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Original Article

Association of the rs4820314 SNV and several exposure factors with lipid-related traits in the Chinese Jing and Han populations

Tao Guo[1], Rui-Xing Yin[1], Feng Huang[1], Jin-Zhen Wu[1], Shang-Ling Pan[2], Wei-Xiong Lin[3]

[1]Department of Cardiology, Institute of Cardiovascular Diseases, The First Affiliated Hospital, Nanning 530021, Guangxi, China; [2]Department of Pathophysiology, School of Premedical Sciences Nanning 530021, Guangxi, China; [3]Department of Molecular Genetics, Medical Scientific Research Center, Guangxi Medical University, Nanning, Guangxi, China

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Abstract: The association of the phospholipase A2 group VI gene (*PLA2G6*) single nucleotide variants (SNVs) and exposure factors with lipid-related traits is little known. This study sought to determine the interactions of the *PLA2G6* rs4820314 SNV and several exposure factors on lipid-associated phenotypes in the Chinese Jing and Han populations. A total of 1629 unrelated participants (Jing, 785 and Han, 844) were randomly selected from our previous stratified randomized samples. Genotypes of the *PLA2G6* rs4820314 were determined by polymerase chain reaction and restriction fragment length polymorphism, and then confirmed by direct sequencing. Analyses were performed to assess the interaction between genotypes and several exposure factors on serum lipid levels. The levels of serum total cholesterol and triglyceride (TG) were higher in Jing than in Han. The genotypic and allelic frequencies of *PLA2G6* rs4820314 were different between the two populations and body mass index (BMI), alcohol consumption and hyperlipidemia subgroups ($P < 0.05$-0.001). The A allele carriers had higher serum TG levels and the risk of hyperlipidemia than the A allele non-carriers. Consistently, in each population, serum TG levels increased more evidently with higher BMI and/or alcohol consumption of *PLA2G6* rs4820314 AG/AA genotype than in carriers of the major genotype (GG). Participants with different genotypes of *PLA2G6* rs4820314 exhibit differential effected from BMI and alcohol consumption for the hyperlipidemia risk. These findings suggest potential critical roles for the gene-exposure (G×E) interaction between *PLA2G6* rs4820314 and BMI/alcohol consumption in regulating lipid-related traits and hypertriglyceridemia risk.

Keywords: Single nucleotide variant (SNV), body mass index (BMI), alcohol consumption, G×E interaction, lipid-related trait

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide [1]. In 2008, 30% of all global death is attributed to CVDs. Death caused by CVDs is also higher in low- and middle-income countries as over 80% of all global death caused by CVDs occurred in those countries. It is also estimated that by 2030, over 23 million people will die from CVDs each year. There are several exposure factors for CVDs: age, gender, tobacco use, physical inactivity, excessive alcohol consumption, unhealthy diet, obesity, CVD family history, raised blood pressure (hypertension), raised blood sugar (diabetes mellitus), raised blood cholesterol (hyperlipidemia), psychosocial factors, poverty and low educational status, and air pollution [2-6]. While the individual contribution of each risk factor varies between different communities or ethnic groups the overall contribution of these exposure factors is very consistent [7]. Some of these exposure factors, such as age, gender or genetic are immutable; however, many important cardiovascular risk factors are modifiable including prevention of obesity and control alcohol intake.

Obesity is an important component of the pathophysiology of chronic diseases including dyslipidemia and CVDs [8]. Identifying genetic/epigenetic modifications associated with elevated adiposity may point to genomic/epigenomic pathways that are dysregulated in nu-

merous conditions [9]. The risk of CVD is known to be lower in light-to-moderate alcohol drinkers than in abstainers [10]. The effects of alcohol on lipid metabolism, especially the high-density lipoprotein cholesterol (HDL-C)-elevating effects, are thought to greatly contribute to the cardioprotective action of alcohol [11]. On the other hand, excessive alcohol consumption has been shown to cause hypertriglyceridemia [12, 13], which is a prevalent exposure factor for CVD [14-16]. With regard to mechanisms underlying the effects of alcohol on lipid metabolism, alcohol consumption has been shown to increase the activity of lipoprotein lipase and decrease the activity of cholesteryl ester transfer protein, resulting in elevation of HDL-C [17]. The hypertriglyceridemia induced by excessive alcohol drinking may be mainly due to an increase in the synthesis of large very low-density lipoprotein (VLDL) particles in the liver.

Recent genome-wide association studies (GWAS) have identified > 30 novel genetic loci associated with lipid-related traits [18]. The phospholipase A2 group VI gene (*PLA2G6*) and the protein encoded by this gene is an A2 phospholipase, a class of enzyme that catalyzes the release of fatty acids from phospholipids and participate in the pathophysiology of lipid metabolism. (http://www-ncbi-nlm-nih-gov.ezp-prod1.hul.harvard.edu/gene/8398).

As acoastal ethnic minority of China, Jing continue to speak the Vietnamese language and persist in fishing for a living until today. And Jing is small minority with a population of 22,517 (in 2000 the fifth national census statistics of China) compared with Han, which the biggest one. Jing population is a relatively conservative and isolated minority, and preserves their custom of intra-ethnic marriages. Thus, their genetic background may be less heterogeneous within the population [19].

