Apolipoprotein E Gene Polymorphism and Serum Lipid Levels in the Guangxi Hei Yi Zhuang and Han Populations

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Hei Yi Zhuang is an isolated subgroup of the Zhuang minority in China. Little is known about the distribution of apolipoprotein (apo) E genetic variations and its role in lipid metabolism in this population. The present study was undertaken to compare the effect of apoE gene polymorphism on serum lipid levels between the Guangxi Hei Yi Zhuang and Han populations. A total of 873 subjects of Hei Yi Zhuang and 867 participants of Han Chinese were surveyed by a stratified randomized cluster sampling. Genotyping of apoE was performed using polymerase chain reaction and restriction fragment length polymorphism. The frequencies of ε2, ε3, and ε4 alleles were 15.23%, 79.84%, and 4.93% in Hei Yi Zhuang, and 9.23%, 81.43%, and 9.34% in Han ($P < 0.001$); respectively. The frequencies of ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4 genotypes were 4.70%, 17.86%, 3.21%, 68.16%, 5.50%, and 0.57% in Hei Yi Zhuang, and 2.54%, 9.23%, 4.15%, 70.70%, 12.23%, and 1.15% in Han ($P < 0.001$); respectively. Total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and apoB levels were lower in Hei Yi Zhuang than in Han ($P < 0.01$–0.001), but high-density lipoprotein cholesterol (HDL-C) levels and the ratio of apoA-I to apoB were higher in Hei Yi Zhuang than in Han ($P < 0.001$ for each). There were significant differences in TC, HDL-C, LDL-C, and apoB levels among the six genotypes in both ethnic groups ($P < 0.01$–0.001). Hyperlipidemia was positively correlated with age, body mass index, hypertension, alcohol consumption, and apoE allele in both populations ($P < 0.05$–0.001). TC, LDL-C, and apoB levels were positively correlated, and HDL-C levels were negatively associated with apoE genotypes in both ethnic groups ($P < 0.001$ for all). The differences in the lipid profiles between Hei Yi Zhuang and Han Chinese might partly attribute to the differences in apoE genotypic and allelic frequencies. Exp Biol Med 233:409–418, 2008

Key words: lipids; apolipoprotein E; polymorphism; gene

Introduction

Lipid disorders such as elevated serum levels of total cholesterol (TC)(1), triglyceride (TG)(2), low-density lipoprotein cholesterol (LDL-C)(3), apolipoprotein (apo) B (4), and low levels of high-density lipoprotein cholesterol (HDL-C)(5) are well-established risk factors for coronary heart disease. It is also well known that dyslipidemia is determined by both environmental and genetic factors. ApoE is an important structural constituent of several serum lipoprotein classes, including very-low-density lipoprotein (VLDL), chylomicrons, and HDL-C and serves as a ligand for the LDL receptor and LDL receptor-related protein (6). Therefore, it plays an important role in lipid metabolism both by promoting efficient uptake of triacylglycerol-rich lipoproteins from the circulation and by taking part in cellular cholesterol efflux and reverse cholesterol transport (7). It is also involved in cholesterol absorption from the intestine (8). The gene coding for apoE (Online Mendelian Inheritance in Man database: 107741) is located on the long arm of chromosome 19 (19q13.2)(9). Three common polymorphisms of apoE have been described in humans (10). The isoforms differ at amino acid residues 112 and 158. Isoform E2 has cysteine residues at both sites (Cys 112, Cys 158), E4 has arginine residues at both sites (Arg 112, Arg 158), and E3 has a cysteine at position 112 and an arginine at position 158 (Cys 112, Arg 158). These phenotypes are the result of a single apoE gene locus with

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three common alleles, designated ε2, ε3, and ε4, respectively (11). The presence of three alleles leads to the formation of six different phenotypes: E2/2, E2/3, E2/4, E3/3, E3/4, and E4/4. Different populations exhibit various frequencies in the distribution of apoE isoforms, and thus far, the most frequent allele in all populations examined is the isoform apoε3 (12). Three alleles have quantitative effects on lipid and lipoprotein levels. Many studies have shown that ε2 allele is associated with low levels of TC, LDL-C, and apoB, whereas for ε4 allele the opposite is observed (10, 12). Additionally, a positive association of the ε2 allele with serum concentrations of HDL-C has been suggested (13, 14).

