Author's response to reviews

Title: Association between HLA-DRB1 alleles polymorphism and hepatocellular carcinoma: A Meta-analysis

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Author's response to reviews: see over
Dear Miss Sonia Aguera,

Thank you very much for your comments and suggestions.

We have revised the manuscript according to the comments and suggestions of reviewers and editor, and responded point by point to the comments as listed below. And we have highlighted the amendments in the revised manuscript.

I would like to re-submit this revised manuscript to BMC Gastroenterology, and hope it will be acceptable for publication in the journal.

Looking forward to hearing from you soon.

With kindest regards,
Yours Sincerely
Shi-Ying Xuan (Corresponding author)
Zhong-Hua Lin (First author)

Replies to the reviewer’s suggestions:

Minor Essential Revisions

1. - Methods section, Search Strategy:
   Specify if terms in addition to HLA such as MHC or HLA-DRB1 were used to search for the studies.

   **Answers:** Thank you very much for the suggestion. This section has been revised to: The search strategy was based on combinations of the terms: HLA-DRB1 AND (Hepatocellular carcinoma or HCC) AND (variants or polymorphism or alleles).

   (Page 4)
2. - Methods section, inclusive selection criteria:
Specify detailed study characteristics including the criteria that the diagnosis of patients with HCC was based on and give the age range of patients that were included. Move the information that is in the results section (Page 7) “The diagnosis of HCC was based on at least one of the following criteria: classical histological characteristics or a serum a-fetoprotein (AFP) level higher than 500µg/ml together with radiological findings (ultrasound and/or CT) consistent with HCC” to the inclusive selection criteria part of the methods section.
In addition, specify if the criteria for HCC diagnosis are author based or if they are based on specific reported guidelines. If so, include the specific reference.

**Answers:**
I have followed your suggestion to move this information to the inclusive selection criteria part of the methods section, and added the reference.
This section has been revised to:
(5) inclusion of patients according to the diagnosis standard of HCC defined in 2002, based on at least one of the following criteria: classical histological characteristics or serum a-fetoprotein (AFP) level higher than 400ng/ml together with radiological findings (ultrasound and/or CT) consistent with HCC [6]. A single study, Donaldson et al, done before 2002, was included in the meta-analysis given that the inclusion criteria of patients were similar to the diagnosis standard.


(Page 5)

3. - Statistical analysis, first paragraph:
State if I² was used as an additional method for heterogeneity because it is shown in the author’s attached forest plots. Furthermore, it is important to state if Tau squared was used to evaluate heterogeneity because it is also reported on the forest plots.
Answers:
This section has been revised to:
Heterogeneity was calculated by means of Cochran's Q test ($\alpha=0.05$) and Higgins's ($I^2$) tests. $I^2$ values of 25%, 50% and 75% were assigned as low, moderate, and high estimates, respectively.

(Page 6)
In our meta-analysis, we did not take Tau squared as an index to evaluate heterogeneity.
The corresponding sections of the results are also adjusted accordingly.

4. - Statistical analysis:
Clarify if the crude number of patients and controls was used to calculate ORs and if specific allele frequencies were not used. Indicate what the reason was for not doing it. Specify that heterozygosity and homozygosity (allelic dosage) were not taken into account for these calculations.

Answers:
I have added this information to the method sections. Shown as follows:
Allelic frequency was calculated as the number of cases or controls harboring at least one allele type (HLA-DRB1) divided by the total number of chromosomes included in each of the corresponding groups.

(Page 6)

5. - Results section, Association between HLA-DRB1 alleles with HCC:
Clarify if the results by random effect model were the same as the results from the fixed effect model in the article in the case of HLA-DRB1*07 where $I^2$ shows some heterogeneity other than the Q statistics results (54%).

