Author’s response to reviews

Title: Exposure to prenatal secondhand smoke and early neurodevelopment: Mothers and Children’s Environmental Health (MOCEH) study

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Author’s response to reviews:

Associate Editor: The manuscript reports on an interesting cohort from Korea, testing a hypothesis of public health importance & impact.

1. I think it would be helpful 1) to provide your definition of SHS in the abstract, mention that you excluded active smokers based on their cotinine levels exceeding 42.7 ng/ml and 2) to provide the betas and confidence intervals for both strata (e.g., both for those who were breast fed for 6 months exclusively vs those who were not, and GST mu and theta null vs not null).

>> Response:

We thank the Associate Editor for these great suggestions. We agree with the Editor. Therefore, we have added the following sentences into the Abstract.
1) Abstract, Methods, Line 8-10.

“We quantified urine cotinine concentrations in mothers once from 12th to 20th gestational weeks and excluded mothers whose urine cotinine levels exceeding 42.7 ng/ml to represent SHS exposure in early pregnancy.”

2) Abstract, Results, Line 17-23.

“This negative association was more pronounced in children whose mothers had both Glutathione S-transferases mu 1 (GSTM1) and theta 1 (GSTT1) null type [β = -5.78; 95% CI: -10.69 to -0.87], but not in children whose mothers had any present type of GSTM1/GSTT1 [β = -1.64; 95% CI: -4.79 to 1.52]. The association was no longer significant when children received breast milk exclusively for up to 6 months [β = -0.24; 95% CI: -4.69 to 4.20] compared to others [β = -3.75; 95% CI: -7.51 to 0.00].”

2. Adding the statistical significance of the interactions would also be helpful to the body of the text (Results section) and table.

>> Response:

We appreciate your comments and we agree with the Editor. Taking your suggestions into account, we tested the interaction term between maternal urinary cotinine level at early pregnancy and each stratum in Table 3. We found that none of the interaction term was statistically significant where the significance level was 0.05. However, it is well known that the interaction term with a p-value lower than 0.20 or 0.25 should then be investigated to determine if it is substantively important.

Please find the Table 3.
We have added this into the Methods (Statistical Analyses) and Results section of the revised manuscript.

Methods, Page 10, Line 18-19.

“We then tested the significance of interaction term between maternal urinary cotinine level at early pregnancy and each stratum.”

Results, Page 12, Line 9–10.

“However, we found that none of the interaction terms between urinary cotinine level and each stratum was statistically significant.”

3. On the GAM figure 3, it would helpful to the readers to see the GAM plots stratified by your genotype and breast feeding variables as another illustration of the effect modification.

>> Response:

We thank the Editor for this great suggestion and we agree with the Editor. Therefore, we have provided GAM plots stratified by genetic polymorphisms (both null vs any present in GSTM1/GSTT1) and breastfeeding behaviors (breastfeed up to 6 months or not) as a supplementary figure. In children whose mothers’ urinary cotinine levels were greater than 1.90 ng/ml at early pregnancy, we found the pattern that we had already observed in Table 3. That is, a negative association between their mothers’ urinary cotinine level and MDI score at 24 months was found when their mothers had both GSTM1 and GSTT1 null type. However, no association was found in children whose mothers had GSTM1/GSTT1 with any present type. MDI score at 24 months was not associated with maternal urinary cotinine level when children received breastmilk up to 6 months after birth whereas negative association was found in children who did not exclusively receive exclusive breastfeeding up to 6 months old.
Please find the Supplementary Figure.

We have added this into the Result section of the revised manuscript.

Results, Page 12, Line 3–5.

“The effect of maternal urinary cotinine level at early pregnancy on infant’s MDI score at 24 months of age differed depending on genetic polymorphism and breastfeeding behavior (Supplementary Figure).”

Reviewer #1: The topic of the study is of great importance and the paper is generally interesting. There are several issues which in my opinion need to be address before paper can be considered for publication:

1. By "direct" (in the first sentence of introduction) you mean active smoking? If yes please change it into more standard word.

>> Response:

We thank the reviewer for pointing this out and we agree with the reviewer. Therefore, we have modified the first sentence of the introduction as shown below.

Background, Page 4, Line 1.

“Exposure to active smoking and secondhand smoke (SHS) causes health concern.”
2. As in my previous review (stated for other journal) I recommend to add the study by Polanska et al. "Environmental Tobacco Smoke Exposure during Pregnancy and Child Neurodevelopment" (2017) - It is not clear to me why the oldest studies are quoted and that new one is still omitted - this study is focusing on the same topic (almost the same time of the study, ETS by cotinine measurement and Bayley for child psychomotor development at age of 1 and 2 years)

>> Response:

We thank the reviewer for pointing this out and we agree with the reviewer that we need to cite the new one. Therefore, we have added the article (Polanska et al., 2017) as reference 7 in the following sections of the revised manuscript.

Introduction, Page 4, Line 16–18.

“Lower development scores in cognition, language, and fine motor scales (6, 7), gross motor scores (8), and MDI scores (9) have been reported in children with prenatal SHS exposure.


“Such negative association has also been observed by using cord blood cotinine level (6) and cotinine level in saliva during pregnancy (7).”

Discussion, Page 15, Line 16–18.

