Reviewer's report

Title: Gene expression profiles in Finnish twins with multiple sclerosis

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Reviewer: Anat Achiron

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Gene expression profiles in Finnish twins with multiple sclerosis

This is an interesting paper trying to identify genes responsible for MS by studying monozygotic twin pairs discordant for MS. However there are major flaws in the study methods, leading to problems with the results.

CRITIC:

1. Patients: It is not clear if the 8 MS patients studied were treated. This is of utmost importance since the only differentiated gene found was interferon inducible protein which could be related to treatment.
2. Patients were evaluated for gene expression after disease duration between 1 to 23 years. Thus, the interpretation of data is problematic, at least for the pairs of twins with short disease duration in which the second twin still may develop the disease. Moreover, analysis of gene expression after many years of disease may signify many confounders and the comparison to patients with short diseases duration is non substantial.
3. The statistical evaluation is far from sufficient. It is not clear how the differentiating genes were identified as significant. According to the paper (page 6) it was enough if one gene in one subject was different. This should be evaluated for all the genes in all subjects.
4. No scoring methods either parametric or non parametric like T-test or info were used in the data analysis.
5. The statistical analysis is unclear (page 7). What does it mean to evaluate the differences in the proportions of up and down regulated genes within the groups? The evaluation should include differences not in the proportions but rather in the differentiating genes between groups according to statistical tests. The way the data was analyzed does not mean anything.
6. Results: The authors state that the differences between the MS pairs are presented in table 2. However, no p-values are given and the reader has no indication if this number has any statistical differences.
7. In the results (page 8) it is stated that the proportion of down regulated genes had a p=0.023 for the difference but it is not explain what difference the authors mean. Moreover, the authors state that one gene - interferon alpha inducible protein - appeared to be up regulated in 50% (4/8) of the MZ MS twin pairs. However, This gene does not appear in the list of table 3. the authors conclude that this implies 50% change. It can be stated on the same line of thinking that in 50 % there is no change.
8. The authors refer to MRI finding but no data is given related to what MRI protocol they used, when the MRI was preformed, what MRI parameters they looked at and how did they measured lesion load, if at all.
9. Figure 2 is not clear. There is not indication which line related to the patients and which to the healthy twin.
10. The presentation include only 4 twins and again it is not clear if the results are significant. Moreover the results in twin 4 may be related to the fact that it is the only one that did not receive treatment. Taking together, these results show variability of the patients more than differences.
between patients and their twin pair.

11. Figure 1 does not show the healthy twin pair. Verification was done only for one gene (G1P3) and only in 4 patients.

12. Over abundance analyses suggest that the expected results will be 415 differentiating genes for all patients while the authors' results were 308 in one patient. This should be further explained.

**What next?:** Reject because scientifically unsound