Interactions of the apolipoprotein C-III 3238C>G polymorphism and alcohol consumption on serum triglyceride levels

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Abstract

Background: Both apolipoprotein (Apo) C-III gene polymorphism and alcohol consumption have been associated with increased serum triglyceride (TG) levels, but their interactions on serum TG levels are not well known. The present study was undertaken to detect the interactions of the ApoC-III 3238C>G (rs5128) polymorphism and alcohol consumption on serum TG levels.

Methods: A total of 516 unrelated nondrinkers and 514 drinkers aged 15-89 were randomly selected from our previous stratified randomized cluster samples. Genotyping of the ApoC-III 3238C>G was performed by polymerase chain reaction and restriction fragment length polymorphism combined with gel electrophoresis, and then confirmed by direct sequencing. Interactions of the ApoC-III 3238C>G genotype and alcohol consumption was assessed by using a cross-product term between genotypes and the aforementioned factor.

Results: Serum total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C), ApoA-I and ApoB levels were higher in drinkers than in nondrinkers (P < 0.05-0.001). There was no significant difference in the genotypic and allelic frequencies between the two groups. Serum TG levels in nondrinkers were higher in CG genotype than in CC genotype (P < 0.01). Serum TC, TG, low-density lipoprotein cholesterol (LDL-C) and ApoB levels in drinkers were higher in GG genotype than in CC or CG genotype (P < 0.01 for all). Serum HDL-C levels in drinkers were higher in CG genotype than in CC genotype (P < 0.01). Serum TC, HDL-C, ApoA-I levels and the ratio of ApoA-I to ApoB in CC genotype, and TC, TG, LDL-C, ApoA-I and ApoB levels in GG genotype were higher in drinkers than in nondrinkers (P < 0.05-0.01). But the ratio of ApoA-I to ApoB in GG genotype was lower in drinkers than in nondrinkers (P < 0.01). Multivariate logistic regression analysis showed that the levels of TC, TG and ApoB were correlated with genotype in nondrinkers (P < 0.05 for all). The levels of TC, LDL-C and ApoB were associated with genotype in drinkers (P < 0.01 for all). Serum lipid parameters were also correlated with age, sex, alcohol consumption, cigarette smoking, blood pressure, body weight, and body mass index in both groups.

Conclusions: This study suggests that the ApoC-III 3238CG heterozygotes benefited more from alcohol consumption than CC and GG homozygotes in increasing serum levels of HDL-C, ApoA-I, and the ratio of ApoA-I to ApoB, and lowering serum levels of TC and TG.
Introduction
Coronary artery disease (CAD) is the most common cause of death in industrialized countries with evidence that high plasma or serum triglyceride (TG) concentration is an independent risk factor [1-5]. It is well known that plasma TG concentration is modulated by both environmental and genetic factors [6]. Numerous studies have evaluated the influence of alcohol intake, an index of lifestyle, on plasma lipid and lipoprotein concentrations. Alcohol consumption can promote lipogenesis [7] and accordingly increase serum TG levels [8,9]. Alcohol in doses > 30 g/day in both sexes can augment the TG level. It has been found that the alcohol intake of 60 g/day increases the TG level by about 0.19 mg/dl per 1 gram of alcohol consumed [10].

Plasma apolipoprotein (Apo) C-III is a major component of TG-rich lipoproteins (chylomicrons and very low density lipoprotein) and a minor component of high density lipoprotein. The mature 79-amino-acid ApoC-III protein is synthesized predominantly in the intestine but also to a lesser extent in the liver. In vitro studies have indicated that ApoC-III is a noncompetitive inhibitor of lipoprotein lipase, thereby suggesting an important role in the catabolism of TG-rich lipoproteins [11]. Plasma ApoC-III concentrations were positively correlated with plasma TG levels, both in the normal population as well as in hypertriglyceridemic patients [12] or in transgenic animals [13]. ApoC-III gene has been mapped to chromosome 11q23.3 [14] and is flanked by the genes for ApoA-I and ApoA-IV in a 15-kb gene cluster [15]. Several polymorphic sites have been detected within and around the ApoC-III gene. The most extensively studied is the SstI polymorphism, due to a C→G substitution at nucleotide 3238, in the 3′ untranslated region of the gene. Numerous studies have found an association between the presence of a polymorphic SstI site in the untranslated region of the ApoC-III gene with raised ApoC-III and TG concentrations [16-53] and with an increased risk of CAD [53-62]. However, little is known about the interactions of the ApoC-III gene polymorphism and alcohol consumption on serum lipid concentrations. Therefore, the aim of the present study was to determine the interactions of the ApoC-III 3238C>G (rs5128) polymorphism and alcohol consumption on serum lipid levels.

Materials and methods
Study subjects
A total of 1030 unrelated subjects who reside in 16 villages in Napo County, Guangxi Zhuang Autonomous Region, People’s Republic of China were randomly selected from our previous stratified randomized cluster samples [63]. The age of the subjects ranged from 15 to 89 years, with an average age of 43.30 ± 17.69 years. There were 516 nondrinkers and 514 drinkers. All of the subjects were peasants. The study subjects were essentially healthy and had no evidence of diseases related to atherosclerosis, CAD and diabetes. 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, 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 and >25. 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-minute 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.

Biochemical analysis
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. A part of the sample (2 ml) was transferred into glass tubes and allowed to clot at ambient temperature, and used to determine serum lipid levels, and another part of the sample (5 ml) was transferred into 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 deoxyribonucleic acid (DNA). Immediately following clotting serum was separated by centrifugation for 15 minutes at 3000 rpm. The levels of serum total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) in samples were determined by enzymatic methods with commercially available kits, Tcho-1, TG-LH (RANDOX Laboratories Ltd., Ardmere, Diamond Road, Crumlin Co. Antrim, United Kingdom, 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.

**DNA amplification and genotyping**

Total genomic DNA was isolated from peripheral blood leukocytes using the phenol-chloroform method. The extracted DNA was stored at 4°C until analysis. Genotyping of the ApoC-III 3238C>G was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) according to the previous reports [19]. The sequence of the forward and backward primers used was 5'-CCTAGGCCAGAGAGGAGGTGCC-3' and 5'-CTGAGCCAGCGCGCAGCTAAA-3' (Sangon, Shanghai, China). Each reaction system of a total volume of 25 μL, comprised 0.2 μg of genomic DNA; 1.0 μL of each primer (10 pmol/μL); 2.5 μL of 10 × buffer solution; 1.5 μL of MgCl2 (25 mM/mL); 2.0 μL of dNTP (2.5 mM/mL); and 1.5 U of Taq polymerase (Takara). For the amplification, initial denaturation at 94°C for 5 minutes was followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 61°C for 30 s, and extension at 72°C for 45 s, with final extension at 72°C for 4 min. Each restriction enzyme reaction was performed with 8 μL of amplified DNA; 2 μL of 10 × buffer solution; and 0.2 U SstI restriction enzyme in a total volume of 25 μL digested at 64°C for 4 h. The digestive products were separated by electrophoresis on 2% sepharose gel for 60 min. 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 ultraviolet light and photographed. Genotypes were scored by an experienced reader blinded to epidemiological and lipid results.

**DNA sequencing**

Six samples (CC, CG and GG genotypes in two, respectively) detected by the PCR-RFLP were also confirmed by direct sequencing. The PCR product was purified by low melting point gel electrophoresis and phenol extraction, and then the DNA sequence were analyzed by using an ABI Prism 3100 (Applied Biosystems) in Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., People's Republic of 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 [63]. Hypertension was diagnosed according to the criteria of 1999 The World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [64]. 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/m², respectively [63,64].

**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 (serum TG levels were presented as medians and interquartile ranges). The difference in general characteristics between nondrinkers and drinkers was tested by the Student's unpaired t test. The allele frequencies of the ApoC-III 3238C>G were determined by gene counting. A chi-square analysis was used to evaluate the allelic and genotypic frequencies that were calculated from the observed genotypic counts and to assess Hardy-Weinberg expectations. Interaction between the ApoC-III 3238C>G genotype and alcohol consumption was assessed by using a cross-product term between genotypes and the aforementioned factor. Statistical significance was evaluated with analysis of covariance (ANCOVA). The co-variables include sex, age, BMI, hypertension, and cigarette smoking. In order to evaluate the association of serum lipid parameters with several environmental factors and genotypes, unconditional logistic regression analysis was also performed in the combined population, nondrinkers, and drinkers; respectively. The statistical analyses were performed with the statistical software package SPSS 13.0 (SPSS Inc., Chicago, Illinois). A P value of less than 0.05 was considered statistically significant.

**Results**

**General characteristics between nondrinkers and drinkers**

Table 1 gives the general characteristics between the nondrinkers and drinkers. The ratio of male to female, the mean age, the levels of systolic blood pressure, diastolic blood pressure and pulse pressure, and the percentages of subjects who smoked cigarettes were higher in drinkers than in nondrinkers (P < 0.05-0.001). There was no significant difference in the BMI between the two groups (P > 0.05).

**Serum lipid levels between nondrinkers and drinkers**

The levels of TC, TG, HDL-C, ApoA-I and ApoB were higher in drinkers than in nondrinkers (P < 0.05-0.001).
There were no significant differences in the levels of LDL-C and the ratio of ApoA-I to ApoB between the two groups ($P > 0.05$ for each).

**Results of electrophoresis and genotyping**

After the genomic DNA of the samples was amplified by PCR and imaged by 2% agarose gel electrophoresis, the purpose gene of 596 bp nucleotide sequences could be seen in the samples (Figure 1). The genotypes identified were named according to the presence or absence of the enzyme restriction sites, when a C to G transversion at nucleotide position 3238 of the ApoC-III gene. The presence of the cutting site indicates the 3238G allele, while its absence indicates the 3238C allele. Thus, the GG genotype is homozygote for the presence of the site (bands at 371 bp and 225 bp), CG genotype is heterozygote for the presence and absence of the site (bands at 596 bp, 371 bp and 225 bp), and CC genotype is homozygote for the absence of the site (band 596 bp; Figure 1). The genotype distribution was consistent with the Hardy-Weinberg equilibrium.

**Results of sequencing**

The results shown as CC, CG and GG genotypes by PCR-RFLP, CC, CG and GG genotypes were also confirmed by sequencing (Figure 2).

**Genotypic and allelic frequencies**

The genotypic and allelic frequencies of the ApoC-III 3238C>G are shown in Table 2. The frequencies of CC, CG and GG genotypes were 45.7%, 43.0% and 11.3% in nondrinkers, and 45.2%, 45.5% and 9.3% in drinkers ($P > 0.05$); respectively. The frequencies of C and G alleles were 67.2% and 32.8% in nondrinkers, and 67.9% and 32.1% in drinkers ($P > 0.05$); respectively.

