Author's response to reviews

Title: Low level maternal smoking and infant birthweight reduction: genetic contributions of GSTT1 and GSTM1 polymorphisms

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Author's response to reviews: see over
Cover letter for editors

Please find revised research article Low level maternal smoking and infant birth-weight reduction: genetic contributions of GSTT1 and GSTM1 polymorphisms” prepared by Asta Danileviciute, Regina Grazuleviciene, Algimantas Paulauskas, Ruta Nadisauskiene, Mark J Nieuwenhuijsen.

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and answers for reviewers:

Reply to Reviewer Xinguang Chen
Thank you very much for reviewing our manuscript and the possibility to improve our manuscript.

There are some explanations.

Major Compulsory Revisions
Abstract: Information related to method part is included in the background part (e.g. sample size, specific genes to be examined). Method part needs to be streamlined and revised to include key information (e.g., sample, analytical method). Conclusion part also needs to be revised. The effect of the two genes on birth weight has been reported in a number of studies, findings of this study provide additional data supporting previous findings. According your suggestion we revised all parts of abstract, Page 2.

Introduction section Need more through literature study – data relating GSTM1, GSTT1, prenatal and post natal tobacco exposure and birth weight and related developmental measures (lung growth) have been reported (e.g., Chen et al, 2011, Environmental Research; Lee et al, 2010, Environmental health perspective; to name a few). The authors need to review and abstract findings, including both positive and negative, from all related studies to demonstrate the need for their study. Almost all reported studies around this topic area have examined the interplay among all the related genes and tobacco exposure on outcome variables if sample size is allowed. A relatively larger sample is the strength of this study, but the authors did not make this part standing out. We rewrite the Background making all possible improvements by the suggestions, Page 3-6.

Methods Section
More information is needed regarding the study cohort. How the study subjects were defined and selected? For which population is this sample representative? What is the refusal rate?
On their first visit to a general practitioner, all pregnant women living in Kaunas were invited to join the cohort. Participation was on a voluntary basis and the women were enrolled in the study only if they consented to participate in the cohort. Pregnant women of the cohort were asked to answer questionnaires provided to them at the clinic before delivery. Multiple births or newborns with major births defects were excluded. About 36 percent of women refused to participate in the study. This sample is representative for reproductive age woman.

Description of variable measurement is problematic. Measurement of maternal smoking is poorly defined. It is unclear how “light smoker” was defined. No measurement of smoking BEFORE pregnancy was found. Exposure to environmental smoking (particularly husband smoking) was not included.

Exposure to smoking now is described. To assess smoking level (light smokers) we calculated the mean number cigarettes smoked per day. Page10 Result section.

Maternal smoking before pregnancy and passive (husband) smoking are presented on Page 10 Result section. Before pregnancy smoked 140 (25.7%) study subjects, among them 42.9% continuous smokers during pregnancy and 57.1% non-smokers during pregnancy. Prevalence of passive smoking at home (husband smoking) among continuous smokers during pregnancy was 91% and among non-smokers during pregnancy it was 50.4%.

Birth weight is not an exposure variable but was included under the subheading “Exposure Assessment”. This reviewer is looking for a well organized section on variable assessment, including outcome variable, genetic variables, variables for tobacco exposure, and other control variables. Genotype frequency is part of the statistical analysis, but it was treated not as statistical analysis (the first sentence in the statistical analysis section on page 7). We reorganized and appended Method section, Page 6. Method section we divided into: Participant and outcome assessment, Smoking exposure, Genotyping and Statistical methods.

Results Section

Missing data which are obvious but are not indicated in any of the tables or text. The reasons and impact of missing data on the study findings should be added and discussed as limitation.

We have included in the text the missing data on non-participants, Page 10 Results section: We also conducted analyses comparing questionnaire data and birth certificate data on various characteristics among participants and non-participants. The mean birthweight and gestational duration were similar among the two groups. These two groups did not differed by ethnic group, however, non-participating mothers were younger, less educated (did not graduate from university, 46.6% vs. 54.3%), more often smokers (smokers, 9.6% vs. 6.9%), and did have fewer prior births (no child, 64.1% vs. 45.1%), than that of participants.


