Author's response to reviews

Title: Genetic and functional evidence for a locus controlling otitis media at chromosome 10q26.3

Authors:

Marie S Rye (mrye@ichr.uwa.edu.au)
Elizabeth S.H. Scaman (lizzie_scaman@hotmail.com)
Ruth B Thornton (rthornton@meddent.uwa.edu.au)
Shyan Vijayasekaran (shyan@entkids.com.au)
Harvey L Coates (harveyc@cyllene.uwa.edu.au)
Richard W Francis (rfrancis@ichr.uwa.edu.au)
Craig E Pennell (cpennell@meddent.uwa.edu.au)
Jenefer M Blackwell (jblackwell@ichr.uwa.edu.au)
Sarra E Jamieson (sjamieson@ichr.uwa.edu.au)

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Author's response to reviews: see over
The Editors
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Dear Editors

Re: Reply to Reviewer Comments – MS: 1131937485828818

Genetic and functional evidence for a locus controlling otitis media at chromosome 10q26.3

Thank you for your e-mail dated 09/05/2013. We have addressed the reviewers’ comments for this manuscript as follows (reviewer comments in italics).

Reviewer: Elisabet Einarsdottir

1. The methods section is very long, and detailed, which I appreciate. The Illumina 660W-Quad data has been described previously, but maybe a very short summary of the QC and Imputation steps performed on that dataset would be informative.
   • A summary of the QC and imputation methods used has been added to the Methods section on page 8.

2. Did the authors do any CNV analysis on the region? I believe there is some CNV data in the 660W-Quad chip (if I am wrong, disregard this comment), would have been interesting to see if more information could have been squeezed out about the region.
   • The reviewer is correct, the Illumina 660W Quad Beadchip does contain probes that tag known CNV regions. Specifically in the 10q26 region there are around 560 probes that tag known CNVs. However, this CNV data has not been subjected to the stringent calling procedures required to define the CNV breakpoints by the Raine Study Team. As such we do not have access to this data for analysis.

3. The chromosome 10 locus was not identified in a recent GWAS by the same authors. It would be interesting to see a short discussion of this.
   • None of the SNPs within this 10q26.3 region, or indeed from any other region, achieved genome-wide significance in our recently published GWAS paper. In view of this we presented the results for the top 25 SNPs identified via our GWAS. However, as outlined in our GWAS paper there were several other SNPs/regions that showed association at $P_{adj-PCA} < 10^{-5}$ but limitations on manuscript length prohibited...
us from discussing all of these regions. The SNP rs7922424 reported here was identified by our GWAS but it was ranked at number 29 in the fully adjusted analysis and so was not included in the presented tables. However, brief results from a gene based analysis across this chromosome 10 region were presented and discussed in the GWAS paper. In the work presented here we have now replicated the previously reported linkage in the 10q26 region providing greater confidence that this region does indeed harbor an OM disease susceptibility locus. Therefore, we now present greater detail on the association analyses undertaken across this region.

Reviewer: Carol J MacArthur

4. The population is largely Caucasian, but does include about 7% non-Caucasian. This may add genetic variability of allele frequency and SNP variation. Please discuss this limitation in the Discussion section.

• We have added information to the manuscript to detail how we took differences in population ancestry into account. Briefly, for the linkage analysis we repeated the analysis using only those families that self-identified as Caucasian and found that there was no substantial difference in results. This information has been added to the manuscript on page 12. For the Raine Study cohort we included the first two principal components of a PCA analysis as covariates to take account of population differences. This information was already provided in the manuscript in the Methods section (page 9). In view of this we do not believe that ancestry differences will have had an effect on the results of our study and as such does not represent a limitation that requires specific discussion.

5. The WAFSOM group was used for the linkage analysis for the 5 chromosome of interest in the study. This is a group primarily identified by otolaryngology/physician-diagnosed episodes of rAOM (greater than or equal to 3). However, the parents or siblings of the probands were also invited to participate if they had a history of tube insertion for COME as well as for rAOM. The mixture of the rAOM and COME phenotype may also dilute the ability to identify candidate genes. Please include a discussion of this limitation.

