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Adenosine deaminase and adenosine receptor polymorphisms in aspirin-intolerant asthma

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Summary
In asthmatic airways, adenosine is a potent bronchoconstrictor with either pro- or anti-inflammatory effects depending on receptor interactions. While aspirin has been suggested to mediate adenosine action, the roles of adenosine and its receptors in aspirin-intolerant asthma (AIA) are not well-defined. Therefore, we evaluated associations between genetic polymorphisms of adenosine deaminase and the four adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) with the AIA phenotype. The genes for adenosine deaminase (*ADA*) and the four adenosine receptors (*ADORA1*, *ADORA2A*, *ADORA2B*, and *ADORA3*) were screened by direct sequencing, and 13 single nucleotide polymorphisms (SNPs) were selected among 23 polymorphisms. Using multivariate logistic regression analysis, we compared the frequencies of SNP genotypes and haplotypes among 136 patients with AIA, 181 patients with aspirin-tolerant asthma (ATA), and 183 normal individuals. We found significant differences between normal and patients with AIA in the *ADORA1* SNP genotype frequencies for 1405C > T ($P = 0.001$) and A102A ($P = 0.013$). No other significant associations were detected for the other SNPs. In the haplotype analysis, ht[C–T–G] ($P = 0.003$) and ht[A–C–G] ($P = 0.032$) in *ADORA1* and ht[A–T] in *ADORA2*

Abbreviations: ADA, adenosine deaminase; AHR, airway hyperresponsiveness; AIA, aspirin-intolerant asthma; AMP, adenosine monophosphate; ATA, aspirin-tolerant asthma; COX, cyclooxygenase FEV_1, forced expiratory volume in 1 s; GPR, G protein-coupled receptors; LD, linkage disequilibrium; NSAID, nonsteroidal anti-inflammatory drug; SNP, single nucleotide polymorphism; UTR, untranslated regions.

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($P = 0.013$) were significantly associated with AIA. Genetic polymorphisms of adenosine receptors A_1 and A_{2A} were associated with AIA, suggesting that adenosine might play a crucial role in the development of AIA through interactions with the A_1 and A_{2A} receptors.

Introduction

Aspirin-intolerant asthma (AIA) is a unique clinical syndrome found in 10—20% of adult patients with asthma[1] and characterized by acute bronchoconstriction following the administration of aspirin and/or nonsteroidal anti-inflammatory drugs (NSAIDs). Suggested molecular mechanisms for AIA are increased production and/or expression of cysteinyl leukotrienes and their receptors, dysregulation of cyclooxygenase and prostaglandins, and more severe eosinophilic inflammation.[2] Additionally, genetic polymorphisms in these mediators and receptors have exhibited significant associations with aspirin intolerance in patients with asthma.[3]

The purine nucleoside adenosine is endogenous in human tissues at low concentrations but adenosine accumulates markedly in the extracellular space during tissue hypoxia and inflammation.[4] Adenosine levels are elevated following exercise,[5] challenge with allergens,[6] and in patients with asthma.[7] In addition, inhalation of adenosine induces acute bronchoconstriction in those with asthma, and this is used to evaluate airway hyperresponsiveness (AHR).[8] The biological activities of adenosine are primarily mediated through its interactions with the 7-transmembrane G protein-coupled receptors (GPRs) A_1, A_{2A}, A_{2B}, and A_3, which transduce signals by interaction with G proteins to modulate intracellular cyclic adenosine monophosphate (AMP) levels.[9] Recently, the specific roles of these receptors have been extensively explored and these four GPRs are important targets for developing antiasthma therapies.[10] Extracellular adenosine is produced by alkaline phosphatase from adenosine triphosphate. Adenosine is catabolized by adenosine deaminase (ADA), and blocking ADA induces the accumulation of adenosine and severe pulmonary inflammation.[11]

The role of adenosine and its receptors in AIA pathogenesis is not well clarified. In subjects with asthma, lysine—aspirin inhalation attenuates the bronchoconstrictor response induced by AMP inhalation, and this protective effect is linked to cyclooxygenase (COX) inhibition and reduced production of contractile prostaglandins and thromboxanes.[12] While aspirin and NSAIDs reduce inflammation through inhibition of COX and prostaglandin synthesis, they also produce anti-inflammatory effects that are mediated through adenosine metabolism and adenosine-receptor interactions.[13—15] In addition, the elimination of extracellular adenosine from inflammatory exudates using ADA reverses the anti-inflammatory effects of aspirin.[13] Moreover, the A_{2A} adenosine receptor is reported to have a protective role in aspirin-induced gastric mucosal inflammation.[16] Taken together, these findings suggest that adenosine-mediated mechanisms are involved in the development of AIA. Although a few studies have reported on genetic associations of ADA with asthma and IgE-mediated responses,[17—19] no trial has examined the associations between adenosine-related genes and aspirin intolerance in asthma. Therefore, in the present study, we evaluated whether genetic polymorphisms in ADA and the four known adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) are associated with the development of AIA in a Korean population.

Materials and methods

Subjects

We enrolled 136 patients with AIA and 181 patients with aspirin-tolerant asthma (ATA) from five general hospitals (Ajou University Hospital, Seoul National University Hospital, Dankook University Hospital, Hanyang University Hospital, and Eulji University Hospital) in Korea. As normal controls, 183 healthy subjects without respiratory symptoms and with normal lung function were recruited. This study was approved by the institutional review board of each hospital and informed consent was obtained from all subjects. Asthma was defined as the presence of typical asthmatic symptoms and the demonstration of AHR to methacholine ($PC_{20} < 16$ mg/ml) and/or reversible airway obstruction as evidenced by a bronchodilator test involving inhalation of a short-acting β2 agonist [>15% of predicted value and a 200 ml increase in forced expiratory volume in 1 s (FEV_1)].[20] Subjects with asthma were diagnosed as AIA if they exhibited a positive response to the lysine—aspirin bronchoprovocation test according to a previously described protocol.[21,22] The smoking status, rhinitis symptoms, onset time of asthmatic symptoms, and previous history of aspirin intolerance were observed for all patients with asthma. Those exhibiting a negative response to the aspirin challenge test and no history of aspirin and/or NSAID intolerance were classified as having ATA. Normal controls were recruited from volunteers lacking asthmatic symptoms, a history of aspirin intolerance, AHR to methacholine, and a positive response to the aspirin challenge test. Atopy was defined as one or more positive responses to locally common aeroallergens in a skin prick test and/or a positive specific IgE response to house dust mite allergens. Spirometry was performed to measure basal FEV_1 and forced vital capacity. PNS X-ray and rhinoscopic examinations were carried out to evaluate rhinosinusitis and the presence of nasal polyps. The serum levels of total IgE were measured by the CAP system (Pharmacia, Uppsala, Sweden). The clinical characteristics of the three study groups are summarized in Table 1.

Identification of SNPs

Genomic DNA samples were isolated from the peripheral blood of 24 healthy subjects using the QIAamp DNA blood kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). For SNP identification, up to 2 kb of the

Table 1 Clinical characteristics of the subjects.

Characteristic	AIA ($n = 136$)	ATA ($n = 181$)	NC ($n = 183$)
Male* (%)	49 (36.0)	81 (44.8)	88 (48.1)
Age (years)[†]	44.1 ± 12.7	40.0 ± 14.1	38.6 ± 14.3
Asthma duration (years)	6.6 ± 6.1	6.4 ± 5.9	NA
Baseline FEV_1 (% of predicted value)[‡]	83.3 ± 23.2	89.2 ± 15.4	94.4 ± 8.2
PC_{20} (mg/ml of methacholine)	4.67 ± 13.0	6.7 ± 10.3	NA
Rhinosinusitis (%)	81/101 (80.2)	133/177 (75.1)	NA
Nasal polyp[§] (%)	47/91 (51.6)	10/177 (5.6)	NA
Total serum IgE (IU/ml)	309.3 ± 415.4	362.5 ± 672.7	NA
Atopy[‖] (%)	66/104 (63.5)	120/177 (67.8)	56/180 (31.1)

Mean $\pm$ SD.
AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; NC, normal control; NA, not applicable; FEV_1, forced expiratory volume in 1 s.
*$P = 0.031$ for AIA versus NC by chi-square test;. [†]$P = 0.007$ for AIA versus ATA, $P < 0.001$ for AIA versus NC by Student's t-test;. [‡]$P = 0.017$ for AIA versus ATA, $P = 0.024$ for ATA versus NC, $P < 0.001$ for AIA versus NC by Student's t-test;. [§] $P < 0.001$ for AIA patients versus ATA patients by chi-square test; [‖]$P < 0.001$ for AIA versus NC, $P < 0.001$ for ATA versus NC by chi-square test.

