A genome-wide association and gene–environment interaction study for serum triglycerides levels in a healthy Chinese male population

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Triglyceride (TG) is a complex phenotype influenced by both genetic and environmental factors. Recent genome-wide association studies (GWAS) have identified genes or loci affecting lipid levels; however, such studies in Chinese populations are limited. A two-stage GWAS were conducted to identify genetic variants that were associated with TG in a Chinese population of 3495 men. Gene–environment interactions on serum TG levels were further investigated for the seven single nucleotide polymorphisms (SNPs) that were studied in both stages. Two previously reported SNPs (rs651821 in APOA5, rs328 in LPL) were replicated in the second stage, and the combined P-values were 9.19 × 10−26 and 1.41 × 10−9 for rs651821 and rs328, respectively. More importantly, a significant interaction between aldehyde dehydrogenase 2 (ALDH2) rs671 and alcohol consumption on serum TG levels were observed (P = 3.34 × 10−5). Rs671 was significantly associated with serum TG levels in drinkers (P = 1.90 × 10−10), while no association was observed in non-drinkers (P > 0.05). For drinkers, men carrying the AA/AG genotype have significantly lower serum TG levels, compared with men carrying the GG genotype. For men with the GG genotype, the serum TG levels increased with the quantity of alcohol intake (P = 1.28 × 10−8 for trend test). We identified a novel, significant interaction effect between alcohol consumption and the ALDH2 rs671 polymorphism on TG levels, which suggests that the effect of alcohol intake on TG occurs in a two-faceted manner. Just one drink can increase TG level in susceptible individuals who carry the GG genotype, while individuals carrying AA/AG genotypes may actually benefit from moderate drinking.

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INTRODUCTION

Metabolic syndrome is a combination of medical disorders that increase the risk of developing cardiovascular disease (CVD) (1) and type 2 diabetes mellitus (2). Elevated serum triglyceride (TG) is a key atherogenic lipid phenotype of the metabolic syndrome. And hypertriglyceridemia is a common lipid disorder in China, with a prevalence of 11.9% in individuals aged 18 and over (3).

Current evidence suggests that blood lipids are complex phenotypes, influenced by both environmental and genetic factors. Heritability estimates for TG ranged from 35 to 48% (4). These estimates indicate that DNA sequence variants play an important role in explaining inter-individual variation of TG levels. In fact, genome-wide association studies (GWAS) and human genetic studies have identified a number of genes and gene regions affecting TG (5–7). Moreover, a recent meta-analysis of lipid GWAS comprised of 96,598 individuals of European descent identified another 16 novel loci that were associated with TG (8). GWAS offer a powerful approach to gene discovery and have shown recent success in the identification of novel variants associated with hypertriglyceridemia.

However, the current GWAS for lipid profiles have been predominantly conducted in populations of European ancestry, and data for blood lipid in Chinese populations are limited. Given the difference in allele and genotype frequencies, and linkage disequilibrium (LD) patterns across the populations, we conducted a GWAS in a Chinese population in search of population-specific genetic variations associated with serum TG levels. We also investigated gene–environment interactions on serum TG levels.

RESULTS

The demographics of the samples used in the genome-wide association analysis are provided in Table 1. Except for alcohol consumption (P = 0.02), no significant difference in age distribution, smoking or body mass index (BMI) was observed between the two stages.

The Q–Q plot of adjusted P-values indicates no systematic bias, with an inflation factor of 1.03 (Supplementary Material, Fig. S1). When the top two Eigens were added to other covariates in the GWAS analysis, similar results were obtained; the inflation factor was 1.03 (Supplementary Material, Fig. S1), which indicates that no population substructure was observed in our GWAS population (Supplementary Material, Fig. S2). The genome-wide association results were presented in the Manhattan plot (Fig. 1). In the first stage, we identified seven loci on six chromosomes that reached a P-value of 1.0 × 10⁻⁵. After testing the independence of the associated single nucleotide polymorphisms (SNPs) at each of the seven loci using the multiple regression analysis, one SNP per region remained to be followed in the second stage (rs16835705, rs328, rs10504457, rs651821, rs671, rs1532085 and rs157581).