Materials and methods

Ethical considerations

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Written informed consent for all the participants is obtained as per the guidelines.

Study populations

A total of 1629 individuals, including 785 Jing (387 males, 49.3% and 398 females, 50.7%; mean age 54.91 ± 10.93 years) and 844 Han (418 males, 49.5% and 426 females, 50.5%; mean age 54.75 ± 11.97 years). They were selected randomly from previous stratified randomized samples [20]. All subjects were agricultural workers and/or fishermen from Dongxing City, Guangxi Zhuang Autonomous Region, People's Republic of China [21].

The presence of CVD exposure factors was determined using the criteria of the European society of cardiology [22]: a hypertensive condition was attributed when systolic blood pressure values were ≥ 140 mmHg or diastolic blood pressure values ≥ 90 mmHg in at least two separate measurements. 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 and > 25; obesity was defined as body mass index (BMI, kg/m^2): underweight (BMI < 18.5), normal weight (18.5 ≤ BMI < 24), overweight (24 ≤ BMI < 28) and obesity (28 ≤ BMI); or waist circumference > 85 cm in men or > 80 cm in women. Hypercholesterolemia was considered for total cholesterol (TC) > 5.17 mmol/l, and hypertriglyceridemia was triglyceride (TG) > 1.70 mmol/l. The presence of metabolic syndrome (MetS) was determined using the 2001 NCEP-ATP-III definition [23]. The participants were not taking medications known to affect serum lipid levels (lipid lowering drugs such as statins or fibrates, beta-blockers, diuretics, or hormones). They did not show any signs of CVD from their health questionnaires and clinical examinations.

Serum lipid phenotype analyses

Blood samples were drawn from all subjects after an overnight fast. Sera were separated immediately and stored at -20°C. Serum lipid levels were measured. The levels of TC, TG, HDL-C and low-density lipoprotein cholesterol (LDL-C) in the samples were determined by enzymatic methods with commercially available kits. Serum apolipoprotein (Apo) A1 and ApoB levels were assessed by the immunoturbidimetric immunoassay.

Isolation of DNA

Genomic DNA was extracted from EDTA whole blood sample using aspin column method according to the protocol (QIAamp Blood Kit; Qiagen GmbH, Hilden, Germany). DNA was stored at -20°C till the time of use.

Table 1. Anthropometric and metabolic characteristics in the Chinese Jing and Han populations

Parameter	Jing (n = 785)	Han (n = 844)	*t* (X^2)	*P*
Male/Female	387/398	418/426	0.008	0.927
Age (year)	54.91 ± 10.93	54.75 ± 11.97	0.275	0.784
Height (cm)	158.86 ± 7.19	157.97 ± 7.70	2.676	0.008
Weight (kg)	59.11 ± 9.22	57.39 ± 9.27	3.764	0.000
Body mass index (kg/m^2)	23.36 ± 3.11	22.96 ± 3.15	2.551	0.011
Waist circumference (cm)	80.02 ± 8.87	78.17 ± 8.89	4.189	0.000
Cigarette smoking [n (%)]				
Non-smoker	620 (79.0)	646 (76.5)		
≤ 20 cigarettes/day	38 (4.8)	34 (4.0)		
> 20 cigarettes/day	127 (16.2)	164 (19.4)	3.328	0.189
Alcohol consumption [n (%)]				
Non-drinker	611 (77.8)	588 (69.7)		
≤ 25 g/day	78 (9.9)	54 (6.4)		
> 25 g/day	96 (12.2)	202 (23.9)	40.426	0.000
Systolic blood pressure (mmHg)	129.38 ± 18.38	131.07 ± 17.69	-1.892	0.059
Diastolic blood pressure (mmHg)	80.81 ± 10.23	81.26 ± 9.67	-0.906	0.365
Pulse pressure (mmHg)	48.57 ± 14.73	49.82 ± 14.44	-1.721	0.085
Glucose (mmol/L)	6.55 ± 1.31	6.55 ± 1.01	-0.140	0.889
Total cholesterol (mmol/L)	5.11 ± 0.92	4.83 ± 0.81	6.668	0.000
Triglyceride (mmol/L)	1.41 (1.14)	1.27 (1.03)	-4.524	0.000
HDL-cholesterol (mmol/L)	1.78 ± 0.49	1.80 ± 0.44	-0.755	0.451
LDL-cholesterol (mmol/L)	2.85 ± 0.40	2.84 ± 0.43	0.870	0.385
Apolipoprotein A1 (g/L)	1.31 ± 0.22	1.33 ± 0.20	-1.164	0.245
ApoB (g/L)	1.06 ± 0.25	1.04 ± 0.24	1.630	0.103
ApoA1/ApoB	1.31 ± 0.39	1.35 ± 0.39	-1.901	0.057

HDL, high density lipoprotein; LDL, low density lipoprotein; Apo, apolipoprotein.

Genetic polymorphism detection

Genotyping of the *PLA2G6* rs4820314 polymorphism was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). PCR amplification was carried out with forward primer 5'-AGGAGCCG-TCACAACACTG-3' and reverse primer 5'-TGCAC-ATAAGGAATCCCATT-3'. After initial denaturing at 95°C for 5 min, the reaction mixture was subjected to 33 cycles of 45 s denaturation at 95°C, 30 s annealing at 58°C and extension 60 s at 72°C, followed by a final 10 min extension at 72°C. After restriction enzyme (*Ban*I [G]) digestion of the amplified DNA, the genotypes were identified by electrophoresis on 2% agarose gels and visualized with ethidium-bromide staining ultraviolet illumination. Three genotypes were detected: GG (226- and 103-bp), AG (329-, 226- and 103-bp) and AA (329-bp). Parts of genotyping were also confirmed randomly by direct sequencing. The PCR products were purified by low melting point gel electrophoresis and phenol extraction, and then the DNA sequences were analyzed using an ABI Prism 3100 (Applied Biosystems) in Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., People's Republic of China.

Statistical analyses

Descriptive parameters are presented as mean ± SD (serum TG levels were presented as medians and interquartile ranges) and categorical variables were presented using frequency counts. Comparisons between groups of means were compared by the Student's unpaired *t*-test. Chi-square test (χ^2) was used to compare categorical variables between the groups. Genotype frequencies in Jing and Han were tested for Hardy-Weinberg equilibrium, and any deviation between the observed and expected frequencies was tested for significance using the χ^2 test. The association between genotypes and serum lipid parameters was performed using analysis of covariance (ANCOVA). Age,

gender, BMI, waist circumference, smoking, and alcohol consumption were adjusted for the statistical analysis. A factorial design covariance analysis was performed to assess the interaction between genotypes and ethnic groups, BMI, waist circumference, alcohol consumption, hypercholesterolemia and hypertriglyceridemia after controlling for potential confounders including age, gender, BMI, smoking, and alcohol consumption. Multivariable linear regression analyses with stepwise modeling were used to determine the correlation between genotypes (GG = 1, AG = 2, AA = 3) or alleles (the A allele non-carrier = 1, the A allele carrier = 2) and several environmental factors with serum lipid phenotypes in subgroups. Two sided *P* value < 0.05 was considered statistically significant. All data were evaluated using SPSS version 21.0 (SPSS Inc., Chicago, Illinois) of windows 10.

Results

General characteristics

The general characteristics of the two ethnic groups are shown in **Table 1**. The levels of height, weight, BMI and waist circumference were higher in Jing than in Han populations (*P* < 0.05-0.001), whereas the percentages of subjects who consumed alcohol were lower in Jing than in Han populations (*P* < 0.001). There were no significant differences in the ratio of male to female, age, the percentages of subjects who smoked cigarettes, the levels of systolic blood pressure, diastolic blood pressure, pulse pressure and blood fasting glucose levels between the two groups (*P* > 0.05 for all).

Serum lipid phenotypes

Serum lipid phenotypes between Jing and Han populations are also shown in **Table 1**. The levels of TC and TG were higher in Jing ethnic minority than in Han nationality (*P* < 0.001 for each). There were no significant differences in serum HDL-C, LDL-C, ApoA1, ApoB levels and the ratio of ApoA1 to ApoB between the two ethnic groups (*P* > 0.05 for all).

Genotypic and allelic frequencies

The genotypic and allelic frequencies of *PLA2G6* rs4820314 are shown in **Table 2**. The distribution of genotypes followed the Hardy-Weinberg equilibrium in both Jing and Han populations. The frequencies of G and A alleles were 82.68% and 17.32% in Jing, and 86.02% and 23.98% in Han (*P* < 0.01); respectively. For the Han population, the frequencies of G and A alleles were 87.25% and 12.75% in underweight, 87.15% and 12.85% in normal weight, 85.77% and 14.23% in overweight, and 75.89% and 24.11% in obesity subgroups (*P* < 0.05); 87.41% and 12.59% in nondrinkers, 85.19% and 14.81% in light-moderate alcohol consumption, and 82.18% and 17.82% in heavy alcohol consumption subgroups (*P* < 0.05); 87.10% and 12.90% in non-hypertriglyceridemia, 82.87% and 17.13% in hypertriglyceridemia subgroups (*P* < 0.05); respectively. The frequencies of G and A alleles in the Jing population were 85.40% and 14.60% in non-hypertriglyceridemia, and 76.37% and 23.63% in hypertriglyceridemia subgroups (*P* < 0.001); respectively.

The genotype frequencies of the *PLA2G6* rs4820314 were also different between Jing and Han populations (*P* < 0.05), between Jing non-hypertriglyceridemia and hypertriglyceridemia (*P* < 0.001), among Han different BMI subgroups (*P* = 0.001), and among Han different alcohol consumption subgroups (*P* < 0.05).

Genotypes and serum lipid phenotypes

Serum TG, HDL-C, LDL-C and ApoB levels in Jing were different between the two genotypes (*P* < 0.05-0.001), the participants with AG/AA genotypes had higher serum TG, LDL-C and ApoB levels and lower serum HDL-C levels than the subjects with GG genotype. Serum TG, HDL-C and ApoA1 levels in Han were different between the two genotypes (*P* < 0.01 for all), the participants with AG/AA genotypes had higher serum TG levels and lower serum HDL-C and ApoA1 levels than the subjects with GG genotype (**Figure 1**).

Genotypes and BMI on serum lipid phenotypes

For the Jing population, the levels of TC, LDL-C, ApoA1, ApoB and the ratio of ApoA1 to ApoB in underweight; TG, HDL-C and LDL-C in normal weight; ApoB in overweight; and TC in obesity subgroups were different between the two genotypes (*P* < 0.05-0.001).

For the Han population, the levels of TG in underweight; TG and HDL-C in normal weight; TC, ApoB and the ratio of ApoA1 to ApoB in

Table 2. Genotype and allele distributions

Group	N	Genotype			Allele		HWE
		GG	AG	AA	G	A	
Jing	785	542 (69.05)	214 (27.26)	29 (3.69)	1298 (82.68)	272 (17.32)	0.175
Han	844	628 (74.41)	196 (23.22)	20 (2.37)	1452 (86.02)	236 (23.98)	0.316
X^2			6.636		6.910		
P			0.036		0.009		
Jing	785						
Underweight (BMI < 18.5)	24	15 (62.50)	9 (37.50)	0 (0)	39 (81.25)	9 (18.75)	0.258
Normal Weight (18.5 ≤ BMI < 24)	474	334 (70.46)	123 (25.95)	17 (3.59)	791 (83.44)	157 (16.56)	0.184
Overweight (24 ≤ BMI < 28)	215	146 (67.91)	58 (26.98)	11 (5.11)	350 (81.40)	80 (18.60)	0.109
Obesity (28 ≤ BMI)	72	47 (65.28)	24 (33.33)	1 (1.39)	118 (81.94)	26 (18.06)	0.283
X^2			5.792		0.999		
P			0.447		0.801		
Han	844						
Underweight (BMI < 18.5)	51	41 (80.39)	7 (13.73)	3 (5.88)	89 (87.25)	13 (12.75)	0.006
Normal Weight (18.5 ≤ BMI < 24)	498	374 (75.10)	120 (24.10)	4 (0.80)	868 (87.15)	128 (12.85)	0.091
Overweight (24 ≤ BMI < 28)	239	180 (75.31)	50 (20.92)	9 (3.77)	410 (85.77)	68 (14.23)	0.027
Obesity (28 ≤ BMI)	56	33 (58.93)	19 (33.93)	4 (7.14)	85 (75.89)	27 (24.11)	0.586
X^2			22.173		10.759		
P			0.001		0.013		
Jing	785						
Male (Waist circumference ≤ 85)	254	185 (72.84)	60 (23.62)	9 (3.54)	430 (84.65)	78 (15.35)	0.146
Male (Waist circumference > 85)	133	84 (63.16)	43 (32.33)	6 (4.51)	211 (79.32)	55 (20.68)	0.868
X^2			3.874		3.475		
P			0.144		0.062		
Female (Waist circumference ≤ 80)	240	171 (71.25)	62 (25.83)	7 (2.92)	404 (84.17)	76 (15.83)	0.634
Female (Waist circumference > 80)	158	102 (64.56	49 (31.01)	7 (4.43)	253 (80.06)	63 (19.94)	0.720
X^2			2.159		2.226		
P			0.340		0.136		
Han	844						
Male (Waist circumference ≤ 85)	344	247 (71.80)	92 (26.75)	5 (1.45)	586 (85.17)	102 (14.83)	0.274
Male (Waist circumference > 85)	74	51 (68.92)	21 (28.38)	2 (2.70)	123 (83.11)	25 (16.89)	0.926
X^2			0.699		0.404		
P			0.705		0.525		
Female (Waist circumference ≤ 80)	262	207 (79.01)	48 (18.32)	7 (2.67)	462 (88.17)	62 (11.83)	0.048
Female (Waist circumference > 80)	158	117 (74.05)	35 (22.15)	6 (3.80)	269 (85.13)	47 (14.87)	0.116
X^2			1.450		1.615		
P			0.484		0.204		
Jing	785						
Nondrinker	611	419 (68.58)	168 (27.49)	24 (3.93)	1006 (82.32)	216 (17.68)	0.172
≤ 25 g/day	78	56 (71.79)	22 (28.21)	0 (0)	134 (85.90)	22 (14.10)	0.147
> 25 g/day	96	67 (69.79)	24 (25.00)	5 (5.21)	158 (82.29)	34 (17.71)	0.163
X^2			3.898		1.256		
P			0.420		0.534		
Han	844						
Nondrinker	588	455 (77.38)	118 (20.07)	15 (2.55)	1028 (87.41)	148 (12.59)	0.033
≤ 25 g/day	54	38 (70.37)	16 (29.63)	0 (0)	92 (85.19)	16 (14.81)	0.201
> 25 g/day	202	135 (66.83)	62 (30.69)	5 (2.48)	332 (82.18)	72 (17.82)	0.496
X^2			12.074		6.923		
P			0.017		0.031		
Jing	785						
Non-hypercholesterolemia	444	310 (69.82)	115 (25.90)	19 (4.28)	735 (82.77)	153 (17.23)	0.053

Hypercholesterolemia	341	232 (68.04)	99 (29.03)	10 (2.93)	563 (82.55)	119 (17.45)	0.886
X^2			1.730		0.013		
P			0.420		0.910		
Han	844						
Non-hypercholesterolemia	568	430 (75.70)	127 (22.36)	11 (1.94)	987 (86.89)	149 (13.11)	0.651
Hypercholesterolemia	276	198 (71.74)	69 (25.00)	9 (3.26)	465 (84.24)	87 (15.76)	0.331
X^2			2.325		2.161		
P			0.313		0.142		
Jing	785						
Non-hypertriglyceridemia	548	403 (73.54)	130 (23.72)	15 (2.73)	936 (85.40)	160 (14.60)	0.255
Hypertriglyceridemia	237	139 (58.65)	84 (35.44)	14 (5.91)	362 (76.37)	112 (23.63)	0.782
X^2			18.150		18.838		
P			0.000		0.000		
Han	844						
Non-hypertriglyceridemia	628	478 (76.11)	138 (21.98)	12 (1.91)	1094 (87.10)	162 (12.90)	0.581
Hypertriglyceridemia	216	150 (69.44)	58 (26.85)	8 (3.71)	358 (82.87)	74 (17.13)	0.426
X^2			4.788		4.786		
P			0.091		0.029		

HWE, Hardy-Weinberg equilibrium; BMI, body mass index.

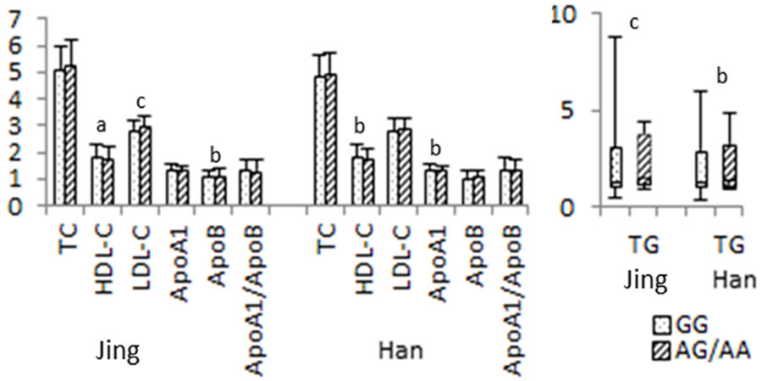

Figure 1. Association of the rs4820314 genotypes and lipid-related traits in the Chinese Jing and Han populations ([a]*P* < 0.05; [b]*P* < 0.01; [c]*P* < 0.001).

overweight; and TG and ApoA1 in obesity subgroups were different between the two genotypes (*P* < 0.05-0.001; **Figure 2**).

Genotypes and alcohol on serum lipid phenotypes

Serum TG, LDL-C, ApoB levels and the ApoA1/ApoB ratio in Jing nondrinker; TC levels in Jing light and moderate drinker (≤ 25 g/day); and HDL-C levels in Jing heavy drinker subgroups (> 25 g/day) were different between the two genotypes (*P* < 0.05-0.001). Serum TG and ApoA1 levels in Han nondrinker; TC, TG, ApoB levels and the ApoA1/ApoB ratio in Han light and moderate drinker subgroups were different between the two genotypes (*P* < 0.05-0.001; **Figure 3**).

Genotypes and hypertriglyceridemia on serum lipid phenotypes

The levels of LDL-C in Jing non-hypertriglyceridemia and HDL-C in Jing hypertriglyceridemia subgroups were different between the two genotypes (*P* < 0.05-0.001). The levels of TG, HDL-C, ApoA1 and the ratio of ApoA1 to ApoB in Han hypertriglyceridemia subgroup were different between the two genotypes (*P* < 0.05-0.001; **Figure 4**).

Genotypes and ethnic groups on serum TG levels

As shown in **Figure 5**, the two populations of different ancestries were divided into non-hypertriglyceridemia, general, and hypertriglyceridemia subgroups according to serum TG levels. The subjects with AG/AA genotypes of the *PLA2G6* rs4820314 in non-hypertriglyceridemia and general subgroups had higher serum TG levels in Jing than in Han populations. But this result was not found in hypertriglyceridemia subgroup.

Interaction of PLA2G6 rs4820314 AG/AA genotype with BMI on serum TG level

BMI interacts with *PLA2G6* rs4820314 SNV on serum TG levels in two populations of different

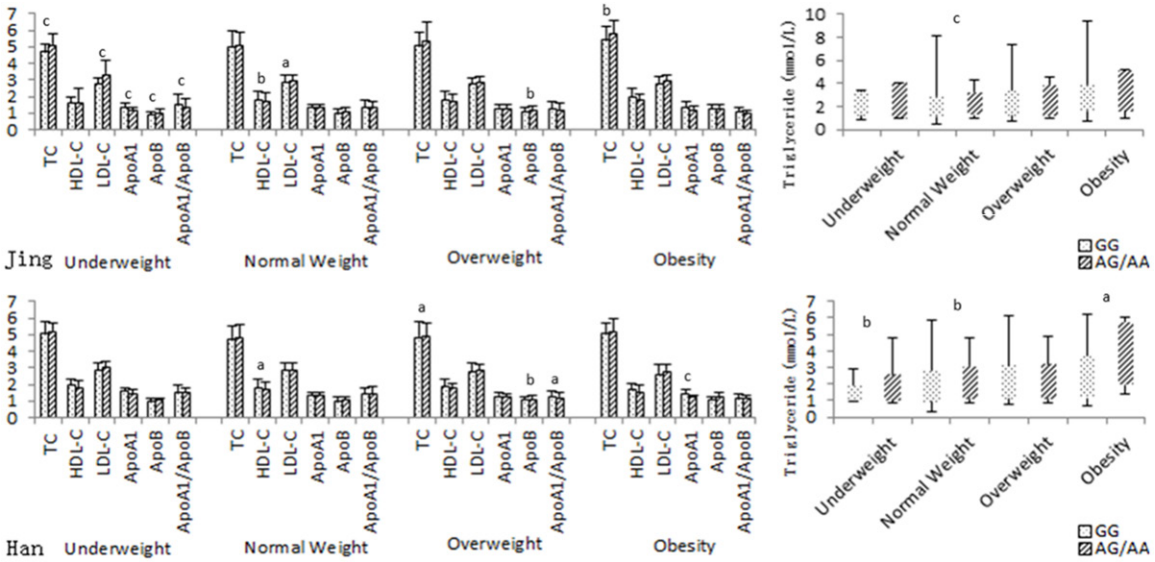

Figure 2. Association of the rs4820314 genotypes and BMI with lipid-related traits ([a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$).

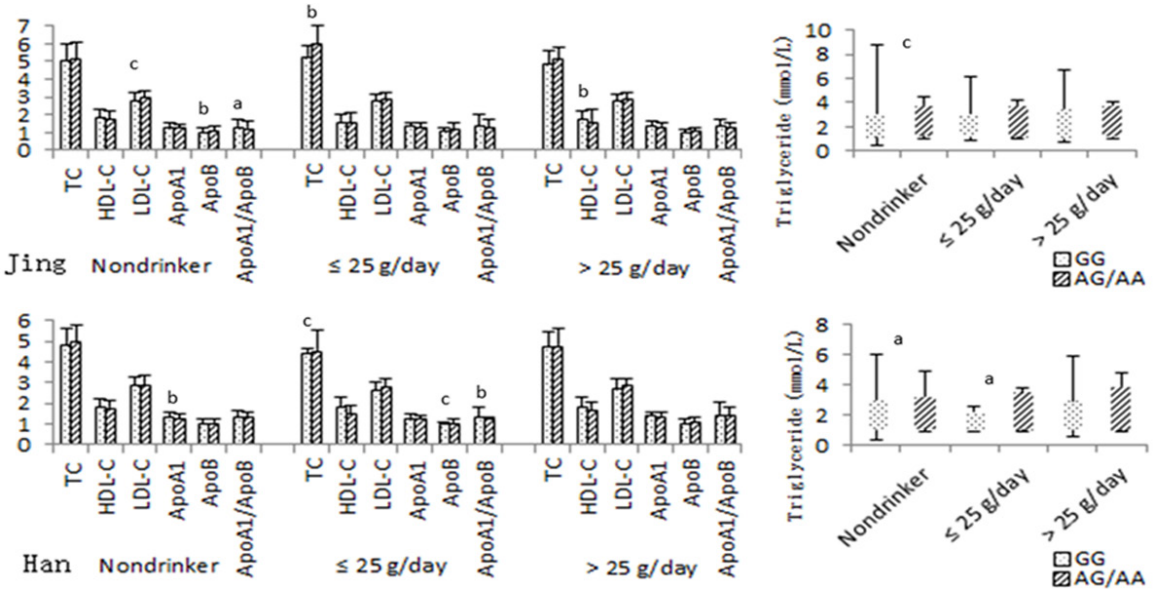

Figure 3. Association of the rs4820314 genotypes and alcohol consumption with lipid-related traits ([a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$).

ancestries are shown in **Figure 6**. With higher BMI, predicted serum TG level decreased more evidently in the *PLA2G6* rs4820314 SNV minor allele homozygotes than the major allele carriers in each population.

Interaction of PLA2G6 rs4820314 AG/AA genotype with alcohol consumption on serum TG level

Alcohol consumption interacts with *PLA2G6* rs4820314 SNV on serum TG levels in two populations of different ancestries are shown in **Figure 7**. With higher alcohol consumption, predicted serum TG level decreased more evidently in the *PLA2G6* rs4820314 SNV AG/GG genotype than the AA genotype carriers in each population.

Discussion

To the best of our knowledge, this is the first study identifying the association of *PLA2G6* rs4820314 and exposure factors including BMI and alcohol consumption with lipid-related traits. In both Jing and Han, higher BMI and

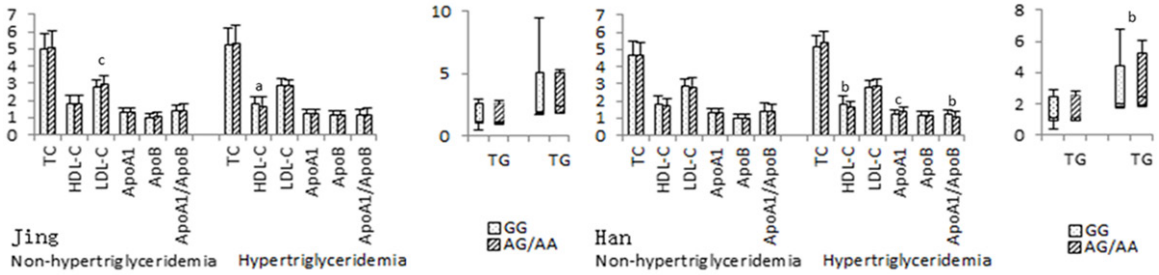

Figure 4. Association of the rs4820314 genotypes and lipid-related traits between non-hypertriglyceridemia and hypertriglyceridemia subgroups ($^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$).

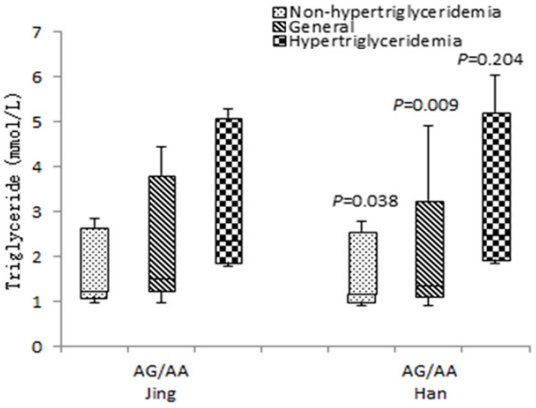

Figure 5. Correlation of *PLA2G6* rs4820314 AG/AA genotype and serum triglyceride levels.

alcohol consumption was associated with higher TC, TG, LDL-C, ApoB levels and lower HDL-C, ApoA1 and the ratio of ApoA1 to ApoB in the rs4820314 AG/AA genotype, but not in the GG genotype. This trend of interaction was further confirmed in the BMI, alcohol and hypertriglyceridemia subgroups. Present results suggest that participants with different genotypes of *PLA2G6* rs4820314 exhibit differential effects from high BMI and alcohol consumption for increasing dyslipidemia risk.

Although the strong risk association of *PLA2G6* rs4820314 with type 2 diabetes mellitus (T2DM) and plasma TG levels [24] has been established in previous reports, and potential of this SNV has been proposed [25], other studies have shown inconsistent results [26] between this SNV and dyslipidemia. A previous genome-wide association meta-analysis showed association of *PLA2G6* rs4820314 and adiposity and cardio-metabolic disease risk [27]. In addition, evidence from observational and intervention study showed that the *PLA2G6* rs4820314 may influence plasma TG levels during a supplementation with n-3 polyunsaturated fatty acids (PUFA) [28]. These discrepancies could be explained by the interactions between *PLA2G6* rs4820314 and BMI and alcohol consumption, as indicated in the current study. Significant associations of BMI and alcohol consumption with lipid-related traits especially TG were observed only in the *PLA2G6* rs4820314 AG/AA genotype. Consistent with those observations, the results were replicated in two populations of different ancestries. The genetic effect of *PLA2G6* rs4820314 SNV on lipid-related traits especially TG was susceptible to various exposure factors.

The gene-nutrient interaction between *PLA2G6* rs4820314 with diet, such as plasma omega-6 fatty acid (supplementation with fish oil) on serum lipid levels [29, 30] has been reported. Another study [31] also reported an interaction between *PLA2G6* and cigarette smoker on coronary artery disease. These studies together with our present study suggest that biological differences may influence the lipid-related traits effects of gene-exposure (G×E) interactions. Although these situations, or others described below, may facilitate the *PLA2G6* rs4820314-BMI and alcohol consumption interactions on lipid-related traits and risk of dyslipidemia, the precise mechanism is still unclear.

Although the same interaction pattern was observed in the Jing and Han populations, slightly different strengths of associations were found between the two populations. This may be because of the proxy markers of *PLA2G6* rs4820314 used in different genetic backgrounds and demographic and cultural characteristics of the two populations. For example, TC and TG were substantially higher in Jing compared with the Han population. Although

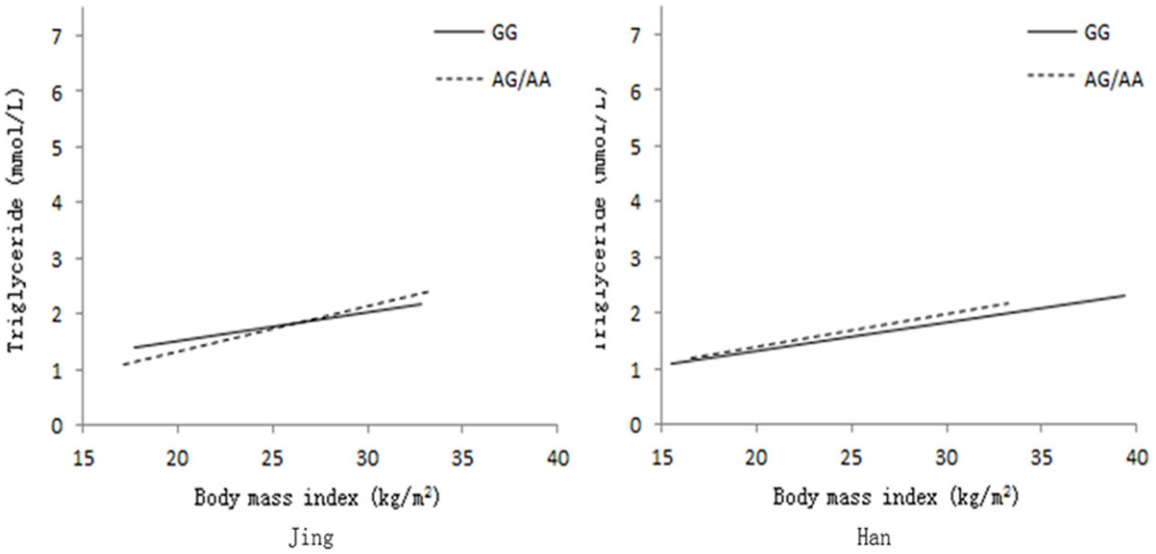

Figure 6. The *PLA2G6* rs4820314 AG/AA genotype was associated with serum TG levels, depending on BMI.

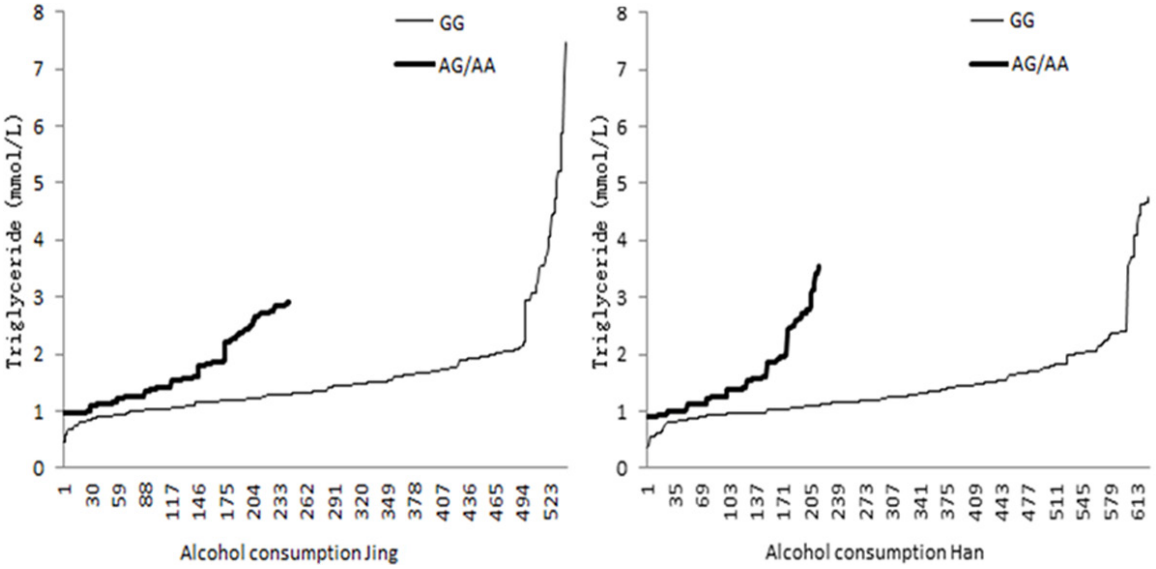

Figure 7. The *PLA2G6* rs4820314 AG/AA genotype was associated with serum TG levels, depending on alcohol consumption.

we adjusted for these differences in the interaction testes, those adjustments may not have been completed.

Nevertheless, there are several limitations with this study. First, a causal relation between *PLA2G6* rs4820314 and BMI and alcohol consumption on lipid-related traits and risk of hypertriglyceridemia cannot be concluded because of the observational study design. Second, just one tag SNV was used in just two populations of different ethnicities for replication, which may lead to underestimation of the genetic effect. Third, our results may be influenced by other genetic and environmental factors, and these may interact with *PLA2G6* rs4820314, which was not considered in this study. These unknown genetic factors may affect the estimated effect of G×E interaction on serum lipid levels. Therefore, future work is needed to establish a causal relation between BMI and/or alcohol consumption and hyperlipidemia or related traits, including genotype-selected randomized controlled traits, as well as gene-by-gene and gene-by-environment interaction analyses at the genome-wide level.

The present study is important in public health implications. Obesity was defined as BMI (kg/m^2): underweight (BMI < 18.5), normal Weight (18.5 ≤ BMI < 24), overweight (24 ≤ BMI < 28) and obesity (28 ≤ BMI). In our study, the prevalence of high BMI was higher in Jing than in Han populations. It is same to dyslipidemia. Given the high BMI individuals recommendation to achieve BMI < 24. However, our study indicates one recommendation may not be optimal for all adults because of genetic background. In terms of personal treatment, developing some recommendations based on personal genotype information could, one day, improve for the prevention of dyslipidemia.

Conclusions

In conclusion, BMI and alcohol consumption modulated the associations of *PLA2G6* rs4820314 SNV with lipid-related traits. Higher BMI and alcohol consumptions were associated with higher risk of hypertriglyceridemia in AG/AA genotypes, but the associations were greatly attenuated in the other genotype groups. Replication was successfully achieved in the participants of two different populations. This study suggests potential critical roles for the gene-exposure (G×E) interaction between the *PLA2G6* rs4820314 and BMI/alcohol consumption in regulating lipid-related traits and hypertriglyceridemia risk.

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Disclosure of conflict of interest

None.

Address correspondence to: Rui-Xing Yin, Department of Cardiology, Institute of Cardiovascular Diseases, The First Affiliated Hospital, Guangxi Medical University, 22 Shuangyong Road, Nanning 530021, Guangxi, China. Tel: +867715358832; E-mail: yinruixing@163.com

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