Author's response to reviews

Title: The Effect of Perinatal Anxiety on Bronchiolitis is Influenced by Polymorphisms in ROS-related Genes

Authors:

Eun Lee (unelee@daum.net)
Hyoung Yoon Chang (hyoungyoon@hotmail.com)
Kyung-Sook Lee (debkslee@daum.net)
Dong In Suh (wellcontrolled@hotmail.com)
Ho-Sung Yu (planetho@naver.com)
Mi-Jin Kang (007jokbal@daum.net)
In Ae Choi (inae0920@daum.net)
Jinah Park (pijah1230@hanmail.net)
Kyung Won Kim (kwkim@yuhs.ac)
Youn Ho Shin (epirubicin13@gmail.com)
Kang Mo Ahn (kmaped@skku.edu)
Ja-Young Kwon (jaykwon@yuhs.ac)
Suk-Joo Choi (drmaxmix.choi@samsung.com)
Kyung-Ju Lee (drki4094@gmail.com)
Hye-Sung Won (hswon@amc.seoul.kr)
Song I Yang (qijit222@naver.com)
Young-ho Jung (youngdr1@naver.com)
Hyung Young Kim (hawmok77@hanmail.net)
Ju-Hee Seo (juheesea@hanmail.net)
Ji-Won Kwon (pedas@naver.com)
Byoung-Ju Kim (drkbj@hanmail.net)
Hyo-Bin Kim (hbkim1126@naver.com)
So-Yeon Lee (imipenem@hanmail.net)
Eun-Jin Kim (lular1@naver.com)
Joo-Shil Lee (jooshil@korea.kr)
Katherine M. Keyes (kmk2104@cumc.columbia.edu)
Yee-Jin Shin (yjshin@yuhs.ac)
Soo-Jong Hong (sijhong@amc.seoul.kr)

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Author's response to reviews: see over
Dear Editor

Please find enclosed a revised version of our manuscript entitled ‘The Effect of Perinatal Anxiety on Bronchiolitis is Influenced by Polymorphisms in ROS-related Genes’ for submission in *BMC Pulmonary Medicine*.

We would like to thank the reviewers for all their helpful comments and we provide a point-by-point response to their comments below.

We look forward to hearing from you at your earliest convenience

Yours sincerely

Soo-Jong Hong, MD, PhD
Yee-Jin Shin, MD, PhD

Reply to reviewers’ comments

Editors’ Comment:

Thanks for the revised version of the manuscript. Most of the reviewer comments have been addressed appropriately. However, further revisions are required:

IN GENERAL

There are two expressions used for ?LRTI? (LRTI and LRTI including bronchiolitis) throughout the manuscript. Please be consistent.

Response: Thank you for your comments. We have removed the phrase ‘LRTIs including’ in line 306 and 361 and changed it to ‘LRTIs, especially bronchiolitis’ to maintain consistency throughout the manuscript.
Since GST subfamily genes are expressed mainly in the respiratory tract and oxidative stress is increased during LRTIs, especially LRTIs including bronchiolitis [1], genetic variants of the GST subfamily may modify the risk of LRTIs, especially LRTIs including bronchiolitis.

The manuscript needs to be carefully proofread, preferably by a native speaker as some sentences are hard to follow.

Response: Thank you for your comments. A specialized editing company has proofread the manuscript. We attached the certificate.

Introduction/Conclusion: In the introduction as well as in the conclusion you mention the economic and medical burden of bronchiolitis early in infancy and a possible relationship with asthma later in childhood. As this turned out to be an important factor in your conclusion you might think to add a respective paragraph in the discussion section on the economic/medical burden and the possible association with asthma including references.

Response: Thank you for your comments. We have added the following paragraph to the discussion section: Hospitalization expenditures because of bronchiolitis during infancy has been increased. A history of bronchiolitis in infancy is associated with adverse respiratory outcome such as reduced lung function and development of asthma in later life. With the prevention of bronchiolitis during early life, health care costs and adverse respiratory health can be preventive.

ABSTRACT

Objective: Please shorten this paragraph, starting with Studies on gene-environment interactions with respect to exposure to perinatal anxiety are lacking

Response: Thank you for your comments. We have revised the objectives in the abstract as follows:
Exposure to perinatal anxiety affects disease susceptibility in offspring but studies on the association between perinatal anxiety and gene polymorphisms are lacking.

