), and sense and antisense primers, 0.5 μM of each. We used 100 ng DNA per PCR reaction. The PCR products were digested with the restriction enzyme HhaI (CfoI) overnight at 37°C. The E2, E3, and E4 alleles were determined as follows: no HhaI restriction site for the E2 allele; one HhaI restriction site at position 158 for the E3 allele; and two HhaI restriction sites at positions 112 and 158 for the E4 allele (25).

Statistical Analysis

Allele frequencies of *apo E* gene 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 *apo E* gene polymorphism on the variation of quantitative variables of lipid, lipoprotein, 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 11.0 statistical software.

RESULTS

The lipid and lipoprotein profile for both overweight/obese and control groups are shown in Table 1. Serum TG, apo B100, apo C-II, apo C-III, and apo E levels were significantly higher, and HDL-C was significantly lower in overweight/obese group compared to those in the control group ($P < 0.05$ or $P < 0.01$).

The PCR-amplified fragments from each sample were digested with restriction enzyme HhaI (CfoI) for apo E site, and analyzed by electrophoresis on an 8% polyacrylamide nondenaturing gel. Subjects with the E2E2, E2E3, and E2E4 genotypes were grouped as E2, subjects E3E3 were classified as E3, and E3E4 and E4E4 were grouped as E4.

TABLE 1. Clinical and Biochemical Characteristics of Overweight/Obese and Control Subjects ($\bar{x} \pm s$)

	Overweight/obese ($n = 282$)	Controls ($n = 172$)	P
Age (years)	56.68 ± 9.73	54.32 ± 10.79	(0.016)
Gender (M/F)	169/113	95/77	(0.303)
TG (mmol/l)	2.36 ± 1.61	1.57 ± 1.43	(0.000)
TC (mmol/l)	5.70 ± 1.06	5.52 ± 1.24	(0.087)
HDL-C (mmol/l)	1.19 ± 0.32	1.34 ± 0.43	(0.000)
LDL-C (mmol/l)	3.52 ± 0.94[a]	3.40 ± 1.05[b]	(0.395)
apo A-I (mg/dl)	127.51 ± 27.67	130.12 ± 24.25	(0.294)
apo A-II (mg/dl)	28.99 ± 5.08	28.80 ± 6.54	(0.663)
apo B100 (mg/dl)	92.07 ± 18.41	85.47 ± 22.50	(0.001)
apo C-II (mg/dl)	7.03 ± 3.33	5.71 ± 4.17	(0.000)
apo C-III (mg/dl)	16.06 ± 6.75	13.28 ± 5.93	(0.000)
apo E (mg/dl)	5.36 ± 1.97	4.80 ± 2.12	(0.014)
BMI (kg/m^2)	25.66 ± 2.22	20.89 ± 2.20	(0.000)

Note: Subjects with TG > 4.516 mmol/l (400 mg/dl) were excluded when calculating the serum LDL-C levels.
[a] $n = 262$.
[b] $n = 163$.

Genotypes of apo E polymorphism were found to be in Hardy–Weinberg equilibrium in both the overweight/obese and control groups, respectively. The frequency data are presented in Table 2. The frequency of apo E genotypes in overweight/obese and control groups were: E2E2, 0.4% and 1.7%, E2E3, 16.3% and 9.3%, E2E4, 2.5% and 1.2%, E3E3, 66.0% and 71.5%, E3E4, 14.2% and 15.7%, and E4E4, 0.7% and 0.6%, respectively. The allele frequencies in overweight/obese and normal control groups were: E2, 9.8% and 7.0%, E3, 81.20% and 84.0%, and E4, 9.0% and 9.0%, respectively. Over half of subjects had apo E3 (E3/E3, 66.0 and 71.5% in overweight/obese and control groups, respectively), which is in general well consistent with data reported in other ethnic groups. The genotype and allele frequencies of the polymorphism in overweight/obese subjects were not different from those in the controls ($P > 0.05$, $P > 0.05$) (Table 2).

To assess the possible impact of the polymorphism in *apo E* gene on lipid metabolism, we analyzed the serum lipid, lipoprotein, and apolipoprotein levels in different genotypes of apo E polymorphism in both overweight/obese and normal control groups (Table 3).

In nonobese control group, subjects with apo E4 and E3 allele had the higher serum LDL-C, and lower TG and apo E concentrations than those with apo E2 allele carriers. The apo E4 allele carriers showed the highest levels for LDL-C levels among the three allele carriers. In addition, apo C-II levels were higher in apo E2 allele carriers than apo E3 allele carriers (Table 3).

In overweight/obese group, genotype-related LDL-C variations were also evident, with the changes being in a same direction as the effect in the controls, and apo B100 levels were decreased and apo E increased in apo E2 allele

TABLE 2. Genotype and Allele Frequencies of apo E Polymorphisms in Overweight/Obese and Control Groups

	Frequencies		
	Overweight/obese ($n = 282$)	Controls ($n = 172$)	P
Genotype			0.168
E2E2	0.004 (1)	0.017 (3)	
E2E3	0.163 (46)	0.093 (16)	
E2E4	0.025 (7)	0.012 (2)	
E3E3	0.660 (186)	0.715 (123)	
E3E4	0.142 (40)	0.157 (27)	
E4E4	0.007 (2)	0.006 (1)	
Allele			0.351
E2	0.098 (55)	0.070 (24)	
E3	0.812 (458)	0.840 (289)	
E4	0.090 (51)	0.090 (31)	

Note: Numbers in parentheses indicate number of subjects with each genotype or number of alleles of each type.

