Author's response to reviews

Title: The association of HLA-DQB1, -DQA1 and -DPB1 alleles with anti-GBM disease in Chinese patients

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

Re: MS: 4979173634920508

Title: The association of HLA-DQB1, -DQA1 and -DPB1 alleles with anti-glomerular basement membrane (GBM) disease in Chinese patients

BMC Nephrology

Thank you for giving us an opportunity to revise our above manuscript. We have revised the manuscript carefully according to the Editor’s and Reviewers’ comments and recommendation. The revision was underlined in the text according your requirement. Briefly speaking, the revision included the following issues:

1. The aim of the study has been clarified in the introduction section.

2. The reason to choose HLA-DQB1, -DQA1 and -DPB1 alleles had been stated.

3. The combination analysis including DRB1 and discussion of the potential relation between DP and DR has been added.

4. SKDM software has been employed to analyze the data.

The response to the each question or comment was listed point-by-point in the attached pages. We hope the revised form will be suitable for publication in your journal.

Thank you for your consideration.

Yours sincerely,

Min Chen
Answer to Reviewer 1

Major Compulsory Revisions:

1) *The Authors should clarify the aim of the study.*

**Answer:** Yes, the aim of the study has been clarified in the introduction section according to the recommendation.

2) *How did the onset of anti-GBM is defined? This information should be reported in Material and Methods section.*

**Answer:** The onset of anti-GBM disease was defined as renal or extra-renal signs and symptoms of anti-GBM disease, or abnormalities related to anti-GBM disease were detected by various examinations, including hemoptysis, oliguria or anuria, proteinuria, hematuria and elevated serum creatinine, etc. It has been added in Material and Methods section according to the recommendation.

3) *In this study were typed: 11 HLA-DQB1 alleles, 9 HLA-DQA1 alleles and 38 HLA-DPB1 alleles. Why did the Authors choose these alleles?*

**Answer:** The current study aimed to analyze the distribution of HLA class II alleles in patients with anti-GBM disease and investigate their potential associations with clinical and pathological parameters. For HLA class II loci, HLA-DRB1 and -DPB1 encode relatively more variable gene products for HLA-DR and -DP molecules.
respectively, while both HLA-DQB1 and DQA1 are variable in human population.

Besides, previous studies have located some HLA-DRB1, -DQB1 and –DPB1 alleles with association with anti-GBM disease in Caucasian as well as Asian population [Huey B, et al. Kidney Int. 1993;44:307-12; Fisher M, et al. Kidney Int. 1997;51:222-9]. But in Chinese patients, few studies have been done for this topic. Therefore we choose to type HLA-DQB1, -DQA1 and -DPB1 loci in this study, on the basis of our previous study on HLA-DRB1 [Yang R, et al. Clin Immunol. 2009;133: 245-50]. The reason for choosing these alleles has been added in the text according to the recommendation.

4) The Authors reported: “no significant difference of gender, age, level of anti-GBM autoantibodies, serum creatinine, other clinical manifestations and pathological parameters were found between anti-GBM patients with and without HLA-DPB1*0401”. In Table 2, they reported that hemoptysis is statistically different between DPB1* 0401 positive and DPB1*0401 negative (p=0.042). Could they explain this incongruity?

Answer: I apologize for this incongruity. The different proportion of patients having hemoptysis between DPB1*0401-positive and DPB1*0401-negative patients has been added in the text according to the recommendation.
5) Are data normally distributed?

**Answer:** The data of anti-GBM antibodies level, interval between onset and diagnosis and the level of Scr were normally distributed, while the data of patients’ age and percentage of crescents in glomeruli were non-normally distributed. Therefore, those non-normally distributed data were expressed as “median (1st and 3rd quartile)”.

6) The Authors reported: “the association of anti-GBM disease and other HLA class II genes, including HLA-DQB1, -DQA1,-DPB1 alleles, has rarely been investigated in Asian, especially Chinese patients”. They should introduce in the text the references of these articles as if they are “rare” and the results should be discussed, in “Discussion” section.

**Answer:** I have added the references [4, 5] in the text according to the recommendation. Moreover, in the “Discussion” section, I have discussed their results.

Minor Essential Revisions:

1) The last sentence of the “Introduction” section seems to be incomplete.

**Answer:** It has been revised in the text according to the recommendation.

2) In the title “anti-GBM” should be written “anti-glomerular basement membrane"
(GBM)”

**Answer:** Yes, it has been revised according to the recommendation.

3) In the “Background” of Abstract section the Authors should add “genes” to the end of last sentence.

**Answer:** Yes, it has been revised according to the recommendation.

4) In the “Results” of Abstract section the Authors should complete the sentence.

**Answer:** Yes, it has been revised according to the recommendation.

5) In “Results’ section, the Authors reported that the mean age of diagnosis in patients was 27, but they should add the standard deviation.

**Answer:** Thank you for the recommendation. Since the data of age was not normally distributed (tested by Kolmogorov-Smirnov, p=0.029), mean± standard deviation was not a good way to describe them. Therefore, I reported the median and 1st and 3rd quartile instead of the mean± standard deviation.

6) In Table 2 the mean± standard deviation of the parameters should be reported.

**Answer:** As described above, tested by Kolmogorov-Smirnov analysis, I found the data of anti-GBM antibodies level, interval between onset and diagnosis and the level
of Scr were normally distributed, while the data of patients’ age and percentage of crescents in glomeruli were more likely skewed distributed. So I only described the former three variables in Table 2 by mean ± standard deviation form, and described the other three variables by median and 1st and 3rd quartile.

7) They should specify Scr in Table 2.

**Answer:** Yes, it has been revised in Table 2 according to the recommendation.

8) The numbers at the beginning of the sentence should be written in letters.

**Answer:** Yes, it has been revised according to the recommendation.

9) In the “Introduction” section the following sentence should be deleted: “HLA class I molecules present peptides to CD8-positive cytotoxic T cells, while HLA class II molecules are responsible for presentation of antigenic peptides to CD4-positive T-cells”.

**Answer:** Yes, it has been deleted according to the recommendation.
Answer to Reviewer 2

Major Compulsory Revisions:

1) It appears that this population is the same population studied when they previously reported the DRB1 association. If indeed it is so, the recent work needs to be presented in the context of these findings as well. The authors need to evaluate whether individuals characterized by the combined presence of DRB1*1501 and absence of DPB1*0401 have an even higher risk, whether there are any individuals with both alleles present, discuss these findings depending on the genotypes of the different individuals and emphasize the DP, DR relationship.

Furthermore the subjects of this study have been typed at the high resolution level and it would be worth it to examine the association not only at the allele level but also at the amino acid level. There are software programs that can facilitate this analysis; see (Kanterakis et al SKDM HLA Tool: A Comprehensive HLA and Disease Associations Analysis Software. Human Immunol. 69: 522-525, 2008). That may reveal individual residues of importance rather than individual alleles.

Answer: Yes, this population is the same population studied when we previously reported the DRB1 association. I have added the combination analysis including DRB1 and discuss the potential relation between DP and DR in anti-GBM disease. Besides I have used software program SKDM to analyze my data and added its result and related discussion in the article.
2) The authors in the Results section mention that in their population of 244 individuals they have identified 9 DQB1, 9 DQA1 and 34 DPB1 alleles. However in Table 1 they only report the frequency of only a limited subset of alleles for each of these loci. Why? They should prepare a table for each of the loci with all of the alleles identified in their population. A somehow related point. The p corrected value for the DPB1*0401 is multiplied by a factor that is not 34 as the number of reported DPB1 alleles. Why?

Answer: I didn’t list all alleles because the table would be very large. So I only selected some alleles with significance or once reported significant. But in the revised form, I have added all the alleles we have typed in Table 1 according to the recommendation.

I didn’t multiply the correction factor as 34 because by definition of Bonferroni correction, we should multiply the number of all the alleles we have typed. Because I added the DRB1 result in this revision, according to your above suggestion, the number now should be 36+9+9+34=88.