There are 56 ethnic groups in China. Han is the largest group, and Zhuang is the largest minority. Hei Yi (means black-worship and black dressing) Zhuang is the most conservative subgroup of the Zhuang minority. The color black is the symbol of Hei Yi Zhuang; they hold that the color black is beautiful and typically wear black garments and pants. The population size is 51,655. Because of their isolation from other ethnic groups and their special customs and culture, including their clothing, the customs of intra-ethnic marriages and a traditional diet have been preserved in their entirety to the present. We have reported that the levels of TC, TG, LDL-C, and apoB were significantly lower**,** and the levels of HDL-C and the ratio of apoA-I to apoB were significantly higher in Hei Yi Zhuang than in Han (15, 16). We hypothesize that there may be significant differences in apoE allelic frequencies between the two ethnic groups. Therefore, the aim of the present study was to determine the polymorphism of apoE gene and its association with serum lipid levels in the Guangxi Hei Yi Zhuang and Han populations.

Materials and Methods

Subjects. A total of 873 Hei Yi Zhuang subjects, residing in seven villages in Napo County, Guangxi Zhuang Autonomous Region, were surveyed by a stratified randomized cluster sampling. Subjects ranged in age from 16 to 80 years, with an average age of 46.15 ± 16.06 years. There were 452 males (51.78%) and 421 females (48.22%), and all participants were farmers. At the same time, a total of 867 people of Han Chinese living in nine villages in Napo County were also surveyed by the same method. The mean age of the subjects was 45.58 ± 15.57 years (range 16 to 82). There were 449 males (51.79%) and 418 females (48.21%), and they were also farmers. All study subjects were essentially healthy and had no evidence of diseases related to atherosclerosis. None of them had been treated with β-adrenergic blocking agents and lipid-lowering drugs such as statins or fibrates. The present study was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Informed consent was obtained from all subjects after they received a full explanation of the study.

Epidemiological Survey. The survey was carried out using internationally standardized methods, following a common protocol. Information on demographics (age, gender, and residential area), socioeconomic status (education level achieved, marital status, and annual household income), cigarette smoking, alcohol consumption, and physical activity was collected with standardized questionnaires. Smoking status was categorized into groups of cigarettes per day: $\leq$20 and $>$20. Alcohol consumption was categorized into groups of grams of alcohol per day: $\leq$25 g and $>$25 g. The physical examination included several anthropometric parameters such as blood pressure, body height, body weight, and waist circumference, etc., and body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Resting blood pressure was measured three times with the use of a mercury sphygmomanometer after the subject rested 5 mins, and the average of the three measurements was used for the level of blood pressure. Systolic pressure was determined by the first Korotkoff sound, and diastolic pressure by the fifth Korotkoff sound.

Measurements of Lipids and Apolipoproteins. A venous blood sample of 8 ml was obtained from subjects between 0800 and 1100 hrs, after at least 12 hrs of fasting, from a forearm vein after venous occlusion for few seconds in a sitting position. Three milliliters were collected into glass tubes and allowed to clot at room temperature and used to determine serum lipids, and the remaining 5 ml was transferred to tubes with anticoagulate solution (4.80 g/L citric acid, 14.70 g/L glucose, and 13.20 g/L tri-sodium citrate) and used to extract DNA. Immediately following, clotting serum was separated by centrifugation for 15 mins at 1760 *g*. The levels of TC, TG, HDL-C, and LDL-C in samples were determined by enzymatic methods with commercially available kits: Tcho-1 and TG-LH (RANDOX Laboratories Ltd., Ardmore, United Kingdom) and Cholestest N HDL and Cholestest LDL (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan), respectively. Serum apoA-I and apoB levels were assessed by the immunoturbidimetric immunoassay using a commercial kit (RANDOX Laboratories Ltd.). All determinations were performed with an autoanalyzer (Type 7170A; Hitachi Ltd., Tokyo, Japan) in the Clinical Science Experiment Center of the First Affiliated Hospital, Guangxi Medical University.

Determination of the apoE Gene Polymorphism. DNA was extracted from the peripheral blood leukocytes by the phenol-chloroform method as described in our previous reports (17, 18). The extracted DNA was stored at 4°C until analysis. Genotyping of apoE was performed using polymerase chain reaction and restriction fragment length polymorphism (19). A 244-bp fragment of the apoE gene covering the codons for amino acids 112 and 158 was amplified by polymerase chain reaction using the primer pair F4 (5′-ACAGAATTCGCCCCGGCCTGGTACAC-3′) and F6 (5′-TAAGCTTGGCACGGCTGTCCAAGGA-3′; Institute of Biochemistry and Cell Biology,

Table 1. Comparison of General Characteristics and Serum Lipid Levels Between the Hei Yi Zhuang and Han Populations

Parameters	Daily use	Hei Yi Zhuang	Han Chinese
Number		873	867
Male/female		452/421	449/418
Age (year)		46.15 ± 16.06	45.58 ± 15.57
Body mass index (kg/m^2)		21.27 ± 2.30	22.56 ± 2.56*
Systolic blood pressure (mm Hg)		125.96 ± 17.36	121.62 ± 16.35*
Diastolic blood pressure (mm Hg)		77.11 ± 11.35	76.57 ± 10.73
Pulse pressure (mm Hg)		49.36 ± 13.75	45.15 ± 11.24*
Cigarette smoking [No. (%)]	Nonsmoker	548 (62.77)	571 (65.85)
	≤20 cigarettes/day	177 (20.27)	149 (17.19)
	>20 cigarettes/day	148 (16.95)	147 (16.96)
Alcohol consumption [No. (%)]	Nondrinker	381 (43.64)	400 (46.14)
	≤25 g/day	346 (39.63)	339 (39.10)
	>25 g/day	146 (16.72)	128 (14.76)

* $P < 0.001$, in comparison with Hei Yi Zhuang.

Shanghai Institute for Advanced Studies, Chinese Academy of Sciences, Shanghai, China) described by Emi et al. (20). Each amplification reaction contained 200 ng of genomic DNA, 10 pmol/μl of each primer, 10% dimethyl sulfoxide, and 0.75 units of *Taq* polymerase in a final volume of 30 μl. Each reaction mixture was heated at 94°C for 5 mins for denaturation and subjected to 30 cycles of amplification by primer annealing (62°C for 45 secs), extension (72°C for 1 min), and denaturation (95°C for 1 min). After amplification, 8 μl of the PCR product were directly digested with 12 units of the restriction enzyme *Hha*I (Promega, Madison, WI) for 4 hrs at 37°C. Gene fragments were separated using 8% polyacrylamide nondenaturing gel electrophoresis (3 hrs, 45 mA) and detected by ethidium bromide staining under ultraviolet illumination (0.2 mg/l, 30 mins), using a known DNA size marker.

Diagnostic Criteria. The normal values of serum TC, TG, HDL-C, LDL-C, apoA-I, apoB, and the ratio of apoA-I to apoB in our Clinical Science Experiment Center were 3.10–5.17, 0.56–1.70, 0.91–1.81, 1.70–3.20 mmol/L, 1.00–1.76, 0.63–1.14 g/L, and 1.00–2.50, respectively. The individuals with TC > 5.17 mmol/L and/or TG > 1.70 mmol/L were defined as hyperlipidemic (15, 16). Hypertension was defined as a systolic pressure of 140 mm Hg or higher and/or a diastolic pressure of 90 mm Hg or higher (21, 22). The diagnostic criteria of overweight and obesity were according to the Cooperative Meta-analysis Group of China Obesity Task Force. Normal weight, overweight, and obesity were defined as a BMI <24, 24–28, and >28 kg/m^2, respectively (23).

Statistical Analysis. Epidemiological data were recorded on a predesigned form and managed with Excel software. Levels of the quantitative variables are presented as mean ± standard deviation (SD). The difference of general characteristics between Hei Yi Zhuang and Han was tested by the Student's unpaired *t* test. The allelic and genotypic frequencies of apoE were estimated by counting alleles and genotypes and calculating sample proportions; the statistical significance of differences of frequencies between groups was compared by chi-square test. The distribution of apoE polymorphism was tested for Hardy-Weinberg equilibrium using chi-square goodness-of-fit test. The association of apoE genotypes/alleles with lipid variables was tested by analysis of covariance (ANCOVA). The covariables include age, BMI, hypertension, and alcohol consumption. All significant associations were corrected for multiple testing by applying a Bonferroni correction. In order to evaluate the association of hyperlipidemia and ethnic group (Hei Yi Zhuang = 0; Han Chinese = 1), sex (female = 0; male = 1), age (<20 = 1; 20–29 = 2; 30–39 = 3; 40–49 = 4; 50–59 = 5; 60–69 = 6; ≥70 = 7), BMI (≤24 kg/m^2 = 0; >24 kg/m^2 = 1), blood pressure (normotensives = 0; hypertensives = 1), alcohol consumption (nondrinkers = 0; ≤25 g/day = 1; >25 g/day = 2), cigarette smoking (nonsmokers = 0; ≤20 cigarettes/day = 1; >20 cigarettes/day = 2), or allele (ε2 = 1; ε3 = 2; ε4 =3), unconditional logistic regression analysis was also performed in combined population of Hei Yi Zhuang and Han, Hei Yi Zhuang, and Han; respectively. The backward multiple logistic regression method was used to select the risk factors significantly associated with hyperlipidemia. In addition, Spearman rank correlation analysis was also performed between lipid parameters and apoE genotypes in both ethnic groups. All statistical analyses were done with the statistical software package SPSS 10.0 (SPSS Inc., Chicago, Illinois). $P < 0.05$ was considered significant.

