The peroxisome proliferator-activated receptor delta +294T > C polymorphism and alcohol consumption on serum lipid levels

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Abstract

Background: The single nucleotide polymorphism (SNP) of peroxisome proliferator-activated receptor delta (PPARD) gene affects serum lipid profiles, but to what extent alcohol consumption interferes with this association remains unknown. The present study was undertaken to compare the association of PPARD +294T > C (rs2016520) polymorphism and serum lipid levels in the nondrinkers and drinkers.

Methods: A total of 685 unrelated nondrinkers and 497 drinkers aged 15-82 were randomly selected from our previous stratified randomized cluster samples. Genotyping of the PPARD +294T > C was performed by polymerase chain reaction and restriction fragment length polymorphism. Interactions of the PPARD +294T > C genotypes and alcohol consumption on serum lipid levels were detected by using a factorial regression analysis after controlling for potential confounders.

Results: The levels of triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), apolipoprotein (Apo) A1, and the ratio of ApoA1 to ApoB were higher in drinkers than in nondrinkers (P < 0.05-0.001). There were no significant differences in the levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and ApoB between the two groups (P > 0.05 for all). The frequencies of TT, TC and CC genotypes were 56.0%, 36.4% and 7.6% in nondrinkers, and 57.2%, 38.0% and 4.8% in drinkers (P > 0.05); respectively. The frequencies of T and C alleles were 74.2% and 25.8% in nondrinkers, and 76.2% and 23.8% in drinkers (P > 0.05); respectively. There was also no significant difference in the genotypic and allelic frequencies between males and females in both groups (P > 0.05 for all). The levels of TC in nondrinkers were different among the three genotypes (P = 0.01), the C allele carriers had higher serum TC levels than the C allele noncarriers. The levels of all seven lipid traits in drinkers were not different among the three genotypes (P > 0.05 for all). The interactions of PPARD +294T > C genotypes and alcohol consumption on serum lipid levels were not detected in the drinkers (P > 0.05 for all). Multiple linear regression analysis showed that serum TC, HDL-C, LDL-C, ApoA1, and ApoB levels were correlated with genotypes in drinkers but not in nondrinkers (P < 0.05-0.01).

Conclusions: These results suggest that the great majority of our study populations are beneficial from alcohol consumption. But there is no interaction between the PPARD +294T > C genotypes and alcohol consumption on serum lipid levels in the drinkers.

Introduction

Abnormalities in lipid metabolism such as elevated total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and apolipoprotein (Apo) B levels, together with decreased high-density lipoprotein cholesterol (HDL-C), and ApoA1 levels, are considered as major risk factors for coronary artery disease (CAD) [1-4]. It is well known that serum lipid levels are modulated by multiple environmental and genetic factors and their interactions [5-11]. Numerous studies have evaluated the influence of alcohol consumption on CAD and serum lipid concentrations. Low to middle amounts of alcohol when taken on a regular basis have been shown to protect against CAD and death [12,13], whereas heavy drinking constitutes a severe risk condition. These...
results probably due in part to a dose-dependent increase in HDL-C and ApoA1 [14-17]. A decrease in LDL-C with increased alcohol intake has also been reported in some studies [18]. However, alcohol in doses > 30 g/day in both sexes can augment serum TG levels [15,19].

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear hormone receptors that act as transcriptional regulators and are involved in glucose and lipid metabolism. Three different PPARs, PPAR-alpha (PPARA), PPAR-gamma (PPARG), and PPAR-delta (PPARD) have been characterized [20], and they are distinguished from each other by tissue distribution and cell activation. PPARA is mainly expressed in liver, muscle, kidney and heart, PPARG is most abundant in adipocytes, intestinal cells and macrophages and PPARD is expressed in many tissues [21-23]. PPARs are encoded by separate genes and characterized by distinct tissue and developmental distribution patterns. The PPARD gene was mapped to 6p21.2-p21.1 with 11 exons spanning 35 Kbp and is expressed ubiquitously [24]. The PPARD +294T > C (rs2016520) polymorphism in the 5'-untranslated region in exon 4 of the PPARD gene is located 87 nucleotides upstream of the start codon. It was shown that the single nucleotide polymorphism (SNP) influenced binding of Sp-1 resulting in higher transcriptional activity for the rare C allele than the common T allele [25]. Several previous studies have showed that the PPARD +294T > C polymorphism was associated with modifications of serum lipid concentrations in the general population and the risk of CAD [25-34] in dyslipidemic women and hypercholesterolemic men and cholesterol metabolites in Alzheimer's disease patients [35]. But the results are inconsistent in diverse populations [33,36]. Furthermore, little is known about the interactions of PPARD +294T > C polymorphism and alcohol consumption on serum lipid concentrations. Therefore, the aim of the present study was to compare the association of PPARD +294T > C (rs2016520) polymorphism and serum lipid levels in the nondrinkers and drinkers.

Materials and methods

Study population

A total of 685 unrelated nondrinkers and 497 drinkers were randomly selected from our previous stratified randomized cluster samples [5,6]. The age of the subjects ranged from 15 to 82 years, with an average age of 43.46 ± 16.50 years. All of the subjects were rural agricultural workers. The subjects with evidence of diseases related to atherosclerosis, CAD and diabetes have been excluded. 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.

Epidemiological survey

The survey was carried out using internationally standardized methods [37]. Information on demographics, socioeconomic status, and lifestyle factors was collected with standardized questionnaires. The alcohol information included questions about the number of liangs (about 50 g) of rice wine, corn wine, rum, beer, or liquor consumed during the preceding 12 months. Alcohol consumption was categorized into groups of grams of alcohol per day: < 25 and ≥ 25. Smoking status was categorized into groups of cigarettes per day: < 20 and ≥ 20. At the physical examination, several parameters including height, weight, and waist circumference were measured. Sitting blood pressure was measured three times with the use of a mercury sphygmomanometer after the subjects had a 5-minute rest, and the average of the three measurements was used for the level of blood pressure. Systolic blood pressure was determined by the first Korotkoff sound, and diastolic blood pressure by the fifth Korotkoff sound. Body weight, to the nearest 50 grams, was measured using a portable balance scale. Subjects were weighed without shoes and in a minimum of clothing. Height was measured, to the nearest 0.5 cm, using a portable steel measuring device. From these two measurements body mass index (BMI, kg/m²) was calculated.

Biochemical analysis

A venous blood sample of 5 mL was obtained from all subjects after at least 12 hours of fasting. A part of the sample (2 mL) was collected into glass tubes and allowed to clot at room temperature, and used to determine serum lipid levels. Another part of the sample (3 mL) was transferred to tubes with anticoagulate solution (4.80 g/L citric acid, 14.70 g/L glucose, and 13.20 g/L tri-sodium citrate) and used to extract DNA. The levels of TC, TG, HDL-C, and LDL-C in samples were determined by enzymatic methods with commercially available kits, Tcho-I, TG-LH (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin Co. Antrim, United Kingdom, BT29 4QY), Cholestest N HDL, and Cholest- est LDL (Daichii Pure Chemicals Co., Ltd., Tokyo, Japan); respectively. Serum ApoA1 and ApoB levels were detected 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 our Clinical Science Experiment Center [5,6].

DNA amplification and genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the phenol-chloroform method [7].
Genotyping of the PPARD +294T > C polymorphism was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) [31]. PCR amplification was performed using 5′-CATGGTAT AGCAGCAAGAA-3′ and 5′-CTCTCCTCCTGTG GCTGCTC-3′ (Sangon, Shanghai, People’s Republic of China) as the forward and reverse primer pairs; respectively. Each amplification reaction was performed using 100 ng genomic DNA in 25 μL of reaction mixture consisting of 1.0 μL of each primer (10 μmol/L), 12.5 μL 2 x Taq PCR MasterMix (constituent: 0.1 U Taq polymerase/μL, 500 μM dNTP each and PCR buffer). After initial denaturation at 95°C for 5 min, the reaction mixture was subjected to 30 cycles of 45 s denaturation at 94°C, 45 s annealing at 62°C and extension 45 s at 72°C, followed by a final 8 min extension at 72°C. After electrophoresis on a 2.0% agarose gel with 0.5 μg/mL ethidium bromide, the amplification products were visualized under ultraviolet light. Then 5 U of BsnI restriction enzyme was added directly to the PCR products (6 μL) and digested at 55°C overnight. After restriction enzyme digestion of the amplified DNA, the genotypes were identified by electrophoresis on 2.5% agarose gels and visualized with ethidium-bromide staining under ultraviolet illumination. The genotypes were scored by an experienced reader blinded to epidemiological data and serum lipid levels.

Diagnostic criteria
The normal values of serum TC, TG, HDL-C, LDL-C, ApoA1, ApoB levels, and the ratio of ApoA1 to ApoB in our Clinical Science Experiment Center were 3.10-5.17, 0.56-1.70, 0.91-1.81, 2.70-3.20 mmol/L, 1.00-1.78, 0.63-1.14 g/L, and 1.00-2.50; respectively. The individuals with TC > 5.17 mmol/L and/or TG > 1.70 mmol/L were defined as hyperlipidemic [5,6]. Hypertension was defined as an average systolic blood pressure of 140 mmHg or greater and/or an average diastolic blood pressure of 90 mmHg or greater, and/or self-reported pharmacological treatment for hypertension within the 2 weeks prior to the interview [38,39]. Normal weight, overweight and obesity were defined as a BMI < 24, 24-28, and > 28 kg/m²; respectively [40].

Statistical analyses
Quantitative variables were expressed as mean ± standard deviation (serum TG levels were presented as medians and interquartile ranges). Qualitative variables were expressed as percentages. Allele frequency was determined via direct counting, and the standard goodness-of-fit test was used to test the Hardy-Weinberg equilibrium. Difference in genotype distribution between the groups was obtained using the chi-square test. The difference in general characteristics between nondrinkers and drinkers tested by the Student’s unpaired t-test. The association of genotypes and serum lipid parameters was tested by analysis of covariance (ANCOVA). Sex, age, BMI, blood pressure, and cigarette smoking were adjusted for the statistical analysis. In order to evaluate the association of serum lipid parameters and genotypes (TT = 1, TC = 2, CC = 3), multiple linear regression analysis with stepwise modeling was also performed in the combined population of nondrinkers and drinkers, nondrinkers, and drinkers; respectively. All statistical analyses were done 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 nondrinkers and drinkers. The ratio of male to female, the levels of mean age, body height, weight, BMI, waist circumference, systolic blood pressure and diastolic blood pressure, and the percentages of subjects who smoked cigarettes were higher in drinkers than in nondrinkers (P < 0.001 for all). There was no significant difference in the levels of pulse pressure between the two groups (P > 0.05).

Serum lipid levels between nondrinkers and drinkers
The levels of serum lipid parameters between nondrinkers and drinkers are also shown in Table 1. The levels of TG, HDL-C, ApoA1, and the ratio of ApoA1 to ApoB were higher in drinkers than in nondrinkers (P < 0.05-0.001). There were no significant differences in the levels of TC, LDL-C and ApoB between the two groups (P > 0.05 for all).

Results of electrophoresis and genotyping
After the genomic DNA of the samples was amplified by PCR and imaged by 2.0% agarose gel electrophoresis, the PCR products of 269 bp nucleotide sequences could be found in all samples. The genotypes identified were named according to the presence or absence of the enzyme restriction sites, when a T to C transversion at +294 locus of the PPARD. The presence of the cutting site indicates the C allele, whereas its absence indicates the T allele (cannot be cut). Thus, the TT genotype is homozygote for the absence of the site (band at 269 bp), TC genotype is heterozygote for the absence and presence of the site (bands at 269-, 167- and 102-bp), and CC genotype is homozygote for the presence of the site (bands at 167- and 102-bp). The genotype distribution was consistent with the Hardy-Weinberg equilibrium.

Genotypic and allelic frequencies
The genotypic and allelic frequencies of PPARD +294T > C polymorphism in the nondrinkers and drinkers are
shown in Table 2. The frequencies of TT, TC and CC genotypes were 56.0%, 36.4% and 7.6% in nondrinkers, and 57.2%, 38.0% and 4.8% in drinkers (P > 0.05); respectively. The frequencies of T and C alleles were 74.2% and 25.8% in nondrinkers, and 76.2% and 23.8% in drinkers (P > 0.05); respectively. There was also no significant difference in the genotypic and allelic frequencies between males and females in both groups (P > 0.05 for all).

Table 1 The general characteristics and serum lipid levels in the nondrinkers and drinkers

| Parameter                        | Nondrinker (n = 685) | Drinker (n = 497) | t (χ²) | P       |
|----------------------------------|----------------------|-------------------|--------|---------|
| Male/female                      | 187/498              | 362/135           | 239.775| 0.000   |
| Age (years)                      | 39.80 ± 16.37        | 44.93 ± 13.79     | -5.691 | 0.000   |
| Height (cm)                      | 152.03 ± 7.75        | 155.34 ± 7.82     | -7.181 | 0.000   |
| Weight (kg)                      | 51.05 ± 7.88         | 54.74 ± 8.21      | -7.763 | 0.000   |
| Body mass index (kg/m²)          | 22.04 ± 2.57         | 22.67 ± 2.91      | -3.894 | 0.000   |
| Waist circumference (cm)         | 69.80 ± 7.17         | 74.69 ± 7.09      | -11.657| 0.000   |
| Systolic blood pressure (mmHg)   | 118.33 ± 16.94       | 122.79 ± 16.85    | -4.475 | 0.000   |
| Diastolic blood pressure (mmHg)  | 74.57 ± 9.82         | 77.70 ± 10.01     | -5.357 | 0.000   |
| Pulse pressure (mmHg)            | 43.76 ± 11.78        | 45.10 ± 12.74     | -1.842 | 0.066   |
| Cigarette smoking [n (%)]        |                      |                   |        |         |
| Nonsmoker                        | 682 (89.2)           | 225 (45.3)        |        |         |
| < 20 cigarettes/day              | 34 (4.4)             | 120 (24.1)        |        |         |
| ≥ 20 cigarettes/day              | 49 (6.4)             | 152 (30.6)        | 287.105| 0.000   |
| Alcohol consumption [n (%)]      |                      |                   |        |         |
| Nondrinker                       | 685 (100.0)          | -                 |        |         |
| < 25 g/day                       | -                   | 380 (76.6)        |        |         |
| ≥ 25 g/day                       | -                   | 116 (23.4)        |        |         |
| Total cholesterol (mmol/L)       | 4.53 ± 0.94          | 4.57 ± 1.07       | -0.712 | 0.477   |
| Triglyceride (mmol/L)            | 0.98 (0.59)          | 1.02 (0.77)       | -2.923 | 0.003   |
| HDL-C (mmol/L)                   | 1.77 ± 0.47          | 1.83 ± 0.47       | -2.102 | 0.036   |
| LDL-C (mmol/L)                   | 2.61 ± 0.72          | 2.56 ± 0.82       | 1.187  | 0.235   |
| Apolipoprotein (Apo) A1 (g/L)    | 1.33 ± 0.27          | 1.41 ± 0.30       | -4.928 | 0.000   |
| ApoB (g/L)                       | 0.87 ± 0.22          | 0.86 ± 0.23       | 0.806  | 0.420   |
| ApoA1/ApoB                       | 1.60 ± 0.49          | 1.77 ± 0.77       | -4.600 | 0.000   |

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. The value of triglyceride was presented as median (interquartile range). The difference between the two groups was determined by the Wilcoxon-Mann-Whitney test.

