Author's response to reviews

Title: Innate immunity in ocular Chlamydia trachomatis infection: contribution of IL8 and CSF2 gene variants to risk of trachomatous scarring in Gambians

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Author's response to reviews: see over
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Dear Dr Zauner,

Re: MS: 5242425472635317 “Innate immunity in ocular Chlamydia trachomatis infection: contribution of IL8 and CSF2 gene variants to risk of trachomatous scarring in Gambians”

Thank you for your letter with the enclosed referees’ comments. We are grateful to the editor and referees for their thoughtful and encouraging reviews.

Please find below our responses to the referees’ questions/comments.

REFEREE #1

What do the authors feel the significance of trichiasis in addition to scarring is of interest in this context? Are additional pathophysiologic mechanisms implied? I wouldn’t think so.

The rationale for stratifying genetic analyses of the severe sequelae in trachoma by clinical phenotype is as follows:
1) trichiasis may be less subject to clinical misclassification than scarring trachoma. Therefore a case-control study of trichiasis may have more power to detect associations than one of scarring alone.
2) it is plausible that molecular mechanisms operate in the progression from scarring to the more severe phenotype of trichiasis which are additional to those which initiate scarring. Accordingly, a stratified analysis by clinical phenotype will identify genetic risk factors that are exclusive to the development of trichiasis.

We acknowledge that this rationale may have been less than clear in the manuscript. A sentence has been inserted in the discussion to clarify this point:

“The genetic risk effects for scarring and trichiasis observed in this genomic region are similar, which suggests that the above molecular mechanism may be common to the development of both phenotypes, rather than implicated in the progression of scarring to trichiasis”

Why were there two phases? Related to that, why the epidemiologic and clinical differences (different ethnic mix and the much lower prevalence of trichiasis (so maybe I am wrong and trichiasis has separate implications from scarring, but I don’t see that reflected in the genetic analyses.)
The 2 phases described in the methodology and result sections of this manuscript refer to the genetic strategy followed to map disease genes for severe trachoma. In the first phase we took advantage of the increased power of LD mapping approaches to fine-map a risk candidate locus to a large genomic region using a relatively small study sample size. In the second phase of the study, each observed risk locus was subjected to extended genotyping in a larger sample of cases and controls. This larger sample included both cases and controls from phase one plus additional case-control samples, in order to increase power to detect a true disease association. As the referee points out, just by chance, phase 1 case-control samples did not include one of the four main ethnic groups in the Gambian population, the Fula. The lack of Fula case-control samples during this study phase might have resulted in a reduced power to detect risk effects at these loci if they were exclusive to this ethnic group. However this is unlikely, because the genetic architecture observed in one of the 2 genetic regions studied here has been found to be highly conserved in both African and non-African ethnic groups (reference: D. Kwiatkowski lab and HAPMAP data). We think this is clear from our description in the methods.

Disease association analyses were performed among the pooled case-control study sample (phase two) in which the four main ethnic groups present in The Gambia are appropriately represented. There is no evidence to date to suggest that studies in the Gambian population ought to be routinely stratified by ethnicity and therefore we might conclude that our study allows the generalization of results across the ethnic groups. The differences in the proportion of trichiasis cases in phases one and two is due to the increased difficulty in recruiting study subjects suffering from the most severe phenotype of trachoma. The implication of this is that the power to detect risk effects for trichiasis is variable in each study phase. The rationale for the distinction between scarring and trichiasis is dealt with above.

This study used a case-control design with pair-matching by known risk factors for trachoma (age, sex and location). The subsequent conditional regression model therefore adjusts the estimates for the effects of confounding by these factors. In addition, because of matching by ethnicity and village of residence, the model should control for unknown risk factors such as socio-economic and behavioural factors linked to village of residence and/or ethnicity. Treatment for active trachoma in The Gambia is organised at village or district level through interventional programs aimed to control the disease. Therefore the matching by age and village of residence should have eliminated differences between cases and controls related to different treatment experiences. In addition these programmes are relatively recent and therefore unlikely to have affected the development of scarring among adults due to recurrent episodes of infection in their childhood. To clarify this we have added this sentence in the discussion:
We are not aware of risk factors associated with younger onset of disease and with the IL8-251 genotype that could explain the increased risk effects for scarring at younger age among carriers of the IL251 risk allele. It is well known that in endemic settings the severe phenotypes of trachoma emerge in an age dependent way, with the prevalence of scarring and trichiasis increasing with age and more commonly seen in older adults. Those subjects who develop scarring at younger ages may represent more extreme examples of the phenotype. We therefore argue in the discussion that this study gives support to a potential ‘early scarring’ phenotype and show that, in an analysis stratified by age, the functional promoter variant IL8-251 is associated with a significantly increased risk of scarring among the youngest subjects.

**REFeree #2**

Discretionary Revisions

1: I have only one minor revision. The use of spaces in the 'minor allele' column (table 4) makes the text appear jagged.

Table 4 has been amended accordingly.

Following the Editorial request, a section heading 'Methods' has been included in the abstract. It reads as follows:

Methods: LD mapping was used to investigate risk effects across the 4q and 5q31 cytokine clusters in relation to the risk of scarring sequelae of ocular *Ct* infection. Conditional logistic regression (CLR) analyses were used to assess disease association and epistasis in a population based study of 651 case-control pairs.

We hope that the answers to comments and manuscript changes address the concerns of the referees/editor and fully clarify our submission and that the paper is now acceptable for your further consideration for publication in BMC Medical Genetics

Yours Sincerely