**Genotypes and serum lipid levels**

As shown in Table 3, the levels of TG in nondrinkers were higher in CG genotype than in CC genotype ($P < 0.01$), and the ratio of ApoA-I to ApoB in nondrinkers was higher in GG genotype than in CC genotype ($P < 0.05$).

The levels of TC, TG, LDL-C and ApoB in drinkers were higher in GG genotype than in CC or CG genotype ($P < 0.01$ for all). The levels of HDL-C in drinkers were higher in CG genotype than in CC genotype ($P < 0.01$). The ratio of ApoA-I to ApoB in drinkers was lower in GG genotype than in CC or CG genotype ($P < 0.01$ for each).
Figure 1 Genotyping of PCR products of the samples. Lane M, 100 bp Marker Ladder; Lanes 1 and 2, the PCR products of the samples (596 bp); Lane 3 and 4, CC genotype (596 bp); Lanes 5 and 6, CG genotype (596 bp, 371 bp and 225 bp); and lanes 7 and 8, GG genotype (371 bp and 225 bp).

Figure 2 A part of the nucleotide sequence of the ApoC-III 3238C>G. (A) CC genotype; (B) CG genotype; (C) GG genotype.
Table 2 Genotypic and allelic frequencies between the nondrinkers and drinkers [n (%)]

| Group       | n   | Genotype | Allele |
|-------------|-----|----------|--------|
|             |     | CC       | CG     | GG     | C     | G     |
| Nondrinker  | 516 | 236(45.7)| 222(43.0)| 58(11.3)| 694(67.2)| 338(32.8)|
| Drinker     | 514 | 232(45.2)| 234(45.5)| 48(9.3) | 698(67.9)| 330(32.1)|

\[ \chi^2 = -1.290, P = 0.280 \]

Table 3 Comparison of serum lipid levels among the genotypes and between the nondrinkers and drinkers

| Group       | Genotype | n   | TC (mmol/L) | TG (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) | ApoA-I (g/L) | ApoB (g/L) | ApoA-I/ApoB |
|-------------|----------|-----|-------------|-------------|----------------|----------------|--------------|------------|-------------|
| Nondrinker  | CC       | 236 | 4.49 ± 0.88 | 0.91 ± 0.57 | 1.97 ± 0.42    | 2.41 ± 0.69    | 1.40 ± 0.13  | 0.89 ± 0.21 | 1.70 ± 0.64  |
|             | CG       | 222 | 4.53 ± 1.11 | 0.90 ± 0.66 | 2.00 ± 0.50    | 2.40 ± 0.68    | 1.40 ± 0.13  | 0.91 ± 0.22 | 1.62 ± 0.41  |
|             | GG       | 58  | 4.57 ± 1.03 | 0.93 ± 0.60 | 2.10 ± 0.43    | 2.40 ± 0.83    | 1.42 ± 0.12  | 0.86 ± 0.28 | 1.84 ± 0.75  |
| Drinker     | CC       | 232 | 4.72 ± 0.90 | 0.97 ± 0.63 | 2.07 ± 0.45    | 2.38 ± 0.71    | 1.47 ± 0.14  | 0.91 ± 0.20 | 1.70 ± 0.46  |
|             | CG       | 234 | 4.75 ± 0.86 | 0.95 ± 0.54 | 2.23 ± 0.53    | 2.37 ± 0.66    | 1.49 ± 0.13  | 0.91 ± 0.20 | 1.73 ± 0.47  |
|             | GG       | 48  | 5.29 ± 0.91 | 1.28 ± 0.93 | 2.12 ± 0.49    | 2.77 ± 0.72    | 1.49 ± 0.14  | 1.06 ± 0.18  | 1.44 ± 0.30  |

\[ \chi^2 = -0.830, P = 0.280 \]

Discussion

The results of the present study show that the levels of TC, TG, HDL-C, ApoA-I and ApoB were higher in drinkers than in nondrinkers. There was no significant difference in the levels of LDL-C and the ratio of ApoA-I to ApoB between the two groups. These findings are consistent with those of several previous studies. A moderate intake of alcohol is associated with protection against CAD, probably due in part to a dose-dependent increase in HDL-C [65,66]. According to Rimma et al. [67], a daily dose of 30 g alcohol results in an average HDL level rise of 3.99 mg/dl, and an ApoA-I level rise of 8.82 mg/dl. Alcohol also causes an increase of TG lipase activity and a decrease of the HDL removal from the circulation [68]. A decrease in LDL-C with increased alcohol intake has also been reported in some studies, but this effect is less consistent and probably depends on the combination of one or more unmeasured factors [68].

The present study shows that there was no significant difference in the allelic and genotypic frequencies of the ApoC-III 3238C>G between the nondrinkers and drinkers. The frequency of G allele was 32.8% in nondrinkers, and 32.1% in drinkers, which is quite similar to the results in Taiwanese (0.30-0.43) [46,69], Japanese (0.25-0.48) [70], and Indians (0.36) [27], but is higher than those reported for Caucasians in whom the G allele frequency was 0.00-0.11 [27,55]. These results suggest that there exists significant racial variation of allele frequencies in this locus.

