Reviewer's report

Title: Metabolic dysfunctions in Multiple Sclerosis: implications as to causation, early detection, and treatment, a case control study

Version: 4  Date: 30 April 2015

Reviewer: Deepali Mathur

Reviewer's report:

The study investigated biochemical alterations associated with multiple sclerosis (MS) and its various clinical forms using mass spectrometry. Changes in the levels of lipid metabolites including very long chain fatty acids containing phosphatidyl ethanolamines (PtdEtn), plasmalogen ethanolamines (PlsEtn) and anti-inflammatory gastrointestinal tract acids (GTAs) as compared to controls were correlated with disease subtype and duration. The findings reveal significant changes in the level of metabolites in MS subtypes compared to controls.

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

There are no major compulsory revisions I would like to suggest.

2. Minor Essential Revisions (such as missing labels on figures, or the wrong use of term, which the author can be trusted to correct)

There are some minor changes that are required to the text and figures. Please correct them.

a. Sample size mentioned in abstract is different from the one given in methodology. Study subjects in abstract: Line no. 26, 27 and 28
Study subjects in methodology: Line no. 129 and 130

b. In text (line no. 182), it is mentioned that the levels of Phosphatidyl ethanolamine 16:0/28:0 in RRMS >= 13 years are not significantly different from SPMS. However, in Fig. 1a, it is shown that the levels of this fatty acid in RRMS >= 13 years are not significantly different from PPMS patients. This is indicated by same letters on RRMS>= 13 years and PPMS depicting no significant difference between these two clinical courses. Please clarify.

c. In Fig 1b, it is shown that the levels of Phosphatidyl ethanolamine 16:0/22:6 to phosphatidyl ethanolamine 16:0/18:3 fatty acid ratio are significantly different in RRMS >=13 years compared to controls as shown by asterisk. In text (line numbers 187, 188 and 189) the levels of this fatty acid ratio are seen to be significantly different in SPMS, PPMS and also RRMS<13 years compared to controls. The significant difference in the levels between long term RRMS (RRMS>=13 years) and control are not mentioned anywhere in the text. Please
d. As inferred from Table 2, 1 GTA in PPMS vs control is elevated as indicated by an upward arrow. On the other hand, in text (line number 220) it is mentioned that 1 GTA is reduced in PPMS patient compared to controls.

In table 2, 11 out of 34 GTAs are shown elevated in RRMS<13 years. However in text (line number 217) elevation in RRMS>13 years is described.

In the text (line number) it is mentioned that there is no significant difference between SPMS and RRMS>=13 years. However, in fig 2a, the levels of GTA are not significantly different between PPMS and RRMS>=13 years as indicated by same letters on the clinical subtypes.

3. Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

The methodology of lipidomic analysis can be elaborated.

**Level of interest:** An article of importance in its field

**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.