The authors also need to report genetic equilibrium analysis (e.g., Hardy-Weinberg equilibrium analysis) to ensure the appropriateness of the genotyping results.

For the GSTM1 and GSTT1 polymorphisms, we were unable to determine whether they were in Hardy-Weinberg equilibrium because heterozygous individuals could not be distinguished from homozygous wild type. Page 11, section Results.

Reporting the results regarding genotypes and quitting smoking is highly distractively. This is a totally different area (see paper by Chen and Woodcroft, 2009, Nicotine and Tobacco Research), and could not be adequately addressed in this study.
According your suggestion, we have removed from our manuscript this part.

Several variables not significantly associated with birth weight were included in both Table 1 and Table 2; but a number of key variables included in the regression analysis were not included in Table 1.

The purpose of Table 1 was to indicate the variables associated with the smoking. A table 2 purpose was by using linear regression to study our selected and in literature mentioned variables as potential risk factors on birthweight, associations with the infant’s birthweight in crude and adjusted models.

The headings for Table 2 and Table 3 are insufficient; consider revision to including information about the analytical method, sample size, and type of results, etc.

We revised the titles for Table 2 and Table 3 according your suggestions.

Discussion Section

The key finding of this study is the significant gene-gene interaction as presented in Table 3, but has caught attention of the authors. Findings of this study as reported in the paper appears to indicate no significant interaction between either of the two genes and maternal smoking on birth weight (none of the interaction terms was significant after the main effect was included). This section is gone off line again at the beginning to include other factors rather than genes, maternal smoking and birth weight.

We have changed the discussion section starting from the description of our obtained results and pointed out:

We also found a gene-gene interaction among smokers. A combination of the GSTM1–null and the GSTT1–null genotypes has been found to exacerbate the effect of maternal exposure to tobacco-smoking on the birthweight of infants more than the presence of either genotype alone: −311.2g, P = 0.008 in smokers vs. 10.1 g, P = 0.447 in non-smokers. An interaction effect of maternal smoking, GSTM1 and GSTT1 genotypes was marginally significant on birthweight (−234.5 g, P = 0.078).

We think that statistically non significant interaction effect was because of relatively small cases group with GSTT1–null genotype.

When discussion about GST gene and maternal smoking, the authors only cited one study [9], ignoring a number of other studies, such as the one by Chen and Woodcroft, 2009.

In the Discussion section about GST gene and maternal smoking now we have cited more studies when we first time mentioned, Page 14 first paragraph.

Now we have removed from our manuscript discussion about smoking cessation part.

Most part presented in the discussion section should be moved to the Introduction section to demonstrate the significance of the current study, particularly those with inconsistent results regarding GST genes, maternal smoking and child birth weight.

According your suggestion we have made changes in the Introduction and Discussion sections.

Thank you once more.
Reply to Reviewer Ian Wright

Thank you very much for reviewing our manuscript.

There are some explanations.

*No details of when in pregnancy the interview was undertaken. This is important to help understand the validity of the smoking details. Cotinine or COHb would be an important validation.*

Now we included in the manuscript: On their first visit to a general practitioner, all pregnant women living in Kaunas were invited to join the cohort and answer to the first questionnaire. We recruited these women for the prospective cohort study, enrolling them at 23–35 weeks of gestation at the four prenatal care clinics affiliated to the hospitals of the Kaunas University of Medicine. Pregnant women of the cohort were asked to answer second questionnaire provided to them at the clinic before delivery. (Method, Participant and outcomes assessment, Page 6-7)

We have no possibility to measure cotinine or COHb and validate smoking level.

*Very unclear as to smoking status.....as written is dichotomous non smoker and any smoking but later this latter group is referred to as " continuous smokers" this is inconsistent.*

Now we have changed “smoked any number of cigarettes” to “continue smoking during pregnancy”. Page 8, Smoking exposure section.

