Author’s response to reviews

Title: Lack of associations between two previously identified susceptible single nucleotide polymorphisms of Interleukin-23 receptor gene and ankylosing spondylitis: a replication study in a Chinese Han population

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

Thanks a lot for the comprehensive review of our manuscript entitled “Lack of associations between two previously identified susceptible single nucleotide polymorphisms of Interleukin-23 receptor gene and ankylosing spondylitis: a replication study in a Chinese Han population” (MS: 1255460407843368). In response to the reviewers’ comments, we are submitting a revised version of the manuscript here and the specific points raised by reviewers are addressed as follows:

**Reviewer #1**

The objective of the study was to investigate the possible associations of two SNPs (rs1004819 and rs10889677) of IL-23R gene as potential AS susceptibility in a Chinese Han population, which SNPs had already been confirmed in British, Hungarian, and Portuguese AS population samples. Investigating a total of 195 AS patients and 203 normal controls the authors could not verify susceptibility nature of these SNPs, and concluded that this might be a population specific consequence.

The manuscript in general is well written, the used methods are sound, the statistical approaches are appropriate, the conclusion drawn are clear, and supported by the data presented in the study.

Question 1(Q1): The discussion is somewhat repetitive, as simple summarizes the aims, the results, and the conclusion.

Response 1(R1): We appreciate your suggestion. In the discussion, we deleted some redundant sentences and added the new data on the comparison of allele frequency distribution between European population and Chinese Han population, indicating that the associations between these two SNPs and AS susceptibility in European population need to be verified in Chinese Han population. The related information was described in Line 2 to Line 12, Page 5.

Q2: The authors should also compare their allele frequencies to findings of others references (refs 12-14). Add some comments to the discussion.

R2: Thanks very much for your valuable advice. We agree with you that we should compare the allele frequencies distributions between European and Chinese Han populations as to support the view that the ethnic factor contributes significantly to the population splitting and genetic variation. The SNP rs1004819 is an A/G variation in British, Hungarian and Portuguese populations. However, it is a C/T variation in Chinese Han population. Therefore, the allele frequency distribution of rs1004819 cannot be compared between European and Chinese Han populations. Although the SNP rs10889677 is an A/C variation in both European and Chinese Han populations, the allele frequency distribution of this SNP is significantly different between these populations. The information is added in Line 2 to Line 7, Page 5. The data of allele
frequency distribution of rs10889677 are listed in Table 3.

Q3: Allele frequencies, linkage features from public domains (HapMap, Entrez, Ensemble) should also be reported shortly in the discussion to support the already verified differences of the IL23R gene's naturally occurring variants in different population, with special attention to the 2 SNPs investigated here.

R3: Thanks a lot for your suggestion. The data from the International Hapmap Project (http://hapmap.ncbi.nlm.nih.gov/) demonstrate wide variations of the allele frequencies of these 2 SNPs between different races. This information is added in Line 7 to Line 12, Page 5, so that to support the already verified differences of the IL23R gene's naturally occurring variants between different populations.

Q4: The authors state in the introduction that "Thus, IL-23R gene polymorphism may be implicated in the susceptibility to human autoimmune disease (paper of Safrany, Curr Med Chem. 2009;16(28):3766 should be inserted here, to help the reader with a supporting reference), such as AS."

R4: Thanks very much for your suggestion. This reference is added as Ref 11(Line 12, Page 3).

Reviewer #2
This paper is generally well written and clearly presents the rationale and details of the analysis. One major point, though, is that I couldn't replicate all of the Chi-square values: in the rs1004819 analysis of allele frequency, the Chi-square value I got was 0.69 (p=0.79), compared to your value of Chi-square = 1.9 (p=0.17). The interpretation of the data doesn't differ, but please check all of your calculations.

Minor Essential Revisions:
In addition, information on these additional points would strengthen the paper:

Q1: One major point, though, is that I couldn't replicate all of the Chi-square values: in the rs1004819 analysis of allele frequency, the Chi-square value I got was 0.69 (p=0.79), compared to your value of Chi-square = 1.9 (p=0.17).

R1: Thanks a lot for your careful review. This error has been corrected in Table 2. We also check all of the calculations to make sure that they are correct.

Q2: What was the source of the patients (i.e., what type of clinic? Were they being seen in? - university hospital, general medicine?) How and from where were the controls recruited?
R2: All the subjects investigated in this study were Chinese Han. All these AS patients were recruited from the outpatient clinic of the affiliated Drum Tower Hospital of Nanjing University Medical School while these normal controls were recruited from the medical control center for healthy population of the hospital. Any autoimmune disease or musculoskeletal disease was ruled out for all the normal controls. This information was added in Line 31 to Line 35 and Line 37 to Line 39 on Page 3.

Q3: Background section - last sentence of first paragraph and first sentence of 2nd paragraph seem to be saying the same thing; these could be combined by adding reference 6 to the first paragraph, and then begin the 2nd paragraph with "The etiology of AS...."

R3: Thanks a lot for your advices. Following your suggestion, the first sentence of the second paragraph was deleted and the reference 6 was added to the last sentence of the first paragraph. This modification can be seen in Line 2, Page 3.

Q4: The first paragraph of the Discussion section repeats information that is in the Background section. One of these pieces could be shortened.

R4: Thanks a lot for your suggestion. The redundant sentences (Numerous genetic association studies have been carried out to identify the non-HLA genes involved in AS susceptibility. In a recent genome-wide association study, the researchers identified two genes (ARTS1 gene and IL23R gene) involved in AS, which was considered as a major breakthrough in the current understanding of the pathogenesis of AS) have been deleted in the first paragraph of the discussion.

Q5: It would be interesting to note how the frequency of the alleles in your population compares to those seen in the European populations (references 12-14)? - is the frequency similar in the controls in Europe and China, but in Europe the AS patients differ? Or is the distribution in the controls different between the two ethnic groups? (or did it differ among the European groups, too?)

R5: Following your advice, both the allele frequency distribution of AS patients and that of normal controls were compared between European and Chinese Han populations based on the results of previous studies. The SNP rs1004819 is an A/G variation in British, Hungarian and Portuguese populations. However, it is a C/T variation in Chinese Han population. Therefore, the allele frequency distribution of rs1004819 cannot be compared between European and Chinese Han population. Although the SNP rs10889677 is an A/C variation in both European and Chinese Han populations, the allele frequency distribution of this SNP is significantly different between these populations. These data support the views that the ethnic factor contributes significantly to the population splitting and genetic variation and the associations between the IL-23R gene polymorphisms and AS susceptibility in
European population need to be further confirmed in Chinese Han population. The information is added in Line 2 to Line 7, Page 5. The data of allele frequency distribution of rs10889677 are listed in Table 3.

Q6: Tables 1 and 2 can be combined; Tables 3 and 4 can be combined.

R6: Thanks for your advices. Tables 1 and 2 have been combined into one table (Table 1). Table 3 and Table 4 also have been combined into another table (Table 2).

Q7: Some minor editing is needed (for example, "a lot" is generally not used in this type of writing, and it would be preferable to say "There is substantial evidence..." rather than "There are substantial evidences...")

R7: Thanks for your careful review. These grammar mistakes have been corrected. These revisions can be found in Line 5, Page 2; Line 41, Page 2 and Line 1, Page 5.

Editorial comments:
Please revise your ethics statement to include the name of the ethics committee that approve your manuscript

Response: Following your request, the name of the ethics committee has revised as “Research Ethics Committee of Medical School of Nanjing University”. This information can be seen in Line 42 to Line 43, Page 3.

I sincerely hope that this revised manuscript will meet your requirements for publication. Many thanks to you, editor and the two reviewers, for the time and effort spent on this paper.

Yours sincerely,
Yong Qiu