Reviewer’s report

Title: In utero exposure to mercury and childhood overweight or obesity: counteracting effect of maternal folate status

Version: 0 Date: 22 Jul 2019

Reviewer: Chiho Watanabe

Reviewer's report:

The paper describes the results of a prospective study regarding the effects of in utero Hg exposure on childhood overweight and obesity (OWO) and its modification by folate nutritional status. The results showed that maternal (in utero) exposure to Hg was positively associated with childhood OWO, which was alleviated by adequate nutritional status of folate.

The overall design appears satisfactory, and some interesting results are reported. While this paper appears to contain new information, the reviewer has several concerns on current manuscript, which need to be adequately addressed by the authors. What follows is a list of such concerns.

*Introduction
- p.1, line 10-11 .... Need to explain why this question is 'critical' by providing background knowledge. Toxicity of methylmercury has been examined by many literatures regarding its neurotoxicity as well as it role as a risk factor for cardiovascular diseases. Of these, the latter has been usually associated with adult exposure and not so much with in utero exposure. Therefore, the authors need to explain why they try to elucidate such an relationship; i.e., in utero exposure to cardiovascular risks. In this connection, it would be better to include recent findings that suggest Hg as an obesogen, which is discussed in page 10, line 8 (in Discussion). It is also recommended to include existing evidence that connects childhood obesity and risk of cardiovascular diseases in later life stage.

- p.1, line 13-20 .... Rationale of including folate in this study design is unclear. Before starting the study, did the authors have clear hypothesis about the potential relationship between folate and Hg toxicity, or was it basically an explorative attempt? Reference #14 should be introduced with more details to clarify this point.

* Methods
- p.5, line 2-5 .... Some analytical details need to be given here;
  1) what kind of reference materials were used to assure the absolute value of the measurement? CV can only guarantee reproducibility.
  2) provide the limit of detection; without LOD value, discussion in page 7, line 42 is meaningless.

- p.5, line 8-10 ..... The same as above; how was the absolute value, rather than reproducibility, of folate measurement guaranteed? This point is important since this paper concerns 'adequate' level of folate.
-p.5, line 15 ..... When was the 'pregnancy BMI' measured/calculated? What was the rationale of adopting 25 as the cut-off point for 'pregnancy BMI' (25 is cut point for normal adult overweight)?

-p.6, line 10-14 .... Cite the 'national reference data' that was used to evaluate child BMI; ref #18 does not appear to be a database. Is this database ethnic-group specific or not (this is unclear from the text)?

-p.6, line 19-21 .... Need appropriate citation for 'PROC LOESS'; the reviewer assumes that this is one of a procedure modules in SAS software. Also, provide the basic assumptions (and models) used in this procedure to draw the curves (as in Figure 1).

-p.7, line 7 ....Provide the clear rationale to set 20.4 nmole/L as the cut-point for adequate folate level. Give a brief summary of the rationale, which should be given in ref #12.

* Results
-p.7, line 18 ..... 'The age range of children was 2-15 years'. Do authors mean 'the age range of children at his/her last visit [to the clinic] was 2-15 years'?

-p.7, line 21 .... Please be accurate in citing EPA's value; EPA's reference dose (RfD) for mercury refers to 'dose' and not to 'concentration'. Cite appropriate reference like EPA's 'IRIS' and explain what this value (5.8 ug/L) is.

-p.7, line 22 ..... 'inadequate folate levels'; Does this mean below the cut-point defined in the methods section or any other cut-point suggested in this ref #12? If the latter is the case, then it should be moved to Discussion section.

-p.9, line 2 ..... Meaning unclear; 'no evidence of interaction' between which and which (Hg, OWO, or DM)?

* Discussion
-p.10, line 17 (also related with p.8, line 5).... 'RBC-Hg above 3.7 microg/L increased the risk' is misleading and need to be revised. This was just the minimum Hg value found in the highest quartile group (Q4), not the threshold value as this sentence would suggest. Note that the maximum Hg value of this group was about 9 times higher. Accordingly, discussion that follows this sentence needs to be reconsidered.

-p.11, line 1 .... 'the association'; unclear. Association between what and what?

-p.11, paragraph 1.... This paragraph needs to be rearranged so that the story should be easily understood; what would be the results of lower methylation of PON1 gene; pro- (or, anti-) oxidative response?

-p.11, line 17-18 ..... ng/L should be microg/L ?

-p.11, line 10-24 ..... These two paragraphs are rather lengthy and remote from the results. Instead, it would be recommended to discuss 1) the issue of potential confounders; Hg exposure levels (Table 1) was associated with maternal age, parity, and smoking, all of which may associated with the risks of child OWO. Although these factors have been considered in the statistical treatment, it should be worth discussed about the potential contribution of these factors.
2) existing epidemiological as well as experimental knowledge about the relationship between folate nutrition and methlymercury toxicity.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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