Author's response to reviews

Title: Genetic variants in the TIRAP gene are associated with increased risk of sepsis-associated acute lung injury

Authors:

Zhenju Song (zhenjusong@yahoo.com)
Chaoyang Tong (tong.chaoyang@zs-hospital.sh.cn)
Zhan Sun (sun.zhan@zs-hospital.sh.cn)
Yao Shen (shenyao123@yahoo.com.cn)
Chenling Yao (yao.chenling@zs-hospital.sh.cn)
Jinjun Jiang (jinjundoc@163.com)
Jun Yin (yin.jun@zs-hospital.sh.cn)
Lei Gao (zsgaolei@163.com)
Yuanlin Song (ylsong70@gmail.com)
Chunxue Bai (bai.chunxue@zs-hospital.sh.cn)

Version: 2 Date: 1 October 2010

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

I am very appreciated with your and the reviewers’ insightful comments for our manuscript. Enclosed please find a revised manuscript entitled: “Genetic variants in the TIRAP gene are associated with increased risk of sepsis-associated acute lung injury” (MS: 1994855642424389).

We have incorporated the helpful comments to strengthen the manuscript. We believe that all these efforts as advised by the reviewers should have resulted in substantial improvement of the manuscript on the basis of the previous version. For this, we are grateful. We have highlighted the revisions (blue color font) in the main text and provided a point-by-point response to the referees’ comments as follows.

Thank you very much for your consideration of this manuscript.

Sincerely yours,

Sincerely,
Chunxue Bai, MD, Ph.D
Department of Pulmonary Medicine, Zhongshan Hospital, Fudan University
180 Feng Lin Road, Shanghai 200032, PR China
Tel: +86.21.6404.1990×3077
Fax: +86.21.6418.7165
Email: bai.chunxue@zs-hospital.sh.cn
Reviewer's report 1
Title: Genetic variations in the TIRAP gene are associated with increased risk of sepsis-associated acute lung injury
Version: 1 Date: 3 August 2010
Reviewer: Carlos Flores

Reviewer's report:
Dear Editor,
The manuscript entitled “Genetic variations in the TIRAP gene are associated with increased risk of sepsis-associated acute lung injury” by Song and coworkers describes an association study of selected SNPs in TIRAP gene and acute lung injury (ALI) risk. A strong association was found for two SNPs and haplotypes when comparing ALI patients with either healthy controls or sepsis patients. This is a well conducted study using an appropriate case-control sample size, and with a reasonable hypothesis given the importance of TLR pathways in sepsis and ALI. The following are suggestions to improve the article and to adhere it to STREGA standards for reporting genetic association studies:

Major Compulsory Revisions.
1) Abstract (but also the main text). The study design is not cohort-based but a case-control. The authors should substitute the cohort designation by appropriate terms throughout the text. Done. Thanks.

2) Methods, study enrollment and design. I guess when authors refer to ALI they are actually referring to ALI + ARDS. If so, please, state that with a sentence in Methods. Additionally, it would be interesting to know how many of the ALI patients developed ARDS and if the authors recorded the number of samples with severe sepsis. If available, please provide percentages in Table 1. This should be stated in Table 1. Additionally, the authors should explore if ORs increase when focusing exclusively on ARDS patients instead of all ALI. This might give an idea of whether SNPs are more related to severity than to susceptibility.
In our study, the ALI patients refer to the combined patients with ALI or ARDS. We have added a sentence in Methods. A total of 175 ALI patients developed ARDS. We have provided the information in Table 1. All sepsis subjects enrolled in our study are either severe sepsis or septic shock. The allele and genotype frequencies of five polymorphisms were not significantly different between ALI and ARDS groups. Genetic variants of TIRAP were not associated with ALI severity in our data.

3) Methods, study enrollment and design. It is not clear how healthy controls were recruited. To allow reproducibility, additional information needs to be provided: if sampling was hospital or population-based, if individuals were from the same location or from different parts of the country, etc…
We provided additional information about the sample as follows: To reduce the potential confounding from ethnic backgrounds, we only enrolled people with self-reported origin of central Han Chinese, including indigenous people from Zhejiang Province, Jiangsu Province, Anhui Province and Shanghai. Sex- and age-matched controls were selected from healthy
blood donors.

4) Methods, SNP selection and genotyping, third paragraph. Please give more details of the genotyping method used and if error checks were assessed. Were sequences read by both strands? Was genotyping blind to disease status? Were genotyping calls performed simultaneously for the entire study or was it performed sample by sample? Was all genotyped performed in the same lab (state where)? Did the authors duplicate a fraction of the samples to monitor genotyping concordance besides the doubling scoring by a second operator? What was the rate of genotyping discordance? Were genotype calls performed manually? or did the authors used an automatic calling algorithm part of the Staden package? If so, please clarify and give a summary of the confidence of calls. Please, provide also a reference for the Staden package.

Genotyping was performed by direct sequencing at the Chinese National Human Genome Center in Shanghai, China. The sequencing reactions were performed using Applied Biosystems BigDye (version 3.1) chemistry (Applied Biosystem, Foster City, CA, USA), and the sequences were resolved using an ABI 3730 Genetic Analyzer. Analyses of the sequence traces were performed using the Staden package and double scored by a second operator. A duplicate were added to each 96-well sample plate for quality assurance and quality control validation of inter-plate discordance, and we placed an extra 10 duplicates into our sample set in order to test for experiment-wide discordance. The data completion rate was 99%. We provided a reference for the Staden package.

5) Methods, Statistical analysis. The authors state the use of alleles to calculate the ORs and 95% CIs but report genotype ORs as well. Please, explain how allelic ORs were calculated and which inheritance model was used for genotype tests.

We have reported allele ORs and haplotype ORs in the manuscript. Allele counts in cases and controls were used to calculate the OR and the 95% CI. Each haplotype was compared with all other haplotypes as the reference in calculating the OR. P values for genotypic distributions were calculated using the global genotype test.

6) Methods, Statistical analysis. The sentence of association adjustments needs re-writing. How were covariates added to the logistic regression model? Please, declare the alpha that needed to consider the association significant after Bonferroni adjustment. Did the authors set the alpha based only on the 5 SNPs or the 5 SNPs x 2 comparisons?

The re-writing for association adjustments: Multiple logistic regression was used to evaluate if each SNP was independently associated with ALI when adjusted for the potential confounding effects of important clinical variables. When comparing ALI patients to sepsis alone patients, age, gender, body mass index (BMI), history of smoking, diabetes, liver cirrhosis and APACHE II score were included in the multivariate models because of their established association with ALI. When comparing ALI patients to healthy controls, age, gender, BMI and history of smoking were included in the multivariate models.

For SNP-based multiple testing, a $P$-value of $< 0.01$ (0.05/5) was considered statistically significant after Bonferroni correction. For haplotype-based multiple testing, a $P$-value of $< $
0.025 (0.05/2) was considered statistically significant after Bonferroni correction.

7) Methods, Statistical analysis. Why did authors chose to test haplotype associations with PLINK? Why not continue using Haplovlew?
We used Plink to conduct the global test for the haplotypes, which could not be conduct by Haploview.

8) Results, Associations of the TIRAP gene SNPs with ALI risk. The Hardy-Weinberg equilibrium p-values should be provided in Table 2. This table should be defined as supplementary material as will be useful only for a limited number of readers. We provided the Hardy-Weinberg equilibrium p-values and put the table in supplementary material.

9) Results, Associations of the TIRAP gene SNPs with ALI risk. The authors suggest that associations of rs595209 and rs8177375 might be independent given the $r^2$ value. That might be the case, however, there may be interaction between the two sites as well. This should be explored as well. The SNP-SNP interaction (epistasis) has been investigated between rs595209 and rs8177375 using PLINK. No significant interaction was found. We added this result in the manuscript.

