Lack of Association of Glutathione S-transferase P1 Ile105Val Polymorphism with Aspirin-Intolerant Asthma

Jung-Mi Oh, Seung-Hyun Kim, PhD., Chang-Hee Suh, M.D., Dong-Ho Nahm, M.D., Hae-Sim Park, M.D., PhD., Young-Mok Lee, M.D., June-Hyuk Lee, M.D., Choon-Sik Park, M.D., PhD. and Hyung-Doo Shin, PhD.

Department of Allergy and Rheumatology, Ajou University School of Medicine, Suwon, Korea; Division of Allergy and Respiratory Medicine, Department of Internal Medicine, Soonchunhyang University School of Medicine, Bucheon, Korea; Department of Genetic Epidemiology, SNP Genetics, Inc., Seoul, Korea

INTRODUCTION

Aspirin-intolerant asthma (AIA) is characterized by the development of asthma after taking aspirin and non-steroidal anti-inflammatory drugs (NSAIDs)\(^1\). The pathogenesis of AIA remains unclear, but many investigators have suggested that AIA is likely to be related with abnormal eicosanoid metabolism, in particular overproduction of leukotrienes (LTs)\(^2\) \(^3\)\(^5\). Inflammation of the airways is a characteristic feature of asthma. Oxidative stress, including reactive oxygen species (ROS), may be one of the key components of airway inflammation resulting from the production of cytotoxic products such as lipid hydroperoxides and hydroxyradicals\(^6\). These products are substrates of GSTP1, which are essential in the mobilization of arachidonic acid and regulation of proinflammatory eicosanoid release such as LTs and prostaglandins\(^7\). Inability to detoxify ROS can perpetuate the inflammation process, activate bronchoconstriction response and finally cause asthmatic symptoms.

Glutathione S-transferase (GST) has been considered to be

---

**Background**: Glutathione S-transferase P1 (GSTP1), the abundant isofrom of glutathione S-transferase in lung epithelium, plays an important role in cellular protection against oxidative stress and toxic foreign chemicals. GSTP1 (Ile105Val) polymorphism has been reported to be associated with asthma related phenotypes such as atopy and bronchial hyperresponsiveness. Therefore we investigated whether this polymorphism may be associated with the development of aspirin-intolerant asthma (AIA).

**Methods**: GSTP1 Ile105Val polymorphism was determined using a single based extension method in 88 AIA subjects and compared to 154 aspirin-tolerant asthma (ATA) subjects and 119 normal healthy controls (NC) recruited from the Korean population.

**Results**: No significant differences in allele and genotype frequencies of the GSTP1 Ile105Val polymorphism were observed in the three groups (p>0.05). However, minor G allele frequency of the GSTP1 Ile105Val polymorphism in AIA group (16.5%) tended to be lower than in the NC group (20.6%).

**Conclusion**: These results suggest a lack of association of the GSTP1 Ile105Val gene polymorphism with AIA phenotype in the Korean population (word count: 159).

**Key Words**: Aspirin-intolerant asthma, Hyperresponsiveness, Glutathione S-transferase

---

Received: March 30, 2005
Accepted: May 30, 2005
Correspondence to: Choon-Sik Park, M.D. Ph.D., Division of Allergy and Respiratory Medicine, Department of Internal Medicine Soonchunhyang University Bucheon hospital, 1174 Jung-dong, Wonmi-gu, Bucheon, Gyeonggido, 420-021, Korea Tel : 82-32-621-5105, Fax : 82-32-621-5023, E-mail: mdcspark@unitel.co.kr

*This study was supported by a grant from the Korea Health 21 R&D project, Ministry of Health & Welfare, R.O.K (01-PJ-3-PG6-01GN04-0003).
important in the protection of cells from ROS by utilizing oxidative products as a substrate. GSTs can be categorized into five cytosolic isoforms based on their biochemical, immunologic and structural properties: GST-alpha (GSTA), -mu (GSTM), pi (GSTP), -theta (GSTT) and sigma (GSTS). Among them, the GSTP1 gene is located on chromosome 11q13 which is an asthma related locus. Genetic polymorphism of the GSTP1 gene has been shown to be strongly associated with asthma and asthma related phenotypes such as atopy and bronchial hyperresponsiveness (BHR). Moreover, it has been reported that a Valine (Val) to Isoleucine (Ile) exchange at codon 105 in exon 5 may protect from developing asthma. In this study, we investigated whether GSTP1 Ile105Val polymorphism may be associated with a susceptibility to AIA phenotype in the Korean population.

### MATERIALS AND METHODS

#### Subjects

The study population consisted of 88 AIA, 154 aspirin-tolerant asthma (ATA), and 119 normal control (NC) subjects who visited the Allergy Clinic at Ajou University Hospital, Soonchunhyang University Seoul Hospital and Buchon Hospital, in Korea. Diagnosis of AIA was made based on positive responses to a lysine aspirin (L-ASA) bronchoprovocation test which was performed according to a modified method as previously described. Skin prick tests were performed using 12 common aeroallergens (Bencard, UK), histamine (positive control) and saline (negative control). Atopy was defined as a reactor to one or more common inhalant allergens on skin prick test. All clinical histories were reviewed in detail by the investigator.

Bronchial hyperresponsiveness was assessed by the methacholine bronchial challenge test in all subjects. Asthmatic subjects were divided into three groups according to their degree of airway dysfunction as follows: Group I: FEV1 ≥ 80% predicted and PC20methacholine ≥ 8 mg/mL; Group II: FEV1 1-80% predicted and PC20methacholine < 8 mg/mL; Group III: FEV1 < 80% predicted (Table 3).

#### Determination of GSTP1 genotypes by single-base extension

The exon 5 region of the GSTP1 gene was amplified by primers 5’- TATGGGAAGGACCCAGAGG-3’ and 5’- CTGC ACCCTGACCAAAG-3’. PCR was performed in a mixture containing 1.25 pmol of each primer, 20 ng genomic DNA, 250 uM dNTPs and 0.15U Taq polymerase (Applied Biosystems, Foster City, CA) in the buffer provided by the manufacturer. Amplification was performed in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA). The extension primer (AATCAATGATGACGCTGCAATAC) was
The primer extension reaction was performed using the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, CA) following the manufacturer directions. To clean up the primer extension reaction products, one unit SAP (Shrimp alkaline phosphate) was added to the reaction mixture and the mixture was then incubated at 37°C for 1 h followed by 15 min of enzyme inactivation at 72°C.

**Statistical analysis**

The difference in allele and genotype frequencies of the GSTP1 Ile105Val polymorphism between the AIA group and the other control groups were analyzed using the Chi-square and Fisher’s two-sided exact test. Differences in the mean value of the phenotypic characteristics within the asthmatic subjects were compared using ANOVA test and t-test. All statistical analysis was performed using SPSS Version 11.5 (Chicago, Illinois, USA). A p value of <0.05 was considered to be statistically significant.

**RESULTS**

**Clinical characteristics of the subjects**

Clinical characteristics of the subjects are summarized in Table 1. There were significant differences in mean age and atopy prevalence between AIA and NC groups (p<0.01). The prevalence of rhinosinusitis was significantly higher in the AIA group than in the ATA group (p=0.02). No significant differences in sex, mean age, PC20 methacholine, and basal FEV1% values were noted between the AIA and ATA groups (p>0.05).

**Allele and genotype frequencies of the GSTP1 Ile105Val polymorphism**

There were no significant differences in allele and genotype frequencies of the GSTP1 Ile105Val polymorphism among the three groups (p>0.05) (Table 2). However, minor G allele frequency of the GSTP1 Ile105Val polymorphism in the AIA group (16.4%) tended to be lower than those of the control groups, ATA (19.2%) and NC (20.5%) (Table 2).

**Association of the GSTP1 Ile105Val polymorphism with airway dysfunction in asthmatics**

Clinical characteristics of the asthmatic subjects are summarized in Table 3. There were no significant differences among the three groups (p>0.05). Although no statistically significant differences were observed, the duration of asthma for group III subjects (7.2±6.2 years) tended to be longer than for group I (5.6±5.4 years). The allele and genotype frequencies of the GSTP1 Ile105Val polymorphism are shown in Table 4. The proportion of subjects with GSTP1 Ile105Ile in group III tended to be higher than those in groups II and I, whereas the proportion of GSTP1Val105Val displayed an inverse trend (Table 4, Figure 1).