• We apologise if the phenotypic definitions we utilized for recruitment to WAFSOM (and for subsequent stratified analyses) were not clear in our manuscript. Briefly, we recruited probands with a history of tymanostomy tube insertion due to rAOM or COME. The patients recruited to our study were identified from the records of collaborating ENT specialists. Parents and full siblings with a history of recurrent disease, defined as ≥3 physician diagnosed episodes of AOM or tympanostomy tube insertion for rAOM or COME, were also invited to participate. We have updated the methods section to make this clearer. We would also like to clarify that the inclusion of patients with both rAOM and COME was done to match the phenotype used in the original genome-wide linkage publications that identified the regions we were replicating.

6. For the association mapping population, 35% of participants were qualified solely on the
basis of questionnaire only without ear exam or history of ear tubes, while the rest of the 65% were classified by significant ear exam findings and/or presence of tympanostomy tubes. Certainly, recollection of episodes of AOM by a parent is fraught with bias (in either direction). This population seems potentially more problematic than the WAFSOM group in that assurance of the phenotype is less secure. Please add this limitation of the study to the Discussion.

- We agree with the reviewer that parent recall of episodes can be problematic. However, in this case the parents were asked to complete a questionnaire every 12 months and so the recall of the number of episodes of OM was only over the previous 12 month period. This gives us greater confidence in the data. We have added information to the methods section (pg 6) to make this fact clearer. Nonetheless, the phenotype defined for the Raine Study cohort is a milder OM phenotype than that defined for the WAFSOM. This issue has been added to the discussion section.

7. In the Results section, Linkage Analysis, it is not entirely clear how the data was able to be stratified by rAOM and COME when the Methods did not describe collection of that demographic data. Yet data is presented in Table 1B for families and individuals stratified by rAOM and COME. To stratify into these two groups, actual physical examination to confirm COME diagnosis would add certainty to the phenotype identification. It appears from the Methods section that the vast majority of the WAFSOM population is recruited by rAOM phenotype, not COME phenotype. Please clarify.

- Please see response to point 5 above. Patients were recruited to WAFSOM on the basis of tympanostomy tube insertion for rAOM or COME as diagnosed by an otolaryngologist. We have updated the methods section (pg 5) to more accurately reflect the recruitment strategy. At the time of recruitment, information on the diagnosis (i.e. rAOM or COME) was ascertained and subsequently used for the stratified linkage analysis presented. The number of families that were included in the stratified analysis was already provided in Table 1 (B).

8. In the Results section, Expression analysis to support putative functional genes, the control group for the tonsil and adenoid tissue examination was tissue from patients undergoing adenotonsillectomy for OSD or RAT without OM history. In our previous work, we found that the adenotonsillectomy group with OSD was NOT an adequate control group for otitis-prone studies and that the SNP profile of the adenotonsillectomy (without OM history) matched that of the OM prone group (Carroll SR, Zald PB, Soler ZM, Milczuk HA, Trune DR, MacArthur CJ. Innate immunity gene single nucleotide polymorphisms and otitis media. Int J Pediatr Otorhinolaryngol. 2012 Jul;76(7):976-9. doi:10.1016/j.ijporl.2012.03.011. Epub 2012 Apr 9.). While adenoid and tonsil tissue is widely available due to the frequency of this surgical intervention, the authors may want to consider using another tissue, such as middle ear mucosa in the future (controls could be middle ear mucosa taken at the time of cochlear implantation).

- To date there is little information on the expression pattern of some genes in the 10q26 region in any tissues/cells that could be considered relevant to OM. Therefore our aim in carrying out the expression profiling presented in the paper was to add
data relevant to OM, including analysis of expression in tissue/cells that are part of the immune or respiratory systems. This includes adenoids, part of the secondary lymphoid system, which play a key role in the response to the respiratory pathogens implicated in OM pathogenesis. We have added a sentence to the Results (pg 14) to try and make this clearer. However, we do agree with the reviewer that extending this analysis to include expression profiling of the middle ear mucosa would be the next logical step but not one we were able to undertake as part of this work as we do not have access to this tissue type.

9. In the Results section, in silico Comparative Genomics analysis of the TCERG1L to PPP2R2D region, I am not sure of the location of the data described in the last paragraph of that section. Please clarify.
   • In the last paragraph of this section we outline that none of the SNPs investigated (i.e. rs7922424, rs7087384 and rs7914323) fall within a CpG island. As such, we do not present the results as a table or figure in the paper. We have updated the paragraph to reflect this.

We thank the reviewers’ for these useful comments, and hope that our paper will now be acceptable for publication in BMC Medical Genetics.

Yours faithfully,

Sarra E Jamieson
For the authors