10) Discussion, third paragraph, starting from the fourth sentence till the end of the paragraph. This is mostly a discussion about the possible functional roles of the associated SNPs. Given that these were tagSNPs, it is more likely that these are tagging something else more than having an effect on mRNA stability. Since the authors did not use a reference database to impute untyped variants (e.g. a re-sequencing sample) to figure out tagged SNPs associated with ALI, the whole paragraph should be re-focused to discuss other potential alternatives for the association: 1) that the association might be due to other common or rare variants of the TIRAP gene associated with ALI; 2) that the association might be due to LD with variants from nearby genes.
We have rewritten the paragraph as suggested: Rs595209 and rs8177375 were reported for the first time to be associated with the susceptibility of ALI. These two SNPs were both located in the non-coding region of TIRAP. SNP rs595209 is located in the intron region of TIRAP. Although rs595209 is at the neighboring region of the nonsynonymous SNPs rs8177374 and rs7932766 in the DNA sequence, these SNPs are not in high LD with each other (Figure 1). SNP rs8177375 was located in the 3’ UTR region of the transcript NM_148910 and in the intron region of NM_001039661. It is well known that 3’ UTRs are regulatory elements which can control protein expression, primarily through effects on mRNA stability and also through transcript translatability. Therefore, it is highly probable that rs8177375 alter the structure of the 3’ UTRs, consequently influence the expression of NM_148910. However, given that these were tagSNPs, it is more likely that rs595209 and rs8177375 are tagging other common or rare variants of the TIRAP gene associated with ALI. Another possibility is that the association might be due to LD with variants from nearby genes. Exhaustive resequencing is required to find or rule out the possibility an as-yet-unidentified causal SNP in LD with rs595209 and rs8177375. And further functional
studies are needed to investigate whether the variants have an effect on TIRAP mRNA stability and translatability.

11) Discussion. The manuscript will benefit from a cautious discussion relating the findings of the study with other studies of the field strengthening the relevance of TLR pathways in sepsis and ALI development.
We have given a cautious discussion relating the findings of the study with other studies of TLR pathways in sepsis and ALI development in the first paragraph of discussion.

12) Discussion, last paragraph. The authors claim that one of the strengths of the study is that racial admixture was reduced since all samples were collected from Han Chinese. However, no empirical assessment for the presence of population stratification or a correction for it was considered. This is a limitation of the study that needs to be clearly identified and discussed. Another main limitation of the study that needs to be identified, in the main text but also in the abstract, is that the association needs to be replicated in independent studies.

We have given a further description of our sample enrollment in the methods: To reduce the potential confounding from ethnic backgrounds, we only enrolled people with self-reported origin of central Han Chinese, including indigenous people from Zhejiang Province, Jiangsu Province, Anhui Province and Shanghai. Recent analysis by Genome-wide SNP variation have shown that the central Han Chinese could be regarded as one single homogenous population. We added the references. We pointed out the limitation as suggested.

Minor Essential Revisions.
1) Abstract, third sentence of methods. Introduce “frequencies” after “haplotype”.
   Added. Thanks.

2) Abstract, first sentence of results. Please, provide 95%CI and alleles at-risk along with ORs and p-values.
   Done. Thanks.

3) Abstract, last sentence of results. Substitute “…and Bonferroni correction” by “…and for multiple comparisons”.
   Done. Thanks.

4) Background, first paragraph. Substitute ref 3 by the more recent: Flores et al. Crit Care 2008, 12:R130.
   Done. Thanks.

5) Methods, SNP selection and genotyping. The sentence “Six SNPs in the TIRAP gene region on chromosome 11q24.2 were used to identify tag SNPs” is confusing and can be safely removed.
   The sentence has been removed.

6) Methods, SNP selection and genotyping. The program used for tagging SNP selection
should be provided (I guess was Tagger within Haploview). Additionally, it would be helpful for some readers to have an idea of the size of the tagged area.

**We added a sentence in the Methods:** Three tag SNPs (rs595209, rs3802813 and rs8177375) for the 11.85-kb region encompassing the entire TIRAP gene were identified by Tagger within Haploview.

7) **Methods,** Statistical analysis. Please, state that LD was calculated in terms of $r^2$ values.
The LD between SNPs was calculated in terms of $r^2$ values by the Haploview v4.1 software.

8) **Results,** Associations of the TIRAP gene SNPs with ALI risk. Please, check that MAFs are correct, as those from rs3802813 and rs8177375 seem to be switched with respect to HapMap.
The MAFs of rs3802813 and rs8177375 in our data are in agreement with that of CHB in Hapmap.

9) **Results,** Associations of the TIRAP gene haplotypes with ALI risk. The haplotype analysis is not adding much more information to the manuscript than the analysis of SNPs alone (either in terms of ORs or in terms of p-values risk haplotype is essentially driven by rs595209). Given this, and the fact that haplotype blocks are strongly dependent on the SNPs being tested in the region, this part of the analysis should be removed.
**We have removed the analysis and added the two-locus haplotype analysis involved the two associated SNPs (rs595209 and rs8177375).**

10) **Discussion,** first paragraph. The authors state that no differences were found between healthy controls and sepsis patients but no result is shown. Although not significant, these results may be considered as supplementary results as they might be important for future studies (e.g. meta-analyses).
**We have provided these results in the supplementary files.**

11) **Discussion,** second paragraph. The authors found no association for rs7932766 and rs8177374. The authors declared that the study had limited power given their MAF in East Asians. However, they should provide power calculations to support the statement.
**We added the following discussion:** Assuming the prevalence of 0.01 and the OR of 1.5 and using a significance level of 0.05, our study had only 11.9% and 11.8% power to detect association with rs8177374 (MAF of 1.0%) in 278 acute lung injury patients and 288 sepsis alone patients vs. 298 control respectively, and had 25.0% and 24.7% power to detect association with rs7932766 (MAF of 2.9%) in 278 acute lung injury patients and 288 sepsis alone patients vs. 298 control respectively.

**Discretionary Revisions.**
1) **Abstract,** second sentence of background. Please, introduce “gene” somewhere in the sentence.
Toll like receptors (TLRs) signaling pathways, including the adaptor protein Mal encoded by the TIRAP gene, play a central role in the development of acute lung injury (ALI).
2) Abstract, first sentence of methods. I suggest starting it with “DNA samples from 298 healthy subjects,….” The authors need to make clear here that the samples are from Han Chinese.

A case-control collection from Han Chinese of 298 healthy subjects, 278 sepsis-associated ALI and 288 sepsis alone patients were included.

3) Abstract, second sentence of methods. What do the authors mean with “spanning the entire TIRAP gene”? To make it simpler I suggest re-writing it as “Three tagging single nucleotide polymorphisms (SNPs) of TIRAP gene and two additional SNPs that have previously showed association with susceptibility to other inflammatory diseases…”

We rewrote the sentence as suggested: Three tag single nucleotide polymorphisms (SNPs) of the TIRAP gene and two additional SNPs that have previously showed association with susceptibility to other inflammatory diseases were genotyped by direct sequencing.

4) Methods, SNP selection and genotyping. The second paragraph can be safely removed from the manuscript since it is not adding any information. Relevant data from the supplementary table 1 can be added to table 3 so that this supplementary table can be removed from the manuscript.

Done. Thanks.

5) Methods, SNP selection and genotyping, third paragraph. The authors refer to the supplementary table 2 for PCR methods. I suggest including this information (primer sequences and PCR conditions) as part of the main text in methods and not as a supplement. This will allow the authors to better explain their methods since, as it is now, the reader has to “guess” that the two first SNPs from the table were genotyped together within an assay. The next three were genotyped together as well but with a different assay.

Done. Thanks.

Additionally, other minor issues are:
1) Substitute “variations” by “variants” throughout the text and title.

Done.

2) Please, make sure that TIRAP is in italics when referring to the gene.

Done.

3) Abstract, last sentence of methods. “Logistic” should be written all in lowercase.

Done.

These associations remained significant after adjustment for covariates in multiple logistic regression analysis and for multiple comparisons.

4) Methods, SNP selection and genotyping, first paragraph, last sentence needs re-writing. A suggestion would be: “Additionally, two coding SNPs (rs8177374 predicting Ser180Leu, and rs7932766 predicting Ala186Ala), were also genotyped in this study as they have showed
evidence of association with other inflammatory diseases [15-19]. These were not genotyped as part of the HapMap project”.

We have rewritten the sentence as suggested.

5) Methods, Statistical analysis, second sentence. Substitute “departure” by “departures”.
Done.

6) Methods, Statistical analysis, third sentence. Substitute “appropriated” by “appropriate”.
Done.

7) Results, Characteristics of the study population, third sentence. Substitute “were” by “are”.
Done.

8) Results, Associations of the TIRAP gene SNPs with ALI risk, second paragraph, second sentence. Substitute “The minor allele of…” by “The alleles…”.
Done.

