Author’s response to reviews

**Title:** Meta-analysis of the association of HLA-DRB1 with rheumatoid arthritis in Chinese populations

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Version: 10 Date: 25 July 2013

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Meta-analysis of the association of HLA-DRB1 with rheumatoid arthritis in Chinese populations” (MS: 4931252299603615). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are highlighted in the paper. The main corrections in the paper and the responds to the reviewer’s comments are as flowing:

Responds to the reviewer’s comments:

Reviewer #1: Young Ho Lee

1. Response to comment: (I do not understand the words, VEGF and UM in the result.)
Response: We are very sorry for our incorrect writing “Finally, 22 studies were performed on the association of VEGF expression between UM and normal people.” We have re-written this sentence to “Finally, 22 studies that examined the association between HLA-DRB1 and rheumatoid arthritis in Chinese populations were included and analyzed.” Thanks so much for pointing out our mistake.

2. Response to comment: (Authors need to show forest plots of meta-analysis results, and present funnel plot to test publication bias.)

Response: It is really true as reviewer suggested that we should show forest plots of meta-analysis results and present funnel plot to test publication bias. We add figures of Meta-analysis of the association of HLA-DRB1*04 with rheumatoid arthritis in Chinese populations (Fig.2), Meta-analysis of the association of HLA-DRB1*0405 with rheumatoid arthritis in Chinese populations (Fig.3), Meta-analysis of the differences in erythrocyte sedimentation rate (ESR) between DR4+ and DR4- in RA patients (Fig.4), Meta-analysis of the differences in C-reactive protein (CRP) between DR4+ and DR4- in RA patients (Fig.5), Meta-analysis of the differences of X-ray phases between DR4+ and DR4- in rheumatoid arthritis (RA) patients (Fig.6) and Funnel plot of the association of HLA-DRB1*0405 with rheumatoid arthritis in Chinese populations (Fig.7) to our manuscript. Sincerely thank you for your good comments and valuable suggestions.

Reviewer #2: Xu LIU

Major points:

1. Response to comment: (The way to distinguish northern from southern Chinese is complicated (Xu S et al. Am J Hum Genet 85: 762–774). If the author
wants to do the subgroup analysis, it should be clearly described that how to definite northern and southern Chinese in the materials and methods section.)

Response: It is really true as Reviewer suggested that the way to distinguish northern from southern Chinese should be clearly described. I read the paper by Shuhua Xu et al (Xu S et al. Am J Hum Genet 85: 762–774). We distinguish the geographical populations into northern and southern Chinese with the Yangtze River serving as a geographical boundary. The sentence “The populations studied were classified into the northern Chinese and southern Chinese groups due to significant geographic variation among the populations in China. The Yangtze River was used as the geographical boundary between these groups.” was added to our manuscript.

Due to some reasons, we did not do subgroup analysis according all the method to distinguish Chinese population, which may lead to an inflated rate of false-positive results. Our result and conclusion was just applied to the method to distinguish them with the Yangtze River serving as a geographical boundary. This is the limitation of our study. Or if the reviewer think the distinction do not make much sense, we can cancel it. Sincerely thank you for your good comments and valuable suggestions.

2. Response to comment: (The statistics used in this study is not well described in materials and methods section. How were OR and MD calculated? Dose it calculated by chi-square test or T test?)

Response: We are very sorry for our negligence of describing the statistics used in this study. In the included studies, some of them report the OR and MD in their manuscript, however some not. So we input the original number of positive events
and number of total events \( (n/N) \) of case and control group to the software Review Manager (Revman), the software will calculated the OR and MD. As to OR, Revman calculated the pooled OR by chi-square test. As to MD, Revman calculated the pooled MD by T test. We add the sentence “Dichotomous data were reported as odds ratios (ORs; chi-square test). Continuous data were reported as mean difference (MD)± standard deviation (SD; T test).” to our manuscript. Thanks so much!

3. Response to comment: (Because there may be some influence of non-genetic factors in disease activity, the authors should describe and balance these characteristics (gender, age, medication and disease duration etc.) to avoid bias in table 3. Otherwise, ESR, CRP, Duration of morning stiffness, number of swollen joints and number of tenderness joint should be removed.)

Response: It is really true that we should describe and balance these characteristics (gender, age and disease duration etc.). We do chi-square test or T test to evaluate the balance of average age, gender and disease duration. We performe the combination if \( P > 0.05 \), making sure that the baseline of included studies are balanced. Characteristics of clinical and laboratory parameters between the DR4+ and DR4- in RA patients are in Table 1. We contacted the original authors to get data on medication. There were not obvious imbalance of the baseline for them of the data. The sentence “If there was not an obvious imbalance in the data, the Chi-square test or the T test was used to evaluate whether average age, gender, and disease duration were balanced between the included studies. We contacted the original authors to obtain data on treatments and on the sensitivity of the X-ray scale. There were no
obvious imbalances in the baseline for these data.” was added to our manuscript. Due to the restriction of the space of table 3, we did not include the gender, age and disease duration in it. Maybe we can build another table if it is necessary for our manuscript. And we can remove ESR, CRP, Duration of morning stiffness, number of swollen joints and number of tenderness joints if there is still a suggestion.

Minor point:

1. Response to comment: (Introduction paragraph 2, the definition of SE is not suitable. Only HLA-DRB1 alleles encode (70Q(R)K(R)RAA74) termed the shared epitope (SE).)

   Response: We have made correction according to the Reviewer’s comments. We have change it to “HLA-DRB1 alleles encode (70Q(R)K(R)RAA74) encoding the shared epitope (SE) (RAA amino acid pattern in positions 72 to 74 of the third hypervariable region of the DRβ1 chain) are associated with RA susceptibility[4]. SE contains HLA-DRB1 alleles representing significant genetic risk factor for RA.”

### Table 1: Characteristics of clinical and laboratory parameters between the DR*+ and DR* in RA patients

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Eligible studies</th>
<th>p value (Chi-square test or T test)</th>
<th>Baseline comparability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gender</td>
<td>Average age</td>
</tr>
<tr>
<td>ESR</td>
<td>10</td>
<td>0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>MHC</td>
<td>7</td>
<td>0.06</td>
<td>0.14</td>
</tr>
<tr>
<td>CRP</td>
<td>5</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>MHC</td>
<td>4</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>2</td>
<td>0.17</td>
<td>0.13</td>
</tr>
<tr>
<td>Duration of morning stiffness</td>
<td>5</td>
<td>0.01</td>
<td>0.15</td>
</tr>
<tr>
<td>Number of swollen joints</td>
<td>7</td>
<td>0.34</td>
<td>0.07</td>
</tr>
<tr>
<td>Number of joint tenderness</td>
<td>6</td>
<td>0.14</td>
<td>0.96</td>
</tr>
<tr>
<td>X-ray phases</td>
<td>8</td>
<td>0.21</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>1 to 2</td>
<td>3</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>3 to 4</td>
<td>5</td>
<td>0.19</td>
</tr>
<tr>
<td>Joint function</td>
<td>8</td>
<td>0.21</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>1 to 2</td>
<td>3</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>3 to 4</td>
<td>5</td>
<td>0.34</td>
</tr>
</tbody>
</table>
Thanks so much for pointing out our mistake.

2. Response to comment: (The manuscript is not written in standard English and needed to be polished by native speaker.)

(1) Introduction, line 3: Older medical literature suggests? Previous literature suggests

(2) Materials and Methods, line 26: if there were still have any disagreement? if there were any disagreement

(3) Results, line 16: DRB10405? DRB1*0405

(4) Results, line 18: The summary ORs showed that? The ORs showed that

(5) Results, subtitle 2: Correlation of clinical and laboratory parameters between the DR4+ and DR4- in RA patients? Differences of clinical and laboratory parameters between the DR4+ and DR4- in RA patients.)

Response: We are so sorry for our limited English level. First we have made correction according to the Reviewer’s comments. Second, we have the language in our manuscript edited by a native-English speaker with scientific expertise of Edanz in Beijing.

3. Response to comment: (In table2 and 3, what does n/N mean?)

Response: In table2 and 3 of our manuscript, ‘n’ means number of positive events, ‘N’ means number of total events, so n/N means number of positive events/number of total events. We are so sorry for not stating this clearly. Thanks so much for pointing out our limitation.

Reviewer #3: Wael Muselmani

Major Compulsory Revisions:

1. Response to comment: (Why did you choose to investigate the correlation of clinical and laboratory parameters only between the DR4+ and DR4- in RA patients? Did you try to investigate this correlation between the DR1+ and DR1- or the DR10+
and DR10- patients? You have 2 possible options: a. You can perform this investigation for DR1 and DR10 in addition to DR4. b. You can instead perform the Meta-analysis only on the association of HLA-DR4 with Rheumatoid arthritis. In this case you have to replace “HLA-DRB1” in the title by “HLA-DR4”. That make the title convey what has been studied.)

Response: It is really true as Reviewer suggested that we should not just investigate the correlation of clinical and laboratory parameters between the DR4+ and DR4- in RA patients. We tried to perform the investigation for DR1 and DR10. However we can't find data about the correlation of clinical and laboratory parameters between DR1+/DR10+ and DR1-/DR10- in RA patients in the included studies at present. We investigate the association of the HLA–DRB1*01, HLA–DRB1*0101, HLA–DRB1*0102 and HLA–DRB1*10 with RA susceptibility. Maybe we think these information is also significant. So if there is still a suggestion to perform the meta-analysis only on the association of HLA-DR4 with Rheumatoid arthritis, we will do it. It is a good direction and domain for us to investigate this correlation between the DR1+ and DR1- or the DR10+ and DR10- patients. Sincerely thank you for your good comments and valuable suggestions.

2. Response to comment: (There is a clear conclusion in abstract, but there is no conclusion in the body of article. You have to add a clear conclusion.)

Response: We are very sorry for our negligence of not adding a clear conclusion in the body of article. “Our meta-analysis indicated that HLA-DRB1*04, *0401, *0404, *0405, and *0410 are risk factors for RA. DRB1*0405 is the most common
polymorphism in Chinese populations. ESR, CRP, RF, and anti-CCP levels were higher in DR4+ patients than in DR4- patients. There were no differences between groups for indices of clinical signs.” was added as conclusion for our manuscript. We will modify the conclusion if there is any unsuitable.

3. Response to comment: (In “Materials and Methods” section, “Quality assessment of included studies” paragraph: You mentioned that HLA-DRB1 alleles were considered only when they were typed in at least 2 independent studies. However, I noticed in “Table 2” That HLA-DRB1*0402, HLA-DRB1*0101 and HLA-DRB1*0102 have only one eligible study. You have to modify the text to avoid this unclarity.)

Response: We are very sorry for our mistake of stating “HLA-DRB1 alleles were considered only when they were typed in at least 2 independent studies.” This sentence was deleted from our manuscript. Thanks so much for your suggestion making our manuscript more rigorous.

Minor Essential Revisions

1. Response to comment: (I suggest that abbreviations should be written in full at the 1st appearance, e.g. in the abstract: ESR, CRP, RF, Anti-CCP or in the body: RA, HLA, ORs, MD, VEGF, UM.)

Response: This was done according to Reviewer's suggestion. Special thanks to you for pointing out our shortcomings.

2. Response to comment: (In “Materials and Methods” section, “Literature search and selection” paragraph: a. Replace “The index terms what we used” by “The index terms that we used”.

Response: Done.
b. you have to clarify the following sentence: “we also modified the terms according to the different database”. What were the modified used terms? Please explain this in the text.)

Response: We are very sorry for our incorrect writing of the sentence. We just modified the key word or abstract or free word or others using the same terms. This sentence was removed from our manuscript.

3. Response to comment: (3. In “Materials and Methods” section, “Inclusion criteria” paragraph: Replace “should meet the criteria” by “was required to meet the following criteria”)

Response: Done.

4. Response to comment: (In “Materials and Methods” section, “Quality assessment of included studies” paragraph: I recommend to move the sentence “The frequency of HLA-DRB1 alleles varies according to ethnic and racial background, with some alleles being extremely rare” to Introduction section, as it is not a part of materials and methods.)

Response: Considering the Reviewer’s suggestion, we have moved the sentence to Introduction section. Thanks for your good comments.

5. Response to comment: (In “Materials and Methods” section, “Data Extraction” paragraph:

a. It’s not sound to use “and so on”, it doesn’t clarify adequately what was performed. Please avoid the use it.

Response: The statements were corrected as “The extracted details included first author, year of publication, geographical region, study design, source of cases and controls, frequency of HLA-DRB1 alleles, number of cases and controls, clinical
feature, laboratory index and detection methods.

b. Replace “The details extracted include” by “The extracted details included”).

Response: Done.

6. Response to comment: (In “Materials and Methods” section, “Statistical Analysis” paragraph, 3rd line: Replace “are” by “were”)

Response: Done.

7. Response to comment: (In “Results” section, 9th line: Replace “are” by “is”)

Response: Done.

