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journal homepage: www.elsevier.com/locate/ypuptAssociation of the variants in *AGT* gene with modified drug response in Korean aspirin-intolerant asthma patients

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ABSTRACT

The *angiotensinogen* (*AGT*) gene enhances the effect of several bronchoconstrictors and produces a peptide that is accumulated in the airways of asthma patients; events that may underpin the pathogenesis of aspirin-intolerant asthma (AIA). To carry out a case-control analysis between *AGT* and aspirin-induced bronchospasm following treatment with an anti-asthma drug, montelukast (MLK), 38 single nucleotide polymorphisms (SNPs) in *AGT* were genotyped in 56 AIA cohort. Genotyping was performed with TaqMan assay and haplotypes were inferred using PHASE algorithm ver. 2.0. Statistical analyses of each SNPs and haplotypes were performed using SAS version 9.1. Among 13 variants displaying significant signals, 2 SNPs (+2401C>G and +2476C>T) in the intronic region of *AGT* were significantly associated with modification of drug response even after correction for multiple testing ($P = 0.0009$ – 0.002 ; $P^{\text{corr}} = 0.02$ – 0.03). Furthermore, the two variants also exhibited associations with MLK response rate ($P = 0.0003$ – 0.0006 ; $P^{\text{corr}} = 0.006$ – 0.01). Although our results are preliminary and further replication in a larger-scale group of subjects should be warranted, these observations provide evidence that *AGT* variants might be one of genetic factors involved in the response of anti-asthma drugs in AIA patients.

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1. Introduction

Aspirin-intolerant asthma (AIA) refers to a distinct clinical syndrome that is characterized by bronchial asthma, aspirin sensitivity and nasal polyps. AIA prevalence has been reported to be around 10–20% in adult asthmatics [1,2], primarily affecting women than men [3]. Moreover, ingestion of aspirin may be a possible factor in life threatening asthma attacks since it has been observed that up to 25% of asthma patients who required emergency mechanical ventilation are aspirin-intolerant [4,5]. Up to

date, the exact mechanisms involved in the pathophysiology of AIA are still poorly defined.

Angiotensin II (Ang II), one of angiotensins produced by the *angiotensinogen* (*AGT*) gene, has been known as a potent vasoconstrictor of vascular smooth muscle and a bronchoconstrictor agent that enhances the effect of other bronchoconstrictors *in vitro* [6] and *in vivo* [7]. Previous studies have shown that elevation of Ang II levels has been observed in some acute asthma patients [8,9]. Furthermore, previous evidences implicating that Ang II in the release of leukotriene C4 (LTC4) [10,11] and associations between polymorphisms in the *leukotriene C4 synthase* (*LTC4S*) gene with the onset of AIA [12] suggest that Ang II-LTC4 interactions may be an essential mechanism of AIA.

Overexpression of cysteinyl leukotriene (cysLT) receptor 1 (cysLT-R1), one of the binding proteins for cysLTs, is crucial in the pathogenesis of AIA [13,14] and has been widely studied for disease therapy. Montelukast (MLK), a cysLT-R1 antagonist, is a well-known drug for asthma. Treatment with MLK can improve lung function during airway inflammation and asthma management in AIA patients [15]. This drug relieves asthma symptoms through

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reduction of leukotriene-generated bronchoconstriction by preventing specific cysLTs from binding to the cysLT receptors in the lungs and bronchus [16]. A recent study has shown that MLK treatment for several weeks produces significant decline in FEV1 following aspirin challenge [17], suggesting the drug's effectiveness in the AIA patients.

Considering that AGT facilitates activation of Ang II, an enzyme that is leaked in the airways as a result of increased vascular permeability during airway inflammation [18], and that AGT affects the bronchoconstrictive function of leukotrienes, we hypothesize that the AGT gene may play a significant role in the potentiality of AIA patients to respond to MLK. A case-control analysis was carried out in order to investigate the association between AGT gene variations and anti-asthma drug response among AIA patients in a Korean population.

2. Materials and methods

2.1. Study subjects

A total of 56 aspirin-intolerant asthmatics were recruited among the patients enrolled at the Asthma Genome Research Center of Soonchunhyang University Bucheon hospital in Korea from 2003 to 2008. Each patient showed clinical symptoms that met the criteria for asthma according to the Global Initiative for Asthma (GINA) Global strategy for asthma management and prevention study. Evaluation of the subjects included airway reversibility measured by a positive bronchodilator response of >15% increase in forced expiratory volume in 1 s (FEV1) after inhalation of two puffs of aerosolized albuterol (100 µg) and/or airway hyperreactivity to <10 mg/mL methacholine [19]. The exclusion criteria included asthma duration that is less than a year, acutely exacerbated asthma within 4 weeks, history of brittle asthma, atopy to pollens, parenchymal lung disease apparent on simple chest radiography, and previous use of leukotriene antagonists. Patients with hypertension and diabetes or those who were previously taking angiotensin converting enzyme inhibitors were excluded from this study. After written consents were obtained, MLK (10 mg) was administered to all of the AIA patients daily for 12 weeks before the second aspirin challenge was carried out [17]. From the results of the second aspirin challenge, subjects were classified into the unnormalized group (cases) and normalized group (controls) when the aspirin-induced rate of FEV1 decline was >15% and <15%, respectively. The unnormalized subjects consisted of AIA patients who least responded to MLK treatment, whereas the normalized group was composed of AIA patients who responded well to the treatment. The study protocol was approved by the Institutional Review Board.

2.2. SNP selection, genotyping and haplotype construction

A total of 38 common SNPs in the AGT gene were selected from dbSNP in the National Center for Biotechnology Information (build 36) and International HapMap Project (<http://hapmap.ncbi.nlm.nih.gov/>) based on the minor allele frequency which is over 10% in Asian population and linkage disequilibrium (LD) status. Genotyping was performed with TaqMan assay using the ABI prism 7900HT sequence detection system (Applied Biosystems, CA, USA), and data quality was assessed by duplicate DNAs ($n = 10$). Genotyped data were obtained using the ABI PRISM sequence detection system (SDS) software version 2.3. With an average call rate of 99.9%, a total of 38 SNPs in AGT were successfully genotyped from 56 AIA patients of Korean ethnicity. Using the PHASE algorithm ver. 2.0 [20], haplotypes were inferred from the genotyped SNPs.

2.3. Statistical analyses

Wilcoxon signed ranks sum test was used to compare the effects of MLK treatment according to clinical parameters. To determine the association between the genotype distributions of unnormalized and normalized patients, multivariate logistic analysis was carried out controlling for age (continuous value), gender (male = 0, female = 1), smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2), atopy (absence = 0, presence = 1) and body mass index (BMI) as covariates to eliminate or reduce any confounds that might influence the findings. Association analysis for the haplotypes inferred in this study was managed by SAS version 9.1 (SAS Inc., Cary, NC). Lewontin's D' ($|D'|$) and the LD coefficient r^2 were then examined to measure LD between all pairs of biallelic loci [21]. For further analyses, differences in the MLK response rate among the genotypes and haplotypes were measured using a regression model. To achieve optimal correction for multiple testing of SNPs that are in LD, the effective number (20,541) of independent marker loci in AGT was calculated using the SNPSpD software (<http://genepi.qimr.edu.au/general/daleN/SNPSpD/>), a program that is based on the spectral decomposition (SpD) of matrices of pair-wise LD between SNPs [22].

3. Results

3.1. Characteristics of the study subjects

In the results from a total of 56 AIA patients of Korean ethnicity who were assessed on their response to a twelve-week MLK treatment, the aspirin-induced decline of FEV1 observed in 21 unnormalized cases was more than 15% after the second aspirin challenge, whereas the remaining 35 normalized controls showed less than 15% decline in aspirin-induced FEV1 on the same examination (Table 1). Any side effects from MLK administration that might interrupt the medication did not occur in the duration of the study. The unnormalized patients who least responded to the drug showed a higher PC20 methacholine level and a lower positive rate of skin test compared to the normalized controls (Table 1). The clinical profiles of the study subjects are summarized in Table 1.

Table 1
Clinical profiles of the study subjects.

Clinical profile	Unnormalized	Normalized
Number of subjects	21	35
Age [year, mean (range)]	44.7 (25.6–66.8)	48.1 (18.2–72.4)
Sex (male/female)	2/19	13/22
BMI (kg/m ²)	23.09 ± 2.82	24.05 ± 2.90
Smoker (current smoker/ex-smoker) (%)	28.57 (4.76/23.81)	22.86 (11.43/11.43)
FVC, predicted (%)	82.05 ± 22.25	85.09 ± 18.08
FEV1, predicted (%)	76.95 ± 25.82	79.34 ± 21.38
PC20 methacholine (mg/ml)	6.25 ± 9.23	2.78 ± 6.03
Positive rate of skin test (%)	57.14	64.29
Initial aspirin-induced rate of FEV1% decline	35.37 ± 13.06	25.32 ± 10.03
Second aspirin-induced rate of FEV1% decline	26.06 ± 9.82	3.92 ± 4.65
MLK response rate*	19.49 ± 34.79	80.40 ± 23.18

Unnormalized, AIA induced rate of FEV1 decline after treatment with MLK > 15%; Normalized, AIA induced rate of FEV1 decline after treatment with MLK < 15%. Second aspirin-induced rate of FEV1% decline indicates the decline rate after three months treatment with MLK 10 mg. MLK response rate = (initial aspirin-induced FEV1% decline – second aspirin-induced FEV1% decline)/initial aspirin-induced FEV1% decline × 100. MLK, Montelukast. *P-value < 0.05.

3.2. Genotyping of AGT variants and haplotype construction

With an average call rate of 99.9%, a total of 38 common SNPs in the AGT gene were successfully genotyped in 56 AIA patients who underwent MLK treatment. Among the variants, 7 SNPs are positioned in 5' flanking region; 1 SNP each in 5' and 3' untranslated regions; 24 SNPs in introns; 2 SNPs in exon 2; 3 SNPs in 3' flanking region (Fig. 1A and Supplementary Table 1). Results from Hardy-Weinberg Equilibrium (HWE) test showed no significant differences between the distribution of the observed genotypes and the expected distributions ($P > 0.05$; Supplementary Table 1). The minor allele frequency (MAF) of each SNP is shown in Supplementary Table 1. Using the genotyped SNPs, five major haplotypes with frequencies over 0.05 (Fig. 1B) were obtained and analyzed for a possible association with modification of MLK effects. Furthermore, one tight LD block (Fig. 1C) was established from the genotyped SNPs.

3.3. Association of SNPs in AGT with modification of aspirin-induced bronchospasm after treatment with MLK among AIA patients

Logistic analysis of the SNPs in AGT between unnormalized and normalized AIA patients adjusted for age, sex, smoking status, atopy and BMI as covariates showed that sixteen SNPs initially exhibited association signals with modified response to MLK treatment depending on the genetic models ($P = 0.0009–0.04$; Table 2). However, the significance was decreased to nominal evidence after correction for multiple comparisons. Finally, two SNPs, +2401C>G and +2476C>T in intron 2 emerged to be significantly associated with modified response to MLK treatment even after multiple testing corrections ($P = 0.0009–0.01$; $P^{\text{corr}} = 0.02–0.04$ depending on the models; Table 2). The MAFs of these significant variants were

over two-fold higher in the unnormalized group compared to those of the normalized subjects (Table 2). This suggests that variants in AGT may be involved in persistent aspirin-induced bronchospasm despite MLK medication among AIA patients. Results from the haplotype analyses, however, revealed no significant association between AGT haplotypes and modified responses to MLK treatment.

3.4. Association of the polymorphisms in AGT with MLK response rate

To further investigate whether polymorphisms of the AGT gene might be associated with MLK response rate, regression analysis was performed. Initially, seventeen AGT SNPs revealed association signals with modification of MLK response rate in AIA patients ($P = 0.00004–0.04$ depending on the models, Table 3). Even after correction for multiple testing, the two SNPs (+2401C>G and +2476C>T), which showed significant signals in the case-control analysis, were also associated with the modified MLK response rate ($P = 0.00004–0.003$; $P^{\text{corr}} = 0.0008–0.02$ depending on the models), suggesting that AGT polymorphisms could play a crucial role in the reversibility of lung function abnormalities in AIA patients despite MLK medication.

4. Discussion

One of the serious threats of aspirin-intolerance is the aggravation of acute asthma attacks, leading patients to undergo mechanical ventilation in some cases [4,5]. Since severe sudden asthma attacks are considered to be deadly symptom even when patients are treated with fast-acting medicines, researchers are actively engaged in identifying mechanisms that are involved in the development of AIA and lead to novel targets for treatment of the

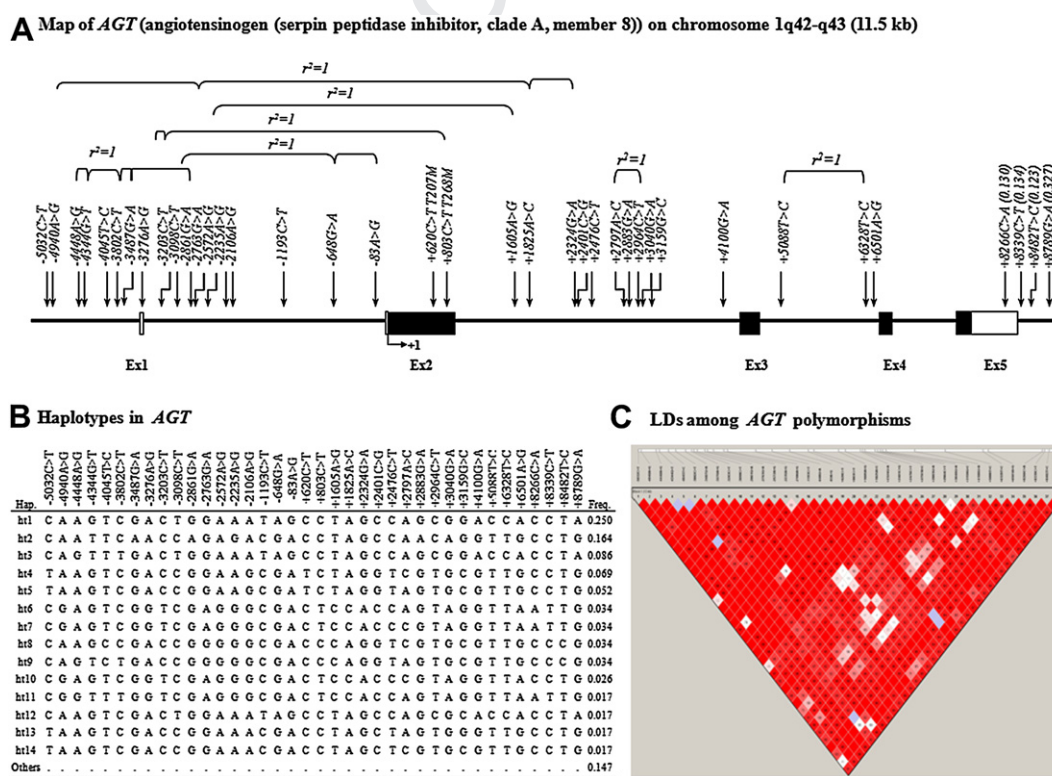


Fig. 1. Physical map, haplotypes, and LD of the AGT gene. (A) Schematic gene map and SNPs in the AGT gene on chromosome 1q42-q43 (11.5 kb). Black blocks represent coding exons and white blocks represent 5' and 3' untranslated regions. The first base of translation site was denoted as nucleotide +1. (B) Haplotypes of AGT. (C) LD coefficient ($|r^2|$) among AGT SNPs.

Table 2
Logistics association of polymorphisms in AGT between unnormalized and normalized subjects depending on the models.

Loci	rs #	MAF		Co-dominant			Dominant			Recessive		
		Unnormalized (n = 21)	Normalized (n = 35)	OR (95% CI)	P*	P ^{corr**}	OR (95% CI)	P*	P ^{corr**}	OR (95% CI)	P*	P ^{corr**}
–5032C>T	rs6683321	0.190	0.186	2.54 (0.63–10.24)	0.19	–	1.25 (0.29–5.38)	0.77	–	–	–	–
–4940A>G	rs2493136	0.119	0.200	0.33 (0.08–1.41)	0.13	–	0.33 (0.07–1.52)	0.15	–	–	–	–
–4448A>G	rs11122580	0.167	0.171	1.03 (0.25–4.26)	0.97	–	1.03 (0.25–4.26)	0.97	–	–	–	–
–4344G>T	rs2071404	0.175	0.171	1.03 (0.25–4.26)	0.97	–	1.03 (0.25–4.26)	0.97	–	–	–	–
–4045T>C	rs2071406	0.238	0.014	–	–	–	–	–	–	–	–	–
–3802C>T	rs5046	0.167	0.171	1.03 (0.25–4.26)	0.97	–	1.03 (0.25–4.26)	0.97	–	–	–	–
–3487G>A	rs5049	0.175	0.171	1.03 (0.25–4.26)	0.97	–	1.03 (0.25–4.26)	0.97	–	–	–	–
–3276A>G	rs5051	0.095	0.200	0.33 (0.08–1.41)	0.13	–	0.33 (0.07–1.52)	0.15	–	–	–	–
–3203C>T	rs2148582	0.095	0.200	0.33 (0.08–1.41)	0.13	–	0.33 (0.07–1.52)	0.15	–	–	–	–
–3098C>T	rs3789679	0.286	0.429	0.22 (0.06–0.84)	0.03	0.56	0.18 (0.04–0.91)	0.04	0.78	0.17 (0.01–2.10)	0.17	–
–2861G>A	rs3827750	0.190	0.171	1.03 (0.25–4.26)	0.97	–	1.03 (0.25–4.26)	0.97	–	–	–	–
–2763G>A	rs2493134	0.119	0.200	0.33 (0.08–1.41)	0.13	–	0.33 (0.07–1.52)	0.15	–	–	–	–
–2572A>G	rs7539013	0.357	0.214	1.93 (0.70–5.32)	0.21	–	2.30 (0.62–8.57)	0.21	–	2.31 (0.23–22.96)	0.47	–
–2235A>G	rs3889728	0.524	0.386	1.78 (0.68–4.63)	0.24	–	0.97 (0.25–3.81)	0.96	–	7.73 (1.13–52.89)	0.04	0.76
–2106A>G	rs2004776	0.524	0.343	2.87 (1.05–7.86)	0.04	0.83	9.58 (1.60–57.48)	0.01	0.28	1.73 (0.35–8.65)	0.50	–
–1193C>T	rs11122578	0.262	0.429	0.22 (0.06–0.84)	0.03	0.56	0.18 (0.04–0.91)	0.04	0.78	0.17 (0.01–2.10)	0.17	–
–648G>A	rs11568028	0.286	0.429	0.22 (0.06–0.84)	0.03	0.56	0.18 (0.04–0.91)	0.04	0.78	0.17 (0.01–2.10)	0.17	–
–83A>G	rs11122576	0.286	0.429	0.22 (0.06–0.84)	0.03	0.56	0.18 (0.04–0.91)	0.04	0.78	0.17 (0.01–2.10)	0.17	–
+620C>T	rs4762	0.167	0.129	3.09 (0.72–13.14)	0.13	–	2.24 (0.49–10.22)	0.30	–	–	–	–
+803C>T	rs699	0.095	0.200	0.33 (0.08–1.41)	0.13	–	0.33 (0.07–1.52)	0.15	–	–	–	–
+1605A>G	rs6687360	0.357	0.214	1.93 (0.70–5.32)	0.21	–	2.30 (0.62–8.57)	0.21	–	2.31 (0.23–22.96)	0.47	–
+1825A>C	rs2478539	0.119	0.200	0.33 (0.08–1.41)	0.13	–	0.33 (0.07–1.52)	0.15	–	–	–	–
+2324G>A	rs2493133	0.119	0.200	0.33 (0.08–1.41)	0.13	–	0.33 (0.07–1.52)	0.15	–	–	–	–
+2401C>G	rs2478544	0.429	0.143	12.56 (2.82–56.01)	0.0009	0.02	12.10 (2.50–58.63)	0.002	0.04	–	–	–
+2476C>T	rs2478545	0.452	0.200	13.52 (2.71–67.43)	0.002	0.03	7.51 (1.61–35.09)	0.01	0.21	–	–	–
+2797A>C	rs3789671	0.500	0.386	2.42 (0.88–6.62)	0.09	–	4.98 (1.02–24.33)	0.05	0.97	1.87 (0.33–10.62)	0.48	–
+2883G>A	rs3789670	0.190	0.171	1.26 (0.32–5.02)	0.74	–	1.26 (0.32–5.02)	0.74	–	–	–	–
+2964C>T	rs3789669	0.500	0.386	2.42 (0.88–6.62)	0.09	–	4.98 (1.02–24.33)	0.05	0.97	1.87 (0.33–10.62)	0.48	–
+3040G>A	rs2493132	0.286	0.371	0.41 (0.13–1.34)	0.14	–	0.28 (0.07–1.10)	0.07	–	1.17 (0.08–16.68)	0.91	–
+3159G>C	rs3789666	0.405	0.206	5.94 (1.67–21.18)	0.006	0.12	5.69 (1.27–25.52)	0.02	0.47	40.57 (1.96–840.03)	0.02	0.34
+4100G>A	rs11568056	0.286	0.457	0.14 (0.03–0.70)	0.02	0.34	0.10 (0.01–0.70)	0.02	0.42	0.17 (0.01–2.10)	0.17	–
+5088T>C	rs2478523	0.595	0.386	23.20 (3.00–179.37)	0.003	0.05	4.06 (0.73–22.66)	0.11	–	–	–	–
+6328T>C	rs11122575	0.310	0.457	0.14 (0.03–0.70)	0.02	0.34	0.10 (0.01–0.70)	0.02	0.42	0.17 (0.01–2.10)	0.17	–
+6501A>G	rs1926723	0.310	0.457	0.14 (0.03–0.70)	0.02	0.34	0.10 (0.01–0.70)	0.02	0.42	0.17 (0.01–2.10)	0.17	–
+8266C>A	rs7079	0.095	0.114	0.48 (0.11–2.07)	0.32	–	0.50 (0.10–2.47)	0.40	–	–	–	–
+8339C>T	rs2067853	0.071	0.114	0.48 (0.11–2.07)	0.32	–	0.50 (0.10–2.47)	0.40	–	–	–	–
+8482T>C	rs11568046	0.214	0.043	–	–	–	–	–	–	–	–	–
+8789G>A	rs11122574	0.262	0.457	0.14 (0.03–0.66)	0.01	0.27	0.09 (0.01–0.61)	0.01	0.28	0.17 (0.01–2.10)	0.17	–
ht1		0.190	0.271	0.56 (0.19–1.64)	0.29	–	0.67 (0.15–3.05)	0.61	–	0.17 (0.01–2.10)	0.17	–
ht2		0.167	0.171	1.03 (0.25–4.26)	0.97	–	1.03 (0.25–4.26)	0.97	–	–	–	–
ht3		0.048	0.114	0.19 (0.02–1.83)	0.15	–	0.19 (0.02–1.83)	0.15	–	–	–	–
ht4		0.095	0.057	4.26 (0.66–27.52)	0.13	–	2.81 (0.35–22.62)	0.33	–	–	–	–
ht5		0.071	0.043	1.44 (0.24–8.66)	0.69	–	1.44 (0.24–8.66)	0.69	–	–	–	–

*P-values for logistic analyses controlling age, smoking status, atopy and BMI as covariates with AIA patients, assuming co-dominant, dominant and recessive models.

**P-values after multiple testing corrections (effective number = 20.541). OR, odds ratio; CI, confidence interval.

disease. Therefore, findings from this study might reveal an important function of the human AGT gene in modification of the anti-asthmatic effects of MLK.

MLK has been one of the widely studied drugs for asthma treatments and has been proven effective in controlling symptoms of AIA [15]. The efficacy of the drug has been summarized by previous studies; that it would take effect after 2 h with a single 10 mg dose and 24 h after the last dose with multiple dosing [23], and that a 12-week MLK treatment provides a protective effect against AIA [17]. Similarly, pretreatment with other cysLT receptor antagonists, such as pranlukast, zileuton and zafirlukast, has prevented the pathophysiological response of asthma after administration of aspirin [24,25]. After conducting different challenges in exercise-induced asthma and AIA, results from recent studies have revealed that all leukotriene-modifying drugs share the ability of preventing bronchoconstriction [26,27], as an additional evidence of the preventive effect of MLK. However, to date there have been minimal studies demonstrating the association between the bronchoconstrictive activity of MLK and specific genetic variants.

The human AGT polymorphisms may be clinically relevant by modulating Ang II, an enzyme that can potentiate the effect of other

bronchoconstrictor agents such as the cysLTs [6,7]. Although the exact mechanism of bronchoconstriction by Ang II remains to be explored, a direct effect on airway smooth muscle or the release of other mediators of bronchoconstriction has been demonstrated by a previous study [9]. Furthermore, elevated levels of Ang II as a byproduct of RAS activation have also been observed in some patients with severe acute asthma [8,28]. This information serves as an appropriate reference for the selection of AGT in our association study with the effectiveness of MLK treatment among aspirin-intolerant asthmatics.

In the present study, 38 common SNPs in the AGT gene were genotyped in 21 unnormalized and 35 normalized AIA patients who underwent anti-asthma drug treatment. Unnormalized and normalized groups were determined according to aspirin-induced bronchospasm exhibited by each subject after two rounds of aspirin challenge. Interestingly, after genotyping SNPs of AGT and performing correction for multiple testing, we found a significant association between two SNPs (+2401C>G and +2476C>T) with modified drug response among AIA subjects. Since the minor allele frequencies of these two variants in unnormalized group were 3-fold and 2.3-fold higher compared to the normalized subjects

Table 3Regression association of polymorphisms in *AGT* with MLK response rate depending on the models.

Loci	rs #	MLK response rate (Mean $\pm$ SD)			<i>Pa</i> *	<i>Pa</i> ^{CORF**}	<i>Pb</i> *	<i>Pb</i> ^{CORF**}	<i>Pc</i> *	<i>Pc</i> ^{CORF**}
		C/C (n)	C/R (n)	R/R (n)						
–5032C>T	rs6683321	37 (56.87 $\pm$ 42.47)	17 (61.39 $\pm$ 40.03)	2 (37.69 $\pm$ 8.42)	0.21	–	0.39	–	0.14	–
–4940A>G	rs2493136	39 (57.92 $\pm$ 40.88)	15 (50.67 $\pm$ 40.95)	2 (102.17 $\pm$ 3.07)	0.40	–	0.70	–	0.12	–
–4448A>G	rs11122580	37 (59.15 $\pm$ 41.07)	19 (54.45 $\pm$ 41.29)	–	0.59	–	0.59	–	–	–
–4344G>T	rs2071404	36 (60.53 $\pm$ 40.78)	19 (54.45 $\pm$ 41.29)	–	0.55	–	0.55	–	–	–
–4045T>C	rs2071406	46 (66.14 $\pm$ 33.60)	9 (15.30 $\pm$ 51.49)	1 (42.86)	0.003	0.06	0.0008	0.02	0.69	–
–3802C>T	rs5046	37 (59.15 $\pm$ 41.07)	19 (54.45 $\pm$ 41.29)	–	0.59	–	0.59	–	–	–
–3487G>A	rs5049	36 (60.53 $\pm$ 40.78)	19 (54.45 $\pm$ 41.29)	–	0.55	–	0.55	–	–	–
–3276A>G	rs5051	40 (56.71 $\pm$ 41.07)	14 (53.61 $\pm$ 40.82)	2 (102.17 $\pm$ 3.07)	0.34	–	0.60	–	0.12	–
–3203C>T	rs2148582	40 (56.71 $\pm$ 41.07)	14 (53.61 $\pm$ 40.82)	2 (102.17 $\pm$ 3.07)	0.34	–	0.60	–	0.12	–
–3098C>T	rs3789679	22 (47.86 $\pm$ 44.62)	26 (58.28 $\pm$ 40.30)	8 (81.88 $\pm$ 18.78)	0.01	0.20	0.01	0.29	0.10	–
–2861G>A	rs3827750	36 (60.53 $\pm$ 40.78)	20 (52.20 $\pm$ 41.42)	–	0.50	–	0.50	–	–	–
–2763G>A	rs2493134	39 (57.92 $\pm$ 40.88)	15 (50.67 $\pm$ 40.95)	2 (102.17 $\pm$ 3.07)	0.40	–	0.70	–	0.12	–
–2572A>G	rs7539013	31 (68.73 $\pm$ 35.60)	20 (39.63 $\pm$ 35.02)	5 (59.95 $\pm$ 70.80)	0.21	–	0.12	–	0.83	–
–2235A>G	rs3889728	18 (67.49 $\pm$ 41.71)	27 (58.89 $\pm$ 27.65)	11 (38.03 $\pm$ 60.18)	0.12	–	0.41	–	0.08	–
–2106A>G	rs2004776	21 (77.82 $\pm$ 24.71)	24 (40.50 $\pm$ 42.63)	11 (56.09 $\pm$ 46.97)	0.04	0.72	0.01	0.22	0.38	–
–1193C>T	rs11122578	23 (46.19 $\pm$ 44.32)	25 (60.23 $\pm$ 39.86)	8 (81.88 $\pm$ 18.78)	0.007	0.15	0.01	0.21	0.10	–
–648G>A	rs11568028	22 (47.86 $\pm$ 44.62)	26 (58.28 $\pm$ 40.30)	8 (81.88 $\pm$ 18.78)	0.01	0.20	0.01	0.29	0.10	–
–83A>G	rs11122576	22 (47.86 $\pm$ 44.62)	26 (58.28 $\pm$ 40.30)	8 (81.88 $\pm$ 18.78)	0.01	0.20	0.01	0.29	0.10	–
+620C>T	rs4762	41 (59.54 $\pm$ 41.58)	14 (53.60 $\pm$ 40.67)	1 (31.73)	0.12	–	0.19	–	0.19	–
+803C>T	rs699	40 (56.71 $\pm$ 41.07)	14 (53.61 $\pm$ 40.82)	2 (102.17 $\pm$ 3.07)	0.34	–	0.60	–	0.12	–
+1605A>G	rs6687360	31 (68.73 $\pm$ 35.60)	20 (39.63 $\pm$ 35.02)	5 (59.95 $\pm$ 70.80)	0.21	–	0.12	–	0.83	–
+1825A>C	rs2478539	39 (57.92 $\pm$ 40.88)	15 (50.67 $\pm$ 40.95)	2 (102.17 $\pm$ 3.07)	0.40	–	0.70	–	0.12	–
+2324G>A	rs2493133	39 (57.92 $\pm$ 40.88)	15 (50.67 $\pm$ 40.95)	2 (102.17 $\pm$ 3.07)	0.40	–	0.70	–	0.12	–
+2401C>G	rs2478544	31 (74.43 $\pm$ 26.25)	22 (35.66 $\pm$ 49.17)	3 (43.70 $\pm$ 12.42)	0.0003	0.006	0.00004	0.0008	0.24	–
+2476C>T	rs2478545	27 (72.99 $\pm$ 26.83)	25 (43.11 $\pm$ 50.11)	4 (43.69 $\pm$ 10.14)	0.0006	0.01	0.0005	0.01	0.17	–
+2797A>C	rs3789671	20 (72.69 $\pm$ 26.19)	24 (40.90 $\pm$ 50.28)	12 (65.65 $\pm$ 27.78)	0.19	–	0.04	0.92	0.97	–
+2883G>A	rs3789670	36 (60.07 $\pm$ 41.27)	20 (53.03 $\pm$ 40.68)	–	0.52	–	0.52	–	–	–
+2964C>T	rs3789669	20 (72.69 $\pm$ 26.19)	24 (40.90 $\pm$ 50.28)	12 (65.65 $\pm$ 27.78)	0.19	–	0.04	0.92	0.97	–
+3040G>A	rs2493132	21 (54.08 $\pm$ 44.39)	32 (57.79 $\pm$ 39.49)	3 (79.41 $\pm$ 33.52)	0.19	–	0.32	–	0.24	–
+3159G>C	rs3789666	29 (66.62 $\pm$ 36.25)	21 (48.15 $\pm$ 49.46)	5 (42.01 $\pm$ 9.55)	0.01	0.20	0.009	0.19	0.20	–
+4100G>A	rs11568056	20 (50.22 $\pm$ 41.00)	28 (55.84 $\pm$ 43.63)	8 (81.88 $\pm$ 18.78)	0.03	0.70	0.08	–	0.10	–
+5088T>C	rs2478523	15 (64.49 $\pm$ 42.14)	30 (61.44 $\pm$ 38.02)	11 (37.50 $\pm$ 43.95)	0.009	0.18	0.14	–	0.007	0.13
+6328T>C	rs11122575	19 (52.37 $\pm$ 40.95)	29 (54.25 $\pm$ 43.70)	8 (81.88 $\pm$ 18.78)	0.04	0.88	0.10	–	0.10	–
+6501A>G	rs1926723	19 (52.37 $\pm$ 40.95)	29 (54.25 $\pm$ 43.70)	8 (81.88 $\pm$ 18.78)	0.04	0.88	0.10	–	0.10	–
+8266C>A	rs7079	45 (59.40 $\pm$ 39.60)	10 (44.58 $\pm$ 45.54)	1 (104.35)	0.57	–	0.92	–	0.10	–
+8339C>T	rs2067853	46 (58.32 $\pm$ 39.84)	9 (48.47 $\pm$ 46.50)	1 (104.35)	0.47	–	0.79	–	0.10	–
+8482T>C	rs11568046	45 (62.34 $\pm$ 37.39)	10 (37.51 $\pm$ 52.61)	1 (42.86)	0.04	0.88	0.03	0.62	0.69	–
+8789G>A	rs11122574	21 (48.97 $\pm$ 40.37)	27 (57.03 $\pm$ 44.00)	8 (81.88 $\pm$ 18.78)	0.03	0.62	0.06	–	0.10	–
ht1		37 (55.16 $\pm$ 42.09)	11 (47.92 $\pm$ 44.13)	8 (81.88 $\pm$ 18.78)	0.14	–	0.29	–	0.10	–
ht2		37 (59.15 $\pm$ 41.07)	19 (54.45 $\pm$ 41.29)	–	0.59	–	0.59	–	–	–
ht3		46 (54.58 $\pm$ 42.65)	10 (71.23 $\pm$ 28.97)	–	0.18	–	0.18	–	–	–
ht4		49 (58.41 $\pm$ 43.17)	6 (54.86 $\pm$ 17.23)	1 (31.73)	0.16	–	0.25	–	0.19	–
ht5		50 (58.90 $\pm$ 38.47)	6 (46.31 $\pm$ 60.55)	–	0.74	–	0.74	–	–	–

Pa*, *P*-values in the co-dominant model; **Pb*, dominant model; **Pc*, recessive model.*P*-values after multiple testing corrections (effective number = 20.541).

C/C, C/R and R/R indicate the homozygote of common allele, and the heterozygote and homozygote of rare allele, respectively.

respectively, the key findings in this study provide evidence that AIA patients carrying either of these polymorphisms may manifest unresponsiveness to the asthma-alleviating effects of MLK and persistent aspirin-induced bronchospasm. Furthermore, pair-wise comparisons of the SNPs in *AGT* gene established one tight LD block in Korean AIA population (Fig. 2A) compared to those of other ethnic groups generated using database of the International HapMap Project (B–D). This unusual LD feature in Korean AIA patients suggests that *AGT* polymorphisms could be pharmacogenetic markers and would benefit further studies for asthma management. Also, in further analyses, it was observed that variants in *AGT* (+2401C>G, +2476C>T and –4045T>C) could affect lung function abnormalities among AIA patients despite ingestion of anti-asthma medication.

Despite the lack of accurate molecular basis for the findings in this study, we speculated that the SNPs displaying association signals may affect roles in the bronchoconstrictive function of the gene. It has been revealed that intronic genetic variants create a new splice site [29] as well as affect efficient intron splicing processivity [30], resulting to production of proteins that influence

the onset of diseases in a complex manner. Although further functional characterizations of the associated *AGT* variants are needed, we performed *in silico* analysis to predict whether the SNPs (rs2478544, rs2478545, rs3789666, rs2478523) could be potential branch point (BP) sites for alternative splicing using EMBL-EBI splice site prediction (<http://www.ebi.ac.uk/asd-srv/wb.cgi?method=2>). However, results showed that the tested SNPs were not estimated to be potential BP sites. On the other hand, previous studies have reported significant associations of the haplotype-tagging +2476C>T SNP with preeclampsia and cardioembolic stroke [31,32], diseases that have also been known to be potentially correlated with asthma [33,34]. These findings provide further evidence that +2476C>T may also play a significant role in non-responsiveness to anti-asthma drug among aspirin-hypersensitive asthmatics.

Another possible explanation for the association between *AGT* polymorphisms and the lack of response to MLK treatment among AIA patients lies in modulations of the angiotensinogen pathway in eosinophilic airway inflammation and bronchoconstriction among AIA patients [35–37]. Inhibition of the angiotensin converting

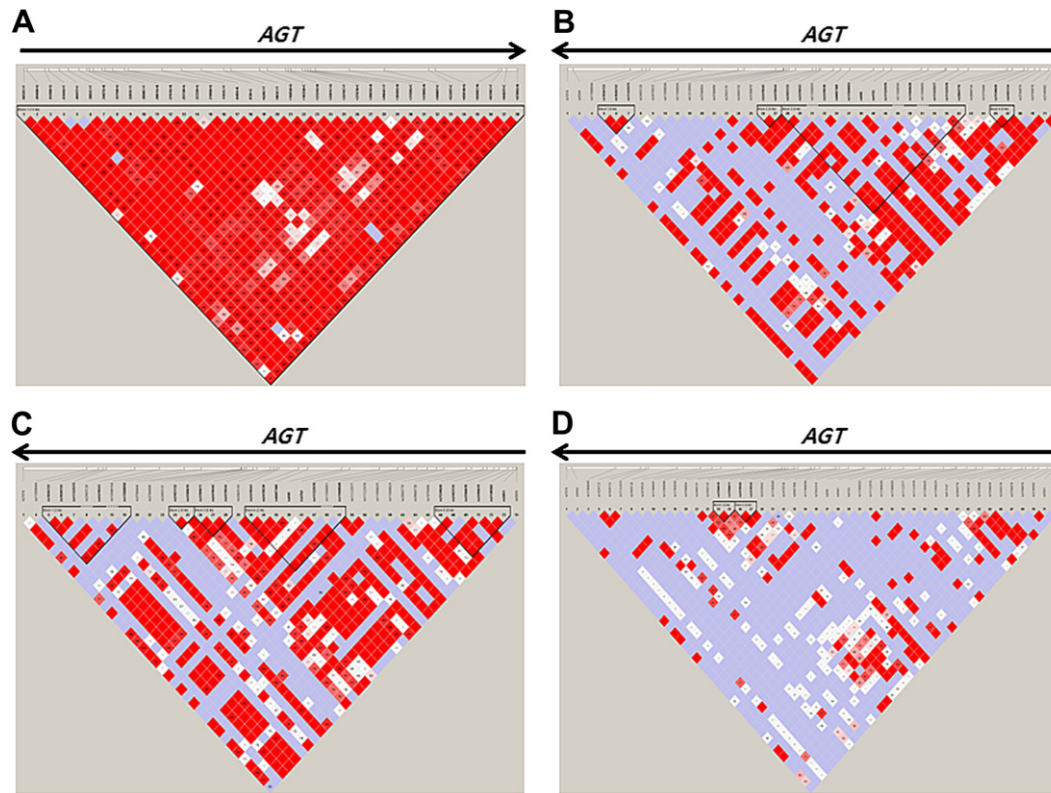


Fig. 2. LD coefficient ($|D'|$) among *AGT* SNPs in (A) Korean, (B) Caucasian, (C) Japanese and Chinese, and (D) African populations. CEU, Caucasian; JPT, Japanese; CHB, Chinese; YRI, African.

enzyme (ACE) leads to suppression of Ang II kinase activity and consequent accumulation of kinins and substance P, leading to aggravation of asthma symptoms [38,39]. Furthermore, activation of the arachidonic pathway as a result of ACE inhibition has also been known to potentiate bronchoconstriction among asthmatics [40]. Considering the interaction between LTC₄ and Ang II [11], we expect that the antagonistic effect of MLK may indirectly play a role in the angiotensinogen pathway.

With AIA being a rare condition, the few sample size may serve as a limiting factor of this study. Therefore, our preliminary findings needed to be confirmed in future replication studies comparing our results to aspirin-tolerant asthmatics and/or healthy controls. On the other hand, despite previous debates on the role of rare variants in disease susceptibility [41], this study employed the common-disease common-variant (CDCV) hypothesis which is applicable especially for complex diseases [42]. However, most importantly, our findings provide evidence that *AGT* variants may lead to modification of the anti-asthma drug response among AIA patients. These reports may contribute to our knowledge on the underlying mechanisms of resistance to asthma medications and may provide useful strategies for aspirin-intolerant asthma management. To our knowledge, these findings are the first to demonstrate the clinical significance of *AGT* variants with response to MLK among AIA patients.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.pupt.2011.05.007.

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