DNA samples were sequenced 5′-upstream of exon 1 in the promoter, and all the exons, including the 3′-untranslated regions (UTRs) of five candidate genes (*ADA*, NM_000022; *ADORA1*, NM_000674; *ADORA2A*, NM_000675; *ADORA2B*, NM_000676; *ADORA3*, NM_000677; Supplementary Fig. 1) were sequenced in both directions based on the reference sequences using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA). From the sequences, we screened informative SNPs with minor allele frequencies of >0.05.

Genotyping and haplotype inference

For selection of tagging SNPs among the informative SNPs, we gave priority to non-synonymous coding SNPs and SNPs that tagged most of the remaining variants after determination of the linkage disequilibrium (LD) patterns (Supplementary Fig. 1). The selected SNPs were genotyped in the study subjects using the high-throughput single base-pair extension method (SNP-IT™ assay) with the SNPstream25K system, which was customized to genotype automatically the DNA samples in 384-well plates and to generate a colorimetric readout (Orchid Biosciences, Princeton, NJ, USA), as described previously.[23] After examining Lewontin's D′ (|D′|) and the LD coefficient r^2 between all pairs of biallelic loci, haplotypes and their frequencies were estimated using Haploview version 3.32 (http://www.broad.mit.edu/mpg/haploview/).

Statistical analysis

Differences in phenotypes among the three subject groups (patients with AIA, patients with ATA, and normal controls) were determined using Student's *t*-test or chi-square test. Hardy–Weinberg equilibrium was assessed using the chi-square test. Genotype frequencies of SNPs and haplotype frequencies were compared among the groups by multivariate logistic regression analysis using age and sex as covariates. We used dominant and recessive analysis models for comparisons of the genotype frequencies. All of the statistical analyses were performed using SAS (version 9.13; SAS Institute, Cary, NC, USA) and *P* values <0.05 were regarded as statistically significant.

Results

Selection of SNPs in *ADA*, *ADORA1*, *ADORA2A*, *ADORA2B*, and *ADORA3*

Through direct sequencing of the *ADA*, *ADORA1*, *ADORA2A*, *ADORA2B*, and *ADORA3* genes and evaluation of minor allele frequencies and LD between SNPs, we selected 13 tagging SNPs in four genes for genotyping as followings: −1130A > T and V178V in *ADA*; −38242C > T, A102A, 1278C > A, 1405C > T, and 1627G > T in *ADORA1*; −1751A > C and Y361Y in *ADORA2A*; −2288A > G, −1050G > T, −564C > T, and A299A in *ADORA3* (Table 2). In *ADORA2B*, no SNP with minor allele frequency >0.05 existed, and thus genotyping of the study subjects was not carried out (Supplementary Table 1).

Associations between SNPs and AIA

Genotype frequencies of two SNPs of *ADA* (−1130A > T and V178V) were not different among subject groups (Table 3). Of five selected SNPs of *ADORA1* (−38242C > T, A102A, 1278C > A, 1405C > T, and 1627 G > T), A102A in exon 5 and 1405C > T in the 3′-UTR of exon 6 showed significant associations with AIA. In A102A, the frequency of the variant allele (G) containing genotypes (TG and GG) was lower in patients with AIA than in normal controls (25.4% versus 39.3%, $P = 0.013$, OR = 0.52, 95% CI = 0.30–0.87), suggesting a protective role of the variant allele in the development of AIA. The frequency of homozygotes of the mutant allele (TT) of 1405C > T was higher in patients with AIA compared to normal controls (18.8% versus 6.6%, $P = 0.001$, OR = 3.22, 95% CI = 1.50–6.92), which implied that 1405C > T conferred a strong risk for AIA. No significant association was observed between the other three SNPs of *ADORA1* (−38242C > T, 1278C > A, and 1627G > T) and the AIA phenotype. In two SNPs of *ADORA2A* (1751A > C and

Table 2 Selected single nucleotide polymorphisms for genotyping.

Gene	SNP	Reference SNP ID	Position	Minor allele frequency	HWE (*P* value)
ADA	−1130A > T	rs11086932	Promoter	0.354	0.733
	V178V	rs244076	Exon 6	0.083	1
ADORA1	−38242C > T	rs6664108	Promoter	0.229	1
	A102A	rs10920568	Exon 5	0.167	1
	1278C > A	rs6427994	Exon 6 (3′-UTR)	0.167	1
	1405C > T	rs16851030	Exon 6 (3′-UTR)	0.375	0.004
	1627G > T	rs12744240	Exon 6 (3′-UTR)	0.125	1
ADORA2A	−1751A > C	rs5996696	Promoter	0.062	1
	Y361Y	rs5751876	Exon 2	0.438	1
ADORA3	−2288A > G	rs2298191	Promoter	0.229	0.452
	−1050 G > T	rs10776727	Promoter	0.396	0.326
	−564C > T	rs1544224	Exon 1 (5′-UTR)	0.292	1
	A299A	rs2229155	Exon 2	0.188	1

HWE, Hardy–Weinberg equilibrium.

Y361Y) and four SNPs of *ADORA3* (−2288A > G, −1050 G > T, −564C > T, and A299A), genotype frequencies were not significantly different among the groups.

Association between gene haplotypes with AIA

Based on the LD between the genotyped SNPs, we inferred the haplotype frequencies for three genes, *ADORA1*, *ADORA2A*, and *ADORA3* (Table 4). In the analysis of the association between haplotypes and AIA, a significant association was found for *ADORA1* and *ADORA2A*. The frequency of ht2[C–T–G] of *ADORA1*, which carried the mutant T allele of 1405C > T, the risk allele in AIA development, was higher in the AIA group than in the normal controls ($P = 0.003$, OR = 3.04, 95% CI = 1.42–6.50) and the frequency of ht3[A–C–G] containing the C allele of 1405C > T was lower in patients with AIA than in those with ATA ($P = 0.032$, OR = 0.56, 95% CI = 0.33–0.95). While no SNP was associated with AIA in the previous single SNP analysis in the adenosine A_2 gene, ht2[A–T] showed a significant association with the AIA phenotype with lower frequency in AIA than in normal controls ($P = 0.013$, OR = 0.33, 95% CI = 0.13–0.82). These findings suggest that ht2[A–T] in *ADORA2A* had a protective role in the development of AIA.

Functional role of 1405C > T in AIA pathogenesis

We evaluated the functional role of 1405C > T of *ADORA1*, which was determined to be a risk allele for AIA by both SNP and haplotype analyses. During aspirin inhalation bronchial challenge in patients with AIA, those with the TT genotype of 1405C > T showed a positive response earlier than those with other genotypes ($P = 0.009$) (Fig. 1).

Discussion

Candidate gene approaches in AIA have been inspired by the current understanding that increased levels of cysteinyl leukotrienes act as the key inflammatory mediators in the development of AIA and the protective prostanoids are decreased in AIA. As a result, many studies in AIA genetics have focused on genes related to eicosanoid metabolism and its receptors, which revealed AIA-associated genetic polymorphisms such as *ALOX5*,[21] *LTC4*,[24] *CYSLTR2*,[25] *TBXA2R*,[26] and prostanoid receptors.[27] In the present study, we explored possible associations between AIA and polymorphisms in adenosine-related genes for the first time and revealed that SNPs in *ADORA1*, but not in *ADA*, *ADORA2A*, *ADORA2B*, and *ADORA3*, were significantly associated with AIA. Additionally, the haplotype of *ADORA1* and *ADORA2A* exhibited significant associations with AIA.

The expression of the A_1 adenosine receptor was elevated in the bronchial epithelium and smooth muscle of subjects with asthma,[28] and this receptor mediated bronchoconstriction induced by exogenous adenosine.[29] Therapeutic effects of blocking the A_1 receptor were evaluated in animal models of asthma using antisense oligonucleoside[30] and specific antagonists.[31] In these experiments, both agents attenuated adenosine- and allergen-induced bronchoconstriction without notable anti-inflammatory effect. In terms of a regulatory role in inflammation, the A_1 receptor showed both pro- and anti-inflammatory effects. Adenosine increased mucus secretion and activated neutrophils and monocytes via the A_1 receptor.[32] In contrast, the A_1 receptor proved to have a protective effect on lung inflammation in ADA and A_1 double-knockout mice.[33] In the present study, the minor allele (T) in 1405C > T in the 3′-UTR conferred susceptibility to AIA, while A102A had a protective effect. Although the functional roles of these variants were not defined, the present data showed more rapid bronchoconstriction and decreased FEV_1 to aspirin inhalation in patients with TT homozygotes in 1405C > T compared to those with CC or CT among patients with AIA. Coupled with the presence of the TT genotype in *ADORA1* with a high risk for AIA, the sensitivity of patients with AIA having the TT genotype to inhaled aspirin also implied that genetic variants of the A_1 receptor could have major clinical effects on modifying bronchoconstriction.

Table 3 Genotype frequencies of single nucleotide polymorphisms in patients with AIA, ATA, and control subjects.

Gene	SNP	Genotype	AIA ($n = 136$)	ATA ($n = 181$)	NC ($n = 183$)	*P* value		
						AIA vs. ATA	AIA vs. NC	ATA vs. NC
ADA	−1130A > T	AA	61 (54.5)	84 (46.9)	104 (57.1)	NS	NS	NS
		AT	41 (36.6)	84 (46.9)	63 (34.6)			
		TT	10 (8.9)	11 (6.2)	15 (8.2)			
	V178V	AA	87 (75.7)	128 (73.6)	139 (78.5)	NS	NS	NS
		AG	26 (22.6)	39 (22.4)	35 (19.8)			
		GG	2 (1.7)	7 (4.0)	3 (1.7)			
ADORA1	−38242C > T	CC	67 (58.3)	90 (51.4)	87 (49.4)	NS	NS	NS
		CT	43 (37.4)	69 (39.4)	80 (45.5)			
		TT	5 (4.4)	16 (9.1)	9 (5.1)			
	A102A	TT	85 (74.6)	114 (63.7)	111 (60.7)	NS	0.013*	NS
		TG	28 (24.6)	60 (33.5)	68 (37.2)			
		GG	1 (0.9)	5 (2.8)	4 (2.2)			
	1278C > A	CC	87 (75.0)	114 (63.7)	128 (70.3)	NS	NS	NS
		AC	27 (23.3)	59 (33.0)	49 (26.9)			
		AA	2 (1.7)	6 (3.4)	5 (2.8)			
	1405C > T	CC	51 (43.6)	91 (51.4)	90 (49.7)	NS	0.001†	NS
		CT	44 (37.6)	67 (37.9)	79 (43.7)			
		TT	22 (18.8)	19 (10.7)	12 (6.6)			
	1627 G > T	GG	89 (77.4)	133 (75.1)	129 (70.5)	NS	NS	NS
		GT	25 (21.7)	42 (23.7)	52 (28.4)			
		TT	1 (0.9)	2 (1.1)	2 (1.1)			
ADORA2A	−1751A > C	AA	76 (66.1)	135 (75.4)	135 (75.8)	NS	NS	NS
		AC	38 (33.0)	44 (24.6)	40 (22.5)			
		CC	1 (0.9)	0 (0.0)	3 (1.7)			
	Y361Y	CC	33 (28.7)	58 (33.1)	48 (26.5)	NS	NS	NS
		CT	64 (55.7)	78 (44.6)	94 (51.9)			
		TT	18 (15.7)	39 (22.3)	39 (21.6)			
ADORA3	−2288A > G	AA	54 (46.2)	74 (41.6)	88 (49.2)	NS	NS	NS
		AG	54 (46.2)	84 (47.2)	79 (44.1)			
		GG	9 (7.7)	20 (11.2)	12 (6.7)			
	−1050G > T	GG	34 (29.8)	53 (29.6)	59 (33.3)	NS	NS	NS
		GT	63 (55.3)	97 (54.2)	93 (52.5)			
		TT	17 (14.9)	29 (16.2)	25 (14.1)			
	−564C > T	CC	60 (51.3)	85 (48.3)	91 (50. 6)	NS	NS	NS
		CT	49 (41.9)	74 (42.1)	71 (39.4)			
		TT	8 (6.8)	17 (9.7)	18 (10.0)			
	A299A	CC	71 (59.7)	106 (59. 9)	106 (58. 9)	NS	NS	NS
		CT	45 (37.8)	63 (35.6)	62 (34.4)			
		TT	3 (2.5)	8 (4.5)	12 (6. 7)			

AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; NC, normal control; NS, not significant.
**P* value in dominant model with OR (95% confidence interval) of 0.52 (0.30—0.87);. †*P* value in recessive model with OR (95% confidence interval) of 3.22 (1.50—6.92).

The molecular mechanisms explaining how a variant in the 3′-UTR could induce changes in bronchial responses to aspirin remain to be explained. However, one possibility is that the nucleotide change induced stability of the mRNA transcript and increased gene expression, thus influencing the development of phenotypes.[34] Our positive association of the 3′-UTR SNP with AIA is coincident with the finding that three polymorphisms in the 3′-UTR of *ADORA1* were associated with specific infarct changes in ischemic cardiomyopathy mediated through mRNA structural differences.[35] Based on these findings, we speculated that aspirin changed the levels of adenosine in the airways of individuals with asthma and the different effects observed with aspirin between normal subjects and patients with AIA was due to altered expression of the adenosine receptor A_1. Currently, we do not know whether adenosine receptor expression in AIA is different from that in ATA. Further studies are needed to clarify this issue and the role of adenosine receptors in AIA.

The adenosine A_{2A} receptor is expressed on inflammatory cells and mediates inhibitory signals by increasing intracellular cAMP upon activation. Stimulation of A_{2A}

Table 4 Haplotype frequencies in patients with AIA, ATA, and control subjects.

Gene	Haplotype*	AIA ($n = 136$)	ATA ($n = 181$)	NC ($n = 183$)	*P* value		
					AIA vs. ATA	AIA vs. NC	ATA vs. NC
ADORA1	ht1[C—C—G]	0.375	0.366	0.400	NS	NS	NS
	ht2[C—T—G]	0.375	0.300	0.283	NS	0.003[†]	NS
	ht3[A—C—G]	0.134	0.203	0.164	0.032[‡]	NS	NS
	ht4[C—C—T]	0.112	0.131	0.153	NS	NS	NS
ADORA2A	ht1[A—C]	0.567	0.554	0.526	NS	NS	NS
	ht2[A—T]	0.255	0.323	0.349	NS	0.013[§]	NS
	ht3[C—T]	0.174	0.123	0.122	NS	NS	NS
ADORA3	ht1[C—C—T—G]	0.319	0.341	0.294	NS	NS	NS
	ht2[C—C—G—A]	0.305	0.267	0.294	NS	NS	NS
	ht3[T—T—G—A]	0.217	0.216	0.221	NS	NS	NS
	ht4[C—C—T—A]	0.102	0.085	0.109	NS	NS	NS
	ht5[C—T—G—A]	0.058	0.085	0.071	NS	NS	NS

AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; NC, normal control; NS, not significant.
*Haplotypes with frequencies >0.05 are listed in descending order; *ADORA1* haplotype of [1278C > A_1405C > T_1627G > T], *ADORA2A* haplotype of [−1751A > C_Y361Y], and *ADORA3* haplotype of [-2288A > G_−1050G > T_−564C > T_A299A]; [†]*P* value in recessive model with OR (95% confidence interval) of 3.04 (1.42—6.50); [‡]*P* value in dominant model with OR (95% confidence interval) of 0.56 (0.33—0.95); [§]*P* value in recessive model with OR (95% confidence interval) of 0.33 (0.13—0.82).

receptor is linked to inhibition of degranulation in neutrophils,[36] eosinophils, and mast cells.[37] Additionally, an A_{2A} receptor agonist attenuated airway inflammation in an allergen-induced animal model of asthma[38] and A_{2A} receptor-deficient mice exhibited enhanced AHR and airway inflammation.[39] In contrast to negative results in single SNP analysis, the haplotype examination of the A_{2A} receptor gene was significantly associated with the AIA phenotype with ht2[A—T], encompassing −1751A > C in the promoter region and Y361Y in exon 2. Although we are not able to explain any functional role of this haplotype in the pathogenesis of AIA, we speculated that levels of gene expression might change with different promoter polymorphisms. The altered expression of A_{2A} receptors in patients with asthma could explain the different inflammatory responses to aspirin administration.

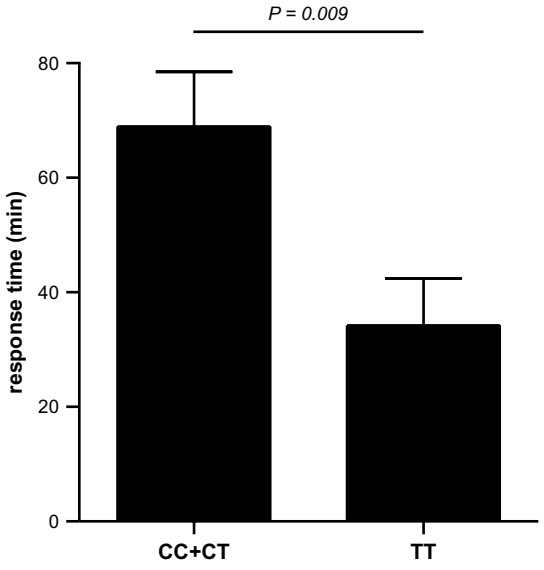

Figure 1 Time for maximal airway obstruction in the aspirin inhalation provocation test according to genotypes in 1405C > T. More prompt bronchospasm after aspirin inhalation in patients with TT homozygotes compared to those with CC or CT among patients with AIA.

In contrast to the anti-inflammatory effects of the A_{2A} receptor, the A_{2B} receptor expressed on mast cells and airway smooth muscle cells has pro-inflammatory effects by way of released inflammatory mediators and cytokines. A selective antagonist of the A_{2B} receptor showed therapeutic effects in a mouse model of asthma by attenuating bronchoconstriction and inflammation.[40] Based on these findings, the adenosine A_{2B} receptor is now an important therapeutic target in asthma. With regard to genetic variations in the A_{2B} receptor, previous studies reported that allelic frequency differed according to ethnicity and that no common SNP occurred in all ethnic populations.[41] Consistent with this finding, we did not identify any genetic variation greater than 5%, and therefore conducted no further genotyping of our study population.

While the A_3 receptor expression was elevated on eosinophils in patients with asthma,[42] the role of A_3 receptors in the airways is somewhat contradictory. Earlier studies reported that A_3 receptor stimulation induced inhibition of eosinophil degranulation and migration.[43] Contrary to this result, inhibition of A_3 receptors with a selective antagonist in ADA-deficient mice attenuated eosinophilic lung inflammation.[44] Although these findings suggest that adenosine acts on pulmonary inflammation via the A_3 receptor, no significant association was detected between genetic polymorphisms in A_3 receptors and aspirin tolerance in asthma.

While more severe eosinophilic inflammation was observed in the airways of subjects with AIA, whether differences of either adenosine concentrations or metabolism exist between individuals with AIA and ATA remains unclear. Among enzymes involved in adenosine metabolism,

we targeted ADA because it is the primary catabolic enzyme for adenosine and ADA-deficient mice exhibited a marked elevation of adenosine and early death accompanied by asthmatic phenotypes, including eosinophilic lung inflammation, mucus production, and elevation of Th2 cytokines.[11] Additionally, significant effects of ADA polymorphisms in atopy and asthma have been reported.[18] However, we could not find an association between ADA polymorphisms and AIA development in this study.

In summary, the present study revealed that genetic polymorphisms of *ADORA1* and *ADORA2A* were significantly associated with AIA in a Korean population. These findings suggest that adenosine might play a crucial role in the development of AIA by mediation of A_1 and A_{2A} receptors. Further studies will be necessary to elucidate the functional mechanisms of these genetic variations and to determine their presence in other ethnic populations.

Conflict of interest

All the authors have no conflicts of interest to disclose.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.rmed.2008.10.008.

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