Author’s response to reviews

Title: HLA class II allele polymorphism may influence susceptibility to adult dermatomyositis and polymyositis in a Han Chinese population

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Version: 2 Date: 30 January 2014

Author’s response to reviews: see over
Dear Dr. Henderson:

We would like to express our appreciation to you and the reviewers for the constructive comments and suggestions. Now the manuscript has been revised according to reviewer’s recommendations. The changes made in the revision and our responses to reviewer’s comments are listed as follows:

**Reviewer 1**

**Reviewer’s comments:**
This is a well-presented manuscript which is easy to read and comprehend. However, many of the HLA associations described are weak and this is partly the result of relatively small numbers in some of the groups, for example polymyositis, which only consists of a patient cohort of 20. The numbers are my main concern and they need to be expanded to give confidence in the findings. There are difficulties with this as idiopathic inflammatory myopathies are not conditions with a high incidence. However, multicentre studies have been of benefit in the past when required in the study of these conditions.

Authors’ response:
We agree with the reviewer that the number of the DM and PM cases included in the study is relatively small and the best way to increase the number is to conduct multi-center study. As the reviewer pointed out that IIM including DM and PM are rare diseases. The number of the DM and PM cases reported in the manuscript is the largest study so far on Chinese patients with the conditions. Many of the patients enrolled in our study were referred from several local hospitals to the IIM clinics at the Huashan Hospital where the study was conducted. We recognize that the relatively small number is one of the limitations of the study and the results present in this manuscript remain to be validated by larger cohorts. Nevertheless, we believe the data reported in the manuscript would still provide useful information to the filed.

**Reviewer’s comments:**
The use of the type 1 correction error is very conservative and has in fact rendered all the associations described statistically insignificant. So strictly speaking using the criteria outlined this manuscript has demonstrated no statistically significant HLA associations with PM or DM. However, an argument could be put that since PM and DM have demonstrated HLA associations in other studies there is an a priori reason for looking at HLA alleles in PM and DM particularly alleles such as DRB1*07 which has been implicated in other studies.

Authors’ response:
We are grateful that the reviewer appreciated our findings in recognizing that the type 1 correction error adopted in our study is very conservative and the data from our study remains valuable in the context of other previous reports.

**Reviewer’s comments:**
The functional unit of the DQA1 and DQB1 genes is the dimeric DQ molecule. When analysing the effect of DQA and DQB alleles on disease susceptibility the combination of genes should also be considered in both cis and trans confirmation. This data should be included in table 2.
Consideration should be given to just including alleles at all loci showing statistically significant differences between the groups studied to shorten the size of the table and focus on the relevant alleles.

Authors’ response:
We appreciate reviewer’s comment. In this cohort, we analyzed frequencies of the HLA alleles and haplotypes in DM and PM and controls to study role of the alleles and haplotypes in DM and PM susceptibility. The same approaches have been used in many similar studies reported in the literature. The allele frequency analysis suggest that it is DQA1 allele but not DQB1 that may influence DM/PM and DM susceptibility while it is DQB1 but not DQA1 that may have impact on PM/DM with ILD. Our data do not seem to suggest that allele interactions may have effects on disease susceptibility. However, we recognize that there are multiple genes and genetic variants, acting in a variety of biological pathways, which may be involved in susceptibility to DM and PM. Our study was focused on only some of the variants. The data from our study should be validated by further analysis of combined effects of multiple genes and polymorphisms on the disease susceptibility. We have included this aspect in the Discussion (page 10).

We are appreciative for reviewer’s suggestion to show only alleles with significant differences in Table 2. However, we think that it may be beneficial to readers by showing which alleles were analyzed in our study since there are a lot of alleles in these loci and various studies analyzed different sets of the alleles with different levels of resolution. In order to better highlight the alleles with significant differences, only the ORs with significant differences are shown in the table while the rest is shown either n/s or n/a.

Reviewer’s comments:
On page 7 it is stated that DQB1*0303 is lower in frequency in ILD than controls. In fact the frequency is similarly low in the PM group but is not significant due to the small number. However this can impact on the number in the ILD group since they are drawn from both the PM and DM groups. It might be preferable to compare frequencies of alleles in those with ILD with patients without ILD to confirm this finding.

Authors’ response:
We compared the frequencies of HLA-DRB1*04; HLA-DRB1*12 and HLA-DQB1*0303 in the patients with ILD with those in patients without ILD, and the differences are not statistically significant with p values of 0.31, 0.54 and 0.24, respectively. However, the ORs for the PM/DM group with ILD are statistically significant as shown in the Table 2.

