Author's response to reviews

Title: An investigation of polymorphisms in the 17q11.2-12 CC chemokine gene cluster for association with multiple sclerosis in Australians

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Author's response to reviews: see over
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The Editorial Board
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To The Editorial Board,
Subject: An investigation of polymorphisms in the 17q11.2-12 CC chemokine gene cluster for association with multiple sclerosis in Australians

Thank you for this review of our manuscript and for the opportunity to respond to the comments of the reviewers. Overall, we believe that it is now a stronger manuscript.

Following is a summary of our responses to the comments and details of alterations made to the manuscript. The reviewers’ comments are copied verbatim below in quotation marks and our responses are bold and italicised to facilitate the review process. Changes have been underlined in the manuscript.

Reviewer: Bernadette Kalman

“Bugeja and colleagues performed a two-stage analysis in the 17q11.2-12 region. After sequencing several DNA pools of MS patients and controls, 48 known and 2 new SNPs were identified within the CCL genes, and served to define minor allele frequencies of markers. Twelve candidate SNPs were selected from this list based on functional considerations for further studies. Seven of these SNPs were genotyped by using the SNaPshot method in 204 MS trios, while 5 of them were genotyped by SNPlex in 373 MS trios. The investigators assessed the distribution of LD, defined the most common haplotypes and determined the minor allele frequencies using the 12 markers. Four individual SNPs showed borderline significance (0.05>p>0.02) in TDT, and without correction for multiple comparisons. A two-marker haplotype within CCL2 and CCL11 also showed transmission distortion, but with a p=0.04 and p=0.05 in the original and in an independent set of trio families, respectively.

“Comments:
“This is a well illustrated and clearly written study of the 17q11.2-12 region in Australian cohorts of MS families, which presents findings similar to those in two previously published studies of North-American Caucasian cohorts (Ref 42 and 65). Reinvestigation of this well justified candidate region in another (predominantly Caucasian) cohort is very important. However, the interpretation of data appears to be somewhat overstated considering the very weak outcome. Although the outcome was also modest in the
North-American cohorts, a marker between CCL2 and CCL7 approached the required p-value after correction for multiple comparisons using 232 SNPs, and transmission distortions of some haplotypes remained significant even after correction for 693 independent tests (reference 65). Nevertheless, as stated in the manuscript, the similar trend of findings is reassuring in the Australian and North-American studies. Another potential criticism concerns the use of relatively few markers (even considering the extensive LD), because of the structural complexity of this chromosome.

“Suggestions:
“1) Discretionary revision: Add a table to summarize the cohorts studied.”

We believe that the short summary of our cohort details in the Materials & Methods section is sufficient, and that addition of a further table, on top of the current four tables and two figures, would be excessive. Thus we have chosen not to make this Discretionary revision.

“2) Minor revision: Tune down interpretation of data; the paper will still remain an important observation and represent difficulties in complex trait disorders.”

We have made a substantial effort, in our belief, in the preparation of this manuscript, to make no claims as to the identification of significant results. However, a number of changes have been made to the language of the manuscript in a number of locations (underlined), to further prevent any over-statement of the results.

Reviewer: Richard M Ransohoff

“General
“This is an exemplary BMC manuscript, because it's interesting, well done, lucidly presented and contains information that will be useful beyond the immediate community of MS-genetics or chemokine-genetics researchers. The inclusion of foundation work for other genetic studies of the CC locus is admirable.

“Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
“None

“Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
“1. Most of the introduction and discussion are very well-written, accurate and comprehensive. However, the discussion of chemokines important in EAE (p.2 of Intro) lacks rigor. Most studies of CCL3, CCL4 and CCL5 in EAE have been negative. They are certainly present in affected tissue, but knockout and interventional studies have mainly been negative.”

An additional sentence has been added, citing the work of Tran, et al finding that CCL3 knockout had no effect on MOG-induced EAE susceptibility. In the interests of keeping the introduction brief, we believe that this is sufficient to counter-balance the previous references to positive associations of chemokines involvement in EAE.

“2. Levels of CC chemokines are not uniformly 'elevated' as stated, in the CSF of MS patients. Rather, some are 'altered', and one chemokine of interest for the current study is reduced as a reflection of MS disease state (Sorenson et al. J Clin Invest, 1999) and MS disease activity (Sorenson, Eur J Neurol, 2001), possibly because of consumption by CCR2+ migrating cells (Mahad et al. Brain, 2006).”
The word “elevated” has been replaced with “altered”. In addition, an extra sentence has been added to address the reduction in CCL2 in the CSF of MS patients compared to controls and a reference to the work of Mahad, et al has been added.

“3. Authors note that 14 of ’24’ CC chemokines are encoded at Chr 17q11.2-12, while 28 CC chemokines have been described.”

This has been corrected.


An additional sentence has been added in the Introduction, at the end of the paragraph discussing 17q chemokine genetics, addressing this additional reference.

Discretionary Revisions (which the author can choose to ignore)

“None”

We are thankful for being given the opportunity to address the concerns of the reviewers and believe that we have suitably done so. We concede that it is now a stronger manuscript.

Approval for submission has been obtained from all authors. All guidelines for authors have been met.

Sincerely,
Graeme Stewart