Author's response to reviews

Title: Positive Association between Aspirin-Intolerant Asthma and Genetic Polymorphisms of FSIP1: a Case Control Study

Authors:

Jason Y Kim (imblue870227@gmail.com)
Jeong-Hyun Kim (soykm@sogang.ac.kr)
Tae Joon Park (pires81@naver.com)
Joon Seol Bae (jsbae@sogang.ac.kr)
Jin Sol Lee (shadow2386@naver.com)
Charisse Flerida A Pasaje (charissepass@yahoo.com)
Byung Lae Park (blpark@snp-genetics.com)
Hyun Sub Cheong (chhs@snp-genetics.com)
Jong-Sook Park (js1221@schbc.ac.kr)
Sung-Woo Park (swpark@schbc.ac.kr)
Soo-Taek Uh (uhs@hosp.sch.ac.kr)
Mi-Kyeong Kim (kimmk@chungbuk.ac.kr)
Inseon S Choi (ischoi@chonnam.ac.kr)
Sang Heon Cho (shcho@plaza.snu.ac.kr)
Byoung Whui Choi (bwchoimd@cau.ac.kr)
Choon-Sik Park (mdcspark@unitel.co.kr)
Hyung-Doo Shin (hdshin@sogang.ac.kr)

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Author's response to reviews: see over
Dear Editor:

Thank you very much for giving us the opportunity to submit a revised version of our manuscript.

We are grateful to the two reviewers for their comments. Our responses to them are given below. According to the comments of the reviewers, we faithfully revised our manuscript with additional data (Supplementary Figure 1). The result evinced a significant association between the *FSIP1* and the risk of aspirin-intolerant asthma in Korean subjects.

We hope that our responses below and the associated changes to the manuscript adequately address the reviewers’ concerns, and that the manuscript would now be acceptable for publication.

With my best regards,

Hyoung Doo Shin, PhD.
Department of Life Science, Sogang University,
1 Shinsu-dong, Mapo-gu, Seoul, 121-742
Republic of Korea
E-mail: hdshin@sogang.ac.kr
Reviewer 1

Major comment:
The rationale for investigation of FSIP1 in AIA is quite vague. Definitely it is not the fact, that the gene was discovered as expressed in spermatogenesis. Neither influence of NSAIDs or PAF on spermatocyte mobility is relevant to AIA in the frame of current knowledge on the disease. The Reviewer suggests to limit copious comments and parallels on male gametes as related to asthma. This sounds oddly and no reproduction defects were ever described in AIA.

→ Reply: Thank you for your comments. We have removed the part that described relation between AIA and spermatogenesis. Instead, we have revised the text added following sentence into the introduction:

In Abstract:
“Recently, it has been reported that a peptide derived from APP is cleaved by α disintegrin and metalloproteinase 33 (ADAM33), which is an asthma susceptibility gene. It has also been known that the FSIP1 gene is expressed in airway epithelium.”

In Introduction:
“Fibrous sheath interacting protein 1 gene (FSIP1), also known as HSD10, is a recently discovered gene that was first described in 2003 [11]. With its primary function on protein binding, the FSIP1 gene is expressed in airway epithelium (GSE4498 and GDS2486, GEO database) [12].”

In Conclusion:
“Although the relation between the FSIP1 gene and AIA is not yet clearly understood, our findings suggest that FSIP1-related regulations of APP and ADAM33 may play a role for the development of aspirin hypersensitivity in asthmatics, along with the fact that FSIP1 is expressed in airway epithelium.”

Minor remarks:
1. INTRODUCTION: Most noticeable symptoms of AIA include aspirin sensitivity, bronchial asthma, and chronic rhinosinusitis with nasal polyposis [4-6] when non-steroidal anti-inflammatory drugs such as aspirin are ingested. – should be refrazed because symptoms persist without ingestion of NSAIDs, while the drugs precipitate bronchoconstriction.

→ Reply: We agree with your comment. We have changed the sentence as follows:

“The AIA was first described in 1922 [2, 3], and its most noticeable symptoms include aspirin sensitivity, bronchial asthma, and chronic rhinosinusitis with nasal polyposis [4-6]. When non-steroidal anti-inflammatory drugs such as aspirin are ingested, the drugs cause bronchoconstriction in the patients.”

2. A note in proof for expression of FSIP1 in respiratory epithelium is needed. This can be found in GEO set GSE4498 with appropriate publication reference

→ Reply: We have revised the text in the introduction section to provide proof that FSIP1 is expressed in respiratory epithelium as follows:
“Fibrous sheath interacting protein 1 gene (*FSIP1*), also known as *HSD10*, is a recently discovered gene that was first described in 2003 [11]. With its primary function on protein binding, the *FSIP1* gene is expressed in airway epithelium (GSE4498 and GDS2486, GEO database) [12].”

