Author's response to reviews

Title: Replication of Associations between Three Positionally Cloned Asthma Candidate Genes and Asthma or Asthma-related Phenotypes in A Chinese Population

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

We are grateful to the Journal for the interest in our manuscript entitled “Replication of Associations between Three Positionally Cloned Asthma Candidate Genes and Asthma or Asthma-related Phenotypes in A Chinese Population” and to the reviewers for the thoughtful and comprehensive reviews. Based on the comments of the reviewers, we have revised the manuscript. The changes made in the revised version of the manuscript are marked in red with underline. Our point-to-point responses to the reviewers are summarized.

Response to the editor:

Informed consent must also be documented. Manuscripts may be rejected if the editorial office considers that the research has not been carried out within an ethical framework, e.g. if the severity of the experimental procedure is not justified by the value of the knowledge gained.

Response: Written informed consent was explained to, read and signed by each participant. We have updated this information in Study Population and Phenotype Definition of the Methods section (Page 6, Paragraph 2). A copy of the informed consent can be provided upon request.

Response to Reviewer 1:

The aim of this study was to test the associations between three positional cloned genes and asthma or asthma related phenotypes in a Chinese population. They studied PHF11, DPP10 and HLA-G. The introduction is of interest but probably all not needed.

Response: We have revised the Introduction section by shortening the description of the discovery of the six positional genes and strengthening the current gap on the studies between these genes and the risk of asthma in Asian population (Page 3, Paragraph 2).

The study population and phenotype definition was reviewed. Apparently 2752 index families which included both parents and 2+ offspring who were 8 or older with physician diagnoses of asthma were studied. 270 reference families were randomly selected from the area. The routine data obtained from each patient was selected. The problem of the original study regarding standardization, environmental variables, etc were touched upon by the original paper but not adequately answered.

Response: As mentioned in our previous study [Xu et al., 1999, Am J Respir Crit Care Med], standardized questionnaire (modified ATS-DLD) was used to assess respiratory history and symptoms, occupational and smoking histories, home environment, family history of asthma, and other chronic or genetic diseases for each participant. Distributions of several environmental factors, such smoking status and education level, were also presented in our previous study. Environmental factors play important roles in asthma development. However, the major aim of
the current study was to seek replication of previous reported genetic associations in which gene-environmental interactions (GxE) were not examined. As a result, we did not include GxE in our current study.

The marked decrease in number of that study is not explained. Did this bias their results? They started with 2752 families who I assume had asthma and ended up studying only at a maximum of about ½ of them is a problem.

Response: The phenotypes (BHR, asthma, high IgE, skin prick test) of the reported associations to be replicated in the current study vary. To improve the efficiency and to accommodate different asthma-related phenotypes, we used a case-control design that incorporated a composite case-control definition. Please refer to the Study Population and Phenotype Definition of the Methods section for the definition. Some families did not have members meeting the case or control definition. Furthermore, to ensure the independence among selected cases and controls, only one subject would be selected from families with multiple qualified cases or controls. As a result, a total of unrelated 1183 sample, including 543 asthmatic cases and 640 non-asthmatic controls, were selected from 3022 families. The aim of our study was to seek for replication of the associations, not to estimate the effect size. So estimation bias should not be a concern in this study.

Results: The phenotypic information between the asthmatic sample and the non asthmatic samples is eluded to latter in the text but is still bothersome.

Response: Due to our “extreme sampling” design and the correlations between asthma-related phenotypes and asthma affection status, the distributions of asthma-related phenotypes, including % predicted FEV1, BHR, total IgE, and positive skin reactions, were well separated between asthmatic and non-asthmatic samples, as shown in Table 1. There were slight but statistically significant differences between asthmatic and non-asthmatic samples in terms of age, height, weight and BMI. We have demonstrated in the Discussion section that these factors were not correlated with the genetic variants (Page 13, Paragraph 2); thus, they would not confound the associations between genetic variants and the studied phenotypes.

First PHF11 was evaluated. Certainly the results are not overwhelming especially in view of the phenotypes used. DPP10 was stronger and of interest. Finally the HLA-G was not strong.

Response: Although we did not find any significant associations between PHF11 and asthma, the gene was nominally associated with positive skin reactions in our current study. We acknowledged that this association was mild, considering it was not significant after the adjustment for multiple testing. We also updated the Abstract and Result sections accordingly (Page 2; and Page 9, Paragraph 2). However, the result for PHF11 was still interesting, since this gene has been shown to be associated with atopic dermatitis in a previous study [Jang et al., 2005, Genes Immun]. We agree with the reviewer that the associations of DPP10 were stronger and of interest. More importantly, the associations of DPP10 remained significant after the adjustment for multiple testing. We also demonstrated that HLA-G haplotype was significantly
associated with asthma + positive MTCH challenge, although only marginal association was detected in single-SNP analysis.

The discussion tries to validate their methods and conclusions. The problem of type 1 errors and stratification of the population are problems that will not go away with a few statements.

Response: As shown in Statistical Analyses of the Methods section (Page 8, Paragraph 2), we used a permutation-based procedure, which accounted for the number of all the tested genetic variants and phenotypes within each gene, to adjust inflated type I error rate. We reported adjusted association results for each gene in the Results section. The association of PHF11 was significant only at the nominal level, and became not significant after the adjustment; while the associations of the SNP and haplotype in DPP10 and the association of the haplotype in HLA-G were significant even after the adjustment.

All of our study samples were from a rural area of Anqing, Anhui province of China, a relatively homogenous population with very limited population influx. In a recent asthma candidate gene study we conducted [Hong et al., 2009, Eur Respir J], 119 SNPs from 105 genes were tested for associations with asthma using 170 asthmatic cases and 347 controls selected from the same 3022 families where the current study subjects were sampled. We applied STRUCTURE2.1 to assess the presence of population substructures within cases, controls and all subjects combined, respectively, using a set of 111 SNPs after excluding potentially associated ones (p < 0.05 in association tests). STRUCTURE indicated a single substructure had the highest likelihoods among all possible number of substructures in the samples. So we believe population stratification is unlikely to be an issue in our study.

The policy in China of family size is not adequately addressed in this paper.

Response: The current study was population-based, not family-based. We did not expect that the policy of family size in China was relevant and would affect the study design.

What bout the sex of the asthmatics etc. As there were actually a large number of females at the time of this paper I believe being adopted this may have added a bias in the ascertainment of subjects. This is not mentioned and should be discussed.

Response: As shown in Table 1, there was no significant difference between asthmatic and non-asthmatic samples in terms of gender and there were about 50% males in each group. However, we still include gender as a covariate in the regression models to minimize its potential confounding effects. Additionally, we performed gender-specific analyses and found similar genetic effects between males and females. Therefore, gender did not bias our results. We have updated this information in the Discussion section of the revised manuscript (Page 13, Paragraph 2).

Their rational of statistical analyses was of interest but did contain some leaps of faith and should have been better documented.
Response: The aim of the current study was to replicate previously reported genetic associations. Therefore, for each gene only relevant phenotypes were examined. Alternatively, all the available asthma-related phenotypes can be tested for each gene. However, this will increase the multiple comparison penalties, and we will probably not be able to detect the genetic effects since a lot of them are only small to moderate. We are not quite sure what the reviewer referred to that “did contain some leaps of faith and should have been better documented”. A more specific comment would be helpful.

In the analysis, we first examined the genetic associations with asthma and asthma-related phenotypes, individually. It is important, because asthma-related phenotypes and asthma may not share the same biological pathways, and there could be different genes underlying them. We then further combined dichotomous asthma-related phenotypes and asthma status to create more extreme cases and controls. This was based on the fact that combining asthma-related phenotypes with physician's diagnosis of asthma might lead to a stronger association to the asthma candidate genes [Van Eerdewegh et al., 2002, Nature; Kormann et al., 2005, Am J Respir Crit Care Med]. The new cases and controls were more genetically homogeneous and studying them will increase the power to detect genes predisposing to both asthma-related phenotypes and asthma. Therefore, our rationale of statistical analyses was valid.

It is an interesting paper but certainly not an overwhelming replication.

Response: Our study was the first one to demonstrate that SNPs or haplotypes in PHF11, DPP10, and HLA-G are associated with phenotypes such as skin prick test, BHR, and BHR asthma, in a Chinese population. The associations for DPP10 and HLA-G were strong and significant even after the adjustment for multiple testing.

Response to Reviewer 2:

The paper by Zhou et al. describes an association study between three positionally cloned asthma candidate gene polymorphisms and asthma/asthma-related traits. They confirmed the association of these polymorphisms with asthma/asthma-related traits in the Chinese population. This is a simple candidate gene association study and generally well-conducted. I have following comments.

The authors selected three asthma candidate genes for the replication. A large scale replication study for asthma was conducted and published recently (Daley et al, 2009, Hum Genet). To make the study more valuable, I suggest the authors to genotype other candidate genes.

Response: We agree with the reviewer that genotyping more candidate genes can make the study more valuable. Actually, we recently have reported a large-scale candidate gene study in Our Chinese cohort, which systematically examined the associations of known or suspected novel candidate genes with asthma and asthma-related phenotypes [Hong et al., 2009, Eur Respir J]. To complement these reported results, the aim of this current study was to replicate the associations of positionally cloned asthma candidate genes. At the time we conducted this study,
only six genes were positionally cloned and three of them have already been studied in Asian populations. Therefore, we conducted the current study and genotyped the polymorphisms in the other three genes. Our results on these three genes were different from the results reported by Daley et al., since they found no associations in Caucasian population. We have updated our Introduction Section by citing the study by Daley et al. (Page 4, Paragraph 2).

Thank you for your reconsideration of the manuscript. We are looking forward to hearing from you.

Sincerely,

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