The relationship between the ApoC-III 3238C>G polymorphism and plasma or serum lipid levels in humans has been evaluated in a large number of studies. However, previous findings on the association of this polymorphism with the changes in plasma lipid levels are inconsistent [71-73]. Previous cohort studies, as well as case-control and familial studies have shown significant association between the rare allele of the polymorphic SstI site (3238G) and higher plasma TG levels [16-53] and CAD [53-62]. This association has been reported in studies carried out with Caucasians, Chinese, Mayans, Japanese (living in Japan or living in Southern Brazil), Koreans, Arabs, and Asian Indians [27,49,55,69,70]. However, several reports failed to find a significant
Table 4 Correlative factors for the serum lipid parameters between the nondrinkers and drinkers

| Lipid parameter | Risk factor | Odds ratio | $\chi^2$ | P | 95% CI |
|-----------------|-------------|------------|--------|---|--------|
| **Nondrinkers plus drinkers** | Age | 1.142 | 13.511 | 0.000 | 1.064-1.225 |
| | Body mass index | 1.175 | 14.633 | 0.000 | 1.082-1.277 |
| | ApoC-III 3238C>G genotype | 1.574 | 6.233 | 0.037 | 1.102-2.248 |
| | TC | 1.030 | 45.053 | 0.000 | 1.021-1.039 |
| | Body mass index | 1.144 | 22.000 | 0.000 | 1.081-1.210 |
| | ApoC-III 3238C>G genotype | 1.324 | 6.542 | 0.011 | 1.068-1.642 |
| | TG | 1.018 | 5.255 | 0.022 | 1.003-1.034 |
| | Body mass index | 1.233 | 30.735 | 0.000 | 1.139-1.314 |
| | Pulse pressure | 1.744 | 14.249 | 0.000 | 1.307-2.329 |
| | Alcohol consumption | 0.614 | 4.056 | 0.044 | 0.382-0.987 |
| | Cigarette smoking | 1.517 | 8.448 | 0.004 | 1.145-2.009 |
| | ApoC-III 3238C>G genotype | 1.324 | 6.542 | 0.011 | 1.068-1.642 |
| | LDL-C | 1.039 | 13.847 | 0.000 | 1.018-1.060 |
| | Age | 1.314 | 9.279 | 0.002 | 1.102-1.566 |
| | Body weight | 2.200 | 12.048 | 0.044 | 1.410-3.434 |
| | ApoC-III 3238C>G genotype | 1.574 | 6.233 | 0.013 | 1.102-2.248 |
| | Sex | 0.414 | 9.812 | 0.002 | 0.238-0.719 |
| | Pulse pressure | 0.414 | 9.812 | 0.002 | 0.238-0.719 |
| | Alcohol consumption | 0.327 | 12.749 | 0.000 | 0.177-0.604 |
| | ApoA-I | 0.745 | 12.294 | 0.000 | 0.632-0.878 |
| | Age | 1.898 | 5.549 | 0.018 | 1.114-3.236 |
| | Body weight | 1.107 | 43.419 | 0.000 | 1.074-1.141 |
| | Alcohol consumption | 0.704 | 4.248 | 0.039 | 0.504-0.983 |
| | ApoC-III 3238C>G genotype | 1.709 | 9.600 | 0.002 | 1.218-2.400 |
| | ApoB | 1.036 | 38.386 | 0.000 | 1.025-1.048 |
| | Age | 1.086 | 45.102 | 0.000 | 1.060-1.113 |
| | Sex | 0.639 | 4.668 | 0.031 | 0.425-0.959 |
| | Pulse pressure | 0.974 | 4.009 | 0.045 | 0.949-0.999 |
| | Alcohol consumption | 0.327 | 12.749 | 0.000 | 0.177-0.604 |
| | ApoC-III 3238C>G genotype | 2.319 | 10.577 | 0.001 | 1.397-3.849 |
| | ApoA-I | 0.414 | 9.812 | 0.002 | 0.238-0.719 |
| | Age | 1.039 | 13.847 | 0.000 | 1.018-1.060 |
| | Body weight | 1.131 | 34.897 | 0.000 | 1.086-1.178 |
| | Sex | 0.337 | 9.542 | 0.002 | 1.561-7.317 |
| | ApoC-III 3238C>G genotype | 1.709 | 9.600 | 0.002 | 1.218-2.400 |
| | ApoB | 0.531 | 18.022 | 0.000 | 0.353-0.797 |
| | Age | 1.039 | 13.847 | 0.000 | 1.018-1.060 |
| | Sex | 0.639 | 4.668 | 0.031 | 0.425-0.959 |
| | Body weight | 1.061 | 4.339 | 0.037 | 1.004-1.122 |
| | ApoC-III 3238C>G genotype | 1.709 | 9.600 | 0.002 | 1.218-2.400 |
| | ApoA-I | 0.671 | 14.044 | 0.000 | 0.545-0.827 |
| | ApoC-III 3238C>G genotype | 2.200 | 12.048 | 0.044 | 1.410-3.434 |
| | ApoB | 0.733 | 11.783 | 0.001 | 0.614-0.875 |
| | Sex | 1.667 | 4.219 | 0.040 | 1.024-2.713 |
| | ApoC-III 3238C>G genotype | 2.200 | 12.048 | 0.044 | 1.410-3.434 |
| | ApoB | 0.733 | 11.783 | 0.001 | 0.614-0.875 |
| | Sex | 1.667 | 4.219 | 0.040 | 1.024-2.713 |
| | ApoC-III 3238C>G genotype | 2.200 | 12.048 | 0.044 | 1.410-3.434 |
| | ApoB | 0.733 | 11.783 | 0.001 | 0.614-0.875 |
| | Sex | 1.667 | 4.219 | 0.040 | 1.024-2.713 |
| | ApoC-III 3238C>G genotype | 2.200 | 12.048 | 0.044 | 1.410-3.434 |
| | ApoB | 0.733 | 11.783 | 0.001 | 0.614-0.875 |
| | Sex | 1.667 | 4.219 | 0.040 | 1.024-2.713 |
| | ApoC-III 3238C>G genotype | 2.200 | 12.048 | 0.044 | 1.410-3.434 |