9) Discussion, third paragraph, third sentence. Substitute “This is in accordance with the recent founding…” by “This is in agreement with recent findings…”.
Done.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests
Reviewer's report

Title: Genetic variations in the TIRAP gene are associated with increased risk of sepsis-associated acute lung injury

Version: 1
Date: 4 August 2010
Reviewer: Chiea Khor

Reviewer's report:
Zhenju Song and co-authors describe a genetic study on Mal/TIRAP, a bridging adaptor molecule downstream of TLR2 and TLR4 signaling. They assessed a total of 5 single nucleotide polymorphisms (SNPs) of varying allele frequency in a longitudinal cohort comprising individuals of Han Chinese descent, and found suggestive evidence (univariate P = 10^{-3} to 10^{-4}) of association with acute lung injury for two out of the 5 mutations. Of note, the 3 study groups for comparison appear to be well matched.

Minor comments:
1. The value of the study would be greatly enhanced with the inclusion of a replication sample set; I would indeed recommend this inclusion if the authors have a replication set which is forthcoming. However, I am very mindful of the difficulty in assembling longitudinal cohorts of this nature, where individuals with acute lung injury + sepsis, sepsis per-se, and controls are carefully matched for potential confounders. This is the major limitation of our study. It is difficult to assemble longitudinal cohort in a short time, we continued the enrollment in order to carry out an independent replication study in the future.

2. As single-point P-values are actually more significant than the haplotype P's, may I suggest dropping the haplotype analysis altogether, as it adds very little to the results. Also, haplotype analysis using unrelated (e.g. in a case-control study) individuals could be unreliable, as they are produced using computer algorithms. To be able to discuss haplotypes meaningfully, a family-based cohort should be included and direct, vertical transmission of the haplotypes tested.

We have removed the analysis and added the two-locus haplotype analysis involved the two associated SNPs (rs595209 and rs8177375).

3. For figure 1, as the authors elect to report the r-squared coefficient to reflect LD, may I suggest that they change the colouring of the diamonds to reflect the r-squared scale on haploview? Currently, the colour scheme chosen is indicative of the D-prime scale.

We chose the coloring of the diamonds to reflect the r-squared scale on haploview.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:
Reviewer's report 3
Title: Genetic variations in the TIRAP gene are associated with increased risk of sepsis-associated acute lung injury
Version: 1
Date: 9 August 2010
Reviewer: Bart Ferwerda

Reviewer's report:
Comments:
1. Page 7 describes study enrollment and design. At the end it mentions that the source of infection of patients is known. It would strengthen the article if a brief summary of this information were also included in the article.

We have given a brief summary of this information in the results section.

2. Page 13: “Previous studies showed …….in Asian populations” mentions the difference between the European and Asian frequencies. Here it is shown that the minor rs595209 A allele has association with sepsis related ALI. In Europe and Africa this allele is the major allele having a frequency of 79.2 and 92.5% (Source: HapMap). Hereby I miss the discussion about the differences in the population frequency of the found SNPs and the consequences for other populations. Are the findings likely to be (Han) Chinese population specific or can the finding have a broader implication?

We added a new paragraph in the discussion section: When compared with the genotypes of other populations from HapMap, we found the rs8177375A allele frequency in our healthy controls and those of Asian descent (CHB), Europeans descent (CEU) and African descent (YRI) from Hapmap does not vary significantly. The minor rs595209 A allele associated with sepsis related ALI has the frequency of 29.6% in the current 588 healthy controls, similar to the Hapmap CHB data (34.3%). However, the A allele of rs595209 is the major allele with a frequency of 93.2% in YRI and 83.2% in CEU from Hapmap data. It remains to be determined whether these differences between ethnic groups influence susceptibility to sepsis related ALI. Investigation in other population is also expected to determine whether the findings is Chinese population specific.

3. Although the upset of the research is mainly focusing on genetics, authors have collected data on source of infection, Acute Physiology and Chronical Health Evaluation II scores and mortality (page 7 and table 1). Despite the presentation of the figures in table 1 it would be interesting to see the differences between those data and the found associated genotypes, which can be used for a better translation to the clinical relevance of the findings. Also strong support for the involvement of the SNPs and haplotypes can be strengthened if the higher mortality within the sepsis related ALI group is due to the TIRAP genetic variation? If possible these analysis should be included and also discussed.