In the second stage, two SNPs (rs651821, rs328) were confirmed at P-value cutoff of 7.0 × 10⁻³ (adjusting for seven tests). When the data from the two stages were combined, both of the two SNPs reached a genome-wide significant level of 5 × 10⁻⁸, with P-values of 9.19 × 10⁻²⁶ and 1.41 × 10⁻⁹ for rs651821 and rs328, respectively (Table 2). The two confirmed TG level-associated regions had been previously reported (9). SNP rs651821 at APOA5 5’ untranslated region had the strongest association with TG in our study and is in strong LD (D’ = 0.98, r² = 0.72) with the SNP rs964184 reported (9) at APOA1–APOC3–APOA4–APOA5. The other SNP rs328 was the non-synonymous variant on the LPL gene, the recognized lipid metabolic gene (6). In addition, rs671 was nominally replicated in the second stage with a P-value of 0.04, and the combined P-value was 1.96 × 10⁻⁶ (Table 2). Rs671 was a non-synonymous SNP in the aldehyde dehydrogenase 2 (ALDH2) gene.

We then tested whether the above seven SNPs interacted with alcohol consumption or smoking in determining serum TG. A significant interaction between rs671 and alcohol drinking on serum TG was observed (P = 3.34 × 10⁻⁵ in the combined data). More importantly, the interaction was consistently observed in both the first stage and the second stage (P = 1.26 × 10⁻³, P = 1.62 × 10⁻², respectively). When stratified based on the drinking status (yes or no), rs671 had a significant relationship with TG in drinkers, with P-values of 5.74 × 10⁻³, 5.08 × 10⁻² and 1.90 × 10⁻¹ in the first stage, the second stage and the combined data, respectively (Table 3). However, there was no significant difference in TG levels among ALDH2 rs671 genotypes in non-alcohol drinkers (P > 0.05). We then stratified men who drink into three subgroups, based on the amount of alcohol consumed (<10, 10–30 and >30 g/day). For men who consume <30 g/day, the mean of TG levels for men who carry AA or AG genotypes were significantly lower than men who carry GG genotypes (P = 6.90 × 10⁻⁶, 3.82 × 10⁻² for men who consume <10, 10–30 g/day, respectively, Table 3). While among men consuming 30 g/day or more, only one person carried AA homozygote, for whom the TG level was 3.21 mmol/l. Men with AG genotypes also had significantly lower TG levels, compared with those with GG genotype (1.04 versus 1.58, P = 3.26 × 10⁻⁴, Table 3). For men with GG genotype, the serum TG levels increased with the quantity of alcohol intake (P = 1.28 × 10⁻⁸ for trend). No significant interaction between rs671 and smoking on serum TG was observed, and none of the other six SNPs showed statistically significant interactions with smoking or alcohol consumption (all P > 0.05).

| Table 1. General characteristics of the two-stage GWAS study participants |
|-----------------|-----------------|-----------------|-----------------|
| Characteristics  | First stage      | Second stage    | P-valueab      |
| n               | 1999            | 1496            |                |
| Age (years)     | 37.54 ± 11.10   | 37.31 ± 10.80   | 0.54           |
| Smoking, n (%)  | Yes             | 1015 (50.8)     | 771 (51.5)     | 0.66           |
|                 | No              | 984 (49.2)      | 725 (48.5)     |                |
| Alcohol drinking, n (%) | Yes         | 1704 (85.5%)    | 1165 (82.6%)   | 0.02           |
|                 | No              | 288 (14.5%)     | 246 (17.4%)    |                |
| Body mass index (kg m⁻²) | 23.31 ± 3.44    | 23.46 ± 3.34    | 0.18           |

a-b Test was used to compare means of the continuous variables between the first and the second stages.

b: χ²-Test was used to compare the differences for categorical variables.
DISCUSSION

In this two-stage GWAS of 3495 men, we identified two loci that were significantly associated with serum TG level, including rs651821 in APOA5 with a \( P \)-value of \( 1.41 \times 10^{-29} \), and rs328 in LPL with a \( P \)-values of \( 9.19 \times 10^{-26} \) in the combined data. These two associations are consistent with known pathways for lipid metabolism (10,11). More importantly, a significant interaction between alcohol consumption and the ALDH2 rs671 polymorphism on TG levels was observed (\( P = 3.34 \times 10^{-5} \)). Rs671 was significantly associated with serum TG levels in drinkers, with a combined \( P \)-value of \( 1.90 \times 10^{-10} \), while no association was observed in non-drinkers (\( P > 0.05 \)).

