Reviewer's report

Title: Gene expression profiles and genotyping implicate TGF beta-1 and ALOX5 in multiple sclerosis pathogenesis.

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Reviewer: david dyment

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General

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

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BMC-Medical Genetics

Title: Gene expression profiles and genotyping implicate TGF beta-1 and ALOX5 in multiple sclerosis pathogenesis

Authors: Arthur AT, Armati PJ, Bye C, Southern MS Genetics Consortium, Heard RNS, Stewart GJ, Pollard JP, Booth DR

General comments:

The researchers perform an investigation to identify MS genes by screening for differentially expressed candidate genes. The gene promoter regions were then sequenced to identify common variants. A transmission disequilibrium (TDT) in two independent samples of MS families was performed to further implicate the selected genes.

The strategy has been used by these researchers before. A previous study in PPMS showed IL7R to be a susceptibility gene with modest clinical significance. The IL7R association has now recently been confirmed as a susceptibility gene in a series of high profile publications and the authors of this manuscript should be congratulated on their initial findings.

Comments for the editor (from reviewer instructions):

The questions and goals of the study are clearly defined. The methods appropriate and well described and are there sufficient details to replicate the study. With the exception of the website address (see below-and clearly an accidental oversight) the data are reported well. The manuscript is well-written and the discussion and conclusions well balanced and adequately supported by the data.

Overview of this study:
For this study, the gene expression profiles of 10 RRMS patients (in relapse) showed 989 up-regulated genes compared to a pooled sample of 25 controls. A further 536 genes were down-regulated. Another 10 RRMS (in remission) showed 655 up-regulated genes and 662 down-regulated genes compared to the pooled controls.

The findings were grouped by ontology by a program called Gostat. Not surprisingly inflammatory genes were up-regulated during relapse. Interestingly genes involved in protein transport genes were up-regulated in remission.

Two up-regulated genes were selected for further analysis; TGF#1 and ALOX5. Promoters were sequenced for variants and a TDT was performed in a sample of MS families. This was followed by a second TDT analysis in an independent group of trio families. The authors report a mild association for TGF#1 and no association for ALOX5.

Minor Comments:

1. I would suggest removing “genotyping” from the title as genotyping does not appear to implicate ALOX5 nor does it implicate TGF#1.

2. The researchers have decided to sequence only the promoter region. This is certainly an important region of DNA to sequence, especially given the hypothesis of differential expression. However, the identification of additional SNP’s in coding regions may have proved worthwhile given an altered function (versus altered expression). This may have been highlighted in TDT analysis. I mention this as the authors refer to linkage disequilibrium and a variant at exon 10 within the TGF#1 gene from the Green et al. publication.

3. It would be helpful for a sentence or two on the array system used, i.e. number of genes on the array and how were they selected. It appears as if there may be 10.5K (?) however when I visit the Peter MacCallum Cancer Institute for more detailed information, it is not readily available (at least not on my superficial search) if it is available, perhaps a more detailed web site address would be helpful.

4. Minor point-in the paragraph titled “Screening dysregulated genes for genomic association with MS”. I believe the Sawcer et al manuscript used over 4000 SNPs (versus 400) and over 2600 individuals from 730 families.

5. I am the first to admit, the correction for multiple testing can be considered overly conservative. However if an association is to be claimed, upon stratification, the correction should be appropriate. By my reckoning, in the Westmead sample, the authors tested the total, males, females and then DR2+ and DR2- as well as an older/younger age of onset group. The mild association in the first sample loses significance when all comparisons are included.

6. The second sample, when subtracted out from the first sample shows next to no over-transmission of the putative susceptibility allele. It would be helpful to see the counts presented as “First sample” Second sample" and, if the authors
so wished “Combined”. Also, age of onset, mentioned in the text, should be presented in Table format.

7. I am curious as to the results of IL7R ad IL2R in this studies expression analysis. This was not mentioned in the text. Granted it could have been at the website address mentioned above. Perhaps a sentence or two commenting on these results would be topical.

Major Comments:

1. The researchers used 4 males and 6 females in the remitting patients and 5 males and 5 females for their relapsing patients. For a control sample, the researchers pooled 5 males and 20 females. Since this is a study of differential expression, the authors need to account for any differences in genes expression between males and females; the MS sample is essentially 1:1 males to females and their controls is 4 to 1 females to males. If any gene is up/down regulated in females this may show as a false-positive.

2. The authors report their expression study results are available at http://www.wmi.usyd.edu.au/milinstitute/ms_data.htm. Unfortunately for the reader (and the reviewer) this site is not accessible. It would have been VERY helpful to see a complete listing of those genes shown to be up- and/or down-regulated.

3. I am curious about the gene selection based on expression results. ALOX5 was shown to be up-regulated in patients in relapse and remission while TGF#1 was up-regulated during relapse and not up/down-regulated during remission. Were there any genes up-regulated in relapse and down-regulated in remission? Would the authors consider these to be suitable candidates? I could probably answer this question but for the aforementioned inaccessibility to the results website.

4. The authors refer to the meta-analysis of the GAMES project as a justification for choosing TGF#1. The GAMES meta-analysis (ie ranking of p-values) did identify a microsatellite at 19q13 as showing a mild association across multiple studies. The GAMES meta-analyses, then went further and identified 10 genes within 250kb of D19S552. They sequenced two of these genes (MAP3K10 and AKT2) and unfortunately they were unable to show any association for these genes in a sample of 900+ UK trios. This should be considered weak evidence for the involvement of 19q13 and perhaps not the best justification for TGF#1. Perhaps this would be a good opportunity to demonstrate the GAMES methodology and show significant linkage disequilibrium between the SNPs at TGF#1 and the microsatellite D19S552(?)

5. TGF#1 and ALOX5 do not appear in the recent genome-wide case control study (International MS Consortium) recently performed nor are they included as possible candidates as listed by Gregory et al. (2007)-see supplementary material. When we further add the negative associations of TGF# (He et al 1998; Weinshenker et al., 2001), TGF#1 is not so compelling a candidate.
Overall:
The study is well performed by well respected researchers in the MS field. The author’s present evidence from their expression analysis and the literature to suggest ALOX5 and TGF#1 are involved in MS. This is likely correct and these genes are involved in the inflammatory process. However there are hundreds of genes identified in the author’s expression screen that may also qualify.

With regards to ALOX5 being a susceptibility gene the authors present a convincing negative TDT analysis in two samples. TGF#1 does show a trend to association in the first sample but when tested in a second sample the results are negative. Taken together I interpret the findings as negative with respect to TGF#1 genetic susceptibility.

It was a pleasure reviewing this manuscript for BMC-medical genetics.

Sincerely,

David Dyment
DPhil, MD

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.