

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

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**Background :** Glutathione S-transferase P1 (GSTP1), the abundant isoform 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

## INTRODUCTION

Aspirin-intolerant asthma (AIA) is characterized by the development of asthma after taking aspirin and non-steroidal anti-inflammatory drugs (NSAIDs)<sup>1)</sup>. 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)<sup>2-5)</sup>.

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 hydroxyl radicals<sup>6)</sup>. 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<sup>7)</sup>. 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

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**Table 1.** Clinical characteristics of the subjects

	AIA <sup>†</sup> (N= 88)	NC <sup>†</sup> (N=119)	ATA <sup>†</sup> (N=154)	p value	
				AIA vs. ATA	AIA vs. NC
Age (years) <sup>*</sup>	43.0±14.4	35.5±14.5	44.2±14.0	1.40	<0.01
Sex (F)	54 (61.4%)	58 (48.7%)	107 (69.5%)	0.20	0.07
Atopy	32 (39.0%)	18 (16.2%)	38 (38.4%)	0.21	<0.01
Rhinosinusitis	60 (68.2%)	NA	66 (42.9%)	0.02	NA
FEV1 (%) <sup>*</sup>	81.2±22.6	NA	82.0±20.4	0.06	NA
Serum total IgE (IU/mL) <sup>*</sup>	284.8±317.9	NA	431.1±692.0	0.07	NA
PC20 methacholine (mg/mL) <sup>*</sup>	5.15±10.5	NA	6.4±10.5	0.59	NA

<sup>†</sup> AIA, aspirin-intolerant asthma; NC, normal control; ATA, aspirin-tolerant asthma; N, number; NA, not applicable.

<sup>\*</sup>Data is shown as mean value±S.D.

**Table 2.** Allele and genotype frequencies of the GSTP1 Ile105Val polymorphism

Loci	Genotype	AIA <sup>†</sup> (N=88 )	NC <sup>†</sup> (N=119)	ATA <sup>†</sup> (N=154)	p value <sup>‡</sup>	
					AIA vs. ATA	AIA vs. NC
GSTP1	AA	63 (71.6%)	75 (63.0%)	102 (66.2%)	0.66	0.38
Ile105Val	AG	21 (23.9%)	39 (32.7%)	45 (29.2%)	0.39	0.20
	GG	4 (4.5%)	5 (4.2%)	7 (4.6%)	1.00	0.91
	q.*	0.164	0.205	0.192	0.46	0.29

<sup>†</sup> AIA, aspirin-intolerant asthma; NC, normal control; ATA, aspirin-tolerant asthma; N, number. \*q: rare allele frequency

<sup>‡</sup> Each p values were calculated with co-dominant, dominant, and recessive models. Logistic regression analysis was applied, controlling for age and sex as covariables.

important in the protection of cells from ROS by utilizing oxidative products as a substrate<sup>8</sup>). 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)<sup>9</sup>). Among them, the GSTP1 gene is located on chromosome 11q13<sup>9-11</sup>) which is an asthma related locus<sup>12</sup>). Genetic polymorphism of the GSTP1 gene has been shown to be strongly associated with asthma<sup>13</sup>) and asthma related phenotypes such as atopy and bronchial hyperresponsiveness (BHR)<sup>7, 14</sup>). Moreover, it has been reported that a Valine (Val) to Isoleucine (Ile) exchange at codon 105 in exon 5 may protect from developing asthma<sup>15, 16</sup>). 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<sup>17</sup>). 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.

Clinical characteristics of this study are described in Table 1. 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: FEV<sub>1</sub> ≥ 80% predicted and PC<sub>20</sub> methacholine ≥ 8 mg/mL; Group II: FEV<sub>1</sub> ≥ 80% predicted and PC<sub>20</sub> to methacholine < 8 mg/mL; Group III: FEV<sub>1</sub> < 80% predicted (Table 3)<sup>7, 18</sup>).

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

The exon 5 region of the GSTP1 gene was amplified by primers 5'-TATGGGAAGGACCAGCAGGAG-3' and 5'-CTGC ACCCTGACCCAAGAAG-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 (AATCAATGATGATaggacctccgctgcaatac) was

**Table 3.** Clinical characteristics of the asthmatic subjects according to airway dysfunction

	FEV1 $\geq$ 80% predicted		FEV1 < 80% predicted
	( $\uparrow$ PC20 $\geq$ 8 mg/mL; Group I) (N=31)	(PC20 < 8 mg/mL; Group II) (N=88)	(Group III) (N=78)
Age (years)*	42.7 $\pm$ 13.4	41.7 $\pm$ 13.9	44 $\pm$ 14.1
Sex (F)	22 (71.0%)	53 (60.2%)	68 (68.7%)
Atopy	7 (30.4%)	25 (41.0%)	31 (44.9%)
Total serum IgE <100 IU/mL (%)	3 (50.0%)	10 (40.0%)	6 (20.0%)
Total serum IgE $\geq$ 100 IU/mL (%)	3 (50.0%)	15 (60.0%)	24 (80.0%)
Asthma duration (years)*	5.6 $\pm$ 5.4	6.2 $\pm$ 5.7	7.2 $\pm$ 6.2

\* $\uparrow$  PC20, Provocative dose of methacholine needed to reduce FEV1 by 20%.