Table 2 Genotypic and allelic frequencies of the PPARD +294T > C polymorphism in the nondrinkers and drinkers [n (%)]

| Group        | n   | Genotype | Allele |
|--------------|-----|----------|--------|
|              |     | TT       | CC     | T      | C      |
|              |     | 384 (56.0)| 249 (36.4)| 52 (7.6) | 1017 (74.2) | 353 (25.8) |
| Nondrinker   | 685 | -        | 3.697  | 1.138 |
|              |     | -        | 0.158  | 0.286 |
| Male         | 187 | 105 (56.2)| 68 (36.4)| 14 (7.4) | 278 (74.3) | 96 (25.7) |
| Female       | 498 | 279 (56.0)| 181 (36.4)| 38 (7.6) | 739 (74.2) | 257 (25.8) |
|              |     | -        | 0.004  | 0.003 |
|              |     | -        | 0.998  | 0.959 |
| Drinker      | 497 | 284 (57.2)| 189 (38.0)| 24 (4.8) | 757 (76.2) | 237 (23.8) |
| Male         | 362 | 210 (58.0)| 136 (37.6)| 16 (4.4) | 556 (76.8) | 168 (23.2) |
| Female       | 135 | 74 (54.8)| 53 (39.2)| 8 (6.0) | 201 (74.4) | 69 (25.6) |
|              |     | 0.712   | 0.599  |        |
|              |     | 0.701   | 0.438  |        |
Genotypes and serum lipid levels
As shown in Table 3, the levels of TC in nondrinkers were different among the three genotypes (P = 0.01), the C allele carriers had higher serum TC levels than the C allele noncarriers. There was no significant difference in the remaining serum lipid parameters among the three genotypes in nondrinkers (P > 0.05 for all).

The levels of all seven lipid traits in drinkers were not different among the three genotypes (P > 0.05 for all).

Interactions between genotypes and alcohol on serum lipid parameters
The interaction between PPARD +294T > C genotypes and alcohol consumption on serum TC levels (F = 0.706, P = 0.494) and the remaining serum lipid parameters was not detected by using a factorial regression analysis after controlling for potential confounders.

Correlation between serum lipid parameters and genotypes
Multiple linear regression analysis showed that serum TC, HDL-C, LDL-C, ApoA1, and ApoB levels were correlated with genotypes in drinkers but not in nondrinkers (P < 0.05-0.01; Table 4).

Discussion
The present study showed that the levels of serum TG, HDL-C, ApoA1, and the ratio of ApoA1 to ApoB were higher in drinkers than in nondrinkers. There were no significant differences in the levels of TC, LDL-C and ApoB between the two groups. These results suggest that the great majority of our study populations are beneficial from alcohol consumption. Low to middle amounts of alcohol when taken on a regular basis have been shown to protect against CAD and death [12,13]. A moderate intake of alcohol is associated with protection against CAD, probably due in part to a dose-dependent increase in HDL-C and ApoA1 levels [14-17]. According to a previous meta-analysis, a daily dose of 30 g alcohol results in an average HDL-C level rise of 3.99 mg/dl, and an ApoA1 level rise of 8.82 mg/dl [14]. The harmful effects of heavy alcohol consumption on serum lipid profiles may be due to an increase in plasma TG levels [14,18]. In the previous meta-analysis, 30 g of

Table 3 Genotypes of the PPARD +294T > C polymorphism and serum lipid levels in the nondrinkers and drinkers

| Group     | Genotype | n   | TC (mmol/L) | TG (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) | ApoA1 (g/L) | ApoB (g/L) | ApoA1/ApoB |
|-----------|----------|-----|-------------|-------------|----------------|----------------|-------------|------------|------------|
| Nondrinker| TT       | 384 | 4.44 ± 0.91 | 0.98(0.57)  | 1.74 ± 0.44    | 2.57 ± 0.70    | 1.31 ± 0.26  | 0.86 ± 0.21 | 1.60 ± 0.43 |
|           | TC       | 249 | 4.62 ± 0.92 | 0.99(0.65)  | 1.80 ± 0.48    | 2.64 ± 0.71    | 1.35 ± 0.26  | 0.90 ± 0.24 | 1.59 ± 0.48 |
|           | CC       | 52  | 4.74 ± 1.12 | 0.89(0.57)  | 1.87 ± 0.54    | 2.77 ± 0.92    | 1.39 ± 0.30  | 0.91 ± 0.24 | 1.68 ± 0.88 |
| F         | -        | -   | 4.629       | 0.217       | 2.667          | 2.301          | 2.545       | 2.810      | 0.734      |
| P         | -        | -   | 0.010       | 0.897       | 0.070          | 0.101          | 0.079       | 0.061      | 0.480      |
| Drinker   | TT       | 284 | 4.57 ± 1.01 | 1.01(0.71)  | 1.83 ± 0.49    | 2.60 ± 0.82    | 1.41 ± 0.32  | 0.86 ± 0.23 | 1.77 ± 0.78 |
|           | TC       | 189 | 4.55 ± 1.16 | 1.02(0.89)  | 1.85 ± 0.46    | 2.47 ± 0.79    | 1.42 ± 0.28  | 0.86 ± 0.25 | 1.79 ± 0.69 |
|           | CC       | 24  | 4.74 ± 1.08 | 1.26(0.93)  | 1.68 ± 0.43    | 2.73 ± 0.97    | 1.38 ± 0.31  | 0.93 ± 0.28 | 1.67 ± 0.94 |
| F         | -        | -   | 0.355       | 3.836       | 1.365          | 2.189          | 0.184       | 1.205      | 0.300      |
| P         | -        | -   | 0.702       | 0.147       | 0.256          | 0.113          | 0.832       | 0.301      | 0.741      |

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; ApoA1/ApoB, the ratio of apolipoprotein A1 to apolipoprotein B. The value of TG was presented as median (interquartile range). The difference among the genotypes was determined by the Kruskal-Wallis test.

Table 4 Correlation between serum lipid parameters and the PPARD +294T > C genotypes in the nondrinkers and drinkers

| Lipid       | Relative factor | Unstandardized coefficient | Standard error | Standardized coefficient | t     | P     |
|-------------|-----------------|---------------------------|----------------|--------------------------|-------|-------|
| Nondrinker  | TC Genotype     | 0.123                     | 0.046          | 0.080                    | 2.833 | 0.005 |
|             | ApoB Genotype   | 0.025                     | 0.010          | 0.071                    | 2.527 | 0.012 |
| Drinker     | TC Genotype     | 0.167                     | 0.055          | 0.113                    | 3.058 | 0.002 |
|             | HDL-C Genotype  | 0.068                     | 0.028          | 0.092                    | 2.440 | 0.015 |
|             | LDL-C Genotype  | 0.089                     | 0.042          | 0.078                    | 2.110 | 0.035 |
|             | ApoA1 Genotype  | 0.036                     | 0.016          | 0.086                    | 2.279 | 0.023 |
|             | ApoB Genotype   | 0.030                     | 0.013          | 0.086                    | 2.336 | 0.020 |
alcohol daily was associated with a plasma TG increase of 5.69 mg/dl [14]. The alcohol intake of 60 g/day increases the TG levels by about 0.19 mg/dl per 1 gram of alcohol consumed [18]. The effects of alcohol consumption on LDL-C are inconsistent. A recent study in older Italian subjects (65-84 years old) has found that alcohol intake increases serum LDL-C levels [41]. Another recent study of Turks also found increases in LDL-C, as well as in ApoB and TG, with alcohol in men, while women had decreased TG and no change in LDL-C or ApoB with alcohol [42]. 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 [17].

The genotypic and allelic frequencies of PPARD +294T > C polymorphism were different in diverse populations. Several previous studies have shown that the frequency of the rare allele (+294C) was 18.3% in Russian endurance-oriented athletes and 12.1% in controls (P < 0.0001) [43], 32.0% in Tunisian CAD patients and 18.9% in healthy volunteers (P = 0.001) [32], and 30.8% in Chinese CAD patients and 19.5% in normal controls (P < 0.05) [33]. Other studies, however, showed that there was no difference in its frequency between the patients with type 2 diabetes and the non-diabetic controls (18.7% vs 19.2%) [35], or among the patients with metabolic syndrome, essential hypertension and type 2 diabetes [26]. In the present study, we showed that the frequency of +294C alleles was 25.8% in non-drinkers, and 23.8% in drinkers (P > 0.05). There was no significant difference in the genotypic and allelic frequencies between males and females in both groups. However, the frequency of PPARD +294C allele was higher in our study population than in 543 healthy 50-year-old-men (15.6%) from the northern part of the greater Stockholm area [24], in normal controls (19.5%) from Chinese Anhui Province [33], in healthy Tunisian population (18.9%) [32], and in non-diabetic Germany controls (19.2%) [35]; but it was lower than in Tunisian CAD patients (32.0%) [32] and Chinese CAD patients (30.8%) [33]. These results indicate that the prevalence of the C allele variants of PPARD +294T > C polymorphism may have an ethnic or disease specificity.

