Author's response to reviews

Title: Analysis of eight genes modulating interferon gamma and human genetic susceptibility to tuberculosis: a case-control association study

Authors:

   Marlo Moller (marlom@sun.ac.za)
   Almut Nebel (a.nebel@mucosa.de)
   Paul D van Helden (pvh@sun.ac.za)
   Stefan Schreiber (s.schreiber@mucosa.de)
   Eileen G Hoal (egvh@sun.ac.za)

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

MS 2074982708353219 : Analysis of eight genes modulating interferon gamma and human genetic susceptibility to tuberculosis: a case-control association study

Marlo Möller, Almut Nebel, Paul D van Helden, Stefan Schreiber, Eileen G Hoal

We appreciate the opportunity to revise our manuscript and thank the reviewers for their comments and suggested changes. We think this has improved our manuscript significantly. Please find the reviewers’ comments and our responses and changes listed below. Changes were done in red text in the manuscript file.

List of changes

Editor

1. Please could you also confirm that you obtained informed consent from the subjects included in the study, and include a statement to this effect in your methods section.

Author Response: Informed consent was indeed obtained from all the individuals included in the study. We have added this statement to the methods section of the manuscript (page 5).

Reviewer 1

1. This paper presents a thorough analysis of 8 candidate genes hypothesized to influence IFN-γ response, in their association with TB disease in a South African population. This is a relatively
large study, which is one of its strengths. The conclusion of the abstract states that this study “highlights the importance of using larger sample sizes” – this point should actually be further emphasized in the text, because it is a very important point and likely explanation for discrepancy of results across published studies.

**Author Response**: We have done this and have added the following to the discussion: “Some of those associations were based on extremely low sample numbers (which could lead to false positive associations) or did not correct for multiple testing. Underpowered studies may not detect effect sizes that are small, but reasonable considering the current understanding of the host genetics of complex diseases” (page 13, line 14)

**Minor Essential Revisions**:

2. Most importantly, this study makes the assumption that all the individuals in the study population have latent *M. tuberculosis* infection, based on the high incidence of TB in South Africa. However, studies have shown that some individuals, albeit a minority, do not acquire latent *Mtb* infection despite prolonged and persistent exposure to infectious TB cases. Thus, it is not appropriate to state that this study examined the association of these SNPs in “progression from latent infection to active disease”, and it is a limitation of this study that tuberculin skin testing or interferon-gamma response assays were not conducted to test for latent *Mtb* infection.

**Author Response**: We agree and we have changed this statement to the following: “Our study was more likely to test the possible associations of the SNPs with TB progression from latent infection to active disease only, but we cannot rule out the possibility that some controls were not tuberculin skin test (TST) positive as TSTs were not done. However, our previous study of healthy children and young adults from the control community found that 80% of children older than 15 years had positive tuberculin skin tests, an indication of latent infection with *M.tuberculosis*”. (page 14, line 4)

The majority of the control population is therefore TST positive, and with the average age of the control in this study being 27 years, we estimate a TST positivity of ~90%.

3. The power analysis presented is unclear. In the Methods section, a power analysis is presented, stating that there is 80% power to detect an odds ratio =2.15. (What is meant by 95% confidence – is this an #=0.05? This should be rephrased.) Later, in Additional Table 2, there
are power calculations provided with many different odds ratios. Why are these odds ratios smaller than 2.15? This should be clarified.

**Author Response**: We have rewritten this section to clarify the power calculations: “Since the allele frequencies of the polymorphisms analysed in this study were not known for the SAC population prior to the completion of genotyping and were necessary for power calculations before starting the experiment, we estimated from prior data that each SNP would at least have a minor allele frequency of 5% in our study group. Given this assumption we had 95% confidence (alpha error p = 0.05) and 80% power (beta error = 0.2) to detect an odds ratio of at least 2.15 with the number of samples available (432 cases, and 482 controls). After genotyping, power calculations were done with the experimentally determined allele frequencies of the SNPs previously associated with tuberculosis to confirm that we had enough power to exclude the previously reported genetic effect sizes in the SAC population.” (page 6)

To clarify the many different odds ratios, we have changed the heading “Odds ratio” to “Odds ratio theoretically detectable” and revised footnote “e” in Additional Table 2.

4. Additional Table 1 is a very nice table. The discussion of it is a little spotty – some SNPs are emphasized, others not so much (and study populations not mentioned). Also, the Uganda study did include a haplotype analysis, and found an association between an IL10 haplotype and TB.

**Author Response**: We have added the haplotype analysis of the Uganda study to Additional Table 1 and have added the Uganda (page 10) and other studies not previously mentioned to the text (page 8 -11). We have also added paragraphs discussing genes not previously mentioned in the discussion (page 12).

*Minor discretionary revisions:*

5. It is unclear how WNT5A and FZD5 are involved in IFNg modulation. At least a reference for these genes should be provided.

**Author Response**: We have added a paragraph explaining the involvement of WNT5A and FZD5 in IFNg modulation (page 13)

**Reviewer 2**

*Minor essential revisions*
1. For readers not familiar with this type of genetic analysis, the text of the first sentence of the Abstract Results section on page 2 and of the Conclusions section on page 12 "which was not globally significant" should be qualified to make its meaning clearer to non-specialists.

**Author Response:** We have changed the abstract to: “A haplotype in interleukin 12B was nominally associated with tuberculosis (p = 0.02), but after permutation testing, done to assess the significance for the entire analysis, this was not globally significant.” (page 2)
The conclusion section was changed to: “We found a nominally significant association with an IL12B haplotype which was not considered to be globally significant after permutation testing to determine the significance for the entire analysis.” (page 14)

2. The Conclusions section of the Abstract should also be qualified as to why larger sample sizes are needed (presumably to obtain clear evidence that a particular association is or is not present in a particular population). The text on page 11 gives a better sense as to why previously reported associations may not be valid, such due to small sample size, a lack of stringent correction for multiple testing as used here, or a lack of association with reactivation rather than primary disease in a very highly endemic setting, but also highlights the possibility of ethnic-specific associations, which must remain a possible explanation for such differences.

**Author Response:** We have adjusted the abstract accordingly: “This study highlights the importance of using larger sample sizes when attempting validation of previously reported genetic associations. Initial studies may be false positives or may propose a stronger genetic effect than subsequently found to be the case.” (page 2)

3. On page 4, some additional references might be added in addition to ref 18 to back up the statement that “current experimental data suggest that IFNγ is the best correlate of protection against TB”, as well as a few references to some of those studies in other species such as mice and cattle that do not support the hypothesis that magnitude of the IFNγ response can be used as a correlate of protection, even if its production is necessary for control of infection.

**Author Response:** We have changed the sentence and added additional references: “Some experimental data have suggested that IFN-γ is a correlate of protective immunity against TB, although other studies in humans, mice and cattle do not support this.” (page 4)
We hope these changes meet with your approval.

Yours sincerely

Dr. Marlo Möller

Molecular Biology and Human Genetics, Faculty of Health Sciences, Stellenbosch University
P.O. Box 19063, Tygerberg, 7505, South Africa
Tel: +27-21-938-9403, Fax: +27-21-938-9476, e-mail: marlom@sun.ac.za