\*Data is shown as mean value $\pm$ S.D.

designed for single-base extension (SBE). 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 phosphatase) was added to the reaction mixture and the mixture was then incubated at 37°C for 1h followed by 15min 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, PC<sub>20</sub> methacholine, and basal FEV<sub>1</sub> % 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). Asthma related phenotypes such as atopy, total serum IgE level, predicted basal FEV<sub>1</sub>% and the PC<sub>20</sub> methacholine values were not associated with the GSTP1 Ile105Val polymorphism (*p*>0.05, data not shown).

#### 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 $\pm$ 6.2 years) tended to be longer than for group I (5.6 $\pm$ 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 GSTP1 Val105Val displayed an inverse trend (Table 4, Figure 1). Similarly, allele frequency for GSTP1 Val105Val tended to be lower in groups II and III (18.0% and 15.0%, respectively) than in group I (23.0%).

## DISCUSSION

There have been several studies indicating GSTP1 Ile105Val is associated with asthma and its related phenotypes such as atopy and airway hyperresponsiveness to methacholine<sup>7, 14</sup>. Based on the previous findings that GSTP1 could be involved in the mobilization and regulation of LT release<sup>7</sup>, 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

**Table 4.** Allele and genotype frequencies according to airway dysfunction

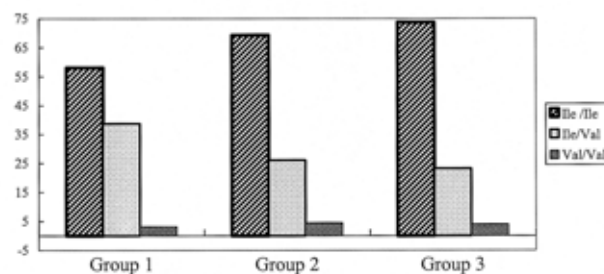
Genotype	Group I (FEV <sub>1</sub> ≥ 80% predicted; PC <sub>20</sub> ≥ 8 mg/mL)	Group II (FEV <sub>1</sub> ≥ 80% predicted; PC <sub>20</sub> < 8 mg/mL)	Group III (FEV <sub>1</sub> < 80% predicted)	* p value		
				Group I vs. Group III	Group I vs. Group II	Group II vs. Group III
Ile105Ile	18 (58.11%)	61 (69.33%)	73 (73.7%)	0.23	0.41	0.75
Ile105Val	12 (38.7%)	23 (26.1%)	23 (23.2%)	0.96	0.75	0.59
Val105Val	1 (3.2%)	4 (4.5%)	3 (3%)	0.10	0.25	0.50
q.*	0.23	0.18	0.15	0.14	0.39	0.44

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

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<sup>19</sup>. However, studies of other diseases such as rheumatoid arthritis<sup>20</sup> and basal cell carcinoma<sup>21</sup> 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 are different based on ethnic population: Asian population (Japanese: 0.14, Taiwanese: 0.18) had a lower Val105 allele frequency compared to African-American (0.42) or European-American (0.33) populations, within the asthmatic cohorts<sup>19, 22, 23</sup>. In this study the allele frequency of the GSTP1 Val105Val in healthy control subjects was similar to Taiwanese subjects.

Asthma is characterized by airway inflammation<sup>12</sup>. Accordingly, we also investigated GSTP1 polymorphism according to the degree of airway dysfunction within the asthmatic group. We selected a concentration of 8 mg/mL of PC<sub>20</sub> methacholine as the cut-off point for current asthma following standards outlined in other studies<sup>7, 18, 24</sup>. The frequency of GSTP1 Val105Val allele tended to be lower in subjects with severe airway dysfunction (Group III, 15.0%) compared to subjects with mild airway dysfunction (Group I, 23.0%; Table 4, Figure 1). This result may imply that the GSTP1 Val105Val polymorphism may be related to 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



**Figure 1.** Association of the GSTP1 Ile105Val polymorphism with airway dysfunction. Subjects were divided into three groups according to their airway dysfunction: group I: FEV<sub>1</sub> ≥ 80% predicted and PC<sub>20</sub> ≥ 8 mg/mL; group II: FEV<sub>1</sub> ≥ 80% predicted and PC<sub>20</sub> < 8 mg/mL; group III: FEV<sub>1</sub> < 80% predicted.

allergic asthma group<sup>14</sup>. 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 GSTP1 Ile105Val gene polymorphism was not significantly associated with the AIA phenotype in the Korean population.

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