Results

General Characteristics. Table 1 gives the general characteristics of the subjects between Hei Yi Zhuang and Han. The levels of systolic blood pressure and pulse pressure were significantly higher in Hei Yi Zhuang than in Han ($P < 0.001$ for each), whereas BMI was higher in Han than in Hei Yi Zhuang ($P < 0.001$). There were no significant differences in body height, diastolic blood

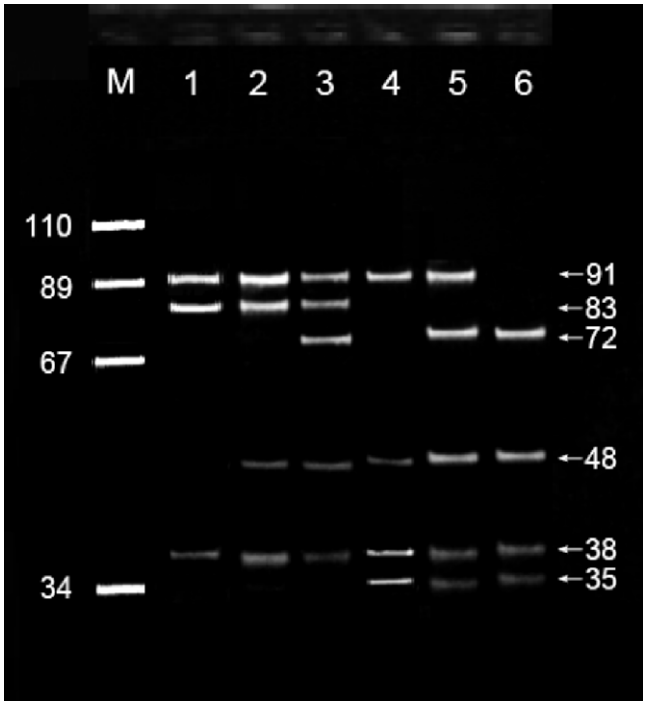

Figure 1. Genotyping of apoE using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). Lane M, marker ladder; lane 1 to 6, ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4 genotypes, respectively.

pressure levels, age structure, or the ratio of male to female between the two ethnic groups ($P > 0.05$).

Genotypic and Allelic Frequencies. Genotypes were scored by an experienced reader blinded to epidemiological and lipid results. The identified genotypes were named according to the results of the enzyme restriction. Figure 1 shows gel-separated products of apoE amplification and *Hha*I digestion from the subjects representing each homozygotic and heterozygotic combination of common apoE alleles. The ε2/ε2 genotype contained 91-, 83- (*Hha*I fragments reflecting the absence of sites at 112 Cys and 158 Cys), and 38-bp fragments (common *Hha*I site at position 38); the ε2/ε3 genotype contained 91-, 83-, 48-, and 38-bp fragments; the ε2/ε4 genotype contained 91-, 83-, 72-, 48-, and 38-bp fragments; the ε3/ε3 genotype contained the 91-bp fragment (112 Cys), as well as 48-, 38-, and 35-bp fragments from cleavage at the *Hha*I site at 158 Arg; the ε3/ε4 genotype contained the 91-bp fragment (112 Cys), as well as 72-, 48-, 38-, and 35-bp fragments; and the ε4/ε4 genotype contained these 48-, 38-, and 35-bp fragments (158 Arg), as well as a unique 72-bp fragment from cleavage at 112 Arg (the 19-bp fragment was too small for detection). The frequencies of apoE alleles and genotypes are shown in Table 2. The frequencies of ε2, ε3, and ε4 alleles were 15.23%, 79.84%, and 4.93% in Hei Yi Zhuang, and 9.23%, 81.43%, and 9.34% in Han ($\chi^2 = 24.870$, $P < 0.001$); respectively. The frequencies of ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4 genotypes were 4.70%, 17.86%, 3.21%, 68.16%, 5.50%, and 0.57% in Hei Yi Zhuang, and

Table 2. Comparison of apoE Genotype and Allele Frequencies Between the Hei Yi Zhuang and Han Populations

Test	Ethnic group	No.	Genotypes					Alleles		
			ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	ε2	ε3	ε4
	Hei Yi Zhuang	873	41 (4.70)	156 (17.86)	28 (3.21)	595 (68.16)	48 (5.50)	5 (0.57)	133 (15.23)	697 (79.84)	43 (4.93)
	Han Chinese	867	22 (2.54)*	80 (9.23)**	36 (4.15)	613 (70.70)	106 (12.23)**	10 (1.15)	80 (9.23)**	706 (81.43)	81 (9.34)**
Chi square		—	—		54.964				24.870		
P		—	—		0.000				0.000		

* $P < 0.05$, in comparison with the same genotype or allele of Hei Yi Zhuang.
** $P < 0.001$, in comparison with the same genotype or allele of Hei Yi Zhuang.