Reviewer’s comments:
Another example of a statistical quirk is the report that DRB1*4 and DRB1*12 are associated with ILD. The frequency of these two alleles in ILD are really no higher in this group than the frequencies found in the other groups (especially for DR12) except for the control group which has the lowest frequency. This leaves open the possibility that these alleles (particularly DR12) are primarily associated with PM and DM.
Authors’ responses:
We appreciate reviewer’s observations. It is known that statistical significance of OR is not
determined by OR number alone that is derived from comparison of frequencies, rather by 95% CI range. For DRB1*12 allele, although the OR for PM is 1.89, 95% CI is 0.6-5.42 which does not reach statistically significant (p=0.2). The same is for OR for DM: 1.58; 95% CI 0.75-3.29 which does not reach statistically significant (p=0.18). Therefore, these numbers do not suggest association of the allele DRB1*12 with DM and PM alone, but OR for DM/PM with ILD does.

Reviewer’s comments:
The statement is made on page 9 that “HLA-DQA1*0301 and HLA-DRB1*03 may provide protection from PM and DM respectively” This is misleading as in table 2 DRB1*03 is shown as being protective for the development of DM while DQA1*0301 is shown as being protective for the combined PM/DM group yet the frequency in this group is higher than that seen in the PM group alone. Again is this just a reflection of small numbers in the PM group or a mistake in the table?

Authors’ response:
We thank the reviewer for pointing out the error. Now the phrase has been corrected to “HLA-DQA1*0301 and HLA-DRB1*03 may provide protection from DM/PM and DM, respectively” (page 9). Although the frequency in DQA1*0301 group is higher than that seen in the PM alone group, the 95% CI is not within the range of statistical significance. As the reviewer suggested, this is likely due to small number of cases in the PM group.

Reviewer’s comments:
I would suggest that the combined PM/DM group is deleted from the tables as PM and DM appear to have different genetic profiles and it again creates doubt due to the small numbers in the PM group. For example in table 3 the DR7 haplotype is shown to be increased in the DM group yet the frequency in the combined group is approximately the same as the DM group. Again this is due to the small contribution of the PM group and the increase in the PM/DM ILD group may be just a reflection of the increased frequency in the DM group.

Authors’ responses:
We agree with the reviewer that it is likely that the frequencies for DM/PM in Tables 2 and 3 are largely influenced by the DM numbers. Therefore, caution should be excised to interpret the DM/PM data. We reiterate this as a limitation of the study in the revision (page 10). Although genetic profiles for DM and PM may be different, both belong to IIM and in most published studies they are grouped together as DM/PM group. We think having all (combined) DM/PM group in our study may be useful to compare our results with those from other studies.

Reviewer’s comments:
DQA1*0103 is mentioned in the conclusions as being “likely” associated with an increased risk of DM and PM. This allele is not shown in table 2 as being associated and no mention is made of this is made in the result section.DRB1*03 is also stated as being associated with lung complications in DM but again this is not shown in table 2 nor is it mentioned in the result section.
Author’s response:
We thank the reviewer for pointing out the inconsistence. These inconsistences in the Abstract and Conclusion now have been resolved (pages 3 and 11).

Reviewer’s comments:
Page 5 –SSA and SSB require definition in the text before the acronyms are used.

Authors’ response:
In the revision SSA and SSB are replaced by anti-Ro and anti-La autoantibodies, respectively (page 5).

Reviewer’s comments
The word haplotype should only be used when the cis configuration of a group of alleles at neighbouring loci has been demonstrated. What is actually shown in table 3 are co-occurring alleles which are designated haplotypes based on population studies. They should be referred to as putative haplotypes particularly in disease studies where linkage disequilibrium seen in the normal population can be perturbed.

Author’s responses:
We thank reviewer for the suggestion. In the revision, “haplotype” is replaced by “putative haplotype”

Reviewer’s comments:
The paper from Han et al Chinese Journal of Microbiology and Immunology 23(3) p225;2003 which reported on DRB1 alleles in PM/DM in Northern Chinese should be recognized and quoted in the manuscript.

Authors’ responses:
The Han’s paper along with another paper on association of HLA alleles with IIM in Chinese populations has been cited in the revision (pages 4 and 9).

Reviewer’s comments:
The “R” has been left out of DRB1 twice on page 7.

Authors’ response:
The errors have been corrected in the revision (page 7).

Reviewer’s comments:
Heading to table 1- “e” has been left out of the word “features” in heading

Authors’ responses:
The typographic error has been corrected (table 1)

Reviewer 2
Reviewer’s comment:
What is already known in the literature about HLA associations in Chinese populations?

Authors’ response:
In the revision, we now include several papers on the related topic in the Introduction and Discussion (pages 4 and 9).