“Previous studies have defined prenatal SHS exposed infants as those with cord blood cotinine level ≥ 0.16 ng/ml (6) or with maternal cotinine level in saliva during pregnancy ≥ 1.5 ng/ml (7).”
3. Many studies report positive associations between breastfeeding and child neurodevelopment and suggest that the longer duration of breastfeeding benefits child psychomotor development. However, in some studies, the correlation between breastfeeding and psychomotor development of children is not statistically significant after considering confounding variables. The latest systematic review by Walfisch et al. (2013) pointed out that much of the reported effect of breastfeeding on child neurodevelopment is due to confounding.

>> Response:

We thank the reviewer for pointing this out and we agree with the reviewer that it is important to control for confounders to investigate the effect of breastfeeding on child neurodevelopment. From our study, we found that the significantly negative association between maternal cotinine level and MDI score at 24 months of age had disappeared when infants received breastmilk exclusively up to 6 months of age after controlling for several confounders such as maternal age, maternal education level, and so on. In addition, another recent systematic review article has showed that breastfeeding is related to improved performance in intelligence tests and the association could be causal. (Horta et al., 2015).

We remained this concern in the Discussion of the revised manuscript.

Discussion, Page 15, Line 4–9.

“Some studies have suggested that the effect of breastfeeding on child neurodevelopment has to be investigated after adjusting for important confounders (51). After controlling for primary confounders such as maternal education level, our study showed that the negative impact of SHS exposure at early pregnancy on MDI score at 24 months of age was diluted when children received breast milk exclusively for 6 months after birth.”

4. The authors need to be aware about variables that should be measured after birth like HOME, mother-child interaction, child ETS exposure etc. which can influence child neurodevelopment and need to be included as confounders (it is mentioned in the discussion as the limitation of the study). In the study only breastfeeding was included.
We thank the reviewer for pointing this out and we agree with the reviewer. Therefore, we have added a covariate (the primary caregiver up to 24 months after birth, mother vs. others) in our analyses that might influence child neurodevelopment after birth based on questionnaires collected at each follow up. We updated all Tables after this additional adjustment of the primary caregiver in our analyses. Results were consistent with previous results.

However, we agreed with the reviewer that this was insufficient considering that many factors should be measured after birth to determine the association between SHS exposure and neurodevelopment. Therefore, this remained a limitation of our study as described in the discussion section.

We have added this into the following sections of the revised manuscript.

Methods, Covariates, Page 9, Line 13–14.

“We also defined the primary caregiver during the first 24 months after birth based on the questionnaire at each follow-up: mother during the whole period or others.”


“For each group, associations between maternal urinary cotinine levels and offspring’s K-BSID-II test scores were separately assessed using a multiple linear regression model after adjusting for ln-transformed urinary creatinine level, sex of a child, maternal age, maternal education, gestational age, region, breastfeeding up to 6 months, and primary caregiver during the first 24 months after birth.”
Fifth, human neurodevelopment continues after birth. However, we did not consider the whole effect of postnatal exposure that could affect children’s neurodevelopment after birth except for breastfeeding behavior up to 6 months and primary caregiver up to 2 years of age.

5. It is also not clear to me why in methods it is indicated as 100 ng/ml (for active smoking) and then ≤ 42.7 ng/ml was used. It need to be clearly explained.

>> Response:

We appreciate your thoughtful comment. The aim of this study was to investigate maternal exposure to secondhand smoke (SHS) during pregnancy on neurodevelopment of infants at age of 24 months. We wanted to restrict subjects to non-smoking pregnant women and exclude mothers who were current smokers during pregnancy. The use of cutoff urinary cotinine level at 100ng/ml has been often suggested or used as a cut-off to differentiate active smokers from the non-active smokers in several studies. We listed some of these studies in R-Table 1. We therefore had applied the 100 ng/ml of urinary cotinine level as a cut-off to distinguish active smokers from nonsmokers in the supplementary analyses in the previous manuscript. We found that our results using cutoff 42.7 ng/ml were robust and consistent compared to results using 100 ng/ml as a cut off level. However, we decided to remove all contents about the analysis using the cut-off level 100 ng/ml which could confuse the reader.

R-Table1.

References [number, author, title, journal, Note]

1 Haufroid et al.,


   ‘Despite rather high levels of passive exposure in some cases, several studies show that urinary cotinine levels in nonsmokers are always less than 100 ng/ml urine.’

2 Florescu A et al.,

In Table 5, Unexposed nonsmokers Passive smokers Active smokers
Urine(ng/ml) <10 10-100 >200

3 Jhun HJ et al.,

‘In urinary cotinine analysis, current smokers were defined as those whose urinary cotinine levels exceeded 100 ng/mL.’

4 Evlampidou I et al.,

‘The cut-off level of 100 ng/mL was used to confirm the self-report smoking habits and select only women with low cotinine levels.’

5 Ko K et al.,

‘We applied a urine cotinine cut-off point of 100 ng/ml in reference to a previous study, in which nonsmoker urine cotinine levels did not exceed 100 ng/mL. This level was lower than the manufacturer’s recommended cut-off point of 500 ng/mL.’


“We restricted mothers whose urine cotinine levels were ≤ 42.7 ng/ml and regarded them as non-active smokers because we focused on the effect of SHS exposure during pregnancy. There has been no standardized cut-off level of urinary cotinine to distinguish active smokers from non-active smokers. Several studies have suggested cut-off levels ranging from 50 to 550 ng/ml (49, 50). These cut-off levels vary by race (51). When we repeated analyses in all mothers without excluding mothers with high urinary cotinine levels, results were consistent (data not shown).”
References