Table 3. Comparison of the Effects of apoE Genotypes on Serum Lipid Levels Between the Hei Yi Zhuang and Han Populations

Ethnic groups	Genotypes	No.	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	apoA-I (g/L)	apoB (g/L)	apoA-I/apoB
Hei Yi Zhuang		873	4.51 ± 0.96	1.15 ± 0.78	2.25 ± 0.62	2.31 ± 0.62	1.45 ± 0.14	0.87 ± 0.20	1.67 ± 0.98
	ε2/ε2	41	3.96 ± 0.88	0.99 ± 0.58	2.48 ± 0.72	1.94 ± 0.53	1.46 ± 0.16	0.78 ± 0.18	1.87 ± 1.14
	ε2/ε3	156	4.12 ± 0.79	1.12 ± 0.62	2.26 ± 0.67	2.26 ± 0.64[a]	1.46 ± 0.14	0.83 ± 0.19	1.76 ± 1.09
	ε2/ε4	28	4.36 ± 0.91	1.23 ± 0.66	2.23 ± 0.58	2.28 ± 0.57	1.44 ± 0.12	0.86 ± 0.22	1.67 ± 0.87
	ε3/ε3	595	4.62 ± 0.86[ab]	1.16 ± 0.79	2.26 ± 0.64	2.33 ± 0.61[a]	1.45 ± 0.15	0.88 ± 0.20[cd]	1.65 ± 0.96
	ε3/ε4	48	4.79 ± 0.99[ab]	1.21 ± 0.89	1.95 ± 0.57[ade]	2.54 ± 0.72[adf]	1.43 ± 0.14	0.93 ± 0.24[ad]	1.53 ± 0.79
	ε4/ε4	5	5.45 ± 1.12[abg]	1.36 ± 0.96	1.87 ± 0.48	2.75 ± 0.67	1.42 ± 0.13	1.06 ± 0.16[c]	1.34 ± 0.85
ANCOVA, *F*		—	9.876	0.633	3.386	4.867	0.521	4.477	0.942
P′		—	0.000	0.630	0.001	0.000	0.796	0.000	0.481
Han Chinese		867	4.77 ± 0.99***	1.27 ± 0.84**	2.13 ± 0.64***	2.47 ± 0.67***	1.44 ± 0.15	1.07 ± 0.21***	1.35 ± 0.79***
	ε2/ε2	22	4.15 ± 1.02	1.01 ± 0.76	2.41 ± 0.75	1.99 ± 0.63	1.46 ± 0.13	0.81 ± 0.21	1.80 ± 0.99
	ε2/ε3	80	4.34 ± 0.87*	1.26 ± 0.69	2.45 ± 0.68*	2.35 ± 0.57[c]	1.45 ± 0.15	0.90 ± 0.20**	1.61 ± 0.86
	ε2/ε4	36	4.62 ± 0.96	1.36 ± 0.82	2.23 ± 0.71	2.41 ± 0.64[c]	1.44 ± 0.14	0.97 ± 0.21[a]	1.48 ± 0.81
	ε3/ε3	613	4.78 ± 1.08[ac]**	1.28 ± 0.86*	2.11 ± 0.63[b]***	2.45 ± 0.65[a]***	1.44 ± 0.15	1.09 ± 0.19[abh]***	1.32 ± 0.78[bc]***
	ε3/ε4	106	5.13 ± 1.12[abeg]	1.23 ± 1.15	1.93 ± 0.59[abeg]	2.78 ± 0.74[abeg]	1.42 ± 0.14	1.18 ± 0.24[abeh]*	1.20 ± 0.66[ab]**
	ε4/ε4	10	5.78 ± 0.99[abeg]	1.52 ± 1.06	1.76 ± 0.61[d]	2.68 ± 0.69[c]	1.43 ± 0.13	1.23 ± 0.23[abh]	1.16 ± 0.68
ANCOVA, *F*		—	6.877	0.668	6.672	7.068	0.544	24.173	4.025
P′		—	0.000	0.651	0.000	0.000	0.731	0.000	0.000