**[Original version]** Although perinatal anxiety affects the developing immune system and susceptibility to diseases in offspring, studies on gene-environment interactions with respect to exposure to perinatal anxiety are lacking.

**Results:** You may change to: Exposure to higher levels of perinatal anxiety increased the risk of bronchiolitis (adjusted odds ratio [aOR] 1.30, 95% confidence intervals [CI] 1.00-1.80), in particular among children with .. genotype.

**Response:** Thank you for your comments. We have revised the results in the abstract as follows:

Exposure to high levels of perinatal anxiety increased the risk of bronchiolitis in the first year of life (adjusted odds ratio [aOR], 1.30; 95% confidence interval [CI]: 1.00–1.80), in particular among children with the AG+GG genotype of *GSTP1* or the *GSTT1* null genotype (aOR 3.36 and 2.79).

**INTRODUCTION**

Page 3, first sentence: needs to be rephrased (? development of physiologic and immune system?)

**Response:** Thank you for your comments. We have revised the first sentence in the introduction as follows:

Environmental factors during early life influence the development of the immune system and physiology, and change susceptibility to disease in later life.

**[Original version]** Environmental factors during early life can influence the development of physiologic and immune system, thereby, modify disease susceptibility later in life

Page 3, line 37: you may delete into the fetus?

**Response:** Thank you for your comments. We have deleted ‘into the fetus’ in previous line 37 as follows:

Perinatal maternal anxiety increases glucocorticoids in pregnant women, which can cross the placental barrier.
Page 3, line 47: change to The GSTP??
Response: Thank you for your comments. To clarify this point, we have started with general information on GST, because the GST subfamily includes GSTP1, GSTT1, and GSTM1 in line 46. The following sentence then mentions genetic polymorphisms of both GSTP1 and GSTT1 as follows: The glutathione S-transferase (GST) subfamily plays an important role in the protection against oxidative stress by catalyzing the conjugation of many compounds with reduced glutathione. Polymorphisms in the GST genes, GSTP1 (rs1695) and GSTT1, affect the ability to deal with excessive oxidative stress due to the resultant altered activity of the GST enzymes.
I hope that our opinion makes enough senses that it’s sufficient to satisfy your question.

Page 4, line 57: rather use occurrence instead of development
Response: Thank you for your comments. This has been changed as follows: This suggests that these genetic variants may affect the occurrence of RTIs and environmental factors may exaggerate the effects of these genetic variations.

Page 4, line 61 : rephrase to The first aim was to??
Response: Thank you for your comments. We have revised line 61 as follows:
The first aim of this study was to examine the association between perinatal anxiety and RTI occurrence during the first year of life.

METHODS
Page 4, line 72-74: I suggest rephrasing, meaning is not clear.
Response: Thank you for your comments. We have revised lines 71–73 as follows:
Women in the third trimester of pregnancy were enrolled from January 2009 to September 2011. The questionnaire used in this study included items on the State-Trait Anxiety Inventory (STAI).
Women in their third trimester of pregnancy were enrolled in this study from January 2009 to September 2011, when the questionnaire included State trait anxiety inventory (STAI) score.

**Page 74, line 74: Rephrase to: Pregnant women with … have been excluded?**

**Response:** Thank you for your comments. We have rephrased the sentence in line 74 as follows:

Pregnant women with pregnancy-associated complications (gestational diabetes and pregnancy-induced hypertension), high risk for early delivery, and delivery earlier than 36 weeks have been excluded.

**Page 7, line 79: Please rephrase, odd sentence**

**Response:** Thank you for your comments. We have rephrased the sentence in line 78 as follows:

More details on this cohort study are described elsewhere.

**Original version**

Additional information on this ongoing cohort study are described elsewhere.

**Page 5, line 82 : Change to Participants included in the study were not different compared to the non-included regarding ??**

**Response:** Thank you for your comments. We have changed lines 81-82 to the following:

Study participants did not differ from those women not included in the study with regard to key demographic covariates, except for the season of offspring’s birth.

**Page 5, line 97: and instead of which**

**Response:** Thank you for your comments. Line 97 describes the characteristics of K-STAI, which consists of two subscales. To clarify this section, we have changed it to the following:

Instead of using STAI, the Korean version of STAI (K-STAI) was used, which consists of two subscales, namely State Anxiety (anxiety about an event) and Trait Anxiety (anxiety levels as a personal characteristic).
Statistical analysis:
Is there a different made between maternal active and passive smoking? (exposure to tobacco smoke?)

