SPANDIDOS PUBLICATIONS

Associations of the apolipoprotein A-I gene polymorphism and serum lipid levels in the Guangxi Hei Yi Zhuang and Han populations

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Abstract. Hei Yi Zhuang is an isolated subgroup of the Zhuang minority in China. Little is known about the effects of the genetic variants on serum lipid profiles in this population. The present study was undertaken to estimate the effects of the apolipoprotein (apo) A-I gene polymorphism adjacent to the initiate transcription site (-75 bp G/A) on the serum lipid levels in the Hei Yi Zhuang and Han populations. A total of 474 subjects of Hei Yi Zhuang and 564 subjects of Han Chinese were surveyed by a stratified randomized cluster sampling. Serum lipid levels were measured, and apoA-I gene polymorphism determined by polymerase chain reaction and restriction fragment length polymorphism. The frequencies of G and A alleles were 70.25 and 29.75% in Hei Yi Zhuang, and 65.96 and 34.04% in Han ($P<0.05$), respectively. The genotypic frequencies in Han were significantly different between males and females, subjects with normal TG (≤1.70 mmol/l) and those with high TG (>1.70 mmol/l), or subjects with normal apoA-I (≥1.20 g/l) and those with abnormal apoA-I (<1.20 g/l; $P<0.05$-0.01), respectively. The levels of LDL-C and apoA-I in Hei Yi Zhuang were higher in GG genotype than in AA or GA genotype ($P<0.05$ for each), but the levels of TG was lower in AA genotype than in GA genotype ($P<0.05$). There were also significant differences in serum TG levels among the three genotypes in Hei Yi Zhuang ($P<0.05$). The levels of HDL-C in Han were higher in GG genotype than in AA genotype ($P<0.05$), but the levels of TG in Han were lower in GG genotype than in GA genotype ($P<0.05$). The levels of apoA-I in Hei Yi Zhuang and the levels of HDL-C and apoB in Han were significantly correlated with genotype ($P<0.05$ for all). Hypertriglyceridemia was negatively associated with genotype in Hei Yi Zhuang ($P<0.01$). There were significant differences in the apoA-I -75 bp G/A between the Hei Yi Zhuang and Han populations. An association of the apoAI -75 bp G/A and serum TG, LDL-C and apoA-I levels in Hei Yi Zhuang and serum TG, HDL-C and apoB levels in Han was also observed in this study.

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Introduction

During the past 20 years, China has experienced remarkable socioeconomic development, with the mean income increasing by several fold. Consequently, the lifestyles of people throughout the country have changed dramatically. The nutritional changes have led to increased intake of fat and cholesterol contents which has gradually increased serum lipid levels. Disorders of lipid metabolism such as elevated serum levels of total cholesterol (TC) (1-3), triglycerides (TGs) (4,5), low-density lipoprotein cholesterol (LDL-C) (6,7), apolipoprotein (apo) B (8-11), or low levels of high-density lipoprotein cholesterol (HDL-C) and apoA-I (12) have been considered to be important risk factors in the pathogenesis of atherosclerosis and coronary heart disease (CHD) (13). Clinical and epidemiological studies have demonstrated that plasma levels of HDL-C (14,15) was negatively correlated with the risk of developing CHD. The protective effects of HDL-C and apoA-I are mediated mainly through the promotion of cholesterol efflux from peripheral cells (16-18). In addition, HDL has both anti-inflammatory and anti-oxidant properties (15,19-21). ApoA-I is the predominant protein component of HDL-C, and is involved in the activation of lecithin: cholesterol acyltransferase, which mediate the reverse cholesterol transport from peripheral tissues to the liver. Human apoA-I gene resides in the apoAI-CIII-AIV gene cluster, a short region on chromosome 11q23-q24 (22). The apoAI-CIII-AIV gene cluster has been identified as an associated region with hyperlipidemia, especially with hypertriglyceridemia (23-25). A common *Msp*I enzyme site 75 bp upstream from the initiate transcription site of human apoA-I gene was reported in 1990 (26,27), relevance between serum lipid patterns and this polymorphism

had been focused on since then. Extensive studies conducted on different races in different nations showed conflicting results (28-32). Serum lipid levels were decided by the interaction between gene variants and other factors such as race, environment exposure, diet, and lifestyle.

There are 56 ethnic groups in China. Han is the largest ethnic group, and Zhuang is the largest minority. Zhuang can be classified into 43 ethnic subgroups according to the differences in habitat and language. Hei Yi Zhuang is a specific subgroup of the Zhuang minority. The population size is 51,655. Because of the isolation from other ethnic groups, the special customs and cultures including their clothing, inter-ethnic marriage, diet and lifestyle are still completely conserved. We have previously reported the differences in the serum lipid parameters (33,34), and the associations of the microsomal triglyceride transfer protein gene, the lipoprotein lipase gene at *Pvu*II locus and the variable number of tandem repeats region 3' of the apoB gene polymorphism with the lipid profiles between the Hei Yi Zhuang and Han populations (35-37). We hypothesize that many genetic polymorphisms may be responsible for the differences in the serum lipid profiles between the two ethnic groups. Therefore, the aim of the present study was to assess the effect of the apoA-I -75 bp G/A polymorphism on serum lipid levels in the Guangxi Hei Yi Zhuang and Han populations.