*This is a very unusual population with very high proportion of tertiary educated mothers amongst other things. Amongst the smokers the smoking use is very low. This is not adequately discussed....the birthweight difference is close to that reported for much heavier smokers. Any dose effect is not discussed enough.*

The women who agreed to participate in the genetic analysis education were higher than National education level and smoking prevalence was lower to compare to less educated woman with lover social status (Table 1).

We can not present dose-response effect because of very few number of women smoking >10 cig or more during pregnancy. Birthweight difference maybe is result not only smoking but also lover social status. The mean birthweight was similar among the two groups (p=0.132). Table 1.

*The postulation of difference in cessation rate being due to metabolite changes is not adequately supported by references or evidence. As this could account for all observed effects this is really important.*

According another reviewer, that our results regarding genotypes and quitting smoking is highly distractively and were removed basing on the recommendation from our manuscript.

- **Minor Essential Revisions**

  2. "Statistical significance was defined as P = 0.05" "less than" I think

We have changed “P = 0.05” to “P < 0.05”, Page 10.

  3. Repetition of classification of smoking and nonsmoking

Now description of smoking is presented only in the Method section, Smoking exposure, Page 7.

  4. Several references are made to this showing a significant effect on adverse pregnancy outcome. This is not really true.....a small decrease in birthweight as a continuous variable but
within the normal range is not actually an adverse pregnancy outcome. No evidence for IUGR, increased SGA, decreased placental wt, cord gases or any other categorical adverse outcome is reported so this terminology is not justified.

Now we have made changes in Conclusion: The study shows the modifying effect of the GSTT1 and GSTM1 genotypes on birthweight among smoking women and presents evidence that carriers of the null genotypes should be treated as an increased susceptibility group for infant birthweight decrease.

Thank you once more.

Reply to Reviewer Mika Gissler

Thank you very much for reviewing our manuscript.

There are some explanations.

Reviewer’s report:
Major compulsory revisions:
- The article should include power calculations, since the sample is relatively small.
We can not calculate the power because there are absent published data on prevalence of light smoking women with GSTT1-null that we should use to calculated ratio of an exposed and unexposed woman to tobacco smoke.

- The authors should include a short description how exactly the information on maternal and paternal smoking was collected. The validity of these self-reports should be discussed.
How the information on maternal and paternal smoking was collected. We described in the Smoking Exposure section (Page 7-8)
Data regarding smoking behaviour were acquired through face-to-face interviewing. The trained research assistant in person in the hospital setting asked the women to report their daily cigarette consumption both before and during pregnancy. Woman had to answer the questions, “How many cigarettes did you smoke before pregnancy?” and “How many cigarettes did you smoke during pregnancy?” A mother was defined as smokers if she reported smoking at least one cigarette per day during pregnancy. In this study, data on mothers were categorised into two groups with respect to their cigarette smoking habits: those who did not smoke and those women who continue smoking during pregnancy. The parent was defined as a smoker if he smoked at least one cigarette per day. To assess smoking level we calculated the mean number cigarettes smoked per day.
We have no possibility to measure cotinine or COHb and validate smoking level. And this is discussed in the Discussion, Page 16: The evaluation of exposure to tobacco smoke was indirect; we used self-reported information on smoking during pregnancy, and thus the possibility of bias in both reporting and exposure classification exists.

Minor revisions:
- Use the term sex instead of gender.
We have changed gender to the sex

- Table 1: Define pregnancy loss in more detail.
We changed Pregnancy history to miscarriage (Table 1). Page 26-27.

- **Table 2: Define maternal stress in more detail.**
Now we defined maternal stress in the Method section, Participant and outcome assessment, Page 7. The self-reported stress of the respondents was assessed by the following thesis: my daily activities are very trying and stressful. Four respondent options were used to define stress: this describes my state (1) very well, (2) fairly well, (3) not very well, (4) not at all. Values 1 and 2 were considered to represent stress; 3 and 4 represented no stress.

Thank you once more.