8. Response to comment: (In “Results; Association of DRB1 alleles with RA susceptibility” section, 1st paragraph, 5th line: Replace “was shown” by “is shown”).

Response: Done.

9. Response to comment: (In “Results; Association of DRB1 alleles with RA susceptibility” section, 4th paragraph: You mentioned that “subjects of all included studies were divided into the southerners and northerners of China”. It’s preferable to mention that in Materials and Methods section rather than results section.)

Response: We move the sentence to Materials and Methods section.

10. Response to comment: (In “Results; correlation of clinical and laboratory parameters between the DR4+ and DR4- in RA patients” section, 2nd paragraph: You mentioned “There is” 2 times, replace it by “There was”).

Response: Done.

11. Response to comment: (In “Discussion” section: The start of discussion needs rearrangement, you started with DR4 then you mentioned that 12 HLA-DRB1
polymorphisms were addressed. I recommend to remove the 1st sentence “DR4 …. of the world” and start from: “In this meta-analysis, 12 HLA-DRB1 …. ”.

Response: Done.

Reviewer #4: Benjamin Fisher

Major:

1. Response to comment: (The authors score the quality of the articles but none of the 22 evaluated are excluded. Many of the studies have very small numbers, and while that does not necessarily imply poor quality, good matching would be important. It would be helpful to know what was be the minimum quality expected in order to be included.)

Response: It is really true as Reviewer suggested that it would be helpful to know what was be the minimum quality expected in order to be included. Quality scores ranged from 0 to 30. We defined 10, 20 and 30 scores as low, moderate and high grade respectively. And articles of low quality score were excluded. We add this sentence "Articles that received a low quality score were excluded from the analysis" to our manuscript. The 22 articles are the included studies of our final meta analysis. So none of them are excluded even many of the studies have very small numbers.

2. Response to comment: (Discussion para 4: It is important that current thinking is for early treatment in all RA patients and not just those who are DR4 positive)

Response: We are very sorry for our unsuitable writing “They should be given an early application of the anti-rheumatic drugs, in order to be able to control the disease development and improve the quality of their life” and “In order to avoid teratogenic disability, patients with DR4+ should be treated as early as possible.” The two sentences were deleted from our manuscript. Thanks so much for your suggestion making our manuscript more rigorous.

3. Response to comment: (There are important limitations that are not discussed. The X-ray data uses a scale that lacks sensitivity to discriminate but, more importantly,
most of these studies will be cross-sectional with varying disease durations and treatments so these outcome data are hard to interpret. Patients with naturally more severe disease may be on more treatments.)

Response: It is really true as Reviewer suggested that most of these studies will be cross-sectional with varying disease durations and treatments so these outcome data are hard to interpret. Chi-square test or T test was took to evaluate the balance of average age, gender and disease duration of included studies, there were not obvious imbalance of the baseline for them. We contacted the original authors to get data of treatments and sensitivity of the scale of X-ray. There were not obvious imbalance of the baseline for them of the data. However this maybe our limitation. Maybe we can withdraw the X-ray comparison in differences of clinical and laboratory parameters between the DR4+ and DR4- in RA patients if it is suggested. Sincerely thank you for your valuable suggestions.

4. Response to comment: (Discussion para 5: “In order to avoid teratogenic disability…” – what does this mean? This may be the wrong message in that current thinking is to treat all RA early and not just DR4+. However it as also hard to establish with the data as this shows subjects with DR4 are less likely to be stage I-II but no more likely to be stages II-IV. Furthermore, some studies have suggested that the HLA SE associations with outcome become non-significant once the presence of anti-CCP antibodies is taken into account. This needs to be discussed.)

Response: We are very sorry for our incorrect writing of teratogenicity to teratogenic. It is really true that current thinking is to treat all RA early and not just DR4+. “In order to avoid teratogenic disability, patients with DR4+ should be treated as early as possible.” was deleted from our manuscript. Our analysis showed that there was a positive association between DRB1*0404 and X-ray phases to RA in phases I ~ II (OR=0.47, 95%CI=0.26 – 0.87), but not in the phases III~IV (OR =1.27, 95%CI=0.84, 1.92). We checked the data carefully, and found no errors. And the reason needs to be explored in the future. Some studies have suggested that the HLA SE associations with outcome become non-significant once the presence of anti-CCP antibodies is taken into account. As to laboratory parameters, our analysis showed that
Anti-CCP in patients with DR4+ were higher than patients with DR4- (MD =177.35, 95%CI =85.06–269.63, p=0.0002). The reason also needs to be explored. In the future, we will do the meta-analysis about them in order to further clarify the reasons. This could be a very interesting study.