Materials and methods

Subjects. A total of 474 subjects of Hei Yi Zhuang who reside in 7 villages in Napo County, Guangxi Zhuang Autonomous Region, China were surveyed by a stratified randomized cluster sampling. The age of the subjects ranged from 15 to 78 years, with an average age of 42.47±17.75 years. There were 234 males (49.37%) and 240 females (50.63%). The subjects were peasants. During the same period, a total of 564 subjects of Han Chinese who reside in 9 villages in Napo County were also surveyed by the same method. The average age of the subjects was 42.03±17.21 years (range 15-89). There were 279 males (49.47%) and 285 females (50.53%). They were also peasants. All study subjects were essentially healthy and had no evidence of diseases related to atherosclerosis. None of them had been treated with ß-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, socioeconomic status, and lifestyle was collected with standardized questionnaires. Smoking status was categorized into groups of cigarettes per day: <20 and ≥20. Alcohol consumption was categorized into groups of grams of alcohol per day: ≤25 g and >25 g. The physical examination included blood pressure, body height, and body weight, and body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Sitting blood pressure was measured three times with use of a mercury sphygmomanometer after the subjects had a 5-min rest, and the average of the three measurements was used in statistical analysis. Systolic blood pressure was determined by the first Korotkoff sound, and diastolic blood pressure by the fifth Korotkoff sound.

Measurements of serum lipid levels. Venous blood samples (8 ml) were drawn from a forearm vein of every subject after venous occlusion for a few seconds in a sitting position, after an overnight fast of 12 h and abstention from alcohol use for at least 12 h. Three ml was collected into glass tubes and allowed to clot at ambient temperature, and used to determine serum lipid levels, and the remaining 5 ml was transferred to tubes with anticoagulate solution (ACD: 4.80 g/l citric acid, 14.70 g/l glucose, and 13.20 g/l tri-sodium citrate) and used to extract deoxyribonucleic acid (DNA). Immediately following clotting serum was separated by centrifugation for 15 min at 3000 rpm. The levels of TC, TG, HDL-C, and LDL-C in samples were determined by enzymatic methods with commercially available kits, Tcho-1, TG-LH (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin Co. Antrim, UK, BT29 4QY), 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.

Deoxyribonucleic acid extraction. Genomic DNA was extracted from the peripheral blood leukocytes by applying phenol/chloroform method as follows: 5 ml gelatine solution (3% Gelatine and 0.5% EDTA in 7.5% NaCl solution) was added into 2 ml whole peripheral blood (with ACD anti-agglutination), mixed and after incubating at 37˚C for 10 min, the supernatant was transferred to a clean tube, centrifuged in 3500 rpm for 5 min, then the supernatant was discarded and the pellet was homogenized with 2 ml TES solution (15 mM Tris-HCl, pH 8.0; 15 mM NaCl and EDTA, pH 7.8). 0.1 ml of 10% SDS was added and mixed, then added 2 ml phenol, mixed and centrifuged 3500 rpm for 5 min. The supernatant was carefully transferred into a clean tube, chloroform/isoamylic (24:1, v/v), 2 ml was added, mixed and centrifuged 3500 rpm for 5 min. The supernatant was transferred into another clean tube, 95% ethanol (5 ml) was gently added, mixed, the precipitated DNA was removed. The DNA was washed twice in 2 ml of 70% ethanol and the pellet was stored at room temperature and suspended in 50-200 μl of ddH_2O depending on the yield of the extracted DNA. The extracted DNA was stored at 4˚C until analysis (35-37).

Determination of the apoA-I gene polymorphism

Amplification of genomic DNA. ApoA-I gene polymorphism was determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) according to previous reports (38). The sequence of the forward and backward primers used was 5'-CACCCGGGAGACCTGCA AGC-3' and 5'-TCTAAGCAGCCAGCTCTTGCA-3'. Each reaction system of a total volume of 25 μl, comprised 100 ng

genomic DNA, 0.8 μl of each primer (10 pmol/μl), 10X buffer solution, 3 μl dNTP, and 0.4 μl (1 U) Taq polymerase. For the amplification, initial denaturation at 95˚C for 5 min was followed by 30 cycles of denaturation at 95˚C for 15 sec, annealing at 61˚C for 1 min, and extension at 72˚C for 1 min, with final extension at 72˚C for 7 min. Genomic DNA in the samples was amplified by PCR and imaged by 2% agarose gel electrophoresis, the target gene corresponding to bands at 259 bp could be identified in the samples (Fig. 1).

Genotyping of the apo AI -75 Msp I locus. A *Msp*I enzyme site formed on the basis of the common G allele at apoA-I -75 bp locus, whereas it disappeared when a G→A mutation happened at the locus. Each restriction enzyme reaction was performed with 15 μl of amplified DNA; 2 μl of 10X buffer solution; and 0.15 μl (1.5 U) *Msp*I restriction enzyme in a total volume of 20 μl digested at 37˚C overnight. The digested products were separated by electrophoresis on 4% sepharose gel for 60 min at 20 mA. The length of each digested DNA fragment was determined by comparing migration of a sample with that of standard DNA marker. Stained with ethidium bromide, the gel was visualized under UV light and photographed. Genotypes were scored by an experienced reader blinded to epidemiological and lipid results. There were two *Msp*I restriction sites on the PCR amplified products. One was at the -75 bp locus and another arose on the forward primer. The digestive products contained 4 bands: 254,176, 78 and 5 bp. The 5-bp fragment was invisible in the gel owing to its fast migration speed. The presence of the restriction site at -75 bp locus (G) resulted in fragments of 254 and 5 bp. The genotypes identified were named after the presence or absence of the -75 bp enzyme restriction sites. Thus, GG was homozygote for the presence of this site (bands at 254,176, 78 and 5 bp), GA was heterozygotes for the presence and absence of the site (bands at 176, 78 and 5 bp), and AA was homozygotes for the absence of the site (bands at 254 and 5 bp).

DNA sequencing. Genomic DNA was chosen from the subjects carrying GG, GA and AA of Hei Yi Zhuang and was amplified by symmetric PCR. The PCR product was purified by low melting point gel electrophoresis and phenol extraction. The products were analyzed by using an ABI PRISM 3100 (Applied Biosystems) in our Medical Scientific Research Center, Guangxi Medical University, China.

