Author's response to reviews

Title: Beta2-Adrenergic receptor promoter haplotype influences the severity of acute viral respiratory tract infection during infancy: a prospective cohort study

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Author's response to reviews: see over
June 13, 2015

RE: MS: 1787793635156298: “Beta2-Adrenergic receptor promoter haplotype influences the severity of acute viral respiratory tract infection during infancy: a prospective cohort study”

Dear Dr. Hui-Qi Qu:

Thank you to the Reviewers for their helpful comments. We are resubmitting for your consideration our manuscript titled “Beta2-Adrenergic receptor promoter haplotype influences the severity of acute viral respiratory tract infection during infancy: a prospective cohort study” together with a point-by-point response to the reviewers’ helpful comments.

Our point-by-point changes in response to reviewers’ comments are described below.

Reviewer #1 (Mingzhi Zhang):

The aim of the article “β 2-Adrenergic receptor promoter haplotype influences the severity of acute viral respiratory tract infection during infancy: a prospective cohort study” is interesting but some corrections should have been done.

Methods:

1. Study population: The authors could write this part (term infants without chronic medical conditions) some more clearly. Which disease are chronic medical conditions? This patient selection should be more informative.

Response: We have revised and clarified the cohort and patient selection in the Methods section (page 6, lines 112-115):

“Term (≥37 weeks) and non-low birth weight (≥2275 grams) infants who were otherwise healthy with no significant co-morbidities or cardio-pulmonary disease were eligible, with oversampling for hospitalization infants. The rationale, methods, and detailed inclusion and exclusion criteria have been reported previously.”

The complete co-morbidity or cardio-pulmonary disease is published previously and includes: congenital or acquired chronic heart or lung disease, prior requirement for mechanical ventilation for cardiac or pulmonary disease, immunodeficiency, neurologic disease with possible aspiration, significant gastroesophageal reflux disease felt to contribute to pulmonary disease, and tracheomalacia.

2. Infant acute respiratory infection severity: Who rated the BSS?

Response: Bronchiolitis severity score (BSS) was determined by experienced research nurses who recruited all study subjects. We have added information to the methods section (page 7, lines 122-125):
“The severity of acute respiratory infection was determined by experienced research nurses through medical record chart review using an ordinal bronchiolitis severity score (BSS). The score is an aggregate of assigned values ranging of 0-3 in categories of respiratory rate, room air oxygen saturation, the presence and extent of wheezing, and the presence and extent of flaring and retractions.”

3. The sequences of the primer and probe should be clear.

Response: We have added the information in the methods section as a table (table 1) (page 8, lines 143-144), and page 24, table 1).

“Primers and probes for all SNPs are listed in table 1”.

**Table 1: Primers and probes of the 5 ADRB2 SNPs.**

<table>
<thead>
<tr>
<th>Loci</th>
<th>rs number</th>
<th>Primers</th>
<th>Probes</th>
</tr>
</thead>
</table>
| -2387 | rs1432623 | Forward Primer 5’-TTCTAAACCACTAAAGTAATTATGAAACTCGTT-3’  
Reverse Primer 5’-GGTAAGCAAGAATTGAATGATATAGTAAGAATATGAAAAA-3’ | Vic-TCACACAAGTAGTTTG  
Fam-CACACAAGTAGTTTG |
| -2274 | rs11168068 | Forward Primer 5’-GGAAGTGACTTTATGCCCCTTTAGA-3’  
Reverse Primer 5’-AGATTCACCAAACTGGAGCTTCT-3’ | Vic-AATCAGAAGTAGCTGATT  
Fam-TCACGAAGTAATTT |
| -1343 | rs2400707 | Forward Primer 5’-TAAGTCACAGACGCCAGAGGT-3’  
Reverse Primer 5’-AACAACCATCCACAGGAATGAAAGGAAT-3’ | Vic-TTCACATGGCAACAACC  
Fam-CACATGGCGCAACC |
| +16   | rs1042713 | Forward Primer 5’-CGGCAGCTTCTCGCTGCAC-3’  
Reverse Primer 5’-TGCCTGACGTGTCGTC-3’ | Vic-CACCCAATGGGAAGCCC  
Fam-CACCCAATAGAAGCCC |
| +27   | rs1042714 | Forward Primer 5’-CCTCTTGGCCTGGCAACCAAT-3’  
Reverse Primer 5’-TGCCCACACCCACAC-3’ | Vic-TCGTCCCTTTGCTGCGT  
Fam-TCGTCCCTTTGCTGCGT |
4. All SNPs met Hardy Weinberg proportions (p>0.48). This should be in Statistical analyses part. In this part there could be described how the Hardy-Weinberg equilibrium was calculated.

Response: We have moved this sentence to the statistical analyses section, and added descriptions how the Hardy-Weinberg equilibrium was calculated, including the reference to the exact method (page 8, lines 154-155).

“SNPs were tested for deviations from Hardy Weinberg proportions, also using Haploview”[1].

Results

5. Caucasian infants were more to have lower respiratory tract infection instead of upper respiratory infection, and have 216 more severe respiratory infections based on the BSS (p<0.05). What about other items’ P value? So all the P value should be added in Table 1.

Response: P values have been added in table 1 (currently table 2, page 25, Table 2).

Table 2 Characteristics of infants and their biological mothers (N=374).

<table>
<thead>
<tr>
<th>Infant characteristics</th>
<th>Caucasian (N=261)</th>
<th>African American (N=113)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (grams) (N=372), median (IQR*)</td>
<td>3345 (3090, 3657)</td>
<td>3147 (2892, 3430)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational age (weeks) (N=371), median (IQR)</td>
<td>39 (38,40)</td>
<td>39 (38,40)</td>
<td>0.498</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>140 (54)</td>
<td>67 (59)</td>
<td>0.313</td>
</tr>
<tr>
<td>Age at enrollment (weeks), median (IQR)</td>
<td>10 (6, 21)</td>
<td>18 (7, 35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Breastfeeding, n (%)</td>
<td>152 (58)</td>
<td>46 (41)</td>
<td>0.002</td>
</tr>
<tr>
<td>Daycare attendance, n (%)</td>
<td>68 (26)</td>
<td>39 (35)</td>
<td>0.096</td>
</tr>
<tr>
<td>Secondhand smoke exposure (N=371), n (%)</td>
<td>158 (61)</td>
<td>64 (57)</td>
<td>0.486</td>
</tr>
<tr>
<td>Having siblings, n (%)</td>
<td>198 (76)</td>
<td>85 (75)</td>
<td>0.894</td>
</tr>
<tr>
<td>Insurance type, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private</td>
<td>111 (43)</td>
<td>9 (8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medicare</td>
<td>135 (52)</td>
<td>101 (89)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>15 (6)</td>
<td>3 (3)</td>
<td></td>
</tr>
<tr>
<td>Season of enrollment, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004 – 2005</td>
<td>42 (16)</td>
<td>21 (19)</td>
<td>0.590</td>
</tr>
<tr>
<td>2005 – 2006</td>
<td>76 (29)</td>
<td>38 (34)</td>
<td></td>
</tr>
<tr>
<td>2006 – 2007</td>
<td>75 (29)</td>
<td>31 (27)</td>
<td></td>
</tr>
<tr>
<td>2007 – 2008</td>
<td>68 (26)</td>
<td>23 (20)</td>
<td></td>
</tr>
<tr>
<td>RSV positive</td>
<td>180 (69)</td>
<td>59 (52)</td>
<td>0.002</td>
</tr>
<tr>
<td>Lower respiratory tract infection vs. upper respiratory infection, n (%)</td>
<td>213 (82)</td>
<td>72 (64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bronchiolitis severity score (n=373), median</td>
<td>6.5 (3, 9)</td>
<td>4 (1, 7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
### Maternal characteristics

| Maternal age at enrollment (years) | 27 (22, 31) | 24 (21, 27) | <0.001 |
| Maternal education, years (n=313), n (%) | | | |
| < 12 | 42 (19) | 16 (17) | 0.004 |
| 12 | 56 (25) | 41 (44) | |
| > 12 | 122 (55) | 36 (39) | |
| Maternal asthma, n (%) | 50 (19) | 27 (24) | 0.298 |