Reviewer’s comments:
Was a “definite” or “probable” diagnosis of myositis made according to Bohan and Peter? The methodology is otherwise well described.

Authors’ responses:
In this study, we included patients met probable or definite PM or DM according to Bohan and Peter’s criteria. We clarify this in the revision (page 5)

Reviewer’s comments:
A limited repertoire of autoantibodies was tested in the study. Therefore, the statement “autoantibodies were more likely present among PM patients” should be rephrased to something like” the tested autoantibodies were more frequent in the PM group”. The data is on a limited number of patients, thus phrases such as “suggesting that patients with this allele are unlikely to develop this lung disorder” should be avoided in the methods. This may be purely down to lack of statistical power.

Authors’ response:
We appreciate the reviewer’s suggestions. Now these suggestions have been adopted in the revision. In the Result “MSA and MAA tested were more frequent in the PM group than in DM group” replaced “autoantibodies were more likely present among PM patients” (page 6). Also the phrase “suggesting that patients with this allele are unlikely to develop this lung disorder” has been deleted in the revision.

Reviewer’s comments:
Please correct “DM and PM patients with HLA-RB1*04, HLA-RB1*12”

Authors’ response:
In the conclusion, the phrase starts with “DM and PM patients with HLA-RB1*04, HLA-RB1*12” is replaced by “HLA-RB1*04, HLA-RB1*12, and DRB1*07-DQA1*01-DQB1*02 were more frequently observed in DM/PM patients with the lung complication than in controls”. (page 11)

Reviewer’s comments:
More should be made of the DRB1*07 association which is also present in Caucasian DM patients, suggesting shared ethno-geographic susceptibility. It is interesting that this allele is present on a different haplotype to that described in this manuscript (DRB1*07-DQA1*02-DQB1*02).
Authors’ response:
We appreciate the reviewer’s suggestion. One paragraph has been added to the Discussion to highlight this point (page 9).

Reviewer 3
Reviewer’s comments:
The manuscript has a major limitation that refers to the small patient sample size. The differences presented did not reach significance after Bonferroni corrections, further emphasizing this problem. As these are rare disease entities collaborations with several centers is often needed for genetic studies.

Authors’ responses:
We agree with the reviewer that the number of the DM and PM cases included in the study is relatively small and the best way to increase the number is to conduct multi-center study. As the reviewer pointed out that IIM including DM and PM are rare diseases. The number of the DM and PM cases reported in the manuscript is the largest study so far on Chinese patients with the conditions. Many of the patients enrolled in our study were referred from several local hospitals to the IIM clinics at the Huashan Hospital where the study was conducted. We recognize that the relatively small number is one of the limitations of the study and the results present in this manuscript remain to be validated by larger cohorts. Nevertheless, we believe the data reported in the manuscript would still provide useful information to the filed.

Reviewer’s comments:
For diagnosis Bohan and Peter criteria were used. Did the authors accept possible, probable and definite diagnosis?

Authors’ responses:
In this study, we included patients met probable or definite PM or DM according to Bohan and Peter’s criteria. We clarify this in the revision (page 5).

Reviewer’s comments:
Interstitial lung disease was checked for, how was this defined.

Authors’ responses:
Interstitial lung disease was defined as idiopathic interstitial pneumonitis. The lung lesions were examined by chest computed tomography and the diagnosis was made by a pulmonologist (Page 5).

Reviewer’s comments:
Which tests were used for autoantibody detection?

Authors’ response:
Autoantibodies were analyzed by an indirect immunofluorescence method using the commercial kits from Euroimmun AG (Lubeck, Germany) (page 5).
Reviewer’s comments:
It may be worthwhile to test your sera using immunoprecipitation or some other novel test to pick up some of the newly identified autoantibodies that are more prevalent in DM.

Authors’ response:
We agree with the reviewer that it would be desirable to test patient sera using additional assays developed recently. Unfortunately, some of the current methodologies for immunoassays were not available at the time the study was conducted. Moreover, the majority of the patients has already received steroid treatments and is no longer suitable for study of autoantibodies. It is our plan to use some of the current methodologies to test more autoantibodies in our future studies.

Editor’s comments:
Please provide the email addresses of all authors on the title page.

Authors’ response:
The email addresses of all authors have been listed on the title page (pages 1 and 2).

Editor’s comments:
Please amend the mistake in this section. The word "declare" should replace "declaim"

Authors’ response:
The correction has been made in the revision (page 11).

We hope that the revision has adequately adopted the suggestions and is acceptable for publication. Once again, we are very grateful for reviewers’ recommendations and editorial guidance to the manuscript preparation.

Please do not hesitate to contact me with questions.

Yours sincerely,

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