

Format for ANSWERING REVIEWERS

February 28, 2014



Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8873-review.docx).

Title: Pooled genetic analysis in ultrasound measured NAFLD in Indian subjects: a pilot study

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Name of Journal: *World Journal of Hepatology*

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The manuscript has been improved according to the suggestions of reviewers:

Reviewer 1

No Comments

Reviewer 2

Comment 1: In the METHODS: 306 subjects were recruited, out of them 156 had fatty liver and 150 were in the control group. But in the MATERIALS AND METHODS section, 450 individuals with fatty liver were recruited. Please explain.

Response: Initially a total of 450 individuals were screened on the ultrasound out of which 198 individuals were excluded on the basis of alcohol intake, viral, autoimmune and Wilson's disease workup. Of the remaining 252 individuals, 96 individuals had to be excluded due to consent issues and drug intake. Finally 156 individuals were included in the present study. Individuals (n=150) who were age, sex and ethnicity matched controls were then selected based on no fatty infiltration as detected on the ultrasound. The same is reflected in Figure 1.

Comment 2: In RESULTS: first paragraph – line 10, change wrt to with.

Response: As suggested "wrt" is changed to "with respect to"

Reviewer 3

Comment 1: Genetics play an important role along with metabolic factors in the development of NAFLD. Ultrasound has low specificity and sensitivity for NAFLD. Further association studies should be done with liver biopsy.

Response: We thank the reviewer for the comment however, as already mentioned and justified in the materials and methods section that although liver biopsy is considered to be the gold standard for identifying NAFLD and NASH, lack of indication for asymptomatic individuals, costs involved, risk of complications and ethical concerns limit

its use in these types of studies. Therefore subjects were recruited based on ultrasound findings of hepatic steatosis. Furthermore, based on previous reports # and work \$ on NAFLD, we initiated genetic studies in Indian NAFLD.

National Center for Health Statistics, Centers for Disease control and Prevention. Hepatic steatosis. Ultrasound Images assessment procedures manual. Available from http://www.cdc.gov/nchs/data/nhanes/nhanes3/Hepatic_steatosis_Ultrasound_Procedures_Manual.pdf.

Hernaes R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology* 2011; **54(3)**: 1082-1090. [PMID:21618575 DOI 10.1002/hep.24452.]

\$ **Peng XE**, Wu YL, Lin SW, Lu QQ, Hu ZJ, Lin X.. Genetic Variants in and Risk PNPLA3 of Non-Alcoholic Fatty Liver Disease in a Han Chinese Population. *PLoS ONE* 2012; **7(11)**: e50256. [PMID: 23226254 doi:10.1371/journal.pone.0050256].

\$ **Hernaes R**, McLean J, Lazo M, Brancati FL, Hirschhorn JN, Borecki IB, Harris TB; Genetics of Obesity-Related Liver Disease (GOLD) Consortium, Nguyen T, Kamel IR, Bonekamp S, Eberhardt MS, Clark JM, Kao WH, Speliotes EK. Association between variants in or near PNPLA3, GCKR, and PPP1R3B with ultrasound-defined steatosis based on data from the third national health and nutrition examination survey. *Clin Gastroenterol Hepatol.* 2013; **11(9)**:1183-1190.e2. [PMID: 23416328 DOI 10.1016/j.cgh.2013.02.011.]

Reviewer 4:

Major comments

Comment 1: According to the Abstract, NAFLD and control groups are recruited on the basis of standard criteria for NAFLD and ultrasound findings of fatty infiltration. The criteria for NAFLD, however, have not been described in the MATERIALS AND METHODS. If Indices of glycolipid metabolism (as shown in Table 2), such as glucose, insulin, total cholesterol, HDL, LDL, etc., reflect the criteria for NAFLD, formula concerning these indice should be clearly mentioned. Otherwise, authors have to make it clear that NAFLD is evaluated solely by the ultrasound findings, in accordance to the description that 'subjects were recruited based on ultrasound findings of hepatic steatosis as per earlier reports' (Page 5).

Response: The NAFLD and control groups have been included in the study solely based on the ultrasound detection of fatty infiltration. The sentence "the basis of standard criteria for NAFLD" in the abstract has been deleted.

Comment 2: SNPs with p value <0.05 are considered to be statistically significant in this study. In contrast, rs738409 of PNPLA3 (p=0.05), and rs2143571 of SAMM50 (p=0.05), is reported to associated with NAFLD and high triglyceride level, respectively. Is there any discrepancy?

Response: We thank the reviewer for the comment. In this study, a p value less than or equal to 0.05 were considered to be significant. The same has been added to the statistics

section.

Minor comments

Comment 1: Nonalcoholic fatty liver disease instead of its abbreviation, namely NAFLD, maybe suitable for the title of manuscript.

Response: As suggested we can expand the abbreviation of NAFLD, however as per the instruction in the manuscript preparation, the title should be less than 12 words and expanding the abbreviation exceeds the limit of 12 words.

Comment 2: SNPs, rather than the genes in which they located, should be listed in Table 3.

Response: The SNPs have also been added along with the gene names.

Comment 3: Rows with header are suggested in Table 5.

Response: Rows with header have been added and we thank the reviewer for the comment.

Reviewer 5:

Major

Comment 1: The authors did not state that how were these cohort recruited? What were the reasons these subjects visited the Hepatology clinics of the hospital? Do they have other metabolic comorbidity such as diabetes, dyslipidemia, or hypertension? Were these subjects recruited consecutively or selected on some basis? These issues are very important because selection bias can occur to distort the study results.

Response: The master health checkup of the hospital includes ultrasound scan of the abdomen and individuals who were detected with fatty infiltration of the liver were referred to the Hepatology clinic of the hospital and these individuals were then recruited for the present study from the Hepatology clinic.

Comment 2: As the authors stated in the **Material and Method** Section, the major limitation of this study is that they use ultrasound as the diagnostic tool for NAFLD, instead of the good standard biopsy or other advanced imaging techniques. Therefore, the authors should describe what the diagnostic criteria were for NAFLD, and how to avoid the inter-observer variability?

Response: The diagnosis of NAFLD was based on standard imaging criteria used for ascertaining liver fat content by ultrasound (brightness of liver) and the ultrasound was performed by two experienced radiologists consistently, strictly adhering to similar standard criteria.

Minor

Comment 1: **Table 2** described the demographic and clinical characteristics of the study subjects. However, no data of lipid profiles, blood pressure, fasting plasma glucose, and percentage of subjects with hypertension and diabetes were given. The authors should show these information.

Response: The data has been added.

Comment 2: In the **Material and Method** Section, the authors define low levels of high density lipoprotein as HDL less than 50 mg/dL in males and 40 mg/dL in females. This is

completely different from the commonly used definition, that is 40 mg/dL and 50 mg/dL in males and females, respectively.

Response: It is a typographical error and it has been corrected. We thank the reviewer for the same.

Comment 3: The legend of **Table 4** is misleading. If the SNPs were independent variables, and BMI, TG and ALT were dependent variables, this table indicated "Significant SNPs associated with higher Odds of obesity, TG, and ALT (but not NAFLD)".

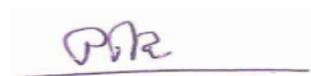
Response: As suggested we have changed the title of the table.

Comment 4: We suggest the authors to merge the information provided in **Figure 2** into **Table 5**.

Response: As suggested we have now deleted figure 2 and only table 5 is retained.

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

Sincerely yours,



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