[a] $P < 0.01$, in comparison with ε2/ε2 genotype.
[b] $P < 0.01$, in comparison with ε2/ε3 genotype.
[c] $P < 0.05$, in comparison with ε2/ε2 genotype.
[d] $P < 0.05$, in comparison with ε2/ε3 genotype.
[e] $P < 0.01$, in comparison with ε3/ε3 genotype.
[f] $P < 0.05$, in comparison with ε3/ε3 genotype.
[g] $P < 0.05$, in comparison with ε2/ε4 genotype.
[h] $P < 0.01$, in comparison with ε2/ε4 genotype.
* $P < 0.05$, in comparison with Hei Yi Zhuang.
** $P < 0.01$, in comparison with Hei Yi Zhuang.
*** $P < 0.001$, in comparison with Hei Yi Zhuang.

Table 4. Comparison of the Effects of apoE Alleles on Serum Lipid Levels Between the Hei Yi Zhuang and Han Populations

Ethnic groups	Alleles	No.	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	apoA-I (g/L)	apoB (g/L)	apoA-I/apoB
Hei Yi Zhuang	ε2	133	4.01 ± 0.83	1.09 ± 0.61	2.33 ± 0.68	2.16 ± 0.60	1.46 ± 0.14	0.82 ± 0.19	1.78 ± 1.08
	ε3	697	4.57 ± 0.86[a]	1.16 ± 0.77	2.25 ± 0.64	2.33 ± 0.62[a]	1.45 ± 0.15	0.88 ± 0.20[a]	1.66 ± 0.97
	ε4	43	4.73 ± 0.98[a]	1.23 ± 0.82	2.03 ± 0.56[bc]	2.48 ± 0.67[a]	1.43 ± 0.13	0.92 ± 0.22[b]	1.55 ± 0.82
ANCOVA, *F*		—	20.242	0.713	3.571	5.292	0.593	5.430	1.072
P'		—	0.000	0.492	0.010	0.001	0.541	0.001	0.396
Han Chinese	ε2	80	4.35 ± 0.93**	1.21 ± 0.74	2.39 ± 0.71	2.27 ± 0.60	1.45 ± 0.14	0.89 ± 0.21*	1.63 ± 0.89
	ε3	706	4.78 ± 1.07[a***]	1.28 ± 0.87**	2.12 ± 0.63[a***]	2.47 ± 0.65[a***]	1.44 ± 0.15	1.09 ± 0.19[a***]	1.33 ± 0.78[a***]
	ε4	81	5.10 ± 1.07[ad]	1.30 ± 1.07	1.98 ± 0.62[a]	2.69 ± 0.71[ad]	1.43 ± 0.14	1.14 ± 0.23[ab***]	1.26 ± 0.70[a*]
ANCOVA, *F*		—	8.224	0.317	7.379	6.960	0.413	33.623	4.813
P'		—	0.000	0.694	0.000	0.000	0.583	0.000	0.001

[a] $P < 0.01$, in comparison with ε2 allele.
[b] $P < 0.05$, in comparison with ε2 allele.
[c] $P < 0.05$, in comparison with ε3 allele.
[d] $P < 0.01$, in comparison with ε3 allele.
* $P < 0.05$, in comparison with Hei Yi Zhuang.
** $P < 0.01$, in comparison with Hei Yi Zhuang.
*** $P < 0.001$, in comparison with Hei Yi Zhuang.

2.54%, 9.23%, 4.15%, 70.70%, 12.23%, and 1.15% in Han ($\chi^2 = 54.964$, $P < 0.001$); respectively.

Effects of apoE Genotypes and Alleles on Serum Lipid Levels. As shown in Table 3, the levels of TC, TG, LDL-C, and apoB in Hei Yi Zhuang were significantly lower than those in Han ($P < 0.01$–0.001), but the levels of HDL-C and the ratio of apoA-I to apoB in Hei Yi Zhuang were significantly higher than those in Han ($P < 0.001$). There were no significant differences of apoA-I levels between the two ethnic groups ($P > 0.05$). There were significant differences in the levels of TC, HDL-C, LDL-C, and apoB among six genotypes or three alleles (Table 4) in the both ethnic groups. There was no significant difference of apoA-I levels among the six genotypes or three alleles in the both populations ($P > 0.05$).

