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Answering Reviewer

December 26, 2016

Dear Editor

Thank you very much for your kind letter of 19 December,2016 with regard to our manuscript together with the comments from the reviewers. The comments of the reviewers have been helpful in allowing us to revise our manuscript. We have studied these comments carefully and have made the corrections requested.

Title: Association between TLR7 copy number variations and HBV infection outcome in Chinese

Author: Fang Li, Xu Li,Gui-Zhou Zou, Yu-Feng Gao, Jun Ye

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 30246

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

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

Reviewer No.1

(1)Introduction: - The study design would be clearer if the introduction included 1-2 sentences why it is important to analyze male and female groups separately (as described in the discussion) and why it is interesting in the framework of the study to compare patient groups based on hepatitis B e-antigen titer.

Reply：

Because TLR7 is a highly conserved gene on the X chromosome,so we separated the groups according to gender and analyze the TLR7 CNVs in this study.HBeAg can play a role of immune regulation which is associated with the outcomes of HBV infection.E antigen was used as an important index of HBV replication and infection.So in our study we divided patients into two groups according to e-antigen titers (0–1 IU/mL vs. >1 IU/ml). Both of them are associated with the body's immune function,so in the study we compare patient groups based on hepatitis B e-antigen titer.

(2)It is stated that 923 individuals were enrolled in the study. Were individuals with chronic HBV somehow selected or were just all individuals enrolled who presented with predefined diagnostic criteria and gave consent for this study?

Reply:

All the individuals enrolled presented with predefined diagnostic criteria and none of these patients had received anti-HBV therapy or had overlapping infections of hepatitis A, C, D, E, or G. CHB patients were also free from drug-induced hepatitis, alcoholic liver disease, fatty liver disease, and pregnancy.All of them have given consent for this study

(3)In the sample demographics section it would be clearer to first state that there was no significant difference in age distribution between AHB and CHB (since cases and controls were matched, as described in the material and methods section), but that there was a difference if the CHB group was divided into CHB, LC and HCC.

Reply:

There were no significant age differences between AHB and CHB patients(including CHB ,LC and HCC groups) (t=0.823P =0.944＞ 0.05)，but that there was a difference if the CHB group was divided into CHB, LC and HCC.（F =83.216,P＜0.01）.We can not completely eliminate the age factor have an effect on the results, but actually we found that the influence of age has no significant correlation of outcome of HBV infection by logistic regression analysis.

(4)In the current manuscript, the authors present copy number data as <1, >1 (males) or <2, >2 (females). It would be interesting to see the copy number distribution (1, 2, 3… and number of individuals) e.g. as supplementary info, if possible?

Reply:

The copy number distribution of individuals can be found from Table 3 and 4,meanwhile there is no missing phenomenon of CNVs in our study,and we consulted previous research to group the CNVs.[Association of TLR7 copy number variation with susceptibility to childhood-onset systemic lupus erythematosus in Mexican population]

(5) Since the Figure legends for Figure 1 and 2 as well as for Figure 3 and 4 are the same (except for gender), I would suggest to combine Figure 1 and 2 as well as Figure 3 and 4. Figure 1A: male patients, Figure 1B: female patients, Figure 2A: male patients, Figure 2B: female patients according to e-antigen titer.

Reply:

Figure 1 and 2 as well as Figure 3 and 4 are combined already.

(6)Please discuss shortly findings for patient groups with different e-antigen titers.

Reply:

According to the previous study ,we found that the replication system is adapted to generate high levels of virions without stimulating the innate immune system. Secreted viral proteins (HBsAg and HBeAg) suppress innate responses through inhibition of TLR signaling, which leads to a weak adaptive immune response with an exhausted phenotype that is incapable of inducing viral

elimination.[Basis of HBV persistence and new treatment options.]This experiment want to study the relationship between the two indicators from gene level ,but we found that e-antigen titer levels did not vary significantly among patient groups ,which showed that there were no correlation from gene level to the two indicators.

Reviewer No.2

(1)One common and possible limitation when including patients with progressive chronic diseases such as chronic hepatitis B, is classification of patients outcome because patients with cirrhosis may develop HCC sometime later. This limitation must be discussed in the manuscript.

Reply:

We used one-way ANOVA to analyze the age between the four groups ,and found that there were no statistical difference between LC and HCC group ,meanwhile the distribution of CNVs were no statistical difference as well between two groups in this study, so this limitation is lowly with the results.

(2)Institutional review board statement has to be included in the methods section.

Reply:

Institutional review board statement has shown in the methods section.

(3)"AHB controls were age and sex matched with CHB cases" statement has been included in materials and Methods section. However, CHB group includes 495/600 males (82.5%) while AHB group includes 165/300 males (55%) as indicated in the text and in table 2, and they are not sex-matched.

Reply:

The gene for Toll-like receptor7 is located on the X chromosome and so experiences sex-linked inheritance.But by the logistic regression analysis we found there is no correlation between gender and the outcome of HBV infection.So the four groups are not need to be sex-matched. Meanwhile there were no significant age differences between AHB and CHB patients(including CHB ,LC and HCC groups) ,but that there was a difference if the CHB group was divided into CHB, LC and HCC.（AS included in Sample demographics)

(4) table 2: please indicate the p value comparing age and gender among groups.

Reply:

The P value has been indicated in table 2.

(5)how many males had 1 TLR7 copy, and how many females carried 2 copies? Authors only indicate <1 and >1 copy for males and <2 or >2 in females. In which group are included carriers of 1 copy (males) and 2 copies (females)?

Reply:

In our study ,there were 12 males had 1 TLR7 copy, and only 3 females carried 2 copies.Meanwhile ,based on the reference category, the risk of disease progression associated with the absolute TLR7 CN was estimated by comparing cases and controls that were categorized as CN > 2 or CN ⩽ 2 for females and as CN > 1 or CN ⩽ 1 for males. And we feel very sorry for this mistake.

(6)table 3 and figures 1 and 2 are redundant. Include only table 3 or figures 1-2.

Reply:

In our study we found that low TLR7 CN was significantly associated with chronic HBV infection in males and females ,and the OR value in table 3 makes the results more intuitive,so I appreciate that the editor can keep this table.

(7)Please revise references have been correctly placed. For example, references 2-5, in page 5, lines 1-5, do not correspond to studies supporting the idea that host genetic susceptibility plays an important role in outcome of HBV infection. Also, update reference 1.

Reply:

References and typesetting were corrected

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

Sincerely yours

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