Diagnostic criteria. The normal values of serum TC, TG, HDL-C, LDL-C, apoA-I, and apoB in our Clinical Science Experiment Center were 3.10-5.17, 0.56-1.70, 1.04-1.81, 1.70-3.37 mmol/l, 1.20-1.60, and 0.63-1.14 g/l, respectively. Individuals with TC >5.17 mmol/l and/or TG >1.70 mmol/l were defined as hyperlipidemic. Hypertension was diagnosed according to the criteria of 1999 The World Health Organization-International Society of Hypertension Guidelines for the management of hypertension (39). 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 (40).

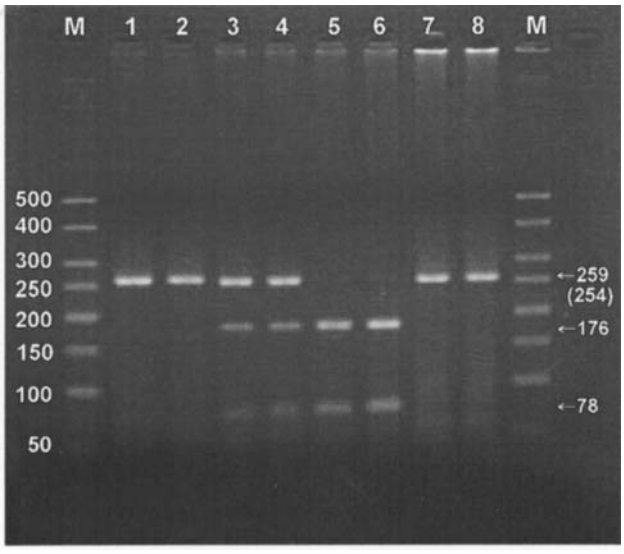

Figure 1. Computer-assisted photodocumentation showing examples of apo A-I -75 bp G/A polymorphic genotyping using Sepharose gel electrophoresis of PCR amplification products. Lane M, 50-bp DNA Ladder Marker; lanes 1 and 2, PCR amplificatied fragments (259-bp bands); lanes 3 and 4, GA genotypes (254-bp, 176- and 78-bp bands); lanes 5 and 6, GG genotypes (176- and 78-bp bands); lanes 7 and 8, AA genotypes (254-bp bands).

Statistical analysis. Epidemiological data were recorded on a pre-designed form and managed with Excel software. The quantitative variables were presented as mean ± standard deviation (SD). The difference in general characteristics between Hei Yi Zhuang and Han was tested by the Student's unpaired t-test. The allele frequencies of the apoA-I -75G/A were determined by gene counting. The χ^2 analysis was used to evaluate the allelic and genotypic frequencies that were calculated from the observed genotypic counts and to assess Hardy-Weinberg expectations. The association of genotypes with lipid variables was tested by analysis of covariance (ANCOVA). Sex, age, BMI, blood pressure, alcohol intake, cigarette smoking were adjusted for the statistical analysis. In order to evaluate the association of serum lipid levels with sex (male = 0; female = 1), age (year), BMI (kg/m^2), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), pulse pressure (mmHg), cigarette smoking (cigarettes/day), alcohol consumption (g/day), A+/A- (A+ = A carriers, A- = non-carriers of A allele), and genotype (GG=0, GA=1, AA=2), multiple linear regression analysis were executed with stepwise modeling. In addition, unconditional logistic regression analysis with forward stepwise modeling was used to analyze the relation between hypertriglyceridemia and sex (male = 0; female = 1), age (<20=1; 20-29=2; 30-39=3; 40-49=4; 50-59=5; 60-69=6; ≥70=7), BMI (kg/m^2), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), pulse pressure (mmHg), cigarette smoking (non-smokers = 0; <20 cigarettes/day = 1; ≥20 cigarettes/day = 2), alcohol consumption (non-drinkers = 0; <25 g/day = 1; ≥25 g/day = 2), A+/A-(A+ = A carriers, A- = non-carriers of A allele), genotype (GG=0, GA=1, AA=2). All statistical analyses were performed with the statistical software package SPSS 13.0 (SPSS Inc., Chicago, IL). A $P<0.05$ was considered significant.

Table I. Comparison of demographic, lifestyle characteristics and serum lipid levels between Hei Yi Zhuang and Han.

Characteristics	Hei Yi Zhuang	Han Chinese	t (χ^2)	P-value
Male/female	234/240	279/285	0.001	0.974
Age (years)	42.47±17.75	42.03±17.21	-0.407	0.684
Height (cm)	151.62±10.93	152.25±8.41	1.030	0.303
Weight (kg)	49.42±9.18	51.59±8.31	3.999	0.000
Cigarette smoking [n (%)]	153 (32.3)	180 (31.9)	0.016	0.901
Alcohol consumption [n (%)]	258 (54.4)	249 (44.1)	10.896	0.001
Body mass index (kg/m^2)	21.33±2.43	22.18±2.61	5.442	0.000
Systolic blood pressure (mm Hg)	124.73±17.14	119.91±15.44	-4.726	0.000
Diastolic blood pressure (mm Hg)	76.35±11.91	75.35±9.12	-1.510	0.132
Pulse pressure (mm Hg)	48.38±12.27	44.59±10.74	-5.243	0.000
Total cholesterol (mmol/l)	4.47±0.85	4.76±0.99	4.999	0.000
Triglycerides (mmol/l)	0.88±0.51	1.095±0.59	-6.859	0.000
HDL-C (mmol/l)	2.12±0.50	1.97±0.45	-4.732	0.000
LDL-C (mmol/l)	2.22±0.63	2.52±0.69	7.288	0.000
Apolipoprotein (apo) A-I (g/l)	1.45±0.14	1.43±0.17	-2.152	0.032
Apo B (g/l)	0.85±0.21	0.94±0.20	7.418	0.000
Apo A-I/Apo B	1.82±0.60	1.57±0.33	-8.224	0.000

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Results

General characteristics. Table I gives the general characteristics of the subjects between Hei Yi Zhuang and Han. The levels of systolic blood pressure, pulse pressure, and the percentage of subjects who consumed alcohol were higher in Hei Yi Zhuang than in Han (P<0.01 for all), whereas body weight and BMI were higher in Han than in Hei Yi Zhuang (P<0.01 for each). There were no significant differences in body height, diastolic blood pressure, age, the percentage of subjects who smoked cigarettes, or the ratio of male to female between the two ethnic groups (P>0.05).

Serum lipid levels. As shown in Table I, the levels of TC, TG, LDL-C, and apoB in Hei Yi Zhuang were lower than those in Han (P<0.01), but the levels of HDL-C, apoA-I and the ratio of apoA-I to apoB in Hei Yi Zhuang were higher than those in Han (P<0.05-0.01).

Genotypic and allelic frequencies. Genotypic and allelic frequencies in the study groups are shown in Table II. The frequencies of G and A alleles were 70.25 and 29.75% in Hei Yi Zhuang, and 65.96 and 34.04% in Han (P<0.05), respectively. The frequencies of GG, GA and AA genotypes were 48.10, 44.30 and 7.60% in Hei Yi Zhuang, and 43.09, 45.74 and 11.17% in Han (P>0.05), respectively. The genotypic frequencies in Han were significant differences between males and females, between subjects with normal TG (≤1.70 mmol/l) and subjects with high TG (>1.70 mmol/l), or between subjects with normal apoA-I (≥1.20 g/l) and subjects with abnormal apoA-I (<1.20 g/l; P<0.05-0.01), respectively. There were no significant differences in the remaining genotypic or allelic frequencies between Hei Yi Zhuang and Han, or between the subgroups of the same ethnic group. The GG, GA and AA genotypes shown by the PCR-RFLP method were also confirmed by direct sequencing (Figs. 2-4).

We also compared the differences in allelic frequencies between Hei Yi Zhuang and other ethnic groups in China, or other races in different countries (28-30,41-46). The frequency of the A allele was higher in Hei Yi Zhuang (0.297) than in Yi nationality (0.220) (41), Australian Caucasian (0.218) (42), Italian Caucasian (0.191) (30), Belgian Caucasian (0.181) (28), American Caucasian (0.157) (43), Finnish Caucasian (0.147) (44), or Icelandic Caucasian (0.124) (29) (P<0.01 for all). However, there were no significant differences in the A allele frequency between Hei Yi Zhuang and Han Chinese in Beijing (45), or Singapore Chinese (46) (P>0.05 for each; Table III).

ApoA-I gene polymorphism and serum lipid levels. As shown in Table IV, the levels of LDL-C and apoA-I in Hei Yi Zhuang were higher in GG genotype than in AA or GA genotype (P<0.05 for each). There were also significant differences in serum TG levels among the three genotypes in Hei Yi Zhuang (P<0.05). The levels of HDL-C in Han were higher in GG genotype than in AA genotype (P<0.05), but the levels of TG in Han were lower in GG genotype than in GA genotype (P<0.05). There were no significant differences in the remaining lipid parameters among the three genotypes in Hei Yi Zhuang, or in Han.

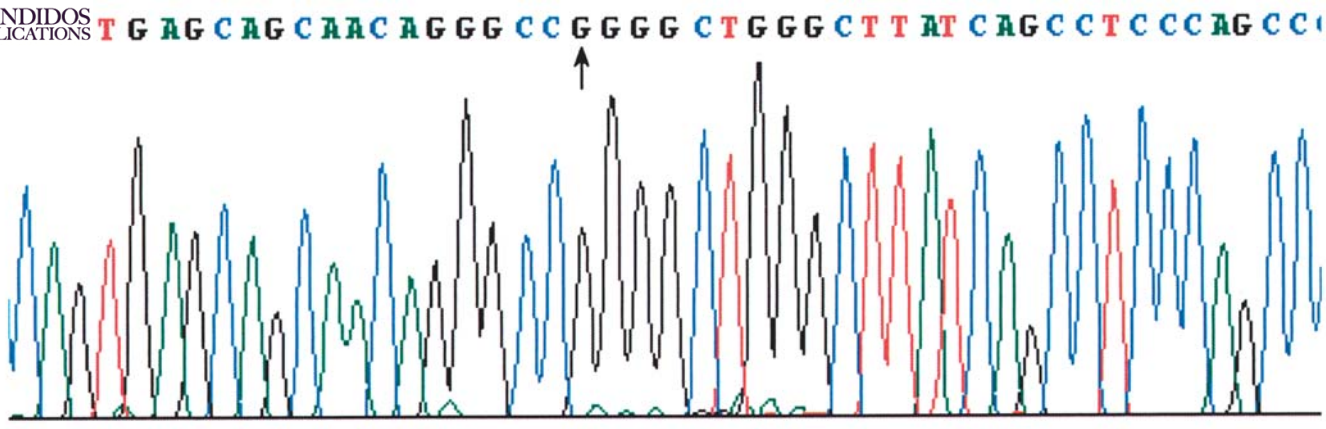

Figure 2. The GG homozygote of apo A-I -75 bp G/A SNP detected by sequencing. Arrow represented the G allele locus at apo A-I gene -75 bp.