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http://www.lipidworld.com/content/9/1/86
genetic effect on TG concentrations [71-73]. In a previous work, Kee and coworkers found no association between variability at the SstI ApoC-III gene site (in the 3%-noncoding region) and lipid, lipoproteins and complex lipoprotein particles in a sample of men from northern France [72]. They thought that the SstI polymorphism is not major contributors to the risk of dyslipidemia in the population of northern France. Results from the current study are consistent with many studies cited above which reported associations of ApoC-III gene polymorphism with altered lipid metabolism. The levels of TG in nondrinkers were higher in CG genotype than in CC genotype, and the ratio of ApoA-I to ApoB in nondrinkers was higher in GG genotype than in CG genotype. The levels of TC, TG, LDL-C and ApoB in drinkers were higher in GG genotype than in CC or CG genotype. The levels of HDL-C in drinkers were higher in CG genotype than in CC genotype. The ratio of ApoA-I to ApoB in drinkers was lower in GG genotype than in CC or CG genotype.

The interactions of the ApoC-III 3238C>G polymorphism and alcohol consumption on serum lipid levels are not well known. In the present study, we showed that serum TC, TG, HDL-C and ApoA-I levels in CC genotype, TC, HDL-C, ApoA-I levels and the ratio of ApoA-I to ApoB in CG genotype, and TC, TG, LDL-C, ApoA-I and ApoB levels in GG genotype were higher in drinkers than in nondrinkers. But the ratio of ApoA-I to ApoB in GG genotype was lower in drinkers than in nondrinkers. The levels of TG were correlated with genotype in nondrinkers, whereas the levels of TG were positively associated with alcohol consumption in drinkers. These findings suggest that the ApoC-III 3238CG heterozygotes benefited more from alcohol consumption than CC and GG homozygotes in increasing serum levels of HDL-C, ApoA-I, and the ratio of ApoA-I to ApoB, and lowering serum levels of TC and TG.

Conclusion
The results of the present study show that there was no significant difference in genotypic and allelic frequencies of the ApoC-III 3238C>G polymorphism between the nondrinkers and drinkers. But the interactions of the ApoC-III 3238C>G polymorphism and alcohol consumption on serum lipid levels are different among the three genotypes. The ApoC-III 3238CG heterozygotes benefited more from alcohol consumption than CC and GG homozygotes in increasing serum levels of HDL-C, ApoA-I, and the ratio of ApoA-I to ApoB, and lowering serum levels of TC and TG.

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Authors’ contributions
YR and LY conceived the study, participated in the design, carried out the epidemiologic survey, collected the samples, performed the statistical analyses, and drafted the manuscript; LM, LK, LX, ZL and LW carried out the biochemical analysis; WJ, YD and LW carried out the statistical analyses, and drafted the manuscript; LM, LK, LX, ZL and LW carried out the biochemical analysis; WJ, YD and LW carried out the epidemiologic survey, collected the samples, and helped to carry out the genotyping. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References
1. Austin MA: Plasma triglyceride as a risk factor for coronary heart disease. The epidemiologic evidence and beyond. Am J Epidemiol 1989, 129:249-59.
2. Hokanson JE, Austin MA: Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiacoas Risk 1996, 3:213-9.
3. Talmud PJ, Hawe E, Miller GJ, Humphries SE: Nonfasting apolipoprotein B and triglyceride levels as a useful predictor of coronary heart disease

Table 4 Correlative factors for the serum lipid parameters between the nondrinkers and drinkers (Continued)

| ApoB       | Age          | 1.040 | 17.141 | 0.000 | 1.021-1.060 |
|------------|--------------|-------|--------|-------|-------------|
| Body weight| 1.084        | 22.706| 0.000  | 1.049-1.121|
| Pulse pressure| 0.974       | 5.365 | 0.021  | 0.952-0.996 |
| Alcohol consumption| 0.360   | 13.214| 0.000  | 0.207-0.624 |
| ApoC-III 3238C>G genotype| 1.863      | 11.039| 0.001  | 1.291-2.689 |

TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; CI, confidence interval.

