Author's response to reviews

Title: Estrogen Receptor alpha Gene Polymorphisms and Risk of HBV-Related Acute Liver Failure in the Chinese Population

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Author's response to reviews: see over
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Dear Editor:

Thank you very much for your letter and advice. Our manuscript titled “Estrogen Receptor alpha Gene Polymorphisms and Risk of HBV-Related Acute Liver Failure in the Chinese Population (MS: 1489728620639554)” has been revised carefully according to the review comments and editorial notes.

We have highlighted the changes as red color text and made the changes where appropriate in the revised manuscript. Our point-by-point responses to the comments of the two reviewers are listed below this letter. We hope that the revised version of the manuscript is now acceptable for publication on your journal.

Thanks for your attention.

Best regards

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Point-by-point responses to the comments

REVIEWER 1 (Zheng Zeng):

1. How to define the asymptomatic HBV carriers (ASC)? The authors should explain more detail of include and exclude criteria. And also, in their study, most patients were HBeAg negative, why? Did these patients have experience of antiviral treatment or BCP and/or PreCore mutation?

Response: As illustrated in the part of methods in our manuscripts, asymptomatic HBV carriers (ASC) were diagnosed according to the following criteria: (1) lack of any clinical symptoms; (2) normal liver enzyme tests; (3) normal peripheral blood leucocyte (4~10×10^9/L) and platelet (100~400×10^9/L) counts; (4) serum albumin >39 g/L, globulin <35 g/L, and the ratio of albumin to globulin (A/G) >1.5; (5) normal prothrombin time and serum total bilirubin; (6) no abnormal findings on abdominal ultrasound scans; (7) no esophageal varix revealed by electronic gastroscopy. The carriers, who had serologic evidence for coinfection with hepatitis C virus, hepatitis D virus, and human immunodeficiency virus, were excluded in our case-control study. The examinations or tests were performed once every 6 months from February 2001 to December 2007. The carriers, who occurred to the hepatitis symptoms or cirrhosis evidences during the follow-up, were also excluded from the ASC group. The included and excluded criteria of ASC also have been used in our previous reported studies (Yan et al., 2011; Yan et al., 2009; Zhang et al., 2008).

In our case-control study, 405 of 1216 HBV carriers (33.3%) were HBeAg positive, and about two third were HBeAg negative. We think the prevalence of HBeAg negative of our case-control is rational. In recent community-based studies from different parts of the world, the prevalence of HBeAg negativity in chronic HBV infection has been found to range between 70% and 100% (Hadziyannis and Vassilopoulos, 2001). HBeAg positivity is highly prevalent only in younger age groups of HBsAg carriers. In previous studies, only a few countries were found to have more HBeAg-negative than HBeAg-positive CHB, but now it is apparent that there is a worldwide increase in the prevalence of HBeAg-negative CHB (Hadziyannis and Vassilopoulos, 2001). For example, in Italy, 41% of patients with CHB during the period between 1975 and 1985 were HBeAg negative but in the last decade this has increased to 90%. The prevalence of
HBeAg-negative CHB seems to vary geographically. Possible contributing factors for its development include vertical transmission of HBV, long duration of infection and male sex. In China, most patients were infected through vertical transmission and became HBV carriers, which may be one of the important reasons that most patients were HBeAg negative.

In our study, the 857 asymptomatic HBsAg carriers (ASC) had no history to receive any form of antiviral treatment. About 86 patients of 359 HBV carriers affected with ALF have experience of antiviral treatment before enrolling in our study. The frequency of precore mutants among HBsAg (+)/HBeAg (-) individuals varies geographically. The frequencies of BCP and/or PreCore mutation are not available in our study. However, in a recent study from China, 38% of the HBeAg-negative patients harbored the precore stop codon, 42% possessed the double BCP mutation and 12% had both mutations (Chan et al., 1999).

2. In HBV-ALF group, what is/are the cause(s) for HBV-ALF? There are more patients with alcohol consumption in HBV-ALF than that in ASC group. Were the patients confection with other virus? The author should make logistic regression analysis with multiple factors, e.g. age, gender, alcohol, HBeAg, HBV DNA, genotype, haplotype, and so on.

Response: In our HBV-ALF group, HBV infection is the cause for HBV-ALF.

Indeed, there are more patients with alcohol consumption in the HBV-ALF group (33.7%) than that in the ASC group (18.7%). However, we also observed that there are more men in the HBV-ALF group (83.0%) than that in the ASC group (59.5%). Since few women drink in China, the difference in the alcohol consumption status between the ASC and HBV-ALF group may due to gender difference. Furthermore, the daily drinkers are few in China and most of drinkers are occasional drinkers. Then, the alcohol consumption status should not be the cause of the ALF in our HBV-ALF group.

As illustrated in the part of methods in our manuscripts, all carriers in our study had no serologic evidence for coinfection with hepatitis C virus, hepatitis D virus, and human immunodeficiency virus.

We highly agreed the view that making a logistic regression analysis with multiple factors (e.g. age, gender, alcohol, HBeAg, HBV DNA, genotype, haplotype, and so on.) is important to get more rigorous relationship between the ESRI polymorphisms and risk of HBV-ALF. Actually, we
have make the logistic regression analysis with adjustment for age, sex, HBeAg status and alcohol consumption indicated significant differences in the distribution of the three haplotype groups between ASCs and HBV-ALF groups, as showed in Table 3 in our manuscripts. In table 2 of our revised manuscript, odds ratios and their 95% CIs also have been were given for the comparison of the geneotype effect between ASC and HBV-ALF groups by by logistic regression analysis with adjustment for covariates, including age, sex, HBeAg status and alcohol consumption.