Factors Influencing Hyperlipidemia and Serum Lipid Levels. Multivariate analysis showed that hyperlipidemia was positively correlated with Han Chinese, age, BMI, hypertension, alcohol consumption, and apoE allele in combined population of Hei Yi Zhuang and Han ($P < 0.05$–0.001), positively correlated with age, BMI, hypertension, alcohol consumption, and apoE allele in Hei Yi Zhuang or Han population ($P < 0.05$–0.001), respectively. There was no association between hyperlipidemia and sex or cigarette smoking in both ethnic groups ($P > 0.05$, Table 5). Spearman rank correlation analysis showed that the levels of TC, LDL-C, and apoB were positively correlated, and the levels of HDL-C were negatively associated with genotypes in both ethnic groups ($P < 0.001$ for all), respectively. There is no significant correlation between the levels of TG or apoA-I and genotypes in both ethnic groups ($P > 0.05$, Table 6).

Discussion

The current study shows that the levels of serum TC, TG, LDL-C, and apoB in Hei Yi Zhuang were significantly lower than those in Han, whereas the levels of HDL-C and the ratio of apoA-I to apoB in Hei Yi Zhuang were significantly higher than those in Han. There was no significant difference in apoA-I levels between the two ethnic groups. These findings are in agreement with those of our previous studies (15, 16). It has been suggested that dyslipidemia is a complex trait caused by multiple environmental and genetic factors. Genetic factors can account for approximately one-half of the variation in plasma LDL-C concentration in humans (24). Hei Yi Zhuang is an isolated subgroup of the Zhuang minority in China. Therefore, we believe that genetic factors are involved in the results of the present study. The hereditary characteristics and genotypes of some lipid genes in the Hei Yi Zhuang population may be different from those in Han Chinese.

Studies on the polymorphism of apoE in several populations have demonstrated the presence of three common apoE alleles, ε2, ε3, and ε4, which code for genetic isoforms and determine six different phenotypes. All six apoE genotypes were observed in the Hei Yi Zhuang and Han population samples studied here. It is different in apoE

Table 5. The Risk Factors of Hyperlipidemia Between Hei Yi Zhuang and Han Chinese

Population	Risk factors	Regression coefficient	Standard error	Wald	*P*	OR
Han plus Hei	Ethnic group	0.196	0.108	3.643	0.035	0.975
	Sex	−0.132	0.133	1.473	0.226	0.872
	Age	0.175	0.033	28.756	0.000	1.192
	Body mass index	0.782	0.125	41.643	0.000	2.278
	Blood pressure	0.478	0.122	17.876	0.000	1.662
	Alcohol consumption	0.171	0.052	9.232	0.002	1.185
	Cigarette smoking	0.112	0.055	0.004	0.977	1.015
	Allele	0.553	0.136	9.997	0.002	1.247
Hei Yi Zhuang	Sex	−0.283	0.186	2.431	0.112	0.767
	Age	0.179	0.044	15.633	0.000	1.213
	Body mass index	0.732	0.204	14.585	0.000	2.147
	Blood pressure	0.472	0.155	8.987	0.004	1.579
	Alcohol consumption	0.162	0.075	4.376	0.032	1.184
	Cigarette smoking	0.035	0.025	0.112	0.721	1.038
	Allele	0.535	0.211	7.264	0.009	1.756
Han Chinese	Sex	0.002	0.193	0.002	0.993	1.012
	Age	0.168	0.045	12.683	0.000	1.218
	Body mass index	0.787	0.169	27.125	0.000	2.397
	Blood pressure	0.568	0.191	9.153	0.003	1.763
	Alcohol consumption	0.195	0.081	5.525	0.016	1.223
	Cigarette smoking	0.038	0.025	0.222	0.645	1.018
	Allele	0.616	0.184	12.231	0.000	1.853

