Reviewer's report

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

Version: 1 Date: 12 January 2013

Reviewer: Carol J MacArthur

Reviewer's report:

1. Is the question posed original, important and well defined?

The question posed in this paper is very important to an increased understanding of otitis media genetics. The question is well-defined. The study is a replication analysis of 5 chromosomes previously identified as potential susceptibility genes for otitis media. Replication studies are important to assess candidate genes for generalizability, independent verification and for identification of causal genes involved and pathway analysis

2. Are the data sound and well controlled?

The data are sound. This study approaches the question at hand from multiple levels: a) microsatellites were selected across each region of linkage previously identified. B) Association mapping utilized the dense SNP data from a longitudinal cohort of children identified as OM prone. C) Regression analysis was performed of the10q2 region after identification of this chromosome as the best statistical candidate. D) RT-PCR was performed on tonsil and adenoid tissue (in cases and controls) to identify the presence or absence of the four candidate genes in relevant tissue, as well as in epithelial and macrophage cell lines. The cell lines were exposed to bacteria to identify response to bacterial stimulation. E) Comparative genomics were used to identify conserved non-coding sequences and transcription factor binding sites potentially affected by associated SNPs.

The methodology of selection of cases/controls is well spelled-out. Controls are in place for the data.

3. Is the interpretation (discussion and conclusion) well balanced and supported by the data?

The Discussion and Conclusion are well balanced and supported by the data. (See #5 for requests of added discussion in the Discussion section)

4. Are the methods appropriate and well described, and are sufficient details provided to allow others to evaluate and/or replicate the work? Yes

5. What are the strengths and weaknesses of the methods?

Minor Essential Revisions: A few areas of limitation should be discussed in the Discussion section:
a. 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.

b. The WAFSOM group was used for the linkage analysis for the 5 chromosomes 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.

c. 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.

d. 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.

e. 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 or 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).

f. 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.

6. Can the writing, organization, tables and figures be improved?
No. The paper is very well written.

7. Are there any ethical or competing interests issues you would like to raise? No

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests