

May 6, 2014

Dear Editor,

Thank you for your letter and the reviewer's excellent critiques. We have carefully revised our manuscript in accordance with the reviewer and editor's comments and we would like to submit this revised manuscript for your consideration. The responses are included below. Comments are indicated in bold, followed by our replies. Please find enclosed the revised manuscript in Word format.

Title: *ApolipoproteinC3* (–455T>C) Polymorphism Confers Susceptibility to Nonalcoholic Fatty Liver Disease in the Southern Chinese Han Population

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Response to the reviewer and editor's comments

1 Format has been updated

Reply: Thanks.

Core tip, author contributions, and comments including background, research frontiers, innovations and breakthroughs, applications, and peer review have been added to the revised manuscript.

2 Revision has been made according to the suggestions of the reviewer

Major criticisms:

(1) The authors diagnosed NAFLD only by ultrasonography. For a diagnosis of NAFLD, they should mention alcoholic consumption and the result of liver biopsy. If they did not collect liver biopsy, they should consider scoring system, such as NAFLC score or FIB4 etc., to indicate how many NAFL and NASH exist in the subjects.

Reply: Thanks.

NAFLD caused by alcohol abuse were excluded from the present study, and alcohol abuse was defined as alcohol consumption >140 gram per week in men or >70 gram per week in women. This has been revised in the “**Subjects**” section of the new manuscript (Page 4).

It is very important to identify NASH from NAFL, because the long-term mortality and liver related complications in patients with NASH are much higher than that in the NAFL patients with the same age and sex.

Liver biopsy remains the gold standard to diagnose NASH, but it was a pity to say that we did not get access to the liver biopsy data of the participants in our study due to patients' rejection. Although numerous noninvasive measures such as MRE, transient elastography, NashTest, cytokeratin-18 have been explored to detect NASH, these indices were not routinely performed in our clinical laboratory. And further studies were needed to investigate the sensitivity and specificity of these indices to diagnose NASH.

Several noninvasive score systems [including: NAFLD fibrosis score (NAFLD-FS), the AST/platelet ratio index (APRI); the BARD score; the FIB-4 index] were created to predict NAFLD related advanced fibrosis. However, the above score systems have not been recommended to diagnose NASH until now ^[a, b].

[a]. Shah AG, Lydecker A, Murray K, et al. Comparison of Noninvasive Markers of Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol H* 2009; 7: 1104-1112.

[b]. Castera L, Vilgrain V, Angulo P, et al. Noninvasive evaluation of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013; 10: 666-675.

(2) In Table 1, they should show aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Reply: Thanks.

The information for ALT and AST has been added in **Table 1** in the revised manuscript.

(3) In this study, the prevalence of NAFLD in C-carriers genotypes (TC or CC) and the wild-type homozygote (TT) genotype was 55.4% and 41.2%. In study of the Asian Indian population the prevalence of NAFLD in C-carriers genotypes (TC or CC) and the wild-type homozygote (TT) genotype was 38.0% and 0%. Previous studies examined Asian Indian, African American, European American, Hispanic, and Finnish populations, but the association between *APOC3* (–455T>C) polymorphism and NAFLD susceptibility was found only in the Asian Indian population. I think there is different frequencies of the *APOC3* (–455T>C) genotypes. I recommend presenting the frequencies of the *APOC3* (–455T>C) genotypes in each ethnicity.

Reply: Thanks.

Frequencies of *APOC3* (–455T>C) in African Americans, European Americans, Hispanics, Finns and Northern Chinese have been shown in **Table 5** in the revised manuscript.

However, the frequencies of *APOC3* (–455T>C) genotypes in Indian population and Caucasians were not accessible, because the polymorphism of *APOC3* (–455T>C) and *APOC3* (–482C>T) were linked together to analyze the association between them and hepatic TG content in the original article ^[c, d]. We could not get access to the independent information for the frequencies of *APOC3* (–455T>C) in Indian population and Caucasians.