http://www.lipidworld.com/content/9/1/86
risk in middle-aged UK men. Arterioscler Thromb Vasc Biol 2002, 22:1918-23.
4. Arau H, Yamamoto A, Matuszewa Z, Saito Y, Yamada N, Oikawa S, Mabuchi H, Teramoto T, Sasaki J, Nakaya N, Itakura H, Ishikawa Y, Ouchi Y, Horihe H. Serum lipid survey and its recent trend in the general Japanese population in 2000. J Atheroscler Thromb 2005, 12:98-106.
5. Satoh H, Nishino T, Tomita K, Tsutsui H. Fasting triglyceride is a significant risk factor for coronary artery disease in middle-aged Japanese men: Results from a 10-year cohort study. Circ J 2006, 70:227-31.
6. Wu LL. Review of risk factors for cardiovascular diseases. Ann Clin Lab Sci 1999, 29:127-33.
7. You M, Crabb DW. Recent advances in alcoholic liver disease II. Minireview: molecular mechanisms of alcoholic fatty liver, Am J Physiol Gastrointest Liver Physiol 2004, 287:G1-G6.
8. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hulley SB, Kagan A, Zukel W. Alcohol and blood lipids. The cooperative lipoprotein phenotyping study. Lancet 1977, 2:153-5.
9. Ruxing Y, Shangling P, Hong C, Hanjun Y, Hai W, Yuming C, Jinzhen W, Feng H, Meng L, Muyan L. Diet, alcohol consumption, and serum lipid levels of the middle-aged and elderly in the Guangxi Bai Ku Yao and Han populations. Alcohol 2008, 42:219-29.
10. Stampfer MJ, Krauss RM, Ma J, Blanche PJ, Holl LG, Sacks FM, Hennekens CH. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 1996, 276:882-8.
11. You M, Crabb DW. Recent advances in alcoholic liver disease II. Minireview: molecular mechanisms of alcoholic fatty liver, Am J Physiol Gastrointest Liver Physiol 2004, 287:G1-G6.
12. Le NA, Gibson JC, Ginsberg HN. Effective polymorphisms in apo CIII gene and lipid and lipoprotein variables in northern France. Arterioscler Thromb Vasc Biol 1997, 17:2753-8.
13. Ito Y, Azolon N, O’Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. Science 1990, 249:790-3.
14. Bruns GA, Karanthisis SK, Breslow JL. Human apolipoprotein AI-CIII gene complex is located on chromosome 11. Arteriosclerosis, 1984; 4:97-102.
15. Talmud PJ, Humphries SE. Apolipoprotein C-III gene variation and dyslipidaemia. Curr Opin Lipidol 1997, 8:154-8.
16. Hegele RA, Connelly PW, Hanley AJ, Talmud PJ. Fasting triglyceride concentrations in very low density and high density lipoproteins: implications for the regulation of the catabolism of these lipoproteins. J Lipid Res 1989, 29:669-77.
17. Helbecque N, Kčeleva F, Genest J Jr, Craig S, Robbins AH, Meade T, Pocovi M, Ruiz-Narváez EA, Yang Y, Nakanishi Y, Kirchdorfer J, Campos H. Effect of SstI polymorphism of the apolipoprotein CIII gene and hypertriglyceridemic subjects, the Columbia University BioMarkers Study. Nutr Metab Cardiovasc Dis 2003, 13:194-201.
18. Minihane AM, Finnegane YE, Talmud PJ, Leigh-Franks WC, Williams CM. Influence of the APOC3-1542T>G polymorphism on plasma lipid levels: effect of age and gender. Biochim Biophys Acta 2002, 1583:311-4.
19. Humphries SE, Benguld L, Otvos JD, Kaluski D, Deckelbaum RJ, Shear S, Talmud PJ. Foci for CETP, LPL, LIPC, and APOC3 affect plasma lipoprotein size and sub-population distribution in Hispanic and non-Hispanic white subjects: the Columbia University BioMarkers Study. Nutr Metab Cardiovasc Dis 2002, 12:163-72.
20. Ruiz-Narváez EA, Yang Y, Nakashima Y, Ishikawa Y, Ouchi Y, AePreps J, Bergeron J. Effect of apoC-III gene polymorphisms on the lipoprotein-lipid profile of viscero-obese men. J Lipid Res 2003, 44:886-93.
21. Espino-Montoro A, Barrios-Artillo M, López-Chozas JM, Cayuela A, Stiefel P, Villar J. Influence of polymorphism (RFLP-sst) at the apolipoprotein C-III gene locus on the lipoprotein metabolism and insulin resistance in essential hypertensive patients. Interaction between gender and genetic polymorphism. Nutr Metab Cardiovasc Dis 2003, 13:194-201.
22. Nalluruch V, Pape GP, Smith L, Patch J, Boenewinkel E. Polymorphic markers in apolipoprotein C-III gene flanking regions and hypertriglyceridemia. Arterioscler Thromb Vasc Biol 1996, 16:941-7.
23. Li GP, Wang JY, Yang SK, Chen BS, Xue H, Wu G. Genetic effect of two polymorphisms in the apolipoprotein A5 gene and apolipoprotein CIII gene on serum lipids and lipoproteins levels in a Chinese population. Clin Genet 2004, 65:470-6.
24. Garenc C, Aubert S, Larocche J, Giraud J, Vohl MC, Bergeron J, Rousseau F, Julien P. Population prevalence of APOE, APOC3 and PPAR-alpha mutations associated to hypertriglyceridemia in French Canadians. J Hum Genet 2004, 49:691-700.
25. Guettier JM, Georgopoulos A, Tsai MY, Radha V, Shankaran S, Deepa R, Grois M, Rao G, Mohan V. Polymorphisms in the fatty acid-binding protein 2 and apolipoprotein C-III gene are associated with the metabolic syndrome and dyslipidemia in a South Indian population. J Clin Endocrinol Metab 2005, 90:1705-11.
26. Garenc C, Couillard C, Lafamme N, Cadelle F, Gagné C, Couture P, Julien P, Bergeron J. Effect of the APOC3 SstI 1 SNP on fasting triglyceride levels in men heterozygous for the LPL P207L deficiency. Eur J Hum Genet 2003, 11:1599-605.
27. Ruiz-Narváez EA, Yang Y, Nakashima Y, Ishikawa Y, Ouchi Y, AePreps J, Bergeron J. Effect of the APOC3 SstI 1 SNP on fasting triglyceride levels in men heterozygous for the LPL P207L deficiency. Eur J Hum Genet 2003, 11:1599-605.
28. Herron KL, Loefgren L, Adcock X, Otvos JD, Fernandez-Fresnedo G, Iida D, Gonzalez-Coronel J, Zimndern, JF, De Francisco AL, Garcia-Fuentes M, Arias M. Apolipoprotein C-III and E polymorphisms and cardiovascular syndrome, hyperlipidemia, and insulin resistance in renal transplantation. Am J Transplant 2002, 2:843-8.
29. Minihane AM, Finnegane YE, Talmud PJ, Leigh-Franks WC, Williams CM. Influence of the APOC3-1542T>G polymorphism on plasma lipid levels: effect of age and gender. Biochim Biophys Acta 2002, 1583:311-4.
30. Humphries SE, Berglund L, Otvos JD, Kaluski D, Deckelbaum RJ, Shear S, Talmud PJ. Foci for CETP, LPL, LIPC, and APOC3 affect plasma lipoprotein size and sub-population distribution in Hispanic and non-Hispanic white subjects: the Columbia University BioMarkers Study. Nutr Metab Cardiovasc Dis 2003, 13:194-201.
31. Espino-Montoro A, Barrios-Artillo M, López-Chozas JM, Cayuela A, Stiefel P, Villar J. Influence of polymorphism (RFLP-sst) at the apolipoprotein C-III gene locus on the lipoprotein metabolism and insulin resistance in essential hypertensive patients. Interaction between gender and genetic polymorphism. Nutr Metab Cardiovasc Dis 2003, 13:194-201.
32. Nalluruch V, Pape GP, Smith L, Patch J, Boenewinkel E. Polymorphic markers in apolipoprotein C-III gene flanking regions and hypertriglyceridemia. Arterioscler Thromb Vasc Biol 1996, 16:941-7.
33. Li GP, Wang JY, Yang SK, Chen BS, Xue H, Wu G. Genetic effect of two polymorphisms in the apolipoprotein A5 gene and apolipoprotein CIII gene on serum lipids and lipoproteins levels in a Chinese population. Clin Genet 2004, 65:470-6.
34. Garenc C, Aubert S, Larocche J, Giraud J, Vohl MC, Bergeron J, Rousseau F, Julien P. Population prevalence of APOE, APOC3 and PPAR-alpha mutations associated to hypertriglyceridemia in French Canadians. J Hum Genet 2004, 49:691-700.
35. Guettier JM, Georgopoulos A, Tsai MY, Radha V, Shankaran S, Deepa R, Grois M, Rao G, Mohan V. Polymorphisms in the fatty acid-binding protein 2 and apolipoprotein C-III gene are associated with the metabolic syndrome and dyslipidemia in a South Indian population. J Clin Endocrinol Metab 2005, 90:1705-11.
36. Garenc C, Couillard C, Lafamme N, Cadelle F, Gagné C, Couture P, Julien P, Bergeron J. Effect of the APOC3 SstI 1 SNP on fasting triglyceride levels in men heterozygous for the LPL P207L deficiency. Eur J Hum Genet 2003, 11:1599-605.
37. Ruiz-Narváez EA, Yang Y, Nakashima Y, Ishikawa Y, Ouchi Y, AePreps J, Bergeron J. Effect of the APOC3 SstI 1 SNP on fasting triglyceride levels in men heterozygous for the LPL P207L deficiency. Eur J Hum Genet 2003, 11:1599-605.
in European Whites, Indian Asians and Afro-Caribbeans with type 2 diabetes. Biochim Biophys Acta 2007, 1772:355-63.