Abbreviations: IQR = interquartile range

6. There was a significant difference between Caucasians and African Americans in the frequency distribution of the two coding SNPs. But the Caucasians and African Americans' BSS are different, so is this frequency distribution comparison right? This should be mentioned.

Response: The frequency distribution of the two coding SNPs in Caucasians and African Americans is correct, and is consistent with the reference population observed in the International HapMap Project[2] and Hawkins et al[3]. Such distribution difference, plus other differences in genetic and social economic factors between Caucasians and African Americans are the reasons that we stratified all the analyses by subjects’ racial/ancestral groups. Therefore, the association between BSS and haplotype frequency observed within each racial/ancestral group minimize the spurious finding due to the unequal distribution of alleles between racial/ancestral groups. We have added one paragraph in the Discussion section (page 13 and 14, lines 279-283):

“Such frequency distributions observed in our study are consistent with the allele frequencies reported in the reference population of the International HapMap Project[2] and Hawkins et. al[3]. Our stratified analyses by race therefore allow us to detect the association between severity of a respiratory illness and haplotype frequency within each racial group, and to minimize the spurious associations due to such population substructure.”

7. This dose dependent relationship persisted ...... (adjusted odds ratio: 0.35, 95% confidence interval [CI]: 0.13, 0.96, p=0.042). The P value is 0.042, is very mild, so the conclusion is not so powerful.

Response: We have updated the Discussion to provide context for a p value of 0.042 (page 14, lines 290-296):

“Lastly, although a p value of 0.042 is at the threshold of statistical significance, this p value reflects sample size of 113 African Americans in our study. Whether the association between BSS and CCA haplotype in African Americans is powerful or not depends upon not only p value, but also upon the effect size such as odds ratio and the clinical significance of the findings. As a difference of 0.5 in BSS is considered as clinically significant, a 65% reduced odds of developing a more severe disease (higher BSS) comparing 2 copies of CCA with 0 copy of CCA might also be clinically significant and meaningful.”

Reviewing this concern also allowed us to correct a mistake we identified in the manuscript: the 0.35 adjusted odds ratio and corresponding confidence internal reported is the result comparing 2 copies of CCA with 0 copy of CCA. We corrected in the manuscript and reported the adjusted odds ratio
(94%CI) with 1 copy difference of CCA (2 vs. 1 copy of CCA or 1 vs. 0 copy of CCA). The adjusted odds ratio is 0.59 (95%CI: 0.36, 0.98). The corresponding p value is the same as it comes from same model.

Discussion

8. The authors could discuss the BSS calculation and the possible problems with it.

Response: We have added the BSS calculation in the Methods section and discussed limitations of this score in the Discussion section.

In Methods section (page 7, lines 122-125):

“The severity of acute respiratory infection was determined by experienced research nurses through medical record chart review using an ordinal bronchiolitis severity score (BSS). The score is an aggregate of assigned values ranging of 0-3 in categories of respiratory rate, room air oxygen saturation, the presence and extent of wheezing, and the presence and extent of flaring and retractions”

In Discussion section (page 14, line 285-290):

“We recognize that the significance of our findings is limited by our small sample size and in classifying infants in this study by a severity score rather than by measuring some direct response to bronchodilator therapy. However, BSS correlated with the severity of disease (classified as upper respiratory and lower respiratory illness) well. Our sensitivity analyses with the severity of disease (upper versus lower respiratory illness) showed consistent results indicated that BSS is a valid surrogate of disease severity.”