The association of PPARD +294T > C polymorphism and serum lipid levels is inconsistent. Skogsberg et al. [24] demonstrated that homozygotes for the rare C allele had a higher LDL-C concentration than homozygotes for the common T allele. There were no associations with the HDL-C levels. In another study in Scottish men, they found that the +294C allele did not influence LDL-C concentrations but was associated with lower HDL-C levels [25]. Aberle et al. [27] also showed a highly significant association between the rare C allele and lower HDL-C levels in dyslipidemic female subjects. In addition, metabolic syndrome patients with CC genotype had significantly higher TC and LDL-C levels than those with TT and TC genotypes [26]. The PPARD +294T > C polymorphism was associated with HDL-C and was dependent on sex among subjects with and without type 2 diabetes [29]. The risk variant of PPARD +294T > C marker was associated with higher LDL-C and increased serum TC [31]. However, Gouni-Berthold et al. [35] found that the presence of the C allele had no effect on TG, HDL-C, and LDL-C levels, both in diabetic and non-diabetic German controls, or both in men and in women. The same result was found by Jguirri-Souissi et al. [32] both in CAD patients and healthy controls. In the present study, we showed that the levels of TC in nondrinkers were different among the three genotypes, the C allele carriers had higher serum TC levels than the C allele noncarriers. But the levels of all seven lipid traits in drinkers were not different among the three genotypes. Serum TC, HDL-C, LDL-C, ApoA1, and ApoB levels were correlated with genotypes in drinkers but not in nondrinkers. These results suggest that the effects of PPARD +294T > C polymorphism on serum lipid levels are different between nondrinkers and drinkers.

The interactions between PPARD +294T > C polymorphism and alcohol consumption on serum lipid levels have not been previously explored. In a previous study, Brand-Herrmann et al. [9] showed that alcohol consumption modulates the relation between the PPAR-gamma 2 (PPARG) Pro12Ala and HDL-C. They randomly recruited 251 nuclear families (433 parents and 493 offspring) in the framework of the European Project on Genes in Hypertension study and genotyped 926 participants in whom all serum lipid variables and information on alcohol consumption were available for PPARG gamma 2 Pro12Ala. The results showed that the Ala12 allele was more frequent in Novosibirsk (17%) than in Cracow (12%) and Mirano (11%, P < 0.01). Italian offspring carrying the Ala12 allele had higher serum HDL-C than noncarriers (P < 0.05). HDL-C levels were on average 0.086 mmol/L (P = 0.001) higher in drinkers than in nondrinkers. As compared with Pro12 homozygotes, Ala12 allele carriers consuming alcohol had higher serum total and HDL-C, with the opposite trend occurring in nondrinkers. This genotype-alcohol interaction was independent of the type of alcoholic beverage and more pronounced in moderate than in heavy drinkers. In the present study, however, we found no interaction between the PPARD +294T > C genotypes and alcohol consumption on serum lipid levels in the drinkers. These findings suggest that increased levels of TG, HDL-C, ApoA1, and the ratio of ApoA1 to ApoB in drinkers were not influenced by the interactions of
PPARD +294T > C polymorphism and alcohol consumption. The effect of different kinds of wine on serum lipid profiles is not well known. In the present study, 90% of the wine drunk by the subjects was corn wine, rice wine or rum, in which the alcohol content is low. Thus, the effects of different kinds of alcohol consumption on serum lipid levels still need to be determined [44,45].

Conclusion
The present study shows that the levels of TG, HDL-C, ApoA1, and the ratio of ApoA1 to ApoB were higher in drinkers than in nondrinkers. There were no significant differences in the levels of TC, LDL-C and ApoB, and the genotypic and allelic frequencies of PPARD +294T > C between nondrinkers and drinkers. The levels of TC in nondrinkers were different among the three genotypes, the C allele carriers had higher serum TC levels than the C allele noncarriers, whereas the levels of all seven lipid traits in drinkers were not different among the three genotypes. The interaction of PPARD +294T > C genotypes and alcohol consumption on serum TC levels and the remaining serum lipid parameters was not detected in the drinkers. Multiple linear regression analysis showed that serum TC, HDL-C, LDL-C, ApoA1, and ApoB levels were correlated with genotypes in drinkers but not in nondrinkers. These results suggest that the great majority of our study populations are beneficial from alcohol consumption. But there is no interaction between the PPARD +294T > C genotypes and alcohol consumption on serum lipid levels in the drinkers.