**DISCUSSION**

There have been several studies indicating GSTP1 Ile105Val is associated with asthma and its related phenotypes such as atopy and airway hyperresponsiveness to methacholine7, 14. Based on the previous findings that GSTP1 could be involved in the mobilization and regulation of LT release7, we hypothesized that the GSTP1 Ile105Val polymorphism might contribute to AIA pathogenesis and investigated the GSTP1 Ile105Val polymorphism in AIA subjects and compared their results to ATA and NC subjects. In this study, although the frequency of minor G allele of the GSTP1 Ile105Val polymorphism tended to...
be lower in the AIA group (0.164) than in the other control groups (0.205 for NC, 0.192 for ATA), no statistically significant association was observed between GSTP1 Ile105Val polymorphism and the AIA phenotype. Moreover, no association between asthma related phenotypes such as atopy and airway hyperresponsiveness of the AIA group was observed. Further studies are needed to verify these findings in a larger AIA cohort and non-Korean population.

Regarding the association between the GSTP1 Ile105Val polymorphism and asthma genetics in other populations, the frequency of the GSTP1 Ile105Ile polymorphism might be increased in patients with allergic asthma and chronic obstructive pulmonary disease. However, studies of other diseases such as rheumatoid arthritis and basal cell carcinomas suggested that the GSTP1 Ile105Ile polymorphism might have a protective role or the Val105Val polymorphism might be associated with a worse prognosis. Previous studies have suggested that the rare allele frequencies of the GSTP1 Val105Val polymorphism might be associated with the progression of airway hyperresponsiveness, which may be comparable to the previous study suggesting that the GSTP1 Val105Val polymorphism was significantly associated with a reduced risk of airway responsiveness and atopy within the allergic asthma group. The discrepancy in this study may be associated with ethnic or phenotypic differences because AIA is a distinct syndrome differing from ASA tolerant or allergic asthma. Further studies are needed to replicate these association studies in non-Korean populations.

In conclusion, the GSTPI Ile1105Val gene polymorphism was not significantly associated with the AIA phenotype in the Korean population.

Table 4. Allele and genotype frequencies according to airway dysfunction

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Group I (FEV1 ≥ 80% predicted; PC20 ≥ 8 mg/mL)</th>
<th>Group II (FEV1 ≥ 80% predicted; PC20 &lt; 8 mg/mL)</th>
<th>Group III (FEV1 &lt; 80% predicted)</th>
<th>( p ) value</th>
<th>Group I vs. Group II</th>
<th>Group I vs. Group III</th>
<th>Group II vs. Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ile105Ile</td>
<td>18 (58.11%)</td>
<td>61 (69.33%)</td>
<td>73 (73.7%)</td>
<td>0.23</td>
<td>0.41</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Ile105Val</td>
<td>12 (38.7%)</td>
<td>23 (26.3%)</td>
<td>23 (23.2%)</td>
<td>0.96</td>
<td>0.75</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>Val105Val</td>
<td>1 (3.2%)</td>
<td>4 (4.5%)</td>
<td>3 (3%)</td>
<td>0.10</td>
<td>0.25</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>q.*</td>
<td>0.23</td>
<td>0.18</td>
<td>0.15</td>
<td>0.39</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( q.* \): rare allele frequency. Each \( p \) value was calculated with co-dominant, dominant, and recessive models. Logistic regression analysis was applied, controlling for age and sex as covariables.

Figure 1. Association of the GSTP1 Ile105Val polymorphism with airway dysfunction. Subjects were divided into three groups according to their airway dysfunction: group I: FEV1 ≥ 80% predicted and PC20 ≥ 8 mg/mL; group II: FEV1 ≥ 80% predicted and PC20 < 8 mg/mL; group III: FEV1 < 80% predicted.


REFERENCES

4) Nasser S, Christie PE, Pfister R, Sousa AR, Walls A, Schmitz-Schumann M, Lee TH. Effect of endobronchial aspirin...
challenge on inflammatory cells in bronchial biopsy samples from aspirin sensitive asthmatic subjects. Thorax 51:64-70, 1996
9) Thomas NS, Wilkinson J, Holgate ST. The candidate region approach to the genetics of asthma and allergy. Am J Respir Crit Care Med 156:S144-S151, 1997
24) Weiss ST. Diet as a risk factor for asthma. Ciba Found Symp 206:244-257, 1997