Reviewer's report

Title: Blood Manganese Concentrations in Jamaican Children with and without Autism Spectrum Disorders

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Reviewer: Birgit Claus Henn

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This manuscript describes a case-control study conducted to evaluate associations between blood manganese levels and risk of autism spectrum disorder (ASD) among Jamaican children. It appears to be one of the first reports to characterize Mn levels in a Jamaican pediatric population and to describe potentially unique dietary Mn exposure sources, and as such, would be a novel contribution to the literature. However, I have several concerns and suggestions for improvement detailed below.

Major Compulsory Revisions

Abstract
1. As it is written, the introduction of the abstract only mentions the essentiality of Mn with no mention of its potential to be neurotoxic. I suggest providing a brief rationale for investigating Mn as a risk factor for ASD. Is there a proposed biological mechanism, for example, or mechanistic link that led authors to this hypothesis?

Background
2. The background lacks text on what is known about environmental risk factors for ASD, and about Mn specifically as a potential risk factor for ASD. In contrast, there is extensive text covering general aspects of Mn, such as general sources of exposure, breakdown in the environment, and environmental fate and transport, which would be better suited in a different publication. The background should be streamlined and improved substantially, e.g., the first paragraph and the last 3 paragraphs of the background contain the crux of the introductory material.

3. Authors describe Jamaican soils as having Mn levels that are >10% higher than “recommended amounts.” While it would be appropriate to describe Jamaican soil Mn levels relative to soil Mn in other areas of the world, I have a hard time understanding “recommendations” for soil Mn concentrations. Please clarify or rewrite.

4. There is a lack of reference values for blood Mn levels in children. I am not aware of a published blood manganese guidance value, or level that is defined to be “elevated,” as stated by authors. Any guidance values that have been recommended by ATSDR are interim values, based on dietary balance studies in adults. Their relevance for children, especially in the age range of children in this
study, is questionable. Therefore, I would urge the authors to omit citing the percentage of children with “elevated” blood Mn levels in their cohort, throughout the paper.

Methods

5. This paper would be of much broader interest and importance if authors reported findings on the other metals they examined (Pb, Hg, As, Cd), in addition to Mn. Especially given the null findings for Mn, I strongly suggest adding results for the other metals that authors have not yet published (e.g., Pb, Cd). Because Cd has been reported to be a concern in Jamaican produce (Howe et al. 2005), I would think this question would be of interest and fit well with the current manuscript. I also urge authors to review Roberts et al. (2013), who created an index for overall measure of metals, and strongly suggest that they consider a similar type of overall measure.

6. In the methods, it is unclear which variables were considered as potential confounders. As written, it seems that authors only considered paternal age, place of child’s birth, and dietary/water intake (though these were only the variables included in final models). Please clarify in text. Were child’s age, gender, and maternal age considered? Blood Mn levels decline with age, and some studies have reported differences in internal Mn levels by sex. Also, authors have previously published on maternal and paternal age being jointly associated with ASD, so could the authors’ published recommendation of jointly modeling maternal and paternal ages be implemented in this study?

7. How did authors take into account the possible beneficial effects of Mn in their models? Using a linear model, we may fail to observe a true association if the association is not linear. Authors should, for example, categorize Mn or model Mn as a smoothed term to allow for possible non-linearity.

8. Authors claim that blood Mn is a “reliable measure” of Mn exposure given the population’s continuous exposure to Mn in the diet. Only a small fraction of oral Mn, however, is absorbed, and this is held under tight homeostatic control. It is not known what blood Mn levels reflect, either environmental exposure or an underlying physiologic need/role or both. I suggest omitting this statement and including text on the limitations of blood Mn as a biomarker in the discussion section. Authors might consider referring to ATSDR, which states that, “elevated blood manganese alone does not constitute an adequate indicator of manganese overexposure”, however, “at high exposure levels, a higher level of blood manganese may be maintained.”

9. The statement that blood and urine are the most frequently used biomarkers of Mn exposure may be true for occupational settings, but is probably not true for studies of environmental Mn exposure, where hair (and water) are also often used to measure exposure. Mn is excreted primarily via the bile and thus urine is only a minor excretion route for Mn. The statement should be qualified, or rephrased to something like, “blood and urine have frequently been used as biomarkers in occupational settings…”.

10. Page 11: authors write, “we used GLMs to evaluate associations between Mn concentrations and exposures of interest.” By “exposures of interest,” do you
mean possible sources of Mn exposure? Please clarify, here and throughout the manuscript.

Results

11. There seem to be several significant differences in characteristics of cases vs controls, including the place of child’s birth. For me, this raises the question of whether controls were appropriately selected to represent the source population that gave rise to the cases. Could there be selection bias? The majority of controls were selected from Kingston parish, while majority of cases selected from other parishes. I’m not familiar with these areas of Jamaica, but is it possible that controls were inadvertently selected in a way that was not independent of exposure, thereby making the control group more similar to cases, and result in a null finding? Can the authors address this concern and provide more information about how controls were selected?

Discussion

12. There is unnecessary repetition between text in the intro and discussion, which should be eliminated. (For example, paragraph on p. 16 on biomarkers is similar to section on assessing Mn exposure on p. 10)

13. This section could be strengthened with additional consideration of possible reasons for failing to detect an association. For example, is it possible that prenatal and/or early life Mn exposure is more predictive of ASD, and that this exposure period was not adequately captured here?

Minor Essential Revisions

14. By GLM, do you mean general linear model (i.e., standard linear model assuming normality of residuals) or generalized linear model (i.e., errors do not need to be normally distributed)? Perhaps describing the model rather than, or in addition to, using this terminology would help clarify.

15. In Statistical Analysis, delete ‘not geometric means.’

16. Reporting odds ratios, an informative estimate of the association between blood Mn and ASD, could be useful for comparison with other case-control studies reporting ORs.

17. We “fit” GLMs, not “fitted”.

18. Blood Mn levels decline with age. Was there an association between child’s age and blood Mn levels? If so, authors could consider reporting age-specific Mn levels, or reporting any observed trend.

Level of interest: An article whose findings are important to those with closely related research interests

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.