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Authors’ contributions
XW participated in the design, helped to carry out the genotyping, and drafted the manuscript. LM undertook genotyping. DFW collaborated to the genotyping and performed the statistical analyses. RXY conceived the study, participated in the design, carried out the epidemiological survey, collected the samples, and helped to draft the manuscript. 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, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. Am J Cardiol 1998, 81:78-128.
2. Castelli WP, Anderson K, Wilson PW, Levy D. Lipids and risk of coronary heart disease. The Framingham Study. Ann Epidemiol 1992, 2:23-8.
3. Brunzell JD, Sniderman AD, Albers JJ, Kuverovich PO Jr. Apoproteins B and A-I and coronary artery disease in humans. Arteriosclerosis 1984, 4:79-83.
4. Holewijn S, den Heijer M, Swinkels DWM, Stalenhoef AHF, de Graaf J. Apolipoprotein B, non-HDL cholesterol and LDL cholesterol for identifying individuals at increased cardiovascular risk. J Intern Med 2010, 268:567-77.
5. Ruixing Y, Qiming F, Deyi Z, Xingjiang L, Lin Z. Alcohol consumption raises HDL cholesterol levels. J Intern Med 2001, 249:167-76.
6. Ruixing Y, Qiming F, Dezhai Y, Xingjiang L, Lin Z. Alcohol intake modulates the genetic association between HDL cholesterol and the PPARgamma2 Pro12Ala polymorphism. J Lipid Res 2003, 44:919-9.
7. Brand-Hermann SM, Kuznetsova T, Wiechert A, Stolarz K, Tikhonoff V, Schmidt-Petersen K, Telgmann R, Casiglia E, Wang JG, Thijs L, Staessen JA, Brand E. European Project on Genes in Hypertension Investigators. Alcohol intake modulates the genetic association between HDL cholesterol and the PPARgamma2 Pro12Ala polymorphism. J Lipid Res 2005, 46:913-9.
8. Tai ES, Corella D, Demissie S, Cupples LA, Coltell O, Schaefer EJ, Tucker KL, Ordovas JM, Framingham heart study. Polymunsaturated fatty acids interact with the PPARA-L126V polymorphism to affect plasma triglyceride and apolipoprotein C-III concentrations in the Framingham Heart Study. J Nutr 2005, 135:397-403.
9. Ruixing Y, Yiyang L, Meng L, Kela L, Xingjiang L, Lin Z, Wanying L, Jing T, Yiyang L. Alcohol intake and serum lipids in a Japanese population. Int J Epidemiol 1994, 23:940-7.
10. Rimm EB, Williams P, Foster K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 1999, 319:1523-8.
11. De Oliveira E, Silva ER, Foster D, McGee Harper M, Seidman CE, Smith JD, Breslow JL, Brinton EA. Alcohol consumption raises HDL cholesterol levels by increasing the transport rate of apoprotein A-I and coronary artery disease in humans. J Lipid Res 2005, 46:913-9.
12. Camargo CA Jr, Hennekens CH, Gaziano JM, Glynn RJ, Manson JE, Stampfer MJ. Prospective study of moderate alcohol consumption and mortality in US male physicians. Arch Intern Med 1997, 157:79-85.
13. Choudhury SR, Ueshima H, Kita Y, Kobayashi KM, Okayama A, Yamakawa M, Hirao Y, Ishikawa M, Miyoshi Y, Kela L, Xingjiang L, Lin Z, Wanying L, Jing T, Yiyang L. Alcohol intake and serum lipids in a Japanese population. Int J Epidemiol 1994, 23:940-7.
14. Rimm EB, Williams P, Foster K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 1999, 319:1523-8.
15. De Oliveira E, Silva ER, Foster D, McGee Harper M, Seidman CE, Smith JD, Breslow JL, Brinton EA. Alcohol consumption raises HDL cholesterol levels by increasing the transport rate of apoprotein A-I and A-II. Circulation 2000, 102:2347-52.
16. Agarwal DP. Cardioprotective effects of light-moderate consumption of alcohol: a review of putative mechanisms. Alcohol Alcohol 2002, 37:409-15.
17. Savolainen MJ, Keranen MI. Effects of alcohol lipoproteins in relation to coronary heart disease. Cur Opin Lipidol 1995, 6:343-50.
18. 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.
19. Kamon J, Yamauchi T, Kadowaki T. PPAR family (PPAR alpha, PPAR delta, PPAR gamma). Nihon Rinsho 2002, 60:5591-5600.
20. Reddy JK, Hashimoto T. Peroxisomal beta-oxidation and peroxisome proliferator-activated receptor alpha: an adaptive metabolic system. Annu Rev Nutr 2001, 21:193-230.
21. Spiegelman BM, Flier JS. Adipogenesis and obesity: rounding out the big picture. Cell 1996, 87:317-22.
22. Skogberg L, Kanno K, Roshani L, Gagne E, Hamsten A, Larnson C, Ehrnberg E. Characterization of the human peroxisome proliferator activated receptor delta gene and its expression. Int J Mol Med 2000, 6:73-81.
23. Yoshikawa T, Brikanez D, Dupont BR, Xing QG, Leach RJ, Detera-Wadleigh SD. Assignment of the human nuclear hormone receptor, UC1 (PPARD), to chromosome 6p21.1-p21.2. Genomics 1996, 35:637-8.
24. Skogsborg J, Kannisto K, Cassel TN, Hamsten A, Eriksson P, Ehnborg E. Evidence that peroxisome proliferator-activated receptor-delta influences cholesterol metabolism in men. Arterioscler Thromb Vasc Biol 2003, 23:637-43.

25. Skogsborg J, Mcmahon AD, Karpe F, Hamsten A, Packard CJ, Ehnborg E, West of Scotland Coronary Prevention Study. Peroxisome proliferator-activated receptor delta genotype in relation to cardiovascular risk factors and risk of coronary heart disease in hypercholesterolaemic men. J Intern Med 2003, 254:597-604.

26. Yan ZC, Shen CY, Zhong J, Wang L, Ni YX, Nie H, Zhu ZM: PPARG delta +294T>C gene polymorphism related to plasma lipid, obesity and left ventricular hypertrophy in subjects with metabolic syndrome, Zhonghua Xin Xue Guan Bing Za Zhi 2005, 33:529-33.

27. Aberle J, Hopfer I, Bel FJ, Seedorf U: Association of the T+294C polymorphism in PPAR delta with low HDL cholesterol and coronary heart disease risk in women. Int J Med Sci 2006, 3:108-11.