allele frequencies between different ethnic groups. Gerdes et al. (25) reviewed 45 different population studies of apoE polymorphism and grouped eight different types of apoE allelic frequencies from these studies. The results of the current study reveal that the frequencies of apoE alleles in the Hei Yi Zhuang population were significantly different from those in Han. The frequencies of apoE alleles and genotypes in Hei Yi Zhuang were also significantly different from those observed in the other previously studied populations (26–52). Among Caucasians, the frequencies of apoE alleles and phenotypes are similar, with the high frequency of the ε4 allele in the Finnish population as a notable exception (34). The ε4 allele frequency in Iceland is only slightly higher than in other Caucasian populations (53). Results from Norway and Denmark also indicate a lower ε4 allele frequency than in Finland (54). In general, Asian populations traditionally have lower apoε4 frequency than Europeans. The cause for this regional variability is still not clear. Notably, the frequency of ε4 appears to be higher in northern regions of Europe than in southern regions (25). In Asia, a similar trend has not been described. Mongolia has been shown to have the highest frequency of apoε4 allele, while India is a country with very low ε4 allele frequency. In China, the frequency of the apoε4 allele is low (46–51, 53). Strict intra-ethnic marriages have been performed in Hei Yi Zhuang from time immemorial. Only a man and a woman who are both Hei Yi Zhuang can marry, and intermarriage with other Zhuang subgroups or other ethnic groups is forbidden. This tradition of intra-ethnic marriages has an absolute binding force for all of Hei Yi Zhuang. They must comply consciously regardless of whether they are involved in subsistence farming or employed outside the home, and also whether it is their first marriage or they are remarrying. The intra-ethnic marriages of Hei Yi Zhuang are not consanguineous marriages. Hei Yi Zhuang cannot marry direct descendants or collateral kin in seven generations. This type of intra-ethnic marriage can partially explain the differences in serum lipid levels between the Hei Yi Zhuang and Han populations, or other studied populations (15–18).

The potential relationships in humans between polymorphisms in the apoE gene and the plasma or serum levels of triacylglycerol-rich lipoproteins have been evaluated in a large number of studies. In healthy individuals, between 5% and 15% of normal interindividual variation in plasma cholesterol levels can be attributed to a common apoE polymorphism (10). In many human populations, it has been found that individuals with apoε2 display high levels of

Table 6. The Results of Spearman Rank Correlation Analysis Between the Lipid Parameters and Genotypes in the Hei Yi Zhuang and Han Populations

Ethnic groups	r_{TC} (*P*)	r_{TG} (*P*)	$r_{HDL\text{-}C}$ (*P*)	$r_{LDL\text{-}C}$ (*P*)	$r_{Apo\ A1}$ (*P*)	$r_{Apo\ B}$ (*P*)	$r_{Apo\ A1/Apo\ B}$ (*P*)
Hei Yi Zhuang	0.294 (0.000)	0.054 (0.129)	−0.167 (0.000)	0.288 (0.000)	0.035 (0.321)	0.193 (0.000)	0.016 (0.648)
Han Chinese	0.229 (0.000)	0.058 (0.075)	−0.199 (0.000)	0.296 (0.000)	0.043 (0.225)	0.299 (0.000)	0.156 (0.000)

apoE and low levels of plasma cholesterol, LDL-C, and apoB, whereas those with homozygotes and heterozygotes for the ε4 allele had higher amounts of LDL-C, which may indicate an increased risk for coronary artery disease (10, 33, 34, 53, 55–62). However, in some populations this has not been the case, and different dietary habits have been suggested to explain this apparent discrepancy (63–65). In the present study, we showed that there were significant differences in the levels of TC, HDL-C, LDL-C, and apoB among six genotypes in both ethnic groups. The levels of serum TC, LDL-C, and apoB increased with the apoE genotype in the order of ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4 in both Hei Yi Zhuang and Han populations. The levels of TC, LDL-C, and apoB were positively correlated, and the levels of HDL-C were negatively associated with genotypes in both ethnic groups, respectively. The results of the current study are in agreement with those of previous studies in Chinese populations (46–51, 53).

Epidemiological studies have provided abundant evidence that dyslipidemia is determined by genetic and environmental factors. In the present study, we also showed that hyperlipidemia was positively correlated with age, BMI, hypertension, and alcohol consumption in both ethnic groups, suggesting that environmental factors such as demographic characteristics, dietary habits, and lifestyle choices may also play an important role in determining the levels of these lipid phenotypes and the prevalence of hyperlipidemia in these populations.

In conclusion, we compared the genotypic and allelic frequencies of apoE between the Hei Yi Zhuang and Han populations. There were significant differences in the genotypic and allelic frequencies of apoE between the two ethnic groups. The frequencies of ε2 allele and ε2/ε2 genotype were significantly higher in Hei Yi Zhuang than in Han, whereas the frequencies of ε4 allele and ε4/ε4 genotype were significantly lower in Hei Yi Zhuang than in Han. There were significant differences in the levels of TC, HDL-C, LDL-C, and apoB among six genotypes in the both ethnic groups. Hyperlipidemia was positively correlated with age, BMI, hypertension, alcohol consumption, and apoE allele in both populations. The levels of TC, LDL-C, and apoB were positively correlated, and the levels of HDL-C were negatively associated with apoE genotypes in both ethnic groups. The differences in the lipid profiles between the Hei Yi Zhuang and Han populations might partly attribute to the difference in apoE genotypic and allelic frequencies.

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