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Cysteinyl leukotriene receptor 1 promoter polymorphism is associated with aspirin-intolerant asthma in males

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Summary

Background Cysteinyl leukotrienes (CysLTs) play important roles in the pathogenesis of eosinophilic airway inflammation characterized by bronchoconstriction, mucus secretion and airway hyper-responsiveness via cysteinyl leukotriene receptor 1 (CysLTR1)-mediated mechanism. CysLTR1-selective antagonists have anti-bronchoconstrictive and anti-inflammatory effects in asthma, particularly aspirin-intolerant asthma (AIA).

Methods To investigate the association of CysLTR1 with AIA development, we identified three single nucleotide polymorphisms (SNPs), $-634C > T$, $-475A > C$, $-336A > G$, in the 5′ upstream region of CysLTR1 gene using a direct sequencing method in 105 AIA patients, 110 ASA-tolerant asthma (ATA) patients and 125 normal healthy controls (NC).

Results Significant differences were observed in allele frequencies of the three SNPs within male subjects; Male AIA patients had higher frequencies of the minor alleles of these three SNPs than male control groups ($P = 0.03$ for AIA vs. NC; $P = 0.02$ for AIA vs. ATA). Moreover, three-SNP haplotype, ht2 [T–C–G], was associated with increased disease risk (odds ratio (OR) = 2.71, $P = 0.03$ for AIA vs. NC; OR = 2.89, $P = 0.02$ for AIA vs. ATA) in males. CysLTR1 haplotypes were also associated with altered gene expression; luciferase activity was significantly enhanced with the ht2 [T–C–G] construct in comparison with the ht1 [C–A–A] construct in human Jurkat cells ($P = 0.04$).

Conclusion These results suggest that genetic variants of CysLTR1 are associated with AIA in a Korean population, and may modulate CysLTR1 expression.

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Introduction

Aspirin acetylsalicylic acid (ASA) -intolerant asthma (AIA) is a distinct clinical syndrome [1, 2] that involves the development of bronchoconstriction in asthmatic individuals following ingestion of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). AIA affects 5–10% of adult asthmatics [3] and appears to be dependent on the overproduction of cysteinyl leukotrienes (CysLTs) [4]. CysLTs such as LTC4, LTD4 and LTE4 are pro-inflammatory lipid mediators that participate in the pathogenesis of eosinophilic airway inflammation characterized by bronchoconstriction, mucus secretion and airway hyper-responsiveness [5–8]. CysLTs are synthesized by the 5′-lipoxygenase pathway from arachidonic acid which composes the phospholipid membrane. CysLTR1-selective antagonists, such as montelukast (Singulair™, Merck & Co., Inc, NJ, USA), zafirlukast (Accolate™, Astra Zeneca Pharmaceutical, Wilmington, DE, USA) and pranlukast (Onon™, Ono Pharmaceutical Co., Chuo-ku, Osaka, Japan), have been known to have anti-bronchoconstrictive and anti-inflammatory effects in asthma [9–13], particularly in AIA patients. A recent report found that the numbers of cells expressing the CysLTR1 in nasal mucosa were significantly higher in AIA patients with chronic rhinosinusitis than that in ASA-

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tolerant asthma (ATA) patients [14], suggesting that over expression of CysLTR1 may be related to the pathogenesis of aspirin hypersensitivity. Other studies have found evidence of extensive interactions between CysLT and other mediators relevant to asthmatic inflammation via a CysLTR1-mediated mechanism [15]. CysLTs promoted generation of T-helper type 2 (Th2) cytokines such as IL-4, IL-5 and IL-13 which also enhanced generation of CysLT by inducing the expression of CysLTR1 [15–18]. These findings suggest that CysLTR1 might be one of the key genes in the pathogenic mechanisms underlying AIA.

The human CysLTR1 gene (MIM300201), a member of the seven-transmembrane G protein-coupled receptor family, is located on the X chromosome at bands 13–21 (Xq13-q21) and encodes a protein of 337 amino acids with a molecular mass of 38 549 Da [19, 20]. Expression of CysLTR1 mRNA has been identified in airway smooth muscle, alveolar macrophages, peripheral blood monocytes, eosinophils and endothelial cells [21–23]. The expression of CysLTR1 mRNA can be increased by inflammatory cytokines such as IL-4 [16], IL-5 [17] and IL-13 [16, 18]. In a Th2 cytokine-rich environment, CysLTR1 expression can therefore be up-regulated; this promotes the pro-inflammatory effects of CysLTs.