28. Hautala AJ, Leon AS, Skinner JS, Rao DC, Bouchard C, Rankinen T: Peroxisome proliferator-activated receptor-delta polymorphisms are associated with physical performance and plasma lipids: the HERITAGE Family Study. Am J Physiol Heart Circ Physiol 2007, 292:H2498-505.

29. Burch LR, Donnelly LA, Doney AS, Brady J, Tommasi AM, Whitley AL, Godlard C, Morris AD, Hansen MK, Palmer CN: Peroxisome proliferator-activated receptor-delta genotype influences metabolic phenotype and may influence lipid response to statin therapy in humans: a genetics of diabetes audit and research Tayside study. J Clin Endocrinol Metab 2010, 95:1830-7.

30. Miao L, Yin YX, Wu DF, Cao XL, Li Q, Hu XJ, Yan TT, Aung LH, Yang DZ, Lin WX: Peroxisome proliferator-activated receptor delta +294 T>C polymorphism and serum lipid levels in the Guangxi Bai Ku Yao and Han populations. Lipids Health Dis 2011, 9:145.

31. Nikitin AG, Chistiakov DA, Minushkina LO, Zateyshchikov DA, Nosikov VV: Association of the CYBA, PPARGC1A, PPARG3, and PPARD gene variants with coronary artery disease and metabolic risk factors of coronary atherosclerosis in a Russian population. Heart Vessels 2010, 25:229-36.

32. Jguririm-Souissi I, Jelassi A, Hrira Y, Najah M, Slimani A, Addad F, Hassine M, Hamda KJ, Maatouk F, Rouis M, Slimane MN: T+294C polymorphism in the PPAR-delta gene is associated with risk of coronary artery disease in normolipidemic Tunisians. Genet Mol Res 2010, 9:1326-33.

33. Wang LF, Tan M, Chang H, Yu H, Shen JJ: Relationship of peroxisome proliferator-activated receptor-delta +294 T>C gene polymorphism with coronary artery disease. Acta Univ Med Anhui 2008, 43:701-5.

34. Holzapfel J, Heun R, Lutjohann D, Jessen F, Maier W, Kolch H: PPAR-delta haplotype influences cholesterol metabolism but is no risk factor of Alzheimer's disease. Neurosci Lett 2006, 408:57-61.

35. Gouni-Berthold I, Giannakidou E, Faust M, Berthold HK, Krone W: The peroxisome proliferator-activated receptor delta +294 T>C polymorphism in relation to lipoprotein metabolism in patients with diabetes mellitus type 2 and in non-diabetic controls. Atherosclerosis 2005, 183:336-41.

36. People's Republic of China-United States Cardiovascular and Cardiopulmonary Epidemiology Research Group: An epidemiological study of cardiovascular and cardiopulmonary disease risk factors in four populations in the People's Republic of China. Baseline report from the P.R.C-U.S.A. Collaborative Study. Circulation 1992, 85:1083-96.

37. Ruixing Y, Weixiong L, Hanjun Y, Dezhai Y, Shuqian L, Shanglei Y, Qiming F, Jinwen H, Jianting G, Yaju D: Diet, lifestyle, and blood pressure of the middle-aged and elderly in the Guangxi Bai Ku Yao and Han populations. Am J Hypertens 2008, 21:382-7.

38. Ruixing Y, Shanglei Y, Dezhai Y, Weixiong L, Qiming F, Jinwen H, Yaju D: Diet, lifestyle, and blood pressure of the middle-aged and elderly in the Guangxi Bai Ku Yao and Han populations. Am J Hypertens 2008, 21:382-7.

39. Cooperative Meta-analysis Group of China Obesity Task Force: Predictive values of body mass index and waist circumference to risk factors of related diseases in Chinese adult population. Chin J Epidemiol 2002, 23:5-10.

40. Akhmetov II, Astranenkova IV, Rogozkin VA: Association of PPARG gene polymorphism with human physical performance. Mol Biol (Mosk) 2007, 41:852-7.

41. Perissinotto E, Buja A, Maggi S, Enzi G, Manzato E, Scalfaro E, Mastrangelo G, Frigo AC, Com A, Crepaldi G, Sergi G, ILSA Working Group: Alcohol consumption and cardiovascular risk factors in older lifelong wine drinkers: the Italian Longitudinal Study on Aging. Nutr Metab Cardiovasc Dis 2010, 20:647-55.

42. Onat A, Hergenc G, Dursunoğlu D, Oрудu S, Can G, Bulur S, Yüksel H: Associations of alcohol consumption with blood pressure, lipoproteins, and subclinical inflammation among Turks. Alcohol 2008, 42:593-601.

43. Akhmetov II, Astranenkova IV, Rogozkin VA: Association of PPARG gene polymorphism with human physical performance. Mol Biol (Mosk) 2007, 41:852-7.

44. Ruixing Y, Shanglei Y, Dezhai Y, Hanjun Y, Yaju D, Yuming C, Jinwen H, Yiyang L, Qiming F, Hanjun Y, Yuming C: Associations of diet and lifestyle with hyperlipidemia for middle-aged and elderly persons among the Guangxi Bai Ku Yao and Han populations. J Am Diet Assoc 2008, 108:970-6.