5. Response to comment: (Discussion: Although not the subject of this report, the relationship between smoking, anti-CCP antibodies and HLA should be mentioned. Some brief comparison with findings in Korean, Japanese and Malaysian Chinese populations (Chun-Lai T, et al Shared epitope alleles remain a risk factor for anti-citrullinated proteins antibody(ACPA)--positive rheumatoid arthritis in three Asian ethnic groups. PLoS One.2011;6(6):e21069) would be desirable.)

Response: It is really true as Reviewer suggested that the relationship between smoking, anti-CCP antibodies and HLA should be mentioned in our manuscript. The sentence “Chun-Lai found that different DRB1 SE alleles are common in Asian patients. These alleles confer a significant risk of developing ACPA-positive RA, but not ACPA-negative RA (i.e., in three Asian populations from Malaysia)\(^{16}\). Fisher found that HLA-DRB1 SE was associated with ACPA fine-specificity in Koreans with RA via one pathogenic pathway, smoking and there was an association between smoking, HLA- DRB1 SE alleles, and Anti-CCP\(^{18,19}\)” was added to our manuscript. We are very sorry for our negligence of study of some comparison.

Minor:

1. Response to comment: (Abstract para 2: “We brought into” should be changed to e.g. We examined…)

Response: Done.

2. Response to comment: (Abstract conclusion: “are susceptible…” would be better as “…are risk factors for RA in Chinese populations.”)

Response: Done.

3. Response to comment: (There needs to be a description of the X-ray phases (Steinbrocker?) referred to and how joint function was measured)

Response: We have re-written this part according to the Reviewer’s suggestion.
“X-ray criteria followed the Steinbrocker classification standard for joint function. Joint function was measured with a triaxial goniometer.” was added in our manuscript. Sincerely thank you for pointing out our limitation.

4. Response to comment: (Methods paragraph 6: MD should be defined)

Response: It is really true as reviewer suggested that MD should be defined in our manuscript. MD means mean difference. The mean difference is the average difference between instrument results and the target reference values. We add the sentence “Odds ratio (OR) and mean difference (MD) estimates with corresponding 95% confidence intervals (CI) were used to describe the relationships.” to Methods in Abstract.

5. Response to comment: (Results paragraph 1 “Finally, 22 studies were performed on the association of VEGF expression between UM and normal people” – what does this mean?)

Response: We are very sorry for our incorrect writing “Finally, 22 studies were performed on the association of VEGF expression between UM and normal people” We have re-written this sentence to “Finally, 22 studies that examined the association between HLA-DRB1 and rheumatoid arthritis in Chinese populations were included and analyzed”. Thanks so much for pointing out our mistake.

6. Response to comment: (Results para 6 and 7: It would read more easily if the MD and CIs were in brackets after each variable rather than in a long list.)

Response: I am very sorry that we are not standardized writing which brings inconvenience to you. Because we had many subgroup analysis for HLA-DRB1. We just wrote $\text{OR}_{\text{DRB1}^*04}=4.19$, 95%CI =3.44–5.11, $p<0.000011$; $\text{OR}_{\text{DRB1}^*0401}=2.53$, 95%CI =1.54–4.16, $p=0.0003$ and so on. In fact, we did not find a suitable expression for it. We are not quite clear how to use the method to express as you suggested. Maybe we can turn to a native English speaker for help. Sincerely thank you for your suggestions.

7. Response to comment: (Discussion para 6: “nationalization” should be nationality.)

Response: Done.
8. Response to comment: (Table 3: The p-value for X-ray phases III-IV cannot be <0.00001, as the CI for OR crosses 1.)

   Response: We are very sorry for our incorrect writing it in our manuscript. The p-value for X-ray phases III-IV is 0.25.

9. Response to comment: (It would be of interest to report associations with HLA*0901 if this data is available.)

   Response: It is really true as Reviewer suggested that it would be of interest to report associations with HLA*0901. We try to find data about that. However, we can't find the data at present. It is a good direction and domain for us. Sincerely thank you for your good comments and valuable suggestions.

   We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. We appreciate for Editors/Reviewers’ warm work earnestly, and hope that the correction will meet with approval. We do really want to publish our manuscript in your journal.

   Once again, thank you very much for your comments and suggestions.