Despite the potentially important pathophysiologic roles of CysLT and CysLTR1, there have been no published investigations of the association of genetic polymorphism of the CysLTR1 gene and asthma, ATA or AIA. The aim of the current study was to investigate whether genetic polymorphism of CysLTR1 is associated with AIA in the Korean population. We first screened for genetic variation in the 5′ upstream region of CysLTR1 by direct sequencing, and finally identified three novel single-nucleotide polymorphisms (SNPs) ($-634C>T$, $-475A>C$, $-336A>G$) located in the 5′ upstream region of the gene at positions -634, -475, -336 from the transcription start site of CysLTR1. Although these three polymorphisms in the CysLTR1 gene have been cited in GenBank (GenBank ID: AL445202; $-634C>T$: rs321029, $-475A>C$: rs 2637204, $-336A>G$: rs 2806489), they have yet been investigated in the context of a genetic association study. Therefore, we conducted a case–control study of these three CysLTR1 SNPs in three groups of Korean subjects classified as ASA-intolerant asthmatics (AIA), ASA-tolerant asthmatics (ATA) and normal healthy controls (NC).

Methods

Subjects and phenotyping

Three groups of the study subjects (105 patients with AIA, 110 patients with ATA and 125 NC) were enrolled from Ajou University Hospital in Korea. Diagnosis of asthma was confirmed using the Global Initiative for Asthma (GINA) guidelines (2002, revised) and diagnosis of AIA was based on those showing positive response to lysine aspirin (L-ASA) bronchoprovocation tests which was performed with increasing doses of aspirin (75–300 mg/mL, Althargyl, Arthromedica, Switzerland) according to a modified method as previously described [24]. Change of forced expiratory volume in 1 s (FEV_1) was followed up to 5 h after the last dose of ASA challenge. 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 normal findings on simple chest radiograms. Atopy was defined as one or more positive reactions on skin prick test, using 12 common aeroallergens (Bencard, West Sussex, UK), as well as positive (histamine) and negative (saline) controls. Methacholine bronchial challenge tests were performed according to a previously described method [24]. The study protocol was approved by the institutional review board of Ajou University Hospital, Korea and all subjects gave the written informed consent.

Single nucleotide polymorphisms identification and genotyping

In order to identify the transcription start site of the human CysLTR1 transcript, 5′ rapid amplification of cDNA ends (RACE) reactions were performed on human fetus marathon-ready cDNA using the SMART™ RACE cDNA Amplification Kit (Clontech Laboratories, Mountainview, CA, USA). For the 5′ RACE experiment, the following primers were used; for first amplification, AP1 and 5′-AAGTAGATCTGCTACTGC-3′, for second amplification, AP2 and 5′-TCGATCTACCAGTCCTTGC-3′. The 5′ upstream region of the CysLTR1 gene, a 2466 bp fragment of the CysLTR1 (corresponding to -2158 to $+313$), was amplified with a pair of primer (5′-CATTTGGGAATGGGT GAATC-3′ and 5′-CTGCTAACT TCAAGGTCCA-3′) by PCR using LA-Taq (Takara Bio Inc., Otsu, Shuzo, Japan) under the following conditions; denaturation at 95 °C for 10 s, annealing at 60 °C for 1 min, extension at 72 °C for 3 min, for 50 cycles. The fragment of the CysLTR1 was scanned for the presence of polymorphisms by direct sequencing. In the end, we identified three polymorphic sites: $-634C>T$, $-475A>C$, $-336A>G$ from the transcriptional start site. The heterozygous genotypes of female subjects were resequenced by SNaPshot in order to minimize mistyping.