Despite the fact that two known variants for TG were replicated in our study (Table 2), many other signals identified in Europeans were not replicated (Supplementary Material, Table S1). Among the 32 SNPs for TG trait that were implicated in the European meta-analysis (8), 8 were significant at a nominal \( P \)-value of 0.05. The SNPs showing no evidence of association in this study, although having previously been established in Europeans, have a statistical power ranging from 5–20%, implying an insufficient sample size for these markers to detect association for TG. Furthermore, similar with the finding from the Korean study (12), the minor allele frequencies are considerably lower in the Chinese population, compared with the European population. Therefore, a larger population is needed to draw a more convincing conclusion about those loci.

Rs671 is a non-synonymous (ns) SNP (rs671, G1510A, Glu504Lys) in the ALDH2 gene located on chromosome 12. Its coding enzyme, ALDH2 is a crucial enzyme in alcohol metabolism, which oxidizes aldehyde to acetic acid mainly in the mitochondria. A genetic variant in the ALDH2 rs671 (G→A, Glu504Lys in exon 12) decreases the activity of ALDH2. Compared with ALDH2 activity in the GG genotype, men with the GA genotype showed 30–40% for the activity and those with AA genotype almost showed negligible activity (13). Thus, individuals with GA/AA are unable to degrade aldehyde efficiently and tend to develop malaise, flushing reaction and other uncomfortable symptoms. These conditions make individuals with inactive ALDH2 have to reduce the alcohol consumption. In our study, the percentage of participants carrying AG or AA decreased with the increase in alcohol intake. For the group of men that consumed 30 g/day of alcohol or more, only one individual carried AA genotype. In addition, the Glu504Lys genetic polymorphism of ALDH2 is common in East Asian (14). The frequency of the Chinese population carrying the A allele in ALDH2 rs671 was 26%, indicating that the alleles influence both health and drinking behavior in this population. Consistent with the recent meta-analysis of association between ALDH2 genetic polymorphism and serum lipids in the Asian population (15), our study found that the \( P \)-value of the main effect of rs671 did not reach a genome-wide significant level of \( 5 \times 10^{-8} \) (\( P = 1.96 \times 10^{-6} \) in the combined data set). However, a significant interaction effect was observed between ALDH2 rs671 and alcohol consumption on affecting TG levels. The association between rs671 and serum levels of TG reached a genome-wide significant level in drinkers, with a \( P \)-value of \( 1.90 \times 10^{-9} \), while no significant association was observed in non-drinkers (\( P > 0.05 \)). Men with mild or moderate drinking habits (<30 g/day) had lower serum TG when carrying AA/AG rather than GG. This means men carrying AA or AG may be able to reduce their serum TG levels with moderate drinking. However, men carrying the GG are more likely to have increased serum TG levels with increased quantity of alcohol intake. High levels of TGs in the bloodstream have been linked to atherosclerosis and, by extension, the risk of
Table 2. SNPs associated with levels of serum TGs

<table>
<thead>
<tr>
<th>Region</th>
<th>Position*</th>
<th>Genes</th>
<th>Alleles</th>
<th>First stage (N = 1496)</th>
<th>Second stage (N = 1496)</th>
<th>Combined P-valuesb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>m</td>
<td>Freq (m)</td>
<td>Mean</td>
<td>Freq (m)</td>
</tr>
<tr>
<td>rs328</td>
<td>19,864,004</td>
<td>LPL</td>
<td>G/C</td>
<td>0.15</td>
<td>1.12</td>
<td>1.07</td>
</tr>
<tr>
<td>rs10504457</td>
<td>70,761,214</td>
<td>SLCO5A1</td>
<td>T/C</td>
<td>0.43</td>
<td>1.09</td>
<td>1.18</td>
</tr>
<tr>
<td>rs651821</td>
<td>116,167,789</td>
<td>APOA5</td>
<td>C/T</td>
<td>0.27</td>
<td>1.59</td>
<td>1.26</td>
</tr>
<tr>
<td>rs671</td>
<td>110,726,149</td>
<td>ALDH2</td>
<td>A/G</td>
<td>0.26</td>
<td>1.11</td>
<td>1.08</td>
</tr>
<tr>
<td>rs1532085</td>
<td>56,470,658</td>
<td>Intergenic</td>
<td>A/G</td>
<td>0.42</td>
<td>1.33</td>
<td>1.22</td>
</tr>
<tr>
<td>rs157581</td>
<td>50,087,554</td>
<td>TOMM40</td>
<td>G/A</td>
<td>0.24</td>
<td>1.46</td>
<td>1.26</td>
</tr>
</tbody>
</table>

TG levels were log-transformed and the values presented were back-transformed.

m, minor allele; M, major allele.

aGenomic position is based on NCBI build 36.

bThe P-values are based on linear regression analysis on the log-transformed TGs and adjusted for population stratification, age and BMI assuming an additive model; the combined P-values are calculated based on the linear regression model, adjusting for covariates and stage information.

Heart disease and stroke (16), and mild or moderate drinking can reduce the risk of CVD (17). It is inferred in our study that the positive effect of alcohol intake on the risk of CVD might be partly depend on the ALDH2 genotype. In contrast to the previous views that alcohol spurs the liver to make more TGs, and even light drinking (two to four ounces of wine a week) can raise blood TGs (Diabetes Organization, February 2000, and Tufts University Health & Nutrition Letter, July 1997), our results showed that just one drink can increase TGs in susceptible individuals who carry GG genotype, while men with AA or AG genotypes can benefit from the moderate drinking.

Our results need to be interpreted with caution. First, there was a potential confounding effect between ALDH2 genotype and drinking. In fact, we observed an attenuation of association of the ALDH2 genotype with TG levels after controlling for alcohol consumption in drinkers (P = 8.65 × 10⁻¹⁴ for univariate analysis, and P = 3.32 × 10⁻¹⁰ for multivariate analysis after controlling for drinking). However, the ALDH2 genotype remained highly significant after controlling for alcohol consumption suggested that the association of the ALDH2 genotype with the TG level was only partially mediated by drinking behavior. This was also supported by the moderately correlation between those two variables (Pearson correlation r = 0.11). Secondly, accuracy of self-reporting drinking behavior may also have a confounding effect and was considered one of our study limitations. The self-reporting approach may underestimate or over-estimate the true intake. However, it has been shown to be useful and has provided important information (18). Therefore, the degree to which the self-reported measurement may represent the true alcohol consumption is still unknown and requires future studies to explore. We hope the publication of our results may encourage more studies in this field to explore other methods which can be used to measure alcohol consumption more precisely.

In summary, our study replicated the associations of SNPs on APOA5 and LPL with TG levels in a Chinese male population. We also identified an interaction between alcohol consumption and the ALDH2 rs671 polymorphism on TG levels. ALDH2 rs671 was only significantly associated with serum TG levels in drinkers, but not in non-drinkers. Men with mild or moderate drinking (<30 g/day) had lower serum TG in AA/AG than in GG. While, for men with GG, the serum TG levels increased with the quantity of alcohol intake. This means the effect of alcohol intake on TG levels is in a two-faceted manner. Just one drink can increase TG in susceptible individuals who carry the GG genotype, while individuals carrying AA or AG genotypes may benefit from the moderate drinking. Further studies are needed to further examine the interaction identified in this study.

MATERIALS AND METHODS

Study participants

Stage 1 of the GWAS included 2012 unrelated healthy Chinese men age 20–69 years old from the Fangchenggang Area Male Health and Examination Survey (FAMHES). The FAMHES is described elsewhere (19). Briefly, it was designed
to investigate the effects of environmental and genetic factors and their interaction with the development of age-related chronic diseases. All men who participated in physical examinations in the Medical Centre of Fangchenggang First People’s Hospital from September 2009 to December 2009 were invited to participate in the study. A total of 4030 participants (98.6%) consented and donated blood samples. The participants in stage 1 were randomly selected from these men who met age criteria. All participants self-reported that they were of southern Chinese Han ethnicity.

Stage 2 of the GWAS consisted of 1496 healthy Chinese men age 20–69 years old. They were randomly selected from male participants who participated in physical examinations from September 2009 to September 2010 in the Medical Centre of Fangchenggang First People’s Hospital, Guigang People’s Hospital and Yulin First People’s Hospital. The stage 2 samples from Fangchenggang First People’s Hospital were independently recruited from the stage 1 samples. Among these participants, 996 were of Han ethnicity and 500 were of Zhuang ethnicity.

The same recruitment strategy was used in stages 1 and 2. Comprehensive health information was collected through clinical examination, and additional demographic information was obtained via a standardized questionnaire. All participants self-reported to be free of diabetes mellitus, coronary heart disease, stroke, hyperthyroidism, rheumatoid arthritis, tumors and impaired hepatic or renal function. We obtained written documentation of informed consent from all study participants, and the research protocol was approved by the local Ethics Committee. Drinking behavior was assessed on the basis of a self-administered life-style questionnaire. Alcohol consumption was classified into three categories: drinkers and non-drinkers. Respondents that reported drinking any beverage more often than ‘less than once a year’ or ‘never’ were coded as drinkers (19,20). Also, according to the daily intake of alcohol, drinkers were further classified into three categories: <10, ≥10 <30 and ≥30 g/day.

### Measurement of TG

Blood specimens were obtained after participants had fasted overnight (≥8 h); TG levels were measured by standard enzymatic methods (19).

### SNP genotyping

Two different platforms were used for SNP genotyping. The Illumina Omni 1 platform was used for a genome-wide assay of samples in stage 1. The Sequenom iPLEX system (Sequenom, Inc., San Diego, CA, USA) was used in the second stage. Polymerase chain reaction and extension primers were designed using Mass ARRAY Assay Design 3.1 software (Sequenom, Inc.). Genotyping procedures were performed according to the manufacturer’s iPLEX Application Guide (Sequenom Inc.). All genotyping reactions were performed in 384-well plates. Each plate included a duplicate for three or four participants selected at random, as well as six to nine negative controls in which water was substituted for DNA. The average concordance rate was 99.8%.

### Statistical analysis

Quality control (QC) procedures were first applied to 2012 individuals that were genotyped using the Illumina Omni-Express platform. A total of 1999 individuals passed the call rate of 95% and were used in the final statistical analysis. We then applied the following QC criteria to filter SNPs: $P < 0.001$ for the Hardy–Weinberg equilibrium test, minor allele frequency ≤0.01 and genotype call rate ≤95%. Based on these criteria, 709211 SNPs were retained. The IMPUTE computer program (21) was then used to infer the genotypes of SNPs (e.g. SNPs catalogued in Hapmap Phase II CHB population release #24) in the genome that was not directly genotyped. A posterior probability of >0.90 was applied to call genotypes that were