[c]. Petersen KF, Dufour S, Hariri A, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. *N Engl J Med* 2010; 362: 1082-1089.

[d]. Sentinelli F, Romeo S, Maglio C, et al. Lack of effect of apolipoprotein C3 polymorphisms on indices of liver steatosis, lipid profile and insulin resistance in obese Southern Europeans. *Lipids Health Dis* 2011; 10: 666-75

(4) In statistical analysis, which model did the authors calculate in additive model and dominant recessive model?

Reply: Thanks.

Additive model was used to calculate the ORs.

In vitro studies suggested that C allele carriers of *APOC3* (–455T>C) promoted *APOC3* transcription and resulted in increased level of circulating APOC3 and hypertriglyceridemia.

In the Indian population, C allele carriers of *APOC3* (–455T>C) contributed to the higher level of hepatic TG contents and plasma TG concentration. In contrast to the conclusion drew from *In vitro* studies and Indian population, studies performed in multi-ethnicities including African Americans, European Americans, and Hispanics revealed that TT genotype showed higher level of hepatic TG contents in African Americans.

Therefore, which allele played as the risk factor for NAFLD remained obscure. To explore the association between *APOC3* (–455T>C) polymorphism and NAFLD in a Han Chinese population, the additive model was carried out in our study.

(5) They should perform multiple logistic regression analysis of the factors

associated with *APOC3* (–455T>C) polymorphism.

Reply: Thanks.

Age (>40 years), gender, and BMI were considered as potential confounders for NAFLD and metabolic disorders. Therefore, age, gender and BMI were also included in the multivariate analyses when we explored the association between *APOC3* (–455T>C) polymorphism and NAFLD susceptibility, and the above metabolic disorders. Results involved this section have been revised in the new manuscript.

After multivariate analysis, C allele carriers were found to be independent risk factors for NAFLD susceptibility. TC and CC genotypes increase the susceptibility to NAFLD compared to that of the TT genotype, with adjusted OR of 1.77 (95%CI: 1.16–2.72) and 2.80 (95%CI: 1.64–4.79), respectively (Table 2).

After multivariate analysis, TC and CC genotypes remained the independent risk factors for hypertension, hypertriglyceridemia, low HDL and IR in NAFLD patients (Table 3 and 4).

(6) In table 2, 3, 4 and 5, the authors should consider the correlation of *APOC3* (–455T>C) polymorphism in the control group as well as in NAFLD patients.

Reply: Thanks.

In the present study, we defined healthy controls with that of “subjects who were free of elevated ALT or AST and have no liver steatosis examined by abdominal ultrasonography, and lacked any sign of metabolic disorder such as hypertension, overweight, obesity, hyperuricemia, and dyslipidemia”. And this definition had been described in the “**Subjects**” section of the manuscript (Page 4).

Therefore, all subjects in the control group were free of any kind of metabolic disorder, and we considered it unreasonable to explore the association between *APOC3* (–455T>C) polymorphism and metabolic disorder in the control group as well as the NAFLD group.

(7) The serum concentration of *APOC3* was not obtained in this manuscript. Apolipoprotein C3 is a major consistent of VLDL. I recommend measuring the

serum concentration of VLDL.

Reply: Thanks.

It was very important to measure the serum concentration of APOC3 or even VLDL to confirm the causality between them and the plasma TG concentration. However, the measurements for APOC3 or VLDL serum concentration have not been routinely performed in our hospital yet, and we are so sorry about that.

Minor criticisms:

(1) In Figure 2, why is there two band in the fragments of 194bp position

Reply: Thanks.

PAGE gel was run again. Only one band occurred in the position of the fragment of 194bp. It was revised in the new manuscript.

(2) In references, fonts are different.

Reply: Thanks.

All the references were revised throughout according to the references format of the *World Journal of Gastroenterology*.

3 References and typesetting were corrected

Reply: Thanks.

PubMed citation numbers and DOI citation have been added to the references. The references have been edited again according to the format of the *World Journal of Gastroenterology*.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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