Response: Thank you for your comments. In Korea, the prevalence of maternal active smoking is very low, 0.9% in this study, compared to 39.2% of passive smoking.

Please comment on how the confounders were selected. Have you performed association analysis?
Response: Thank you for your precise comments. We performed a univariate analysis to evaluate the effect of confounding factors on the occurrence of URTI, LRTI, and bronchiolitis at age 1. With respect to bronchiolitis, sex and season of birth was associated with significantly increased risk. For URTI, maternal education level was associated with a decreased risk. The confounding factors used in this study are considered to be associated with RTI risk in other studies [1-3].

References

Table. Univariate analysis for the confounding factors

<table>
<thead>
<tr>
<th>Variables</th>
<th>URTI OR (95% CI)</th>
<th>P value</th>
<th>LRTI OR (95% CI)</th>
<th>P value</th>
<th>Bronchiolitis OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, female</td>
<td>1.06 (0.68–1.65)</td>
<td>0.787</td>
<td>1.81 (1.05–3.12)</td>
<td>0.032</td>
<td>1.90 (1.02–3.54)</td>
<td>0.044</td>
</tr>
</tbody>
</table>
Page 7, line 134-135: Please rephrase, odd sentence

Response: Thank you for your comments. We have revised lines 133 as follows:

The standard deviation (SD) for the STAI score in the regression analyses was 8.47.

[Original version] The unit of STAI score used in the regression analyses was standard deviation (SD) of samples, which was 8.47.

RESULTS

1st paragraph: Report here also the results of the comparison between included and non-included study population.

Response: Thank you for your comments. We have added the differences in the characteristics of pregnant women included and excluded in lines 147-150 as follows:

There were no significant differences in maternal age, maternal education level, infants’ sex, mode of delivery, presence of infants’ siblings, and daycare attendance of infants between pregnant women included in this study and those excluded.
Page 8, line 170: Please re-think the statistical approach, in particular the reference categories used for all multivariate analysis including genotypes. If you aim to evaluate whether higher levels of perinatal anxiety have different results on the phenotype (respiratory infections), depending on the genotype, should not be the exposure (higher levels of perinatal anxiety) remain the same?

Response: Thank you for your comments. We intended to evaluate the interaction between perinatal anxiety levels and polymorphisms of *GSTP1*, *GSTT1*, *GSTM1*, and *CD14* on RTIs. As you mentioned, if we analyzed the risk of outcome as the perinatal exposure remains the same, the results would show the effect of genotype on the outcome. We wanted to investigate the interaction between perinatal exposure and genotype on the outcome i.e., we wanted to evaluate the effect on the outcome of the association between high perinatal exposure and a risk genotype, compared to infants with low perinatal exposure and a non-risk genotype. If our response does not satisfy your question, please give us an opportunity to offer an additional clarification?

Change of subject numbers from table 3 to table 4: The reason why the study participant number has changed has to be at least mentioned in a footnote below table 4.

Response: Thank you for your comments. We have added the reason for the differences in the number of subjects between Tables 3 and 4 in the footnote below Table 4 as follows: The subject number of this analysis is different from those in Table 3 due to the failure of simultaneous genotyping of *GSTP1* (rs1695) and *GSTT1* in several subjects.

DISCUSSION

Potential limitation, 1st sentence: rephrase to as the current study is ?

Response: Thank you for your comments. We have revised the first sentence of ‘potential limitations’ as follows: The current study has a few limitations.

Please add why exactly further studies are needed, in particular in relation to bronchiolitis (e.g., implications for asthma?).

Response: Thank you for your comments. We have revised the sentence in lines 266-269 as follows:
Further large-scale studies are needed to validate the association between perinatal anxiety and LRTIs during infancy, especially bronchiolitis, which is associated with adverse respiratory outcomes such as asthma in later life.

In addition, we added one paragraph in lines 255-259 as follows:

Hospitalization expenditures because of bronchiolitis during infancy has increased. A history of bronchiolitis in infancy is associated with adverse respiratory outcome such as reduced lung function and development of asthma in later life. With the prevention of bronchiolitis during early life, health care costs and adverse respiratory health can be avoided.