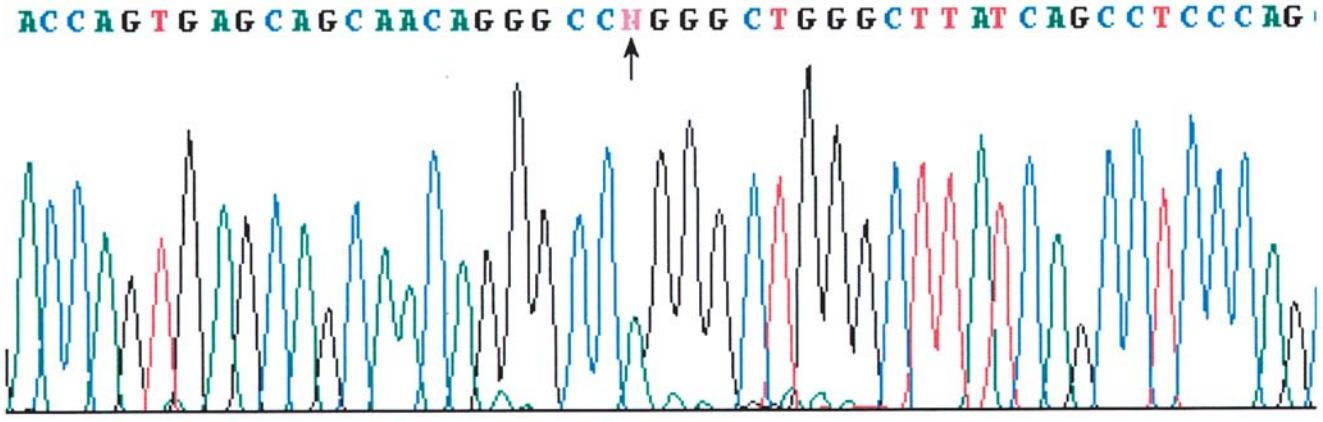

Figure 3. The GA heterozygote of apo A-I -75 bp G/A SNP detected by sequencing. Arrow represented the heterozygosity at apo A-I gene -75 bp locus.

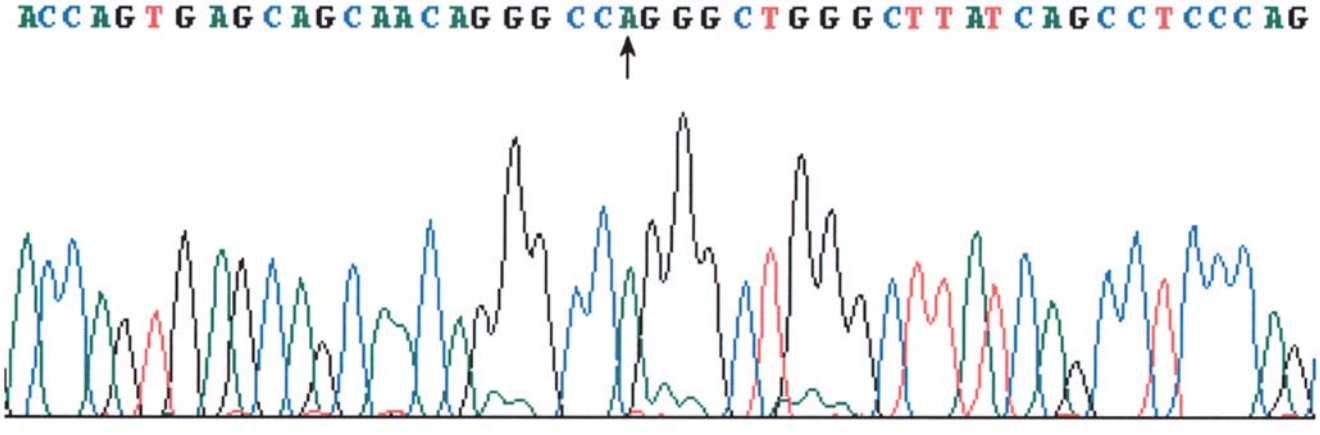

Figure 4. The AA homozygote of apo A-I -75 bp G/A SNP detected by sequencing. Arrow represented the A allele locus at apo A-I gene -75 bp.

Risk factors for serum lipid levels. Table V gives the correlative factors for serum lipid parameters between Hei Yi Zhuang and Han. The levels of TC, HDL-C, LDL-C, apoA-I, and apoB were significantly correlated with several environment factors such as age, sex, BMI, blood pressure, alcohol consumption, and cigarette smoking in both ethnic groups. The levels of apoA-I in Hei Yi Zhuang and the levels of HDL-C and apoB in Han were significantly correlated with genotype ($P<0.05$ for all).

Risk factors for hypertriglyceridemia. Unconditional multiple logistic regression analysis shows that hypertriglyceridemia

Table II. Comparison of genotypic and allelic frequencies between the subgroups divided according to sex and the serum lipid levels.

Groups	n	Genotypes [n (%)]			Alleles [n (%)]	
		GG	GA	AA	G	A
Hei Yi Zhuang	474	228 (48.10)	210 (44.30)	36 (7.60)	666 (70.25)	282 (29.75)
Han Chinese	564	243 (43.09)	258 (45.74)	63 (11.17)	744 (65.96)	384 (34.04)
χ^2	-		4.999		4.362	
P-value	-		0.082		0.037	
Hei Yi Zhuang						
Male	234	108 (46.15)	111 (47.44)	15 (6.41)	327 (69.87)	141 (30.13)
Female	240	120 (50.00)	99 (41.25)	21 (8.75)	339 (70.63)	141 (29.37)
χ^2	-		2.242		0.064	
P-value	-		0.326		0.800	
Normal TG	420	198 (47.14)	186 (44.29)	36 (8.57)	582 (69.29)	258 (30.71)
High TG	54	30 (55.56)	24 (44.44)	0	84 (77.78)	24 (22.22)
χ^2	-		5.333		3.302	
P-value	-		0.070		0.069	
Normal LDL-C	447	210 (46.98)	201 (44.97)	36 (8.05)	621 (69.46)	273 (30.54)
High LDL-C	27	18 (66.67)	9 (33.33)	0	45 (83.33)	9 (16.67)
χ^2	-		5.004		4.688	
P-value	-		0.082		0.030	
Normal Apo A-I	378	174 (46.03)	174 (46.03)	30 (7.94)	522 (69.05)	234 (30.95)
Abnormal Apo A-I	96	54 (56.25)	36 (37.50)	6 (6.25)	144 (75.00)	48 (25.00)
χ^2	-		3.206		2.596	
P-value	-		0.201		0.107	
Han Chinese						
Male	279	135 (48.39)	111 (39.78)	33 (11.83)	381 (68.28)	177 (31.72)
Female	285	108 (37.89)	147 (51.58)	30 (10.53)	363 (63.68)	207 (36.32)
χ^2	-		8.103		2.652	
P-value	-		0.017		0.103	
Normal TG	495	210 (42.42)	222 (44.85)	63 (12.73)	642 (64.85)	348 (35.15)
High TG	69	33 (47.83)	36 (52.17)	0	102 (73.91)	36 (26.09)
χ^2	-		9.902		4.432	
P-value	-		0.007		0.035	
Normal LDL-C	501	219 (43.71)	228 (45.51)	54 (10.78)	666 (66.47)	336 (33.53)
High LDL-C	63	24 (38.10)	30 (47.62)	9 (14.28)	78 (61.90)	48 (38.10)
χ^2	-		1.081		1.038	
P-value	-		0.583		0.308	
Normal Apo A-I	456	189 (41.45)	207 (45.39)	60 (13.16)	585 (64.14)	327 (35.86)
Abnormal Apo A-I	108	54 (50.00)	51 (47.22)	3 (2.78)	159 (73.61)	57 (26.39)
χ^2	-		9.969		6.970	
P-value	-		0.007		0.008	

TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; Apo A-I, apolipoprotein A-I; normal TG, TG ≤1.70 mmol/l; high TG, TG >1.70 mmol/l; normal LDL-C, LDL-C ≤3.37 mmol/l; high LDL-C, LDL-C >3.37 mmol/l; normal Apo A-I, Apo A-I ≥1.20 g/l; abnormal Apo A-I, Apo A-I <1.20 g/l.

Comparison of allelic frequency between Hei Yi Zhang and various races.

Ethnic groups (refs.)	n	Allele [n(%)]		χ^2	P-value
		G	A		
Hei Yi Zhang	474	666 (0.703)	282 (0.297)	-	-
Liangshan Yi (41)	363	530 (0.767)	161 (0.233)	8.423	0.004
Beijing Han (45)	450	668 (0.742)	232 (0.258)	3.623	0.057
Singaporean Chinese (46)	287	416 (0.725)	158 (0.275)	0.858	0.354
Australian Caucasian (42)	243	380 (0.782)	106 (0.218)	10.253	0.001
Italian Caucasian (30)	204	330 (0.809)	78 (0.191)	16.526	0.000
Belgian Caucasian (28)	144	236 (0.819)	52 (0.181)	15.311	0.000
American Caucasian (43)	315	531 (0.843)	99 (0.157)	40.692	0.000
Finnish Caucasian (44)	184	314 (0.853)	54 (0.147)	31.677	0.000
Icelandic Caucasian (29)	315	552 (0.876)	78 (0.124)	64.819	0.000

Table IV. Comparison of lipid levels among genotypes of Hei Yi Zhuang and Han.

Genotypes	n	TC (mmol/l)	TG (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)	ApoA-I (g/l)	ApoB (g/l)
Hei Yi Zhuang							
GG	228	4.56±0.83	0.89±0.48	2.17±0.52	2.32±0.64[b]	1.46±0.13[a]	0.87±0.20
GA	210	4.37±0.88	0.91±0.69[a]	2.07±0.48	2.12±0.65	1.44±0.16	0.83±0.22
AA	36	4.47±0.78	0.69±0.40	2.05±0.51	2.22±0.36	1.41±0.13	0.86±0.14
F	-	1.620	4.413	2.281	5.398	3.999	1.549
P-value	-	0.199	0.013	0.103	0.005	0.019	0.214
Han Chinese							
GG	243	4.71±1.04	1.01±0.54	2.00±0.48[a]	2.50±0.68	1.42±0.18	0.92±0.21
GA	258	4.81±0.97	1.17±0.64[a]	1.97±0.45	2.53±0.70	1.43±0.16	0.95±0.19
AA	63	4.71±0.86	1.01±0.54	1.89±0.31	2.62±0.67	1.42±0.13	0.97±0.18
F	-	0.297	4.892	3.953	1.319	0.729	1.968
P-value	-	0.743	0.008	0.020	0.268	0.483	0.141

TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Apo A-I, apolipoprotein A-I; [a]P<0.05 in comparison with AA genotype of the same ethnic group; [b]P<0.05 in comparison with GA genotype of the same ethnic group.

was positively correlated with age and alcohol consumption, and negatively associated with genotype in Hei Yi Zhuang (P<0.05 for all), whereas it was positively correlated with BMI and alcohol consumption in Han (P<0.001 for each; Table VI).

Discussion

The results of the present study show that serum TC, TG, LDL-C and apoB levels were significantly lower in Hei Yi Zhuang than in Han, whereas the levels of HDL-C, apoA-I and the ratio of apoA-I to apoB in Hei Yi Zhuang were significantly higher than those in Han. These findings are consistent with those of our previous studies in a large population (34-37). Hei Yi Zhuang is an isolated subgroup of the Zhuang minority in China. Strict intra-ethnic marriages have been performed from time immemorial in this ethnic subgroup. Namely, only a man and a woman who are both Hei Yi Zhuang can marry, and can not intermarry with the other subgroups of Zhuang or other ethnic groups. Therefore, we are confident that some genetic polymorphisms may be involved in determing the serum lipid levels in this population.

In the present study, we showed that the frequency of A allele at the apoA-I -75 bp G/A was higher in Hei Yi Zhuang

Table V. Relationship between the lipid parameters and relative factors in Hei Yi Zhuang and Han.