Answers:
This section has been revised to:
The heterogeneity test indicates that the variation of trial-specific ORs was not statistically significant ($\chi^2=10.79$, $I^2=54\%$, $P=0.06$ and $>0.05$). Under the fixed
effect model, the combined OR for the association of HLA-DRB1*07 allele with the risk for HCC in the whole populations was determined to be 1.65 (95% CI: 1.08-2.51; p=0.02), and under the random effects model was 1.77 (95%CI: 0.88-3.56; p=0.11).

(Page 8)

6. - results section, last paragraph:
Specify that fixed effect model was used for the results reported for HLA-DRB1*15 in Asians.

Answers:
This section has been revised to:

(Page 8) Results section:
Subgroup analysis by ethnicity showed that HLA-DRB1*15 allele significantly increased the risk of hepatocellular carcinoma in Asians under the fixed effect model (OR=2.88, 95% CI: 1.77-4.69, P < 0.0001).

(Page 13) Conclusion-Last paragraph:
DRB1*15 allele is only associated with an increased risk of HCC in Asians (under the fixed effect model).

Major Compulsory Revisions
1. - Methods section, selection criteria:
As stated in the author guidelines, authors are requested to make use of the PRISMA checklist (http://www.prisma-statement.org/) when reporting systematic review and meta-analysis. Authors have to refer to this in the methods section and be sure they fit the minimum set of items for reporting in systematic reviews and meta-analyses included there.

Answers:
I have added PRISMA checklist to the methods section. Shown as follows:
The literature review conformed to PRISMA statement standards, and our research fit the minimum set of items for reporting in systematic reviews and
2. Discussion section:

It is important that the authors explain why the DRB1*07 and DRB1*12 could be of interest in HCC. Do these two groups/alleles share some pocket specificities? Are these groups/alleles described in association with specific viral hepatic infections? Do these groups/alleles have a similar preference for specific types of amino acids? Do the authors have a specific hypothesis regarding why these groups/alleles confer susceptibility to HCC? All of the information in the discussion section is of essential importance for understanding functional and biological aspects of HCC and HLA.

Answers:

Thank you very much for this suggestions and I have added a paragraph to the discussion section to clarify the possible mechanism. Shown as follows:

Epidemiological survey showed that Asian countries account for nearly 78% of hepatocellular carcinoma (HCC) reported globally each year, and Hepatitis B Virus (HBV) is the major etiology of HCC in these areas. Although HBV infection plays an important role in HCC, HBV infection alone is not sufficient for progression to HCC. Several lines of evidence suggest that cellular immune surveillance is important in the control of HBV infection and the development of HCC. In 2007, Yang and his colleagues found that HLA-DRB1*07 were markedly higher in the HBV-infected group among people in northwestern China (17.6% of HBV-infected patients vs 9.3% of spontaneously cleared controls, OR = 2.09, P < 0.05) [21]. In 2006, Zhang and his colleagues found that the frequency of HLA-DRB1*12 was significantly higher in the HBV persistent group than in the recovered group among Chinese (0.230 versus 0.063, P = 0.004, OR = 2.09) [22]. In 2003, Amarapurpar and his colleagues found that a positive association of HLA-DRB1*15 to persistence of HBV among Indians (57.6 vs. 25%) [23]. As we know that clearance of acute...
hepatitis B virus (HBV) infection is associated with a vigorous CD4+ T-cell response focusing on the core protein. HLA class II glycoproteins present viral peptides to CD4+ T cells and influence the immune responses. Binding affinities of overlapping peptides covering the core and envelope proteins of HBV were measured to HLA glycoproteins encoded by some HLA-DRB1 molecules and compared with published peptide-specific CD4+ T-cell responses [24]. So we have a hypothesis that HLA-DRB1*07, DRB1*12 and DRB1*15 alleles may be the key host factors to determine the development of diseases from HBV infection to HCC in Asians, basing on our results that HLA-DRB1*07, DRB1*12 and DRB1*15 alleles significantly increased the risk of hepatocellular carcinoma in Asians. Similar Furthermore, the importance of environmental factors and gene-environmental interactions in the development of HCC should not be ignored and is beginning to be delineated.