3. On table 2, the notes showed ASC compare to HBV-LC group (also in text).
What is the HBV-LC group?

Response: Sorry to confuse the reviewer. The notes of table 2 showed ‘ASC compare to HBV-LC group’ (also in text) were slips of the pen. The corrected expression is ‘the ASC group compare to the HBV-ALF group’. In our revised manuscript, the mistake has been corrected. Thank you for reminding to our stupid mistake.

REVIEWER 2 (Ming-Whei Yu):

1. In studies of western populations, it has been demonstrated that acetaminophen overdose and idiosyncratic drug reactions have replaced viral hepatitis as the most frequent apparent causes of ALF. Thus, the authors should clarify how well they excluded drug-induced ALF cases from the analysis and how well they diagnosed HBV-related ALF in this study.

Response: The etiology of ALF shows worldwide variation, prominent causes include drug-induced liver injury, viral hepatitis, autoimmune liver disease and shock or hypoperfusion. In western countries, drug-induced liver injury is the predominant cause of ALF, acetaminophen overdose and idiosyncratic drug reactions have replaced viral hepatitis as the most frequent apparent causes of ALF. However, ALF is one of the end-staged liver diseases caused mainly by HBV infection in China. The use of acetaminophen and idiosyncratic drug in China is not very popular like that in western countries.

In our study, ALF was defined as liver failure with jaundice, coagulation abnormality (usually an INR ≥1.5), and any degree of mental alteration (encephalopathy) in a patient with an
illness of less than 26 weeks duration (Polson and Lee, 2005). All patients with ALF had no evidence with preexisting cirrhosis. The included and excluded criteria of ALF also have been used in our previous reported study (Yan et al., 2009). Information on acetaminophen and idiosyncratic drug and treatment history of these patients was obtained mainly from clinical records and short telephone interviews when necessary. The carriers, who had history of acetaminophen overdose and idiosyncratic drug reactions, were excluded from the HBV-ALF group. We have clarified this point in our revised manuscripts.

2. Two haplotype-tagging SNPs were chosen for analysis. Are there other tagging SNPs that the authors did not select? If so, the authors should discuss the limitation about capturing incomplete genetic information in the genomic region of interest.

**Response:** We have screened SNPs systematically in the ESR1 gene and two linkage disequilibrium (LD) blocks covering the ESR1 gene were identified (Deng et al., 2004). Two polymorphisms, ESR1 c.30T>C (rs2077647, previously reported T29C, exon 1) and c.453-397T>C (rs2234693, previously reported IVS1 T-401C, intron 1), which were identified as haplotype tagging SNPs. The minor allele frequencies (MAF) of other SNPs in the two LD blocks are less than 5% and were not suitable for association analysis for this common disease.

2. In addition to the two ESR1 SNPs tested, it may be better to add a brief introduction on the studies of other genetic polymorphisms and HBV-related ALF.

**Response:** Thanks for your suggestion. We have added a brief introduction on the studies of other genetic polymorphisms and HBV-related ALF in part of the background in our manuscript.

3. It is reasonable to speculate that the effects of the two ESR1 SNPs on the occurrence of ALF may be different between postmenopausal and premenopausal women. Thus, distribution of selected characteristics in Table 1 should be presented according to sex and disease status. Furthermore, in women the SNP-ALF association should be re-analyzed by menopausal status.
Response: Actually, distribution of selected characteristics in Table 1 has been presented according to sex and disease status in our manuscripts. To decrease the bias of age and sex on the effect of the estimate, we conducted stratification analysis for age and sex in total 1216 case and control individuals (Table 2 in our manuscript). Interestingly the association between the two ESR1 polymorphisms (c.453-397T>C and c.30T>C) and HBV-ALF remained significant in both sex and patients at any age (with an age ≥ 40 years or < 40 years).

We highly agreed the reviewer’s view that effects of the two ESR1 SNPs on the occurrence of ALF may be different between postmenopausal and premenopausal women. Of course, to further estimate the effects of the two ESR1 SNPs on the occurrence of ALF between postmenopausal and premenopausal women, it will be better to conduct a stratification analysis for menopausal status (age) in total HBV-ALF women. However, only 61 of 359 cases (17.1%) were women in our HBV-ALF group. Additionally, among the 61 woman with HBV-ALF, only 27 were Age ≥ 40 y (potential postmenopausal women) and 34 were Age <40 y (premenopausal women). Then, if we conduct a stratification analysis for menopausal status (age) in total HBV-ALF women, the number of subgroup women (potential postmenopausal women subgroup vs. premenopausal women subgroup) in the HBV-ALF group is too small to gain enough statistical power which may lead to the confused or even incorrectly results. Therefore, we did not show the results of SNP-ALF association stratified analysis by menopausal status in women.

We have added some sentences in the DISCUSSION part in our manuscript to emphasize this view. The added sentences are: ‘Furthermore, it is reasonable to speculate that the effects of the two SNPs on the occurrence of ALF may be different between postmenopausal and premenopausal women. Then, it will be better to conduct a stratification analysis for menopausal status in total HBV-ALF women. However, the number of potential postmenopausal women (N=27) and premenopausal women (N=34) in the HBV-ALF group is too small to gain enough statistical. In the following study, the expanded numbers of women patients with HBV-ALF will be help clarify the effects of the two SNPs on the occurrence of ALF between postmenopausal and premenopausal women.’

4. Table 2. Odds ratios and their 95% CIs should be added for the genotype effect.

Response: Thanks for your suggestion. In table 2 of our revised manuscript, odds ratios and their
95% CIs have been given for the comparison of the genotype effect between ASC and HBV-ALF groups by logistic regression analysis with adjustment for covariates, including age, sex, HBeAg status and alcohol consumption. We also depicted this data in the RESULT parts of our revised manuscript.

**Reference**