Lipid parameters	Risk factors	B	Standard error	Beta	t	P-value
Hei Yi Zhuang						
TC	Age	0.008	0.002	0.172	3.777	0.000
	Diastolic blood pressure	0.015	0.003	0.213	4.591	0.000
	Gender	-0.213	0.077	-0.125	-2.755	0.006
HDL-C	Diastolic blood pressure	0.010	0.002	0.240	5.360	0.000
	Alcohol consumption	0.009	0.001	0.219	4.200	0.000
	Cigarette smoking	-0.007	0.003	-0.110	-2.131	0.034
LDL-C	Age	0.006	0.002	0.160	3.610	0.000
	Body mass index	0.038	0.012	0.146	3.291	0.001
	A+/A-	-0.179	0.056	-0.141	-3.201	0.001
	Gender	-0.149	0.056	-0.118	-2.645	0.008
ApoA-I	Age	0.002	0.000	0.187	4.167	0.000
	Body mass index	0.010	0.003	0.172	3.885	0.000
	Pulse pressure	-0.001	0.001	-0.102	-2.220	0.027
	Alcohol consumption	0.008	0.005	0.239	5.578	0.000
	Genotype	-0.021	0.010	-0.092	-2.157	0.031
ApoB	Age	0.002	0.001	0.215	4.839	0.000
	Body mass index	0.013	0.004	0.156	3.504	0.001
Han Chinese						
TC	Age	0.012	0.002	0.202	4.853	0.000
	Body mass index	0.071	0.015	0.189	4.673	0.000
	Diastolic blood pressure	0.013	0.005	0.117	2.806	0.005
	Cigarette smoking	-0.014	0.005	-0.119	-2.967	0.003
HDL-C	Age	0.004	0.001	0.168	4.016	0.000
	Body mass index	-0.032	0.007	-0.187	-4.598	0.000
	Diastolic blood pressure	0.007	0.002	0.147	3.473	0.001
	Gender	-0.129	0.037	-0.143	-3.507	0.000
	Genotype	-0.062	0.027	-0.091	-2.280	0.023
LDL-C	Age	0.007	0.002	0.186	4.097	0.000
	Body mass index	0.038	0.011	0.144	3.521	0.000
	Systolic blood pressure	0.004	0.002	0.097	2.104	0.036
	Alcohol consumption	-0.056	0.024	-0.122	-2.886	0.004
ApoA-I	Age	0.002	0.000	0.178	4.242	0.000
	Body mass index	-0.007	0.003	-0.105	-2.591	0.010
	Diastolic blood pressure	0.003	0.001	0.158	3.687	0.000
	Alcohol consumption	0.002	0.000	0.117	2.753	0.006
	Cigarette smoking	-0.003	0.001	-0.155	-3.777	0.000
ApoB	Age	0.002	0.000	0.185	4.606	0.000
	Body mass index	0.016	0.003	0.214	5.277	0.000
	Cigarette smoking	-0.003	0.001	-0.119	-2.950	0.003
	Genotype	0.028	0.012	0.093	2.306	0.021
\						
Hei plus Han						
TC	Age	0.010	0.002	0.186	6.121	0.000
	Gender	-0.151	0.056	-0.080	-2.675	0.008
	Body mass index	0.054	0.011	0.148	4.862	0.000
	Diastolic blood pressure	0.013	0.003	0.140	4.485	0.000

Continued.

Lipid parameters	Risk factors	B	Standard error	Beta	t	P-value
HDL-C	Age	0.003	0.001	0.094	2.904	0.004
	Gender	-0.072	0.034	-0.075	-2.131	0.033
	Body mass index	-0.017	0.006	-0.093	-3.015	0.003
	Diastolic blood pressure	0.009	0.001	0.194	6.039	0.000
	Pulse pressure	-0.003	0.001	-0.064	-2.017	0.044
	Cigarette smoking	-0.005	0.002	-0.079	-2.251	0.025
	Alcohol consumption	0.010	0.002	0.160	4.801	0.000
	Genotype	-0.066	0.022	-0.089	-3.017	0.003
LDL-C	Age	0.006	0.001	0.161	4.981	0.000
	Gender	-0.097	0.042	-0.072	-2.296	0.022
	Body mass index	0.038	0.008	0.144	4.824	0.000
	Systolic blood pressure	0.003	0.001	0.069	2.108	0.035
	Alcohol consumption	-0.099	0.046	-0.074	-2.338	0.020
Apo A-I	Age	0.002	0.000	0.175	5.424	0.000
	Gender	-0.033	0.010	-0.105	-3.349	0.001
	Diastolic blood pressure	0.002	0.000	0.139	4.406	0.000
	Pulse pressure	-0.001	0.000	-0.066	-2.101	0.036
	Alcohol consumption	0.004	0.001	0.190	5. 956	0.000
Apo B	Age	0.002	0.000	0.188	6.448	0.000
	Gender	-0.035	0.012	-0.084	-2.885	0.004
	Body mass index	0.015	0.002	0.182	6.127	0.000

TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Apo A-I, apolipoprotein A-I; ApoB, apolipoprotein B; A+, A carriers; A-, non-carriers of A allele; B, unstandardized coefficient; Beta, standardized coefficient.

Table VI. Risk factors of hypertriglyceridemia in Hei Yi Zhuang and Han.

