Reviewer's report

Title: Methylation of class II transactivator gene promoter IV is not associated with susceptibility to Multiple Sclerosis

Version: 1 Date: 25 March 2008

Reviewer: steven Gray

Reviewer's report:

1. Is the question posed by the authors well defined?
   Yes

2. Are the methods appropriate and well described?
   Yes? Please see "Major Compulsory Revisions"

3. Are the data sound?
   Yes. Please see below "Major Compulsory Revisions"

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
   Yes

5. Are the discussion and conclusions well balanced and adequately supported by the data?
   Yes

6. Are limitations of the work clearly stated?
   Yes

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
   No

8. Do the title and abstract accurately convey what has been found?
   Yes

9. Is the writing acceptable?
   Yes

Major Compulsory Revisions

The results presented have to be taken at face value. There are no figures showing the bisulfite PCR specific product. The authors state that PCR was carried out with universal methylated, universal unmethylated and water as positive, negative and blank controls. Including this figure would help tremendously.

This reviewer has some concerns relating to the methodology used for bisulfite
sequencing. The authors say that PCR products were sequenced (directly?) using an ABI 3700 sequencer. Under normal bisulfite sequencing, each PCR product should be cloned and ~10 clones should be sequenced for each product. In this instance for this manuscript this would represent 1000 sequencing reactions. This strategy allows a researcher to identify small changes in DNA methylation which would be masked if the PCR product was sequenced directly. The authors do not state if they have sub-cloned and sequenced the PCR product, or if they have used direct sequencing of their bisulfite PCR product. This is something which they need to clarify, as unless specific software was used to measure the peaks the authors may be missing low-level methylation patterns., which might be picked up if sub-cloning of the PCR product was utilised.

If the authors can provide details as per methodology in relation to the sequencing, or even include some representative trace files for some of the samples showing complete lack of methylation, this would alleviate this reviewers concerns.

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

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interests