43. Fiegenbaum M, de Andrade FM, Hutz MH: Association between plasma lipid parameters and APOC3 genotypes in Brazilian subjects: effect of gender, smoking and APOE genotypes. Clin Chim Acta 2007, 380:175-81.

44. Miller M, Rhyne J, Chen H, Beach V, Ericson R, Luttrau K, Dwivedi M, Mista A: APOC3 promoter polymorphisms C-482T and T-455C are associated with the metabolic syndrome. Arch Med Res 2007, 38:444-51.

45. Pollex RL, Ban MR, Young TK, Bjergaarda P, Anand SS, Yusuf S, Zimmerman B, Harris SB, Hanley AJ, Connelly PW, Huff MW, Young TK, Bjergaarda P, Hegele RA: Association between the -455T>C promoter polymorphism of the APOC3 gene and the metabolic syndrome in a multi-ethnic sample. BMC Med Genet 2007, 8:80.

46. Chien KL, Fang WH, Wen HC, Lin HP, Lin YL, Lin SW, Wu JH, Kao JT: Apolipoprotein CIII links hyperlipidemia with vascular endothelial cell dysfunction. Circulation 2008, 118:169-71.

47. Ruiz JR, Labajesi A, Ortega FB, Moreno LA, Gonzalez-Lamuno D, Marti A, Nova E, Fuentes MG, Redondo-Figuero C, Martinez JA, Sjostrom M, Castillo MJ, AVENA Study Group: Birth weight and blood lipid levels in Spanish adolescents: influence of selected APOE, APOC3, APOE and PON1 are associated with variation in plasma lipoprotein traits in Greenlanders. Int J Circumpolar Health 2007, 66:390-400.

48. Parzianello L, Oliveira G, Coelho JC: Apolipoprotein CIII polymorphism and triglyceride levels of a Japanese population living in Southern Brazil. Braz J Med Biol Res 2008, 41:462-7.

49. Kawakami A, Osakai M, Tani M, Azuma H, Sacks FM, Shimokado K, Yoshida M: Apolipoprotein CIII links hyperlipidemia with vascular endothelial cell dysfunction. Circulation 2008, 118:731-42.

50. Ruiz JR, Labajesi A, Ortega FB, Moreno LA, Gonzalez-Lamuno D, Marti A, Nova E, Fuentes MG, Redondo-Figuero C, Martinez JA, Sjostrom M, Castillo MJ, AVENA Study Group: Birth weight and blood lipid levels in Spanish adolescents: influence of selected APOE, APOC3 and PPARgamma2 gene polymorphisms. The AVENA Study. BMC Med Genet 2008, 9:6.

51. Ruiz JR, Labajesi A, Ortega FB, Moreno LA, Gonzalez-Lamuno D, Marti A, Nova E, Fuentes MG, Redondo-Figuero C, Martinez JA, Sjostrom M, Castillo MJ, AVENA Study Group: Birth weight and blood lipid levels in Spanish adolescents: influence of selected APOE, APOC3 and PPARgamma2 gene polymorphisms. The AVENA Study. BMC Med Genet 2008, 9:6.

52. Yiang L, Ruixing Y, Meng L, Kela L, Xingjiang L, Lin Z, Wanying L, Ruixing Y, Yuming C, Shangling P, Fengping H, Tangwei L, Dezhai Y, Jinzhao W, Limin X, Weixiong L, Rongshan L, Jiandong H: Effects of demographic, dietary and other lifestyle factors on the prevalence of hyperlipidemia in Guangxi Hei Yi Zhuang and Han populations. Eur J Cardiovasc Prev Rehabil 2006, 13:977-84.

53. Ruixing Y, Yuming C, Shangling P, Fengping H, Tangwei L, Weixiong L, Rongshan L, Jiandong H: Effects of demographic, dietary and other lifestyle factors on the prevalence of hyperlipidemia in Guangxi Hei Yi Zhuang and Han populations. Hypertens Res 2006, 29:423-32.

54. De Oliveira E, Silva ER, Foster D, McGee Harper M, Sandoval CE, Smith JD, Breslow JL, Brinton EA: Alcohol consumption raises HDL cholesterol levels by increasing the transport rate of apolipoproteins A-I and A-II. Circulation 2000, 102:2475-82.

55. Aragval DP: Cardiovascular effects of light-moderate consumption of alcohol: a review of putative mechanisms. Alcohol Alcohol 2002, 37:409-15.

56. Rimm EB, Williams P, Fisher K, Criqui M, Stampfer MJ: Moderate alcohol intake and lower coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 1999, 319:523-8.

57. Savolainen MJ, Vesanen YA: Effects of alcohol lipoproteins in relation to coronary heart disease. Curr Opin Lipidol 1995, 6:243-50.

58. Ko YL, Ko YS, Wu SM, Teng MS, Chen FR, Hsu TS, Chang CW, Lee YS: Interaction between obesity and genetic polymorphisms in the apolipoprotein CIII gene and lipoprotein lipase gene on the risk of hypertriglyceridemia in Chinese. Hum Genet 1997, 100:327-33.

59. Bai H, Saku K, Liu R, Imaiura M, Arakawa K: Association between coronary heart disease and the apolipoprotein A-I/C-III/A-IV complex in a Japanese population. Hum Genet 1995, 95:102-4.

60. Marcil M, Boucher B, Gagné E, Davidson J, Hayden M, Genest J Jr Lack of association of the apolipoprotein A-I/C-III/A-IV gene Xmnl and Sts polymorphisms and of the lipoprotein lipase gene mutations in familial combined hyperlipoproteinaemia in French Canadian subjects. J Lipid Res 1996, 37:309-19.

61. Kee F, Amouyel P, Fumeron F, Arveiler D, Cambou JP, Evans A, Cambien F, Fruchart JC, Ducimetière P, Dallongeville J: Lack of association between genetic variants of apo A-I/C-III/A-IV complex and coronary myocardial infarction in a sample of European male: ECTIM study. Atherosclerosis 1999, 145:187-95.

62. Thu NN, Mai TT, Ohmori R, Kuroki M, Chuyen NV, Hung NT, Kakawaki M, Kondo K: Plasma triglyceride and HDL-cholesterol concentrations in Vietnamese girls are affected by lipoprotein lipase, but not apolipoprotein CIII polymorphisms. J Nutr 2006, 136:1488-92.

63. Ruudvets JB, Ducimetière P, Arveiler D, Amouyel P, Bingham A, Wagner A, Cottel D, Perret B, Ferrieres J: Types of alcoholic beverages and blood lipids in a French population. J Epidemiol Community Health 2002, 56:34-8.

64. Choudhury SR, Ueshima H, Kita Y, Kobayashi KM, Okawaya A, Yamakawa M, Hira Y, Ishikawa M, Miyoshi Y: Alcohol intake and serum lipids in a Japanese population. Int J Epidemiol 1994, 23:940-8.

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