Ethnic groups	Risk factors	Regression coefficient	Standard error	Odds ratio	Wald	P-value
Hei Yi Zhuang	Age	0.197	0.087	1.217	5.069	0.024
	Alcohol consumption	0.554	0.183	1.740	9.202	0.002
	Genotype	-0.534	0.257	0.586	4.324	0.038
Han Chinese	Body mass index	0.171	0.047	1.187	13.262	0.000
	Alcohol consumption	0.737	0.179	2.090	16.904	0.000
Hei plus Han	Gender	-0.470	0.230	0.625	4.192	0.041
	Body mass index	0.090	0.038	1.094	5.550	0.018
	Systolic blood pressure	0.013	0.006	1.013	5.163	0.023
	Alcohol consumption	0.691	0.148	1.997	21.967	0.000

(0.297) than in Liangshan Yi (0.233), the other minority in China (41), and Caucasians (0.124-0.218) in the western countries (28-30,42-44). There were no significant differences in the A allele frequency between Hei Yi Zhuang and Han Chinese in Beijing (0.258) (45), and Singaporean Chinese (0.275) (46). Rare allelic frequency of Caucasians from different nations was similar and significantly lower than that of oriental races, substantiating that this SNP was distinguishing among races, also suggesting that nationalities with similar allelic frequencies may have ancestor homology.

The potential relationships in humans between polymorphisms at the apoA-I -75 bp G/A and plasma or serum lipid levels have been evaluated in a large number of studies. Talmud *et al* (44) found that A allele induced elevated HDL-C

and apoA-I levels. This relationship was also demonstrated in many other studies, but with sex-dependency, Jeenah *et al* (27) and Sigurdsson *et al* (29) found in British and Icelandic male respectively that A allele confered high serum apoA-I level. Xu *et al* (30) reported A carriers had higher mean levels of TC, LDL-C, apoB and apoA-I than G homozygotes in Italian boys. Nevertheless, Pagani *et al* (26) discovered A allele was positively related to HDL-C concentration in Italian female exclusively. In addition, this facilitation of A allele to elevated serum HDL-C and apoA-I levels was affected by lifestyle factors such as smoking and drinking alcohol. Study conducted on 287 healthy Singaporean Chinese found this facilitation in male non-smoker merely (46). Furthermore, a study (41) on Yi-emigrants in China reported that, alcohol users who carried A allele had notably lower serum HDL-C and apoA-I concentrations than G homozygotes, however, this relationship was reversed in non-drinkers. The G→A transition could elucidate 18% of inter-individual variations of serum apoA-I levels in non-drinkers of Yi-emigrants. There are also some studies that detected no correlation between the apoA-I -75 bp G/A and serum lipid patterns (32,47). Considerable studies were undertaken to estimate the effect of the apoA-I -75G/A to serum lipid traits of population with basal diseases. In most research, CHD groups had higher AA genotypic frequencies than healthy controls (48-50), TG level of AA homozygotes was higher than that of GG homozygotes (51). In some subsets of male CHD subjects, AA homozygote conferred decreased serum HDL-C concentration (48,52). Two separate studies performed on Australian CHD patients disclosed association between A allele and coronary artery stenosis, moreover, AA genotypic frequency trended to increase by the growing number of stenosed coronary arteries (42,49). In the present study, we showed that the levels of LDL-C and apoA-I in Hei Yi Zhuang were lower in AA or GA genotype than in GG genotype, but the levels of TG was lower in AA genotype than in GA genotype. There were also significant differences in serum TG levels among the three genotypes in Hei Yi Zhuang, but the levels of TG in Han were higher in GA genotype than in GG genotype. The levels of apoA-I in Hei Yi Zhuang and the levels of HDL-C and apoB in Han were significantly correlated with genotype. Hypertriglyceridemia was negatively associated with genotype in Hei Yi Zhuang but not in Han. These contradictory results of all association studies on this SNP, to some extent, demonstrated the generally accepted hypothesis (32,53), which denies direct effect of the G/A substitution on HDL-C and apoAI concentration, but is due to unknown polymorphism(s), nearby or distant, which is in linkage disequilibrium with this apoA-I -75G/A SNP. The reversed effects of A allele to lipids metabolism between healthy and CHD population indicate that there may be an unknown SNP as genetic marker of CHD absent in normo-subjects, which is in linkage disequilibrium with the apoA-I -75G/A transition.

In addition to the effect of genetic factors, serum lipid levels were also influenced by multiple environmental factors. Some epidemiologic surveys have shown that high-fat diet intake, particularly containing abundant saturated fatty acids, raises the blood cholesterol concentrations and predisposes individuals to cardiovascular disease (54,55). Connor *et al* (56) also showed significantly positive association between serum concentration of cholesterol and cholesterol constituent of food in a cohort taking low-cholesterol diet. As we reported previously (35-37), the people of Hei Yi Zhuang live in the infertile mountainous area. Their earnings derive mostly from planting corn and paddy. Corn gruel and tortillas are their staple food all the year around. Approximately 90% of the beverages were corn wine and rum that they brew themselves. In contrast, rice was the staple food in Han. The standard of living in Han was higher than that in Hei Yi Zhuang. The intake of animal fat was also more than that in Hei Yi Zhuang. About 90% of the beverage was rice wine. Corn was reported as healthy food because it contained abundant dietary fiber and high-quality plant protein (57). High dietary fiber intake can result in a decrease of serum cholesterol levels in healthy and hyperlipidemic subjects (58). Corn oil was a kind of edible oils that is enriched with polyunsaturated fatty acid and monounsaturated fatty acid (59). Suitable intakes of polyunsaturated fatty acids and monounsaturated fatty acids can lower the serum levels of cholesterol and LDL-C (60,61). A potential beneficial effect of dietary monounsaturated fatty acids on HDL-C has also been suggested (61).

In conclusion, the present study shows that there were significant differences in the allele frequency at the apoA-I -75G/A locus between the Hei Yi Zhuang and Han populations. Serum levels of TG, LDL-C and apoA-I in Hei Yi Zhuang were influenced by different genotypes, whereas the levels of TG, HDL-C and apoB in Han were associated with genotypes. The differences in serum lipid levels between the two ethnic groups might be attributed to the interaction of environmental and genetic factors.

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