3. METHODS: The differences in the fall rates in FEV1 following aspirin challenge among the genotypes and haplotypes were examined using logistic model. – this is not clear: a difference is continuous variable, which categorical variables were used exactly?

[Reply: This sentence was wrong and we apologize for the mistake. We have changed the test as follows:]

“The association analyses of differences in the fall rates in FEV1 following aspirin challenge with the genotypes and haplotypes were examined by regression analysis using SAS.”

4. DISCUSSION: rs7179742 SNP is located closer to the gene’s 5’ end. The next gene is only ~20 kb upstream and it is an orphan receptor GPR176. A linkage between SNP and the other gene has to be taken into account.

[Reply: Thank you for your comment. The rs7179742 or *FSIP1* has no LD with GPR176. However, *FSIP1* and thrombospondin-1 (*THBS1* or *TSP-1*) genes were in LD. The *THBS1* gene has been implicated in the network underlying the pulmonary response to oxidative stress in asthma (J Investig Med. 57:756-64, 2009). More interestingly, aspirin, as an inhibitor of THBS1, has recently shown to lead to reduction in THBS1 levels. (J Biol Chem. 285:6770-80, 2010). This suggests that *FSIP1* might have an effect on aspirin hypersensitivity in asthma, with relation to the nearby potential gene of *THBS1*. We have added the text in the discussion part as follows:]

“On the other hand, in additional analysis of LD near *FSIP1* in Asian populations (Chinese and Japanese) from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/index.html.en), the *FSIP1* gene is in LD with the thrombospondin-1 (*THBS1* or *TSP-1*) gene (Supplementary Figure 1). The *THBS1* gene has been implicated in the network underlying the pulmonary response to oxidative stress in asthma [28]. More interestingly, aspirin, as an inhibitor of THBS1, has recently shown to lead to reduction in THBS1 levels [29]. This suggests that *FSIP1* might have an effect on aspirin hypersensitivity in asthma, with relation to the nearby potential gene of *THBS1*.”
Supplementary Figure 1 - LD plot nearby FSIP1
The LD near FSIP1 in Asian populations (Chinese and Japanese) is analyzed from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/). LD coefficient (D’) among SNPs of THBS1, FSIP1, and GPR176 in Asian populations. The FSIP1 is in LD with THBS1 with a LD block.

5. In Table 1 “Fall rate” should be explained in the legend, IgE levels is better described as median and interquartile interval.

⇒ Reply: We have explained the “fall rate” in the legend of Table 1. Also, we have changed the display of IgE levels in agreement with your suggestion. Values are shown as median (25% interquartile interval, 75% interquartile interval). We have revised them in the Table 1 as follows:

Table 1. Clinical profiles of aspirin induced asthma patients and controls.

<table>
<thead>
<tr>
<th>Clinical profile</th>
<th>Asthmatics (all subject)</th>
<th>AIA</th>
<th>ATA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>592</td>
<td>163</td>
<td>429</td>
</tr>
<tr>
<td>Age of first medical examination (mean (range))</td>
<td>46.15 (15.40-77.88)</td>
<td>43.13 (17.22-72.73)</td>
<td>47.30 (15.40-77.88)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.78 ± 8.63</td>
<td>161.72 ± 8.69</td>
<td>160.42 ± 8.39</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.81 ± 10.84</td>
<td>61.25 ± 10.38</td>
<td>63.40 ± 10.97</td>
</tr>
<tr>
<td>Fall rate (%)</td>
<td>9.27 ± 13.24</td>
<td>24.63 ± 16.11</td>
<td>3.54 ± 4.85</td>
</tr>
<tr>
<td>Blood eosinophil (%)</td>
<td>6.01 ± 5.73</td>
<td>5.96 ± 5.21</td>
<td>6.03 ± 5.92</td>
</tr>
<tr>
<td>FVC %, predicted</td>
<td>88.54 ± 14.08</td>
<td>90.35 ± 14.04</td>
<td>87.85 ± 14.05</td>
</tr>
<tr>
<td>FEV1 %, predicted</td>
<td>90.54 ± 16.97</td>
<td>87.58 ± 16.94</td>
<td>91.66 ± 16.87</td>
</tr>
<tr>
<td>PC20, methacholine (mg/ml)</td>
<td>6.43 ± 8.67</td>
<td>5.02 ± 7.83</td>
<td>6.91 ± 8.90</td>
</tr>
<tr>
<td>Total IgE (IU/ml)*</td>
<td>156 (62, 394)</td>
<td>164 (78, 357)</td>
<td>154 (53, 416)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>206/386</td>
<td>59/104</td>
<td>147/282</td>
</tr>
<tr>
<td>Current Smoker (%)</td>
<td>27.70</td>
<td>21.47</td>
<td>30.07</td>
</tr>
<tr>
<td>Positive rate of skin test (%)</td>
<td>56.42</td>
<td>52.76</td>
<td>57.81</td>
</tr>
</tbody>
</table>

Fall rate refers to the decline of FEV1% by aspirin provocation.
*Total IgE value is shown as median (25% interquartile interval, 75% interquartile interval).

AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second.
Reviewer 2

Major Compulsory Revisions:
My concern is about the smaller sample size used in this study (only 163 AIA patients). Authors should discuss this issue with respect to the power of the study and as a limitation of their study.

→ Reply: Thank you for your comments. We have recruited the patients over about 6 years due to the rareness of the AIA condition, resulting in the small number of AIA patients. Also, early publication of our results could provide supporting information. In agreement of your comment, we have revised the text in the Discussion section as follows:

“One of limitation of our study is the small number of AIA patient, which might result in decreasing the statistical power of this study. However, considering the rareness of AIA condition, our results could provide supporting information, with further needs of replication in large number of subjects.”

Minor Revisions:
1. Authors have talked about role of aspirin in spermatogenesis in introduction and discussion. For example ‘Another less known function of aspirin is that it can also lead to increased rate of spermatogenesis’ Are there any evidences of link between spermatogenesis and AIA? Authors should refer to those works, otherwise omit these redundant sentences.

→ Reply: We have removed the part that described relation between AIA and spermatogenesis. Instead, we have added following sentence into the introduction:

In Abstract:
“Recently, it has been reported that a peptide derived from APP is cleaved by α disintegrin and metalloproteinase 33 (ADAM33), which is an asthma susceptibility gene. It has also been known that the FSIP1 gene is expressed in airway epithelium.”

In Introduction:
“Fibrous sheath interacting protein 1 gene (FSIP1), also known as HSD10, is a recently discovered gene that was first described in 2003 [11]. With its primary function on protein binding, the FSIP1 gene is expressed in airway epithelium (GSE4498 and GDS2486, GEO database) [12].”

In Conclusion:
“Although the relation between the FSIP1 gene and AIA is not yet clearly understood, our findings suggest that FSIP1-related regulations of APP and ADAM33 may play a role for the development of aspirin hypersensitivity in asthmatics, along with the fact that FSIP1 is expressed in airway epithelium.”

2. Use of ‘case-control’ study is bit confusing. Current study is rather a case-case study. Authors should avoid the use of ‘controls’ for ATA patients.

→ Reply: I agree with your comment. Then, we have changed ‘controls’ for ATA patients to ‘ATA cases’ throughout the manuscript.
3. In statistical analysis, use of the word 'logistic analysis' is not clear. Do the authors intend to write 'logistic regression analysis'?

→ Reply: We apologize for the mistake. We have used the regression analysis, not logistic analysis, for associations of the fall rates in FEV$_1$ by aspirin provocation (continuous variable) with the genotypes and haplotypes. We have revised the text in the methods section as follows:

“The association analyses of differences in the fall rates in FEV$_1$ following aspirin challenge with the genotypes and haplotypes were examined by regression analysis using SAS.”

4. Though manuscript is well written overall, language needs slight improvement, 'Introduction' section in specific.

→ Reply: We have asked a native English speaker to check overall language, and revised the text throughout the manuscript.

Especially in Introduction:
“Asthma is a disease that affects a large number of people globally, which is estimated to be about 300 million worldwide [1] and about 3 million asthma patients in South Korea.”

“Among these factors, non-steroidal anti-inflammatory drugs such as aspirin are known to cause aspirin-intolerant asthma.”

“Most significant effect of decrease of prostaglandin is the alleviation of inflammation and pain.”

“One function of aspirin is decrease of prostaglandin production, with the most significant effect of decrease in the prostaglandin on the alleviation of inflammation and pain.”

5. Discretionary Revisions: It would be better to add a para about the work carried out at the end on 'Introduction'.

→ Reply: Thank you for your comment. We have revised the text in the end of introduction section as follows:

“Based on these findings, we hypothesized $FSIP1$ gene could have an effect on the mechanism of aspirin on the various levels, including onset of AIA, and conducted association analyses of $FSIP1$ gene polymorphisms between AIA and ATA patients. We also conducted association analyses between SNPs of $FSIP1$ and fall rate of FEV$_1$ by aspirin provocation.”