### Table 3. Interaction between ALDH and alcohol consumption in determining TG concentration

<table>
<thead>
<tr>
<th>Alcohol consumption (g/day)</th>
<th>TG levels (mmol/l) by ALDH2 genotypes(^a)</th>
<th>P-value(^b)</th>
<th>P(^c) for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>First stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-drinking</td>
<td>1.19 (0.26), n = 66</td>
<td>1.61 (0.15), n = 133</td>
<td>1.14 (0.21), n = 89</td>
</tr>
<tr>
<td>Drinking</td>
<td>1.03 (0.21), n = 68</td>
<td>1.07 (0.07), n = 635</td>
<td>1.33 (0.06), n = 1001</td>
</tr>
<tr>
<td>Second stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-drinking</td>
<td>1.33 (0.23), n = 65</td>
<td>1.33 (0.18), n = 113</td>
<td>1.16 (0.23), n = 68</td>
</tr>
<tr>
<td>Drinking</td>
<td>1.21 (0.33), n = 35</td>
<td>1.26 (0.09), n = 436</td>
<td>1.43 (0.07), n = 694</td>
</tr>
<tr>
<td>Combined</td>
<td>1.26 (0.17), n = 131</td>
<td>1.23 (0.12), n = 246</td>
<td>1.15 (0.15), n = 157</td>
</tr>
<tr>
<td>≥0 10</td>
<td>0.90 (0.18), n = 103</td>
<td>1.14 (0.06), n = 1071</td>
<td>1.37 (0.05), n = 1695</td>
</tr>
<tr>
<td>&gt;10 30</td>
<td>1.08 (0.19), n = 94</td>
<td>1.13 (0.06), n = 850</td>
<td>1.28 (0.06), n = 1033</td>
</tr>
<tr>
<td>≥30</td>
<td>1.11 (0.75), n = 6</td>
<td>1.24 (0.16), n = 139</td>
<td>1.49 (0.10), n = 361</td>
</tr>
<tr>
<td>&lt;10</td>
<td>3.21 (1.00), n = 1</td>
<td>1.04 (0.21), n = 72</td>
<td>1.58 (0.13), n = 288</td>
</tr>
</tbody>
</table>

\(^a\)Values are shown as mean (standard errors).
\(^b\)P-value for the ALDH2 genotypes in each stratum after multivariate adjustment for age, body mass index (BMI) and tobacco smoking.
\(^c\)P-value for the interaction term between the ALDH2 genotypes and alcohol consumption (yes/no) in the multivariate adjusted model.
imputed using IMPUTE software. After applying the same QC criteria, as used above, a total of 1,940,243 SNPs remained in the final analysis.

Analysis for TG was performed on log-transformed values. SNP association tests were performed using linear regression implemented in PLINK (22) under the assumption of an additive relationship between the number of copies of the minor allele and the residual lipid values. Population stratification was estimated by a principal component approach, as implemented by EIGENSTRAT software (23). The top two Eigens were adjusted as covariates in the linear regression analysis. Clinical covariates utilized in the linear regression modeling included age at the time of blood lipid measurement, BMI (weight in kg divided by the height in m$^2$), alcohol intake (yes, no) and cigarette smoking (yes, no).

For regions with multiple SNPs that were significant at $P < 10^{-5}$ multivariate linear regression analysis was applied to test the independence of the respective SNPs. Only the SNPs that remained significant at $10^{-5}$ in the multivariate analysis were selected. The combined analysis of two-stage data was performed using a linear regression, adjusting for the covariates and stage information.

The effect of genotypes and environmental factors (alcohol consumption and smoking) on serum TG was investigated using a linear regression model, where the dependent variable was log-transformed TG and the independent variables were the genotypes, the environmental factors and an interaction term between the two independent variables. Covariates that were adjusted in the individual analysis for TG were selected. The combined analysis of two-stage data was performed using a linear regression model, adjusting for the covariates that remained significant at $P < 10^{-5}$ in the multivariate analysis.

For regions with multiple SNPs that were significant at $P < 10^{-5}$ multivariate linear regression analysis was applied to test the independence of the respective SNPs. Only the SNPs that remained significant at $10^{-5}$ in the multivariate analysis were selected. The combined analysis of two-stage data was performed using a linear regression, adjusting for the covariates and stage information.

The effect of genotypes and environmental factors (alcohol consumption and smoking) on serum TG was investigated using a linear regression model, where the dependent variable was log-transformed TG and the independent variables were the genotypes, the environmental factors and an interaction term between the two independent variables. Covariates that were adjusted in the individual SNP analysis were also included in this model. Alcohol consumptions were first tested as binary variables (yes/no), and then was tested as categorical variables (0, ≥10 <30 and ≥30 g/day, respectively). Stratified analysis was also performed. In each stratum based on alcohol consumption, the association of SNP and TG levels was tested with the adjustment for age, BMI and smoking.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG online.

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Conflict of Interest statement. None declared.

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REFERENCES