9. Is there information about the 5 SNPS polymorphisms in different populations?

Response: We have updated the Discussion to provide additional information (page 13, lines 273-279):

The diversity of SNPs of the ADRB2 gene was explored by Drysdale et al., using immortalized lymphocytes from 23 Caucasians, 19 African-Americans, 20 Asians, and 15 Hispanic-Latinos[4]. This report from 2000 included the 2 coding block SNPs (+46 and +79) and a number of promoter SNPs extending to 1023 bp upstream of the translation start site. Hawkins et al. examined a 5.3-kb region of the ADRB2 in 429 Caucasians and 240 African-Americans and identified similar minor allele frequencies in the 3 promoter SNPs (-2387, -2274, and -1343) and in the coding block SNPs discussed in this manuscript[5].

10. The authors should shortly describe how is ADRB2 promoter haplotype alter ADRB2 expression on airway smooth muscle cells and thereby influence basal airway tone, is there a reference about that?

Response: Thank you for this helpful comment. The manuscript has been revised in the Discussion section with greater description and includes 2 additional references (page 12 and 13, lines 255-260):

“McGraw et al. showed that SNPs in the promoter region of the ADRB2 may regulate gene expression[6] Moore et al. demonstrated that ADRB2 genetic variants are associated with
Reviewer #2 (Lahcen WAKRIM):

Summary:

This report documents whether #2-Adrenergic receptor (ADRB2) polymorphisms associated with a response to asthma therapy during an asthma exacerbation is also associated with the severity of viral respiratory infections during infancy. Despite the small size of the studied population, this report has value since it demonstrate that genotype may be predictive of severity of acute respiratory tract infections and allow to potentially identify a subset of infants who may respond to beta-agonist therapy.

1. Major Compulsory Revisions

The authors divided the studied population into two ethnic groups, Caucasians and African Americans, without explaining the reason of this separation. If there is a valid reason, they should be based on this difference to discuss their findings that promoter haplotype CCA was associated with a decreased BSS in African Americans while in Caucasians, no similar protective relationship was identified.

Response: We stratified all analyses into Caucasians and African Americans to minimize spurious findings as population substructure such as race/ancestry impacts genetic study findings[8]. In our study, Caucasians and African Americans served as a surrogate measure of European or African ancestry, and is carefully classified by our genetic epidemiologist (Dr. Emma Larkin) based on questionnaires collected at enrollment. We have added and clarified in the methods section the rationale behind this stratification (page 8, lines 149-152):

“All analyses were stratified by race (Caucasian or African American), as a surrogate measure of European or African ancestry, to reduce the known impact of population substructure which can produce spurious associations[8].”

Please also see reply to reviewer 1 comments #6.

2. Discretionary Revisions

Authors should have included in their study a control population consisting of infants who have the same eligibility conditions and visited the hospital for other diseases than presumed viral bronchiolitis or respiratory tract infection. I think these control population could strengthen the associations between the genetic variants in ADRB2 and the severity of infant acute respiratory viral infections.

Response: We thank the reviewer for these comments. A control population who visited the hospital for other diseases than presumed viral bronchiolitis or respiratory tract infection could certainly help
us better understand ADRB2 genetic distribution in the general population. As noted in the Reply to Reviewer 1 #9, we have updated the Discussion section to provide additional information on what is already known about genetic variants in ADRB2. In the current study, we were focused on whether ADRB2 genotype is associated with the severity of respiratory infection.

Minor Essential Revisions

The following references: 9-10-11-12-15-20-21-22-25-26-27-29-32-38 and 45 must be removed from the list of references. They are not mentioned nowhere in the manuscript.

Response: We thank reviewer and have done a thorough check on references. All references listed are cited in the manuscript.

Thank you very much for your consideration of this revised manuscript for Publication in the BMC Medical Genetics.

Sincerely,

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References


