Author’s response to reviews

Title: Association between KIF1B rs17401966 genetic polymorphism and hepatocellular carcinoma susceptibility: an updated meta-analysis

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Dear Editor,

Thank you very much for your attention and the referee’s evaluation and comments on our paper MGTC-D-18-00016 and advice on our manuscript. We have revised the manuscript according to your kind advices and referee’s detailed suggestions. Enclosed please find the responses to the referees. We sincerely hope this manuscript will be finally acceptable to be published on BMC Medical Genetics. Thank you very much for all your help and looking forward to hearing from you soon.

With best wishes,

Sincerely yours

Dr Ying-ying Luo
Below, please find the comments in black, followed by our responses in red. Exact changes in the manuscript are also presented in red font.

Reviewer 1

1. The reviewer’s comment: There is a previous study that has meta-analyzed the association between rs17401966 and HCC risk (Su M. et al, Clin Mol, Hepatol, 2017). The studies included in the present meta-analysis are roughly the same. This article includes also a stratified analysis like the one performed in the present manuscript. Surprisingly, this previous work of reference is not cited neither in the introduction nor in the discussion. Comparing the results of the present study with meta-analyses previously performed for HCC risk is essential to contextualize the genetic association findings.

The authors’ answer: Thank you for pointing this out. Su M. and his research groups have conducted a meta-analysis of the association between rs17401966 and hepatitis B virus-related (HBV-related) hepatocellular carcinoma (Su M. et al, Clin Mol, Hepatol, 2017). However, the focus of our work is on the association between rs17401966 and hepatocellular carcinoma, which is very different from Su M. ’s work. We focus on the association between rs17401966 and HCC, but not on the association between rs17401966 and HBV-related HCC. As well known, Chinese contribute the largest number to HBV and HBV-related HCC. However, not every HCC patient from China is because of HBV infection. Specially, the search key words and the number of studies included in those two meta-analyses are quite different. It can be proved through the almost same ranges of years, as we searched databases up to June 21 while Su M. ’groups searched databases from January 2010 to April 2016 (Su M. et al, Clin Mol, Hepatol, 2017), and the quite different number of our total studies included. In total, 18 independent case-control studies were included in our study while 12 independent case-control studies were included in Su M. ’study. However, as well known, HBV contributes the biggest to the progress of HCC among all viruses which could intervene the progress of HCC, then we performed stratification analysis by HBV status.

We have added the following discussion.

Although two meta-analyses on the associations between KIF1B rs17401966 polymorphism and hepatocellular carcinoma and one meta-analyses on the associations between KIF1B rs17401966 polymorphism and HBV-related hepatocellular carcinoma have been reported in the last five years. In 2017, Su et al conducted a meta-analysis, with a total of 5 studies containing 12 cohorts
with 4,886 HCC cases and 5,442 controls. Su et al were not able to verify the association between the KIF1B rs1740199 polymorphism and HCC risk. We conducted the present analysis because the conclusions of those studies were controversial (because of different criteria for inclusion of data, different original studies, different stratified facators and articles written in English only).

2. The reviewer’s comment: The stratified meta-analysis is performed on two subgroups of HCC patients, including HBV-positive and Chinese patients. Importantly, it has been previously reported that chronic HBV infection contributes to ≥80% of HCC patients from China (Chen CJ et al, J Gastroenterol Hepatol, 1997). The two stratified analyses here performed are therefore likely to be redundant. Knowing the number of HBV-positive patients from each study included in the meta-analysis would help to rule out this possibility. This could also help to discard that HBV-patients included in the meta-analysis are taken from only one or a very few studies (in such a case, the concept meta-analysis would be strongly compromised). However, these data are hidden in Table 1.

The authors’answer: Thank you for pointing this out. As well known, Chinese contribute the largest number to HBV and HBV-related HCC. However, not every HCC patient from China is because of HBV infection. When the theory from HBV-related HCC was extended to all HCC should be further established using more valid evidences, which we try to do.

3. The reviewer’s comment: In addition to the previous meta-analysis, there is a GWAS that has already found a strong association between the candidate SNP and the risk of developing HCC (P=1.7e-18, Zhang H et al., Nat Genet, 2010). Therefore, the interest and novelty of the present work is quite doubtful.

The authors’answer: Thank you for pointing this out. Hongxing Zhang and his research groups performed a GWAS with 355 chronic HBV carriers with HCC and 360 chronic HBV carriers without HCC, all of Chinese ancestry. The results showed that 1p36.22 locus confers susceptibility to HBV-related HCC. 1p36.22 locus contains KIF1B, phosphogluconate dehydrogenase (PGD) and the 3’ terminal end of the ubiquitination factor E4B (UBE4B) genes. Then Zhang H et al further performed immunohistochemical analysis to determine the protein expression of the KIF1B, PGD and UBE4B genes in the HCC tissues and the corresponding paracarcinoma tissues, and performed quantitative RT-PCR to prove that transcription of KIF1B β was markedly elevated in G allele carriers (P = 3.7 × 10−4, Zhang H et al., Nat Genet, 2010).
So, on the one hand, Zhang H et al’s GWAS research was limited to Chinese, when our meta-analysis included some other ethnicity. On the other hand, Zhang H et al’s GWAS research did not proved their theory by gene silencing or gene knock-out. So their theory should be further established by further valid evidences. We are trying to do that.

4. The reviewer’s comment: In the methods section, it should be written down the range of years used as a threshold to select the studies for meta-analysis. In such a case, the similarity between the present study and the previous meta-analysis would be clearly shown (Su M. et al, Clin Mol, Hepatol, 2017).

The authors’answer: Thank you for pointing this out. We have written down the range of years used. We focus on the association between rs17401966 and HCC, while Su M’s groups focus on the association between rs17401966 and HBV-related HCC. Therefore, ranges of years are almost the same, as we searched databases up to June 21, 2016 while Su M. ‘groups searched databases from January 2010 to April 2016, but total numbers of the total studies included are quite different. In total, 18 independent case-control studies were included in our study while 12 independent case-control studies were included in Su M. ‘study.

5. The reviewer’s comment: In the discussion section, the authors state that the present analysis has been performed because the conclusions of the previous studies are controversial. In my opinion, it would be more informative for the reader if a more detailed explanation of the controversies was described. The addition of this explanation is also necessary to visualize both the novelty and interest of the present meta-analysis.

The authors’answer: Thank you for pointing this out. We have added the following discussion.

Although two meta-analyses on the associations between KIF1B rs17401966 polymorphism and hepatocellular carcinoma and one meta-analyses on the associations between KIF1B rs17401966 polymorphism and HBV-related hepatocellular carcinoma have been reported in the last five years. In 2013, Wang et al performed a meta-analysis, with a total of 5 studies containing 13 cohorts with 5,773 cases and 6,404 controls, under the allele model (G vs. A), the co-dominant models (GG vs. AA; GG vs. AG and AG vs. AA), the dominant model(GG+AG vs. AA), and recessive model (GG vs. AG+AA), which suggests the presence of the G allele at rs17401966 of the KIF1B gene may decrease the risk for HCC. In 2014, Zhang et al performed a meta-analysis, with a total of 15 case-control studies with 7,596 HCC cases and 9,614 controls. And a significant association between KIF1B rs17401966 and HCC risk was detected (OR=0.81, 95 %
CI 0.72 – 0.91, P < 0.001). In 2017, Su et al conducted a meta-analysis, with a total of 5 studies containing 12 cohorts with 4,886 HCC cases and 5,442 controls. Su et al were not able to verify the association between the KIF1B rs1740199 polymorphism and HCC risk. We conducted the present analysis because the conclusions of those studies were controversial (because of different criteria for inclusion of data, different original studies, different stratified factors and articles written in English only).

6. The reviewer’s comment: The present work can have an added value. The meta-analysis stratified by ethnicity could be further performed on Japanese patients. The results of this analysis could be contrasted to the findings obtained in Chinese patients and, hopefully, it could provide new insights into the ethnic-specific associations of the candidate SNP with the risk of developing HCC.

The authors’ answer: Thank you for pointing this out. We have conducted a meta-analysis stratified by ethnicity, that is to say by Japanese and non-Japanese. The results are in the figure below. No statistical differences were found under any models in the Japanese subgroup.

Reviewer 2

1. The reviewer’s comment: In the abstract: HBV ? : not indicated in letter
Key words: you have to write liver cancer but this term is not mentioned in the abstract text

The authors’ answer: Thank you for pointing this out. We have added explanation for the letter “HBV” in the abstract section.

2. The reviewer’s comment: In the background: you indicated that "HCC is a complex process, associated with many factors and co-factors, including genetic predisposition, environmental factors, and viruses" then in the results you mentioned two groups of hepatitis B virus (HBV). Therefore it is necessary to explain more how HBV intervenes in HCC?
The authors’ answer: Thank you for pointing this out. We have added explanation for the interaction between HBV and the progress of HCC in the background section.

3. The reviewer’s comment: Characteristics of included studies should be in the methods and not in the results

The authors’ answer: Thank you for pointing this out. We have moved the Characteristics of included studies to the methods section.

4. The reviewer’s comment: In the Inclusion and exclusion criteria: indicate in addition to solid evidence for HCC, HBV as an HCC subgroup criterion.

The authors’ answer: Thank you for pointing this out. We have added this criterion.

5. The reviewer’s comment: In the results: Meta-analysis

"As shown in Figure 1, a significant allelic association was recorded under a random-effect allelic model, with OR=0.85 (95% CI 0.76-0.94, P=0.003)" but in figure 1: P=0,000

"Similar results were found under the co-dominant genotype models GG vs AA (OR=0.72, 95% CI 0.52-0.99, P=0.044) (Figure 2)" but in figure 2: P=0,000

"and AG vs AA (OR=0.81, 95% CI 0.75-0.87, P<0.001) (Figure 3)". but in Figure 3 : p= 0,016

The authors’ answer: Thank you for pointing this out. We didn't mean these two “p” were the same. P following the 95% CI in the paper refers to the statistical significance of the pooled OR, while P following the I2 in the figures refers to the statistical significance of heterogeneity assay.

6. The reviewer’s comment: In the results: in addition, in the figure 1-5 you did not indicate the test used
The authors’ answer: Thank you for your detailed suggestion. The statistical significance of the pooled OR was determined by the Z test; \( P<0.05 \) was considered statistically significant. All \( p \) values were measured from two-tailed tests of statistical significance with a type I error rate of 5%. We have emphasized it again in the result section based on the reviewer’s comment.

7. The reviewer’s comment: In the results: I do not understand the total case-control of G allele vs A, GG vs AA, AG vs AA and GG vs AG in table 3, 4 and 5 which say that in total, 18,893 participants were selected (8427 HCC boxes and 10,466 controls).

The authors’ answer: Thank you for pointing this out. As we wrote in the manuscript, 10 studies containing 18 independent case-control studies were included. Therefore, 8427 HCC cases and 10,466 controls were included in all. That is to say, 18,893 participants were selected. When the analysis was conducted under the allelic model (G-allele vs A-allele), G-allele contained in GG counted two, and G-allele contained in AG counted one. Then the sum was double in number.

8. The reviewer’s comment: In the results: you mentioned in the results that "Finally, 10 studies [7-16], containing 18 independent case-control studies, based on the inclusion and exclusion criteria, were included." but in the figures 2,3 and 5 containing 15 independent case-control studies.

The authors’ answer: Thank you for pointing this out. Of the 18 independent case-control studies, 3 studies were excluded because of no further data when the meta-analysis was conducted under the co-dominant models (GG vs AA, GG vs AG, AG vs AA).

9. The reviewer’s comment: In additional statistical : you can add a comparison by gender.

The authors’ answer: Thank you for pointing this out. This is a very good idea. It is well known that males are more than females in hepatocellular carcinoma. Unfortunately, not all of the 10 studies included contained detailed information on the effect of rs17401966 on hepatocellular carcinoma stratified by gender. I hope I would do this in the near future.
Finally, we appreciate very much for your time in editing our manuscript and the referees for their valuable suggestions and comments. I am looking forward to hearing from your final decision when it is made.