Reviewer's report

**Title:** Polymorphisms in IL-1beta, Vitamin D Receptor Fok1, and Toll-like Receptor 2 Are Associated with Extrapulmonary Tuberculosis

**Version:** 1  **Date:** 13 August 2009

**Reviewer:** Guy Brock

Reviewer's report:

The authors present a genetic association analysis of 24 extrapulmonary tuberculosis cases, 24 pulmonary tuberculosis controls and 57 PPD+ controls in two stages. The first stage consisted of 22 SNPs and 3 microsatellites which were previously associated with tuberculosis risk, and the second consisted of 613 SNPs in 26 candidate genes involved in tuberculosis pathogenesis. One potential concern with this study is the small sample size. MDR has been previously documented to have adequate power in sample sizes as small as 200 cases and 200 controls (Ritchie, Genet Epidemiol. 2003 Feb;24(2):150-7]. The sample sizes in this study, however, are much smaller, especially when considering just the EP cases (24 patients) vs the PPD+ controls (57 patients), and even MDR will have low power except for markers with large effect sizes. The authors do address this limitation in the Discussion and provide a table with detectable effect sizes for main effects. However, no statement is provided concerning the power to detect gene-gene interactions, which is typically smaller given the number of comparisons involved. Therefore, it is likely not surprising that MDR found no associations with EP tuberculosis in the 2nd stage of analysis. Further, sparse cell counts will be a concern for MDR when assessing 3-way and higher order interactions with these sample sizes (27 cells and only 24 cases). The authors should add an additional statement that addresses these specific limitations.

Another concern is in Table 5 – the average prediction error for this SNP is 52%, which is worse than a coin flip. It is a bit surprising that a SNP with this prediction error has a significant p-value.

Minor Essential Revisions

1. The number of black patients for the sub-analysis should be mentioned in text of the abstract and results, especially since black patients are actually a majority in this study.

2. There is no information on the % missing for each marker, or how missing genotypes were handled / imputed.

3. Table 4: Are the single-locus tests based on the 2 df chi-squared test (for SNPs), the trend test, or allele-test? This should be clarified in the methods. Also, results for significant SNPs should include more information such as
number and percent in each case/control group within each SNP genotype, and odds ratios with 95% CIs relative to a baseline genotype group.

4. In Table 5, the authors should specify whether the ‘Average balanced accuracy’ and ‘average prediction error’ is based on the CV training or testing data. Also, the CV consistency should be reported as a fraction, e.g. 4/10 and 3/10. Why were the MAF and HWE p-value included in the second portion of Table 5 and not the first?

5. The footnote to Table 5 mentions a post-hoc logistic regression analysis of the 2 significant SNPs found be MDR, but gives no details other than the model ‘appears’ to be recessive for both SNPs. It would be more informative for readers to see this model to understand exactly how these two SNPs are interacting with each other.

6. The lower detectable odds ratio in Table 6 is redundant, and should be removed to avoid the value ‘0.00’ which is misleading.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests