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ORIGINAL INVESTIGATION

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Leukotriene-related gene polymorphisms in ASA-intolerant asthma: an association with a haplotype of 5-lipoxygenase

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Abstract A recent study has demonstrated the possible involvement of a leukotriene C4 synthase (LTC4S) gene polymorphism in ASA-intolerant asthma (AIA) in a Polish population, whereas no significant association was noted in other populations. To investigate the role of genetic polymorphism in AIA development, we screened single nucleotide polymorphisms (SNPs) of the key enzymes involved in arachidonate metabolism, and the cysteinyl leukotriene receptor 1 (CYSLTR1) in a large Korean population with AIA: 93 AIA and 181 ASA-tolerant asthma (ATA) patients, and 123 normal controls. The single-base extension method was used to genotype SNPs in 5-lipoxygenase (ALOX5, –1708G→A, 21C→T, 270G→A, 1728G→A), ALOX5-activating protein (ALOX5AP, 218A→G), prostaglandin-endoperoxide synthase 2 (PTGS2, COX2, –162C→G, 10T→G, R228H, V511A), LTC4S (–444A→C), and CYSLTR1 (927T→C). Haplotype analyses were undertaken for the SNPs in ALOX5. No significant differences in allele and genotype frequencies of single SNPs were observed between the patient groups (P>0.05). However, the frequency of the *ALOX5-ht1[G-C-G-A]* haplotype in the AIA group was significantly higher than its frequency in the ATA group with a probability (P) of 0.01, odds ratio (OR) of 5.0, and 95% confidence interval (95%CI) of 1.54–17.9, and in the normal controls (P=0.03, OR=4.5, 95%CI=1.1–18.4), by using a dominant model. These results suggest a lack of association between the ALOX5AP, PTGS2, LTC4S, and CYSLTR1 gene polymorphisms and the AIA phenotype in the Korean population. However, the possible involvement of *ALOX5-ht1[G-C-G-A]* in AIA development is suggested.

Introduction

ASA-intolerant asthma (AIA) refers to the development of bronchoconstriction in asthmatic individuals following the ingestion of ASA and other nonsteroidal anti-inflammatory drugs (NSAIDs). This syndrome is characterized by aspirin hypersensitivity, bronchial asthma, and chronic rhinosinusitis with nasal polyposis, commonly named the "aspirin triad" (Samter and Beers 1967). AIA affects about 5%–10% of adult asthmatics and is found more often in women (Szczeklik and Stevenson 2003).

Although the pathogenesis of AIA is not completely understood, the cyclooxygenase theory is widely accepted (Picado 2002; Szczeklik 1990; Szczeklik et al. 1975). This theory proposes that asthma attacks by ASA and NSAIDs are triggered by the specific inhibition of cyclooxygenase (prostaglandin-endoperoxide synthase) in the respiratory tract, which is followed by a reduction of prostaglandin E2 (PGE2) and overproduction of cysteinyl leukotrienes (cysLTs). CysLTs are important inflammatory mediators in AIA and can mediate bronchoconstriction and increase mucus secretion, vascular permeability, and cellular infiltration (Henderson 1994; Lewis et al. 1990). CysLTs are synthesized through the 5-lipoxygenase (ALOX5; MIM 152390) pathway of arachidonic acid metabolism. The ALOX5 pathway for synthesizing cysLTs has several steps, and distinct enzymes are involved in each steps, namely cytosolic phospholipase A2, ALOX5, ALOX5-activating protein (ALOX5AP, FLAP; MIM 603700), and

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leukotriene C4 synthase (LTC4S; MIM 246530), which is the terminal enzyme for cysLTs production (Foegh et al. 1998). cysLTs exert their biological action by binding two types of G-protein-coupled seven-transmembrane receptors, viz., cysteinyl leukotriene receptor 1 (CYSLTR1; MIM 300201) and CYSLTR2 (Hui and Funk 2002). It remains uncertain which step in the pathway is responsible for the overproduction of cysLTs in patients with AIA.

The expression of LTC4S has been reported to be significantly higher in bronchial biopsies in AIA than in those with ATA and in normal controls (Cowburn et al. 1998). In addition, Sanak et al. (1997, 2000) have shown that AIA is associated with a genetic polymorphism in the LTC4S gene promoter (–444A→C), which creates an additional response element for histone H4 transcription factor-2 and increases the transcription rate of the gene in vitro and in vivo in a Polish population. However, other studies have found no significant association between the LTC4S polymorphism and AIA in other ethnic groups (Kawagishi et al. 2002; Van Sambeek et al. 2000). In the case of the ALOX5 gene, a variable number of tandem repeat (VNTR) in the promoter region, which contains 3–6 tandem repeats of the Sp1-binding motif GGGCGG, have been reported, and alleles containing other than five tandem Sp1 repeats are known to diminish ALOX5 gene expression (In et al. 1997). However, the VNTR has previously been shown not to be related to AIA phenotype in a study of a Japanese population (Kawagishi et al. 2002). Thus, the data reported to date have not been consistent, possibly because of the small sizes of studied samples or ethnic differences between study populations. In addition, few studies have been carried out on the gene polymorphisms of the enzymes in the arachidonic acid pathway in AIA, other than LTC4S and ALOX5. To the best of our knowledge, there has also been no previous study of polymorphisms of the CYSLTR1 gene and no haplotype analysis of candidate genes for AIA.

In this study, we have analyzed SNPs of ALOX5, ALOX5AP, prostaglandin-endoperoxide synthase 2 (PTGS2, COX2; MIM 600262), LTC4S, and CYSLTR1, and their haplotypes in a relatively large number of AIA patients compared with ATA and normal controls in a Korean population in order to investigate the role of genetic polymorphisms of the candidate genes in AIA development.

Materials and methods

Subjects

Ninety-three patients with AIA, 181 patients with ATA, and 123 normal healthy controls were enrolled from the Department of Allergy and Rheumatology, Ajou University Hospital, and the Division of Allergy and Respiratory Medicine, Soonchunhyang University Hospital in Korea; all subjects were Korean. AIA was diagnosed by positive results on lysine-aspirin (L-ASA) bronchoprovocation tests. The L-ASA bronchoprovocation test was performed with increasing doses of aspirin (75–300 mg/ml, Althargyl, Arthromedica, Switzland) according to a modified method as previously described (Park 1995). L-ASA bronchoprovocation tests were performed in all of the patients with ATA to exclude ASA hypersensitivity. Normal controls were recruited from the general population who answered negatively to a screening questionnaire for respiratory symptoms, had no past history of ASA hypersensitivity, and had a forced expiratory volume in 1 s (FEV_1) greater than 80% predicted, PC_{20} methacholine greater than 25 mg/ml, and normal findings on simple chest radiograms. All subjects gave informed consent to the studies, and the protocols were approved by the local ethics committees. Skin prick tests were performed with 12 common aeroallergens (Bencard, UK). Atopy was defined as one or more positive reactions on skin prick test results.

Sequence analyses of the candidate genes

For each of the 11 SNPs in the five genes to be analyzed, pairs of upstream and downstream primers were desgined to amplify the genomic region surrounding the SNP of interest (Table 1). The polymerase chain reaction (PCR) was performed in a final volume of 30 µl containing genomic DNA. Montage PCR96 Cleanup Kit (Millipore) was used for the clean-up of the PCR products, and comparative sequencing of 48 DNA samples from Korean healthy controls was performed by using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kits (Applied Biosystems, Foster City, Calif.) in accordance with the recommendation of the manufacturer.

Procedure for PCR and primer extension reactions

Primer sequences for PCR and primer extension reactions are listed in Table 1. PCR was performed with 1.25 pmol each primer, 20 ng genomic DNA, 250 µM dNTPs, and 0.15 U *Taq* DNA Polymerase (Applied Biosystems) in the buffer provided by the manufacturer. Amplification was performed by using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) under touchdown conditions (Schunck et al. 1995). Primer extension reactions were performed with the SNaPSHOT ddNTP Primer Extension Kit (Applied Biosystems) as recommend by the manufacturer. To clean-up the primer extension reaction products, 1 U SAP was added to the reaction mixture, and the mixture was incubated at 37°C for 1 h, followed by 15 min at 72°C for enzyme inactivation.

Statistical analysis

Differences in clinical phenotypes between the patient groups were determined by Student's *t*-test for nondiscrete variables and the Chi square test for categorical variables. Hardy-Weinberg equilibrium was estimated by Chi-square tests. Haplotypes of ALOX5 were analyzed by using the Haplotyper program based on the Bayesian algorithm (Niu et al. 2002), and linkage disequilibrium between loci was measured by using Lewontin's |D'| (Hedrick 1987). Logistic regression models were employed for the analysis of allele and haplotype frequencies controlling for age, sex, and atopy as covariables with alternative models (co-dominant, dominant, and recessive models). *P* values were corrected for multiple comparisons with the pairs of groups of interest by using the Bonferroni method (Altman 1991). Multiple comparisons were performed by multiplying by eight for ALOX5, since a total of eight comparisons were made on four loci (AIA vs ATA, AIA vs NC) and, in the same manner, multiplying by six for PTGS2, multiplying by two in the other genes, and multiplying by four for ALOX5 haplotype analysis. A P value of 0.05 or less was regarded as significant. All statistical analyses were performed by using software SPSS, version 10.0 (Chicago, Ill.).

Results

Subjects

The clinical characteristics of the study subjects were summarized in Table 2. There were significant differences in

Table 1 Amplifying and extension primers of the candidate genes (*For/Rev* forward/reverse amplifying primers, *R* arginine, *H* histidine, *V* valine, *A* alanine, *UTR* untranslated region)

Gene	Locus	Position		Primers
ALOX5	–1708G→A	Promoter	For/Rev	AAAGAACAGCGTTGGTGGAT/CAAATTCATTGTGTTGCATGTG
			Extension	TAATGATTATATAATCATGATTATAATCAATGATGATCCTGAAGTACATCACGCTGAAGAC
	21C→T	Exon 1	For/Rev	CGCCATGCCCTCCTACAC/CCACGCTCGAAGTCGTTGTA
			Extension	ATGATTATATAATCATGATTATAATCAATGATGATCACGCCCTTGGCAGTCGG
	270G→A	Exon 2	For/Rev	TGTGGACGAGGAACTGGGC/CTCACCGCGTCCATCCCT
			Extension	AATGATTATATAATCATGATTATAATCAATGATGATCACTGCTTGATTACCGCAGTG
	1728A→G	Exon 13	For/Rev	GTGCTCCTGGATCCCCAAT/CTCGTTTTCCTGGAACTGGC
			Extension	ATGATTATATAATCATGATTATAATCAATGATGATGCACTGTATAGCATTGTACATTACAGATCA
ALOX5AP	218A→G	3’	For/Rev	CGGGAACAAAATGATGTCA/AAACACAAACCTGGTCACAAA
			Extension	TCATGATTATAATCAATGATGATGCCCCAAATTTGCTATTCCC
PTGS2	–162C→G	Promoter	For/Rev	GAAGAAAAGACATCTGGCGGA/GGCTGTGGAGCTGAAGGAG
			Extension	TGATGCAGGGTTTTTTACCCACG
	10T→G	5’UTR	For/Rev	GAAGAAAAGACATCTGGCGGA/GGCTGTGGAGCTGAAGGAG
			Extension	AATCAATGATGATTGACGCTCACTGCAAGTCGT
	R228H (G→A)	Exon 6	For/Rev	ATATAAGAAAAACTTCAACAGCAA/AACACATTTTTAGGGATTTTAAA
			Extension	TATAATCAATGATGATGGTGAAACTCTGGCTAGACAGC
	V511A (T→C)	Exon 10	For/Rev	GAGCTGTATCCTGCCCTTCTG/CCACTTCTCCACCAAAAGTGC
			Extension	ATGATGATTCAAGGAGAATGGTGCTCCA
LTC4S	–444A→C	Promoter	For/Rev	CATTCTGAAGCCAAAGGCACT/TGCACCACCCCACTTTCTC
			Extension	AACAGCCTGGATGGGGAC
CYSLTR1	927T→C	Exon 1	For/Rev	AAATCATGTTTTGGTCTTGC/ATTTTCATTGGTTTGGACTG
			Extension	GGGGTAACTTTAGGAAAAGGCTGTCTACATT

Table 2 Clinical characteristics of the study subjects (*AIA* ASA-intolerant asthma, *ATA* ASA-tolerant asthma, *NC* normal controls, *n* number of patients, *NA* not applicable)

Characteristic	AIA (*n*=93)	ATA (*n*=181)	NC (*n*=123)
Age(year)*	42.3±14.2	44.4±13.9	35.4±14.5
Sex(M)*	35 (37.6%)	55 (30.4%)	63 (51.2%)
Atopy*	34 (36.6%)	44 (24.3%)	19 (15.4%)
Asthma duration(year)	7.0±6.2	7.2±9.3	NA
FEV_1(%)	82.1±21.8	81.3±21.1	98.7±13.0
PC_{20}, methacholine (mg/ml)	5.5±11.2	6.1±10.1	NA
Serum total IgE(IU/ml)	352.8±451.5	420.7±686.2	70.0±71.1
Peripheral eosinophil count(/ul)	380.5±355.0	491.2±881.3	143.8±95.8
Rhinitis**	64 (68.8%)	76 (42.0%)	NA
Paranasal sinusitis	40/59 (67.8%)	NA	NA
Nasal polyp	27/40 (67.5%)	NA	NA
Urticaria/angioedema	21/64 (32.8%)	NA	NA

*P<0.01 AIA vs NC, ATA vs NC, **P<0.01 AIA vs ATA

mean age, sex distribution, and prevalence of atopy between normal controls and the other groups (P=0.01, respectively). Sixty-four AIA patients (68.8%) and 76 ATA patients (42.0%) had chronic rhinosinusitis. There were significant differences in the prevalence of rhinosinusitis between the AIA and ATA patients (P<0.01). Of the 40 AIA patients (67.5%), 27 who underwent rhinoscopic examination had nasal polyps.

Allele and genotype frequencies of the ALOX5, ALOX5AP, PTGS2, LTC4S, and CYSLTR1 genes

Genotype distributions of all loci except CYSLTR1 were in Hardy-Weinberg equilibrium (P>0.05). Allele and genotype frequencies of each SNP of the five genes studied in a Korean population are shown in Table 3. The PTGS2 511A allele was not found in any of the patient groups and was therefore excluded from the statistical analysis. There were no significant differences in genotype frequencies among the three groups with alternative models (co-dominant, dominant, and recessive models). The genotype frequency of ALOX5+1728A→G was significantly lower in the AIA group than that in the normal controls with co-dominant and dominant models (P=0.04, respectively). However, the P value was not significant after correction for multiple comparisons. There were no significant differences in allele and genotype frequencies between ATA and NC (data not shown).

Haplotype analysis of ALOX5

There were five haplotypes in ALOX5 showing frequencies of more than 1% among the 11 haplotypes observed (Table 4). All SNPs in ALOX5 showed complete linkage disequilibrium (|D'|=1 and d^2≠1), and haplotypes 2, 3, and 5 are equivalent to ALOX5–1708G→A, ALOX5+270G→A, and ALOX5+1728A>G, respectively. The frequency of *ALOX5-ht1[G-C-G-A]* containing a genotype in the AIA group (92.4%) was significantly higher than that in the ATA group, at 82.3%, with P=0.01, an odds ration (OR) of 5.0, and a 95% confidence interval (95%CI) of 1.54–17.9), or in the normal controls (90.0%, P=0.03, OR=4.5, 95%CI=1.1–18.4) by using a dominant model (Table 5). The P value (AIA vs ATA, P=0.01) remained significant after correction for multiple comparisons (Pc=0.04). The haplotypes of PTGS2 were not analyzed because of the extremely low frequencies of the variant alleles (≤2%).

Discussion

We assessed 11 SNPs in five candidate genes including ALOX5, ALOX5AP, PTGS2, LTC4S, and CYSLTR1, for association with AIA. One SNP in the CYSLTR1 gene was novel. There were no significant associations observed between the AIA phenotype and allele or genotype frequencies of any of the SNPs in the candidate genes studied. However, there was a significant difference observed in the frequency of the *ALOX5-ht1[G-C-G-A]* haplotype between the AIA group and the ATA group and the normal controls, suggesting a possible involvement of this ALOX5 haplotype in the development of AIA.

ALOX5 is the first committed enzyme in the biosynthetic pathway leading to the production of the LTs (Foegh et al. 1998), and clinical studies in patients with asthma have shown a therapeutic benefit with ALOX5 inhibitors (Liu et al. 1996). Recently, analysis of the promoter region of the ALOX5 gene has revealed VNTRs of the Sp1-binding motif, which have potential influence on the transcription rate of the gene and drug response to ALOX5 inhibitors (Drazen et al. 1999a, 1999b; In et al. 1997). Kawagishi et al. (2002), however, have shown no difference in ALOX5 gene variability in AIA patients compared with ATA and normal controls. Moreover, no relationship has been noted between these mutations and the clinical characteristics and urinary LTE4 levels in AIA patients, suggesting that this genetic variation does not seem to contribute to susceptibility to AIA. In this study, although we did not examine VNTRs in the promoter, we screened four known SNPs in the ALOX5 gene. There were no significant associations of each SNPs with AIA. However, the frequency of the *ALOX5-ht1[G-C-G-A]*-containing genotype in the AIA group was significantly higher than those of the ATA group and the normal controls. Haplotype in-

Table 3 Allele and genotype frequencies of the SNPs in candidate genes (*AIA* ASA-intolerant asthma, *ATA* ASA-tolerant asthma, *NC* normal controls, *n* number of patients, *R* arginine, *H* histidine, *NS* not significant)

Gene	Loci	Genotype	AIA *n*=93	ATA *n*=181	NC *n*=123	*P* value[a]	
						AIA vs ATA	AIA vs NC
ALOX5	–1708G→A	G	44 (62.9%)	106(66.3%)	76 (67.9%)		
		AG	25(35.7%)	45(28.1%)	30 (26.8%)	NS	NS
		A	1 (1.4%)	9 (5.6%)	6 (5.4%)		
		Allele frequency	0.20	0.20	0.19	NS	
	21C→T	C	43(55.1%)	104(62.7%)	78 (65.5%)		
		CT	31(39.7%)	48(28.9%)	35 (29.4%)	NS	NS
		T	4(5.1%)	14 (8.4%)	6 (5.0%)		
		Allele frequency	0.25	0.23	0.2	NS	
	270G→A	G	69(89.6%)	151(91.5%)	98 (85.2%)		
		AG	8 (10.4%)	13 (7.9%)	16 (13.9%)	NS	NS
		A	0 (0%)	1 (0.6%)	1 (0.9%)		
		Allele frequency	0.08	0.15	0.08		
	1728A→G	A	70 (94.6%)	142 (89.3%)	98 (86.0%)		*P*=0.04 in co-dominant, dominant model
		AG	4 (5.4%)	15 (9.4%)	16 (14.0%)	NS	
		G	0 (0%)	2 (1.3%)	0 (0%)		
		Allele frequency	0.03	0.06	0.07	NS	
ALOX5AP	218A→G	A	88 (98.9%)	170 (97.7%)	119(98.3%)		
		AG	1 (1.1%)	4 (2.3%)	2(1.7%)	NS	NS
		G	0 (0%)	0 (0%)	0 (0%)		
		Allele frequency	0.01	0.01	0.01	NS	
PTGS2	–162C→G	C	84 (98.8%)	167 (100%)	114(92.7%)		
		CG	1 (1.2%)	0(0%)	4(3.3%)	NS	NS
		G	0 (0%)	0 (0%)	0 (0%)		
		Allele frequency	0.01	0	0.02	NS	
	10T→G	T	87 (98.9%)	168 (100%)	120(98.4%)		
		GT	1 (1.1%)	0 (0%)	2(1.6%)	NS	NS
		G	0 (0%)	0 (0%)	0 (0%)		
		Allele frequency	0.01	0	0.01	NS	
	R228H	G	82 (98.8%)	165 (100%)	114(96.6%)		
	(G→A)	AG	1 (1.2%)	0 (0%)	4(3.4%)	NS	NS
		A	0 (0%)	0 (0%)	0 (0%)		
		Allele frequency	0.01	0	0.02	NS	
LTC4S	–444A→C	A	64(72.7%)	118 (68.6%)	85(72.6%)		
		AC	23(26.1%)	47(27.3%)	25(21.4%)	NS	NS
		C	1(1.1%)	7(4.1%)	7(6.0%)		
		Allele frequency	0.14	0.18	0.17	NS	
CYSLTR1	927T→C	T	39 (48.8%)	89 (55.3%)	54(46.6%)		
		CT	27 (33.8%)	39 (24.2%)	33(28.4%)	NS	NS
		C	14 (17.5%)	33 (20.5%)	29(25.0%)		
		Allele frequency	0.34	0.33	0.39	NS	

[a]Each *P* value was calculated with co-dominant, dominant, and recessive models. Logistic regression analysis was applied to control for age, sex, and atopy as covariables

formation is more informative than individual SNPs in that it can localize specific disease-associated chromosomal regions, and these specific chromosomal regions can be analyzed further to reveal the disease-associated mutations (Kwok and Gu 1999). No previous haplotypic analysis has been reported to have been undertaken to study genetic associations in AIA patients. In et al. (1997) have shown that a VNTR (6-bp del and 12-bp del) in the promoter of ALOX5 is in linkage disequilibrium with ALOX5+21C→T and ALOX5+270G→A, respectively. In this regard, the haplotype *ALOX5-ht1[G-C-G-A]* might be in linkage disequilibrium with the VNTR in the promoter region, which contributes to AIA development. Further studies of the ALOX5 promoter VNTR are needed in

Table 4 Haplotype frequencies of ALOX5gene. Haplotypes with a frequency more than 1% are listed. The haplotype of ALOX5 is in order of –1708G→A-21C→T-270G→A-1728A→G. Rase alleles are given in *bold* (*AIA* ASA-intolerant asthma, *ATA* ASA-tolerant asthma, *NC* normal controls, *ht* haplotype, *n* number of chromosomes)

Gene	Haplotype	AIA (*n*=158)	ATA (*n*=338)	NC (*n*=240)	*P* value[a]	
					AIA vs ATA	AIA vs NC
ALOX5	ht1 (G-C-G-A)	0.66	0.64	0.62	0.36	0.51
	ht2 (**A**-**T**-G-A)	0.19	0.20	0.18	0.64	0.70
	ht3 (G-C-**A**-A)	0.04	0.04	0.03	0.80	0.46
	ht4 (G-**T**-G-A)	0.05	0.04	0.01	0.99	0.16
	ht5 (G-C-G-**G**)	0.03	0.07	0.07	0.07	0.07

[a]Logistic regression analysis was applied to control for age, sex, and atopy as covariables

Table 5 Genotype distributions of haplotype of ALOX5 (*AIA* ASA-intolerant asthma, *ATA* ASA-tolerant asthma, *NC* normal controls, *n* number of patients, *ht* haplotype). Haplotypes 2, 3, and 5 are equivalent to ALOX5–1708G→A, ALOX5+270G→A, and ALOX5+1728A→G, respectively

Haplotype	Genotype	AIA (*n*=93)	ATA (*n*=181)	NC (*n*=123)	*P* value[a]	
					AIA vs ATA	AIA vs NC
ht1 (G-C-G-A)	ht1/ht1	30 (38.0%)	75 (44.4%)	44 (36.7%)	*P*=0.01 in dominant model	*P*=0.03 in dominant model
	ht1/–	43 (54.4%)	64 (37.9%)	64 (53.3%)		
	–/–	6 (7.6%)	30 (17.8%)	12 (10.0%)		
ht4 (G-**T**-G-A)	ht4/ht4	0	0	0		
	ht4/–	6 (7.6%)	11 (6.5%)	2 (1.7%)		
	–/–	73 (92.4%)	158 (93.5%)	118 (98.3%)		

[a]Each *P* value was calculated with co-dominant, dominant, and recessive models. Logistic regression analysis was applied to control for age, sex, and atopy as covariables

our population, including an analysis of the degree of linkage disequilibrium with the associated ALOX5 haplotype and functional evaluation of the effect on gene transcription.

ALOX5AP, which is a 18-kDa nuclear membrane protein, has been postulated to play an essential role in the transfer of arachidonic acid to ALOX5 (Kennedy et al. 1991). In patients with asthma, increased expression of ALOX5AP and ALOX5 mRNAs has been reported in peripheral blood leukocytes by the reverse transcription-polymerase chain reaction (RT-PCR) technique (Koshino et al. 1998). Koshino et al. (1999) have shown that the frequency of the monopolymer A repeats (21 A repeats, 95–73 bp upstream from the ATG transcription start site) in the polyadenyl region of the ALOX5AP promoter gene is significantly higher in asthmatics than in controls, but they have not identified the effects of these monopolymer A repeats of the ALOX5AP promoter gene on its promoter function. In this study, we have screened one known SNP of ALOX5AP+218A→G but have not found any association between ALOX5AP+218A→G and the AIA phenotype. Further study will be needed to screen the monopolymer A repeats in the promoter region as described above and other important novel SNPs.

PTGS2 up-regulation may also have an anti-inflammatory role in some circumstances, particularly in chronic inflammation (Gilroy et al. 1995). Down-regulation of PTGS2 expression has been found in nasal polyps from AIA (Picado et al. 1999). Inadequate regulation of PTGS2 may predispose asthma patients to develop aspirin hypersensitivity. In this study, the allele frequencies of SNPs of PTGS2 gene were low, as described in a previous report (Fritsche et al. 2001). In addition, the non-synonymous SNPs, R228H(G→A) and V511A(T→C) have been shown to be of no functional importance, and the other two SNPs, viz., –162C→G and 10T→G, are not located in any known transcriptional regulatory elements (Fritsche et al. 2001). From these findings, the above-mentioned SNPs of PTGS2 gene are supposedly not associated with AIA development.

LTC4S, the terminal enzyme for cysLTs production, is reported to be highly expressed in bronchial biopsies from the AIA patients compared with ATA and normal controls, with the expression of other enzymes (ALOX5, ALOX5AP, PTGS1, PTGS2) being similar in all subject groups (Cowburn et al. 1998). Increased LTC4S expression in biopsies of AIA is the only change in enzymes or cell markers correlating significantly with bronchial hyperresponsiveness to inhaled L-ASA. AIA development is reported to be associated with the LTC4S –444A→C SNP, which provides an additional transcription-factor-binding site in the promoter, resulting in increased transcription of the gene in a Polish population. However, the –444C allele is not associated with mild asthmatics with AIA who do not require oral steroids to control asthmatic symptoms, suggesting that this polymorphisms may be a marker of severe steroid-dependent AIA (Sanak et al. 1997, 2000; Sanak and Szczeklik 2001). A subsequent study has not demon-

strated any significant association between the variant C allele and the AIA phenotype, although the study subjects were an unstratified population of AIA that included mild, moderate, and severe cases (Van Sambeek et al. 2000). Recently, in a study of a Japanese population (Kawagishi et al. 2002), the frequency of the –444C allele was found to be significantly higher in AIA compared to ATA patients (P=0.042), but there was no significant difference in the dose of inhaled and oral steroid requirements between the AIA patients with the –444C allele (A/C, C/C) and those with the wild-type allele (A/A). In addition, there was no significant relationship observed between the –444A→C polymorphism and LTC4S activity. In our study, the variant C allele was not associated with the AIA phenotype, and there was no significant difference in systemic steroid requirement, which was presented as the prednisolone-equivalent dose for 1 year, between the AIA patients with the variant allele (n=64, 357.9±784.7 mg) and those with wild-type allele (n=24, 556.9±941.4 mg; P>0.05). Although the AIA with the variant allele tended to have a higher steroid requirement than those with the wild-type allele, these results do not seem to support the LTC4S –444A→C polymorphism being associated with severe asthmatics with AIA. Further studies will be needed to elucidate this point in stratified populations with AIA.

In this study, the frequency of the –444C allele in AIA (allele frequency [q]=0.14) was similar to that observed in a Japanese population (q=0.19, Kawagishi et al. 2002) but was lower than the frequncey observed in Polish (q=0.39; Sanak et al. 2000) and American populations (q=0.33; Van Sambeek et al. 2000). These observations are likely to be attributable to differences in ethnicity between study populations. Our study population is larger than previous studies (Kawagishi et al. 2002; Sanak et al. 1997, 2000; Van Sambeek et al. 2000). Accordingly, although further studies are needed with a bigger AIA cohort, the contribution of the LTC4S –444A→C polymorphism to the pathogenesis of of AIA in the Korean population is likely to be low.

The biological actions of cysLTs probably occur as a consequence of binding to their receptors, CYSLTR1 and CYSLTR2, on the surface of target cells (Hui and Funk 2002). CYSLTR1 is a G-protein-coupled seven-transmembrane receptor and is expressed primarily in airway smooth muscle, eosinophils, macrophages, and the spleen. CysLTs bind to CYSLTR1 with a rank order of potency of LTD4>LTC4>LTE4, and the receptor is selectively antagonized by the currently available leukotriene modifiers, such as motelukast, pranlukast, and zafirlukast (Drazen et al. 1999b). Recently, Sousa et al. (2002) have reported that the number of cells expressing CYSLTR1 in nasal mucosa was significantly higher in AIA patients with chronic rhinosinusitis than in ATA patients, suggesting that overexpression of CYSLTR1 is probably fundamental in the pathogenesis of aspirin hypersensitivity. In this study, we could not find any association between CYSLTR1+927T→C and AIA phenotype. Although this novel SNP is a synonymous change, it may affect the efficiency of its transcription or translation or alternatvely be in tight linkage disequilibrium with (an)other polmophism(s) in functionally important genomic elements of the CYSLTR1 gene. Accordingly, we need further studies on other closely linked polymorphisms with this SNP or another novel SNPs in the promoter or coding regions and their functional impacts.

We have performed power calculations for all SNPs when negative results to the AIA phenotype were found. All candidate genes in allele and genotyping studies showed poor power values (power<0.8). When Type II error probability (power>0.8) is applied, the number of study subjects should be more than 1,000. However, it is impossible to enroll more than 1,000 subjects from a homogeneous ethnic group, especially in uncommon diseases such as AIA. To date, the subject numbers in this study is the largest among the previous investigations reporting genetic associations in AIA (Kawagishi et al. 2002; Sanak et al. 1997; Van Sambeek et al. 2000) and is based on a single ethnic group. Further studies with a larger population are needed to confirm these negative associations.

In conclusion, there is a lack of association between the ALOX5 (ALOX5, –1708G→A, 21C→T, 270G→A, 1728G→A), ALOX5AP (218A→G), PTGS2 (–162C→G, 10T→G, R228H, V511A), LTC4S (–444A→C), and CYSLTR1 (927T→C) polymorphisms and AIA development in the Korean population, whereas *ALOX5-ht1[G-C-G-A]* is associated with AIA phenotype. Single-disease-related SNP alleles are neither necessary nor sufficient to cause illness. However, a combined effect of a collection of SNP alleles in sets of key genes plus environmental factors probably together determine whether an individual suffers from such a disease (Brookes 1999). Thus, we need further studies in larger cohorts of AIA patients and of other linked polymorphisms with the SNPs of these genes and their functional impact to confirm clinical significance, especially with *ALOX5-ht1[G-C-G-A]*.

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