TABLE 3. Mean Values ($\bar{x} \pm s$) of Serum Lipid and Apolipoprotein Levels for apo E Gene in Overweight/Obese and Control Groups

	Overweight/obese			Controls		
	E2 ($n = 54$)	E3 ($n = 186$)	E4 ($n = 42$)	E2 ($n = 21$)	E3 ($n = 123$)	E4 ($n = 28$)
TG (mmol/l)	2.61 ± 1.58	2.32 ± 1.56	2.22 ± 1.61	$2.76 \pm 3.14^{c,d,**,*}$	1.68 ± 1.12	1.80 ± 0.97
TC (mmol/l)	5.52 ± 1.01	5.74 ± 1.00	5.76 ± 1.10	5.42 ± 1.47	5.51 ± 1.12	5.67 ± 1.08
HDL-C (mmol/l)	1.16 ± 0.26	1.21 ± 0.32	1.16 ± 0.29	1.32 ± 0.31	1.36 ± 0.30	1.29 ± 0.25
LDL-C (mmol/l)	$3.19^{a} \pm 0.73^{a,b,*}$	3.57 ± 0.93^{b}	3.69 ± 1.11^{c}	$2.86^{d} \pm 0.74^{a,b,*}$	3.45 ± 1.07^{e}	3.60 ± 0.97^{f}
apo A-I (mg/dl)	126.02 ± 23.33	128.10 ± 28.92	126.89 ± 22.48	126.74 ± 26.26	131.09 ± 23.47	128.29 ± 19.53
apo A-II (mg/dl)	28.86 ± 5.42	28.88 ± 4.70	29.69 ± 4.55	28.79 ± 3.65	28.77 ± 4.95	29.00 ± 3.93
apo B100 (mg/dl)	$88.42 \pm 18.84^{e,*}$	92.13 ± 17.85	96.51 ± 19.75	81.26 ± 22.54	84.84 ± 22.28	92.39 ± 22.59
apo C-II (mg/dl)	7.75 ± 3.09	6.79 ± 3.29	7.17 ± 3.94	$7.26 \pm 5.12^{f,*}$	5.37 ± 2.68	6.04 ± 3.20
apo C-III (mg/dl)	16.50 ± 5.94	15.76 ± 6.59	16.83 ± 9.03	15.44 ± 9.59	12.71 ± 5.45	14.09 ± 6.36
apo-E (mg/dl)	$6.47 \pm 2.15^{a,b,**}$	5.10 ± 1.72	5.10 ± 2.01	$6.62 \pm 4.72^{c,d**,*}$	4.59 ± 1.54	4.78 ± 1.56

Note: Subjects with TG > 4.516 mmol/l (400 mg/dl) were excluded when calculating the serum LDL-C levels.
[a,b]Compared with E3 and E4 allele carriers in the same group, respectively, $^{*}P < 0.05$ or $^{**}P < 0.01$.
[c,d]Compared with E3 and E4 allele carriers in the same group, respectively, $^{**}P < 0.01$, $^{*}P < 0.05$.
[e]Compared with E4 allele carriers in the same group, $^{*}P < 0.05$.
[f]Compared with E3 allele carriers in the same group, $^{*}P < 0.05$.
Case numbers in E2, E3, and E4 are 50, 172, and 40, respectively.
Case numbers in E2, E3, and E4 are 20, 116, and 27, respectively.
[g]Age and sex were used as covariates in the analysis to adjust for the genotype effects.

carriers when compared with respective apo E4 and apo E4/apo E3 allele carriers (all $P < 0.05$) (Table 3).

No significant genotype effect on HDL-C levels for the *apo E* polymorphism was observed in our subjects, whatever their weight (BMI) (Table 3). In addition, we further analyzed whether there is any gene–gene interaction between the *apo E* and *CETP (Taq IB)* loci in determining HDL-C concentrations, which was suggested previously in a Southern European population (26). By combining the present *apo E* results with our recent *CETP* genotyping data of the similar subjects (27), we found that there was no such interaction between the polymorphisms of the two genes on HDL-C levels in both the overweight/obese and control Chinese subjects (In overweight/obese group: HDL-C levels in E2/B1B1, E3/B1B1, and E4/B1B1 allele carriers were 1.13 ± 0.26 mmol/l, 1.24 ± 0.34 mmol/l, and 1.19 ± 0.26 mmol/l, respectively; HDL-C levels in E2/B2, E3/B2, E4/B2 allele carriers were 1.25 ± 0.32 mmol/l, 1.24 ± 0.33 mmol/l, and 1.28 ± 0.31 mmol/l, respectively. In control group: HDL-C levels in E2/B1B1, E3/B1B1, and E4/B1B1 allele carriers were 1.03 ± 0.25 mmol/l, 1.26 ± 0.30 mmol/l, and 1.10 ± 0.14 mmol/l, respectively; HDL-C levels in E2/B2, E3/B2, E4/B2 allele carriers were 1.51 ± 0.31 mmol/l, 1.38 ± 0.27 mmol/l, and 1.40 ± 0.25 mmol/l, respectively) (all $P > 0.05$).