No significant associations of TIRAP variants were found with source of infection, PaO₂/FiO₂ and APACHE II scores of all ALI patients. An exploration of the effect of TIRAP SNPs and haplotypes on mortality in ALI patients showed non-significant associations between survivor and non-survivor groups. These data were not shown in our manuscript.
Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests'
Reviewer's report 4
Title: Genetic variations in the TIRAP gene are associated with increased risk of sepsis-associated acute lung injury
Version: 1 Date: 16 August 2010
Reviewer: Thomas Hawn

Reviewer's report:
This study examines where polymorphisms in Mal/TIRP are associated with ALI or sepsis in China. The main finding of the association of 2 TIRAP SNPs with ALI is novel and adds to the literature on disease associations of polymorphisms in this gene. The strengths of the paper are a well-defined case group of the same ethnicity with convincing and statistically associations for the primary endpoints. Weaknesses include unclear rationale and detail for the adjusted analysis and the haplotype analysis.

Major Compulsory Revisions:
1. The main finding is that 2 SNPs (rs595209 and rs8177375) are associated with ALI when compared to healthy controls or sepsis alone patients. The unadjusted data are convincing with statistically significant differences. The rationale and details for the adjusted analysis are not well articulated. The data is adjusted for age, gender, BMI, APACHE score, diabetes, liver cirrhosis, and smoking history. Several questions need to be addressed to clarify this analysis: A. what is the rational for choosing these 7 variables? Although age and gender are commonly done, the others are not. Are the other 5 variables recognized as established risk factors for ALI? Given that none of these variables had a different frequency among the 3 groups (table 1), the rational for adjusting is further questioned. B. By definition, the healthy controls have no history of diabetes, cirrhosis, or APACHE scores. These variables cannot be adjusted for when comparing ALI to controls.

   Previous studies had reported that other 5 variables (BMI, history of smoking, diabetes, liver cirrhosis and APACHE II score) showed association with ALI, therefore we included the variables as covariates. We added the reference in the text.

   When comparing ALI patients to sepsis alone patients, age, gender, BMI, history of smoking, diabetes, liver cirrhosis and APACHE II score were included in the multivariate models because of their established association with ALI. When comparing ALI patients to healthy controls, age, gender, BMI and history of smoking were included in the multivariate models.

2. Haplotype analysis: A 2 SNP haplotype analysis is performed on rs595209 and rs3802813. The rationale for selecting these 2 SNPs is not clear since they have the highest r-squared value of all of the 5 SNPs. A more informative analysis would be to do a 2 SNP haplotype of rs595209 and rs8177375. These are the 2 SNPs associated in a single SNP analysis and they have an r-squared of 0.17—so there is potential to have independent effects that would be further illuminated with a haplotype analysis. The current haplotype analysis could be replaced with an rs595209-rs8177375 analysis.

   We have removed the analysis and added the two-locus haplotype analysis involved the two associated SNPs (rs595209 and rs8177375) as suggested.
Minor essential revisions:
1. Case definition: The definitions based on consensus guidelines are appropriate. A brief summary of the details would be helpful.
   We have given a brief summary about the details in supplementary files.

2. Healthy controls: More details would be helpful. Where were these individuals enrolled? Community, outpatient clinic, inpatient setting? Were they given a health history questionnaire? They have no “recent acute illness or any chronic illness …” Do any have a history of a serious acute illness? It seems unlikely that they could have a history of ALI or sepsis due to the low frequency of these conditions—but it also appears that the case definition would not exclude this possibility. Further details would help clarify this.
   We have given a further description of healthy controls in the methods: Sex- and age-matched controls were selected from healthy blood donors. Questionnaires including smoking, chronic illness and the history of ALI or sepsis were obtained from all control subjects. Healthy controls were defined as individuals without any recent acute illness, any chronic illness and a history of ALI or sepsis.

3. Table 3: The Bonferroni correction notation in the table footnote is not clearly annotated. Which SNPs remain significant after correction?
   We revised the footnote as follows: A $P$-value of < 0.01 (0.05/5) was considered statistically significant after Bonferroni correction. Rs595209 and rs8177375 remained significant after Bonferroni correction.

Minor discretionary revisions
1. Tables 2 and 3 could be combined to save space.
   We moved Table 2 in the supplementary file.

Level of interest:
An article whose findings are important to those with closely related research interests

Quality of written English:
Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests