Author's response to reviews

Title: Obesity-associated gene FTO rs9939609 polymorphism in relation to the risk of tuberculosis

Authors:

Yan Fen (fengyanlanhe@163.com)
Fengliang Wang (xianqu1981@126.com.cn)
Sangsang Qiu (gssyky@126.com.cn)
Jieqiong Lv (ljqljy@163.com.cn)
Ruobing Xu (xuruo_bing@126.com.cn)
Liang Wu (tiger-500@163.com.cn)
Jianming Wang (jmwang@njmu.edu.cn)
Cheng Lu (lucheng66@126.com.cn)

Version: 2
Date: 23 August 2014

Author's response to reviews: see over
Dear editors and reviewers,

We thank the reviewers for their thorough review and constructive comments regarding our manuscript titled “Obesity associated gene FTO rs9939609 polymorphism in relation to the risk of tuberculosis”. We appreciate that the reviewers found this is a potentially important study. We have carefully revised our manuscript according to the comments and suggestions. The following are the point-by-point responses to the reviewers’ questions.

**Reviewer: Adong Shen**

1. **Supplement these results including BCG vaccination, positive tuberculin skin test, radiographic features of TB, culture-positive TB in Table 2.**

   **Reply:** We have added the sputum smear and sputum culture results in Table 2. In China, BCG vaccine has been routinely used. The general population has a high proportion of BCG vaccination history. Thus, we did not collect the data on the BCG vaccination history due to its higher coverage rate in the study area. The main drawback of the TST (tuberculosis skin test) is the lack of specificity due to cross reactivity with proteins present in other mycobacteria such as the BCG vaccine strain, *M. avium* complex organisms and other non-tuberculous mycobacteria. In China, TST is not routinely performed to screen and diagnose TB. Not all study subjects recruited in this study have TST results.

2. **The classification of disease with 1583 cases can be described in an independent Table, such as Primary pulmonary tuberculosis, infiltrative pulmonary tuberculosis, tuberculous pleuritis, et al. So a further stratified analysis may be carried out according to the results of the classification of**
disease with 1583 cases.

Reply: All TB cases recruited in this study were primary pulmonary tuberculosis. Thus we didn’t carry out a stratified analysis according to the clinical classification of disease.

3. The author can carried out some function assays about the expression levels of FTO in lymphocytes with different genotype, and the plasma cytokine levels of subjects with different genotype.

Reply: Both of these two SNPs rs9939609 and rs8050136 are located in the intron region. To determine the potential functional effect of the SNP, we used the prediction tools SNPinfo (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm) and RegulomeDB (http://www.regulomedb.org/). The RegulomeDB showed that rs8050136 was the binding site of transcription factor (TF) EP300. We also searched previous functional studies on the SNP rs9939609. Qi et al. reported that rs9939609 was associated with lower plasma adiponectin (log[e]--means, 1.82 +/- 0.04, 1.73 +/- 0.03, and 1.68 +/- 0.05 for TT, TA, and AA genotypes, respectively; P for trend = 0.02) and leptin (log[e]--means, 3.56 +/- 0.04, 3.63 +/- 0.04, and 3.70 +/- 0.06; P for trend = 0.06) in diabetic women (Qi L, Kang K, Zhang C, et al. Fat mass-and obesity-associated (FTO) gene variant is associated with obesity: longitudinal analyses in two cohort studies and functional test. Diabetes. 2008, 57(11):3145-51).

4. What’s the haplotype of these two SNPs?

Reply: As recommended by the reviewer, we further performed a haplotype analysis. Linkage disequilibrium (LD) was found between these two SNPs of FTO (D’=0.98, r²=0.94). In comparison with the common rs9939609T-rs8050136C haplotype,
rs9939609A-rs8050136C showed a significantly increased risk of TB (OR=6.09, 95% CI: 3.27-12.34) (Table 5).

Reviewer: Thorsten Thye

1. The sample size is sufficiently powered with 1625 TB cases and 1570 controls to demonstrate statistical significance. However, the authors include people with proven alcohol intake. Alcohol is a well-known predisposing factor for TB, and people with alcohol consumption should be excluded from the analyses.

Reply: Yes, previous studies have demonstrated that alcohol drinking is associated with TB, which might be a confounder. There are four common methods to control such a confounder, including subject limitation, matching, stratification and multiple logistic regression analysis. In this study, we used a logistic regression model to adjust the potential confounders including alcohol consumption. In Table 3 and Table 4, we have listed the crude OR(95% CI) based on the univariate analysis and the adjusted OR(95% CI) based on the multiple logistic model.

2. A major concern with this analysis is the strong deviation from the Hardy-Weinberg Equilibrium of the genotypes of SNP rs9939609. With the presented genotype counts of 1258 x TT, 253 x TA and 72 x AA in the case group the calculated deviation from HWE is P=10-27. This strong deviation might indicate either a selection bias or genotyping errors? Therefore the authors should verify the genotyping results for this SNP with a different method.

Reply: Testing Hardy-Weinberg equilibrium (HWE) in the control group is commonly used to detect genotyping errors in genetic association studies. HWE is generally expected to be distorted in the case sample in the region of association.
Wittke-Thompson, Pluzhnikov, Cox (2005) suggest that if a DHWE (departure from Hardy-Weinberg Equilibrium) in cases or in both cases and controls is detected, it does not necessarily imply genotyping errors. Rather than discarding the data, the underlying disease-genotype association should be investigated. The association may explain the observed DHWE. If not, other possible explanations such as “genotyping error, chance, failure of assumptions underlying Hardy-Weinberg expectations” should be explored (Yu C, Zhang S, Zhou C, Sile S. A likelihood ratio test of population Hardy-Weinberg equilibrium for case-control studies. Genet Epidemiol. 2009, 33(3):275-80).

To avoid batch bias in genotyping, we allocated DNA samples of cases and controls in each plate with no discrepancies between amplification reaction conditions. We randomly selected 5% samples to re-genotype and the consistent rate was more than 99%.

**Editorial requirement:**

**Requesting copyedit.**

**Reply:** We have our manuscript copyedited by American Journal Experts (http://www.aje.com/en). The document was edited for grammar, spelling, vocabulary, and phrasing.