DISCUSSION

Our results in the Chinese cohort living in the South West China showed that the polymorphism of the *apo E* gene was associated with LDL-C, TG, apo C-II, apo B, and apo E concentrations in the population; and the TG, apo CII and apo B levels were dependent on BMI. These results provide support for the notion that apo E are involved in the regulation of TG and LDL metabolism and thus might play an important role in lipid and cholesterol transport.

Several studies have reported positive associations of the apo E4 allele with increased LDL-C concentrations or atherosclerosis (12, 18). This is consistent with our present results. A mechanism may account for the association of the E4 allele with hypercholesterolemia (e.g., increased LDL-C). Because of the enhanced catabolism of lipoproteins that contain apo E4, more cholesterol is delivered to liver cells by apo E-mediated uptake in subjects with an E4 allele. A similar but opposite mechanism may account for the association of the E2 allele with relative lower LDL-C. In apo E2 homozygotes, failure of apo E2 to bind the LDL and apo E receptors leads to accumulation of remnant lipoproteins resulting in hyperlipidemia. However, most E2 homozygotes have subnormal rather than elevated cholesterol and low LDL. This is because the delayed catabolism of lipoproteins that contain apo E causes cholesterol of exogenous origin and periphery to enter the liver through apo E-mediated uptake. For compensation, LDL (B/E) receptors may be upregulated, resulting in enhanced uptake of LDL and hence a lowering of LDL in plasma. In addition, a delay in the interconversion of intermediate density lipids (IDL) to LDL may contribute to the low LDL in plasma of E2 homozygotes (28). Our results extended the notion that apo E4 (and E3) allele of apo E gene are associated with elevated LDL-C levels, in contrast to the

decreased levels in apo E2 allele, in overweight/obese and normal weight populations living in South West China.

Numerous studies revealed the influence of *apo E* genotypes on TG levels. The results of a meta-analysis (29) indicated a consistent relationship between plasma TG levels and apo E allele in different populations: TG concentrations were significantly higher in E2E2, E2E3, E3E4, and E2E4 than in E3E3 carriers, which are in general consistent with the results of the present study.

Several studies suggested the association between *apo E* genotype and HDL-C levels (30–32). However, we did not find such association in either overweight/obese or control groups. Sorlí et al., suggested a gene–gene interaction between the *apo E* and *CETP* (*Taq IB*) loci in determining HDL-C concentrations in a Southern European population (26). By combining the present results with our recent data of Taq IB polymorphism in *CETP* gene in similar subjects (27), we found that there was no significant gene–gene interaction for HDL-C levels in both the overweight/obese or control groups. The inconsistent results might be partly explained by the different genetic and environmental factors between the two populations.

This study also demonstrated that relatively higher apo C-II levels in control subjects and lower apo B100 levels in overweight/obese subjects in apo E2 allele carriers, when compared to their respective apo E3 and apo E4 carriers in the corresponding group. The results might be due to different metabolic conditions in control and overweight/obese subjects relating to apo E isoforms. In addition, the genotype effects on apo E variations in both groups are in line with the previous report (33), which reinforced our notion concerning the relationship between *apo E* genotype and plasma apo E levels.

In recent years, Kolovou et al. studied a relationship between *apo E* polymorphism and CHD in patients with different BMI in Greece Caucasians (34). The results suggested that in normal weights subjects' genetic factors might play a greater role than in overweight and obese individuals in predicting CHD risk. Our present study only detected subjects in the Chinese general population. We did not find significant association between *apo E* gene polymorphism and obesity. Further study including CHD patients stratified by BMI levels in our population could make further comparison, and thus determine the potential influence of BMI levels on CHD-risk prediction in subjects with CHD in Chinese population.

We should point out that previous reports on the relationship between genetic components and obesity or related traits mainly focused on single gene locus. In recent years, with the development of high-throughput genotyping technologies, analysis of more complex interaction, such as epistasis, become more widely accessible in genomic research. Indeed, by using genome-wide association studies (GWASs) method, much more candidate genes and new loci have been identified. As genomic research has advantages that give us deeper insight about the complex nature of genetic individuality and heritability, epistatic (as well as epigenetic) interactions are now becoming more significantly associated with complex disorders as obesity and CHD. The data collected from GWASs are beginning to explore through more complex interactions, such as gene–gene and gene–environment interactions. This would lead us understand etiology of chronic diseases further.

In summary, we have shown that polymorphism of the *apo E* gene is associated with altered plasma LDL-C and TG, as well as apo B, apo C-II, and apo E concentrations. The genotype effects on TG, apo B, and apo C-II levels are BMI dependent in Chinese population.

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