Transfection and luciferase assays

A 2466 bp fragment (from -2158 to $+313$) of the human CysLTR1 gene promoter was prepared by PCR using two

haplotype forms (ht1 [C–A–A] and ht2 [T–C–G]; −634C > T, −475A > C, −336A > G) of genomic DNA as a template, each of which was separately subcloned into a *Kpn*I–*Xho*I site of the pGL3-Basic luciferase reporter vector (Promega Corp., Madison, WI, USA). A transfection experiment into Jurkat (human T lymphocytes, No. 40152, KCLB, Korea) cells was undertaken nine times (27 in total) as follows. In each transfection, 2.5 μg of the reporter constructs, 1 μg of pSV-β-galactosidase control vector and 10 μL lipofectamin (Invitrogen, Carlsbad, CA, USA) were used. At 48 h after transfection, cell were lysed and assayed for the Firefly luciferase activity by according to the manufacturer's instruction (Promega Corp.). A second cell line, A549 (human lung carcinoma cell, No. 10185, KCLB, Korea) cells, was cultured. Transfection and luciferase assays were repeated three times (nine in total) according to the method described before.

Statistical analysis

Genotype–phenotype association between CysLTR1 SNPs and asthma-associated phenotypes was analyzed with the SPSS v11 package (SPSS Inc., Chicago, IL, USA). The bi-allelic SNPs were coded into two (dominant, recessive model) or three (additive model) classes and analyzed categorically relative to the most common homozygous genotype for each SNP. Bivariate analysis used analysis of variance (ANOVA) or *t*-tests to compare continuous outcomes across the levels of each genotype and χ^2 tests and calculation of odds ratios (ORs) with 95% confidence intervals (CIs) on contingency tables when comparing genotype to categorical variables.

Among control subjects, Hardy–Weinberg equilibrium was tested at each SNP locus by a χ^2 goodness-of-fit test. Pairwise linkage disequilibrium (LD) between SNP loci was measured using both the absolute value of Lewontin's D' and r^2 [25]. Haplotypes of the CysLTR1 gene were analysed using Haploview v2.0 [26].

Results

Characteristics of the study subjects

One hundred and five ASA-intolerant asthmatics, 110 ASA-tolerant asthmatics and 125 normal healthy control subjects were genotyped at the three CysLTR1 SNPs −634C > T, −475A > C and −336A > G. The clinical characteristics of the study subjects were summarized in Table 1. No significant difference was found in mean age between AIA and control groups ($P = 0.44$ for AIA vs. NC, $P = 0.87$ for AIA vs. ATA). No significant differences were observed in the FEV_1 % predicted, total IgE level, asthma duration and airway hyper-responsiveness to methacholine between the AIA and ATA groups ($P = 0.37$, 0.20, 0.09, 0.22, respectively; data not shown).

Table 1. Clinical characteristics of the study subjects

Characteristics	AIA ($n = 105$)	NC ($n = 125$)	ATA ($n = 110$)
Age (years)	44.1 ± 13.4	45.5 ± 17.6	44.3 ± 14.9
Sex (M)	39 (37.1%)	46 (36.8%)	50 (45.4%)
Atopy	53 (50.5%)	18(18.6%)	58 (52.7%)
Asthma duration (years)	6.7 ± 5.7	NA	5.3 ± 6.2
FEV_1 (%)	83.4 ± 22.9	NA	86.3 ± 23.1
PC_{20} to methacholine (mg/mL)	5.0 ± 8.6	NA	6.1 ± 9.9
Log total IgE (IU/mL)	2.2 ± 0.54	1.9 ± 0.6	2.3 ± 0.6

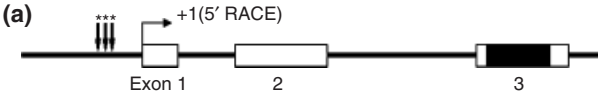
AIA, ASA-intolerant asthma; NC, normal controls; ATA, ASA-tolerant asthma. *n*, number of patientsl; NA, not applicable; M, male. Data are expressed as the mean ± SD.

As the CysLTR1 gene is on the X chromosome, the distribution of the CysLTR1 promoter genotypes were hemizygous within male control subjects, and deviated from Hardy–Weinberg equilibrium ($P < 0.01$). The distribution of the CysLTR1 promoter genotypes within female controls was consistent with HWE ($P = 0.38$). Therefore, all analyses were stratified by sex.

Case–control association analysis

After screening the 5′ upstream region of CysLTR1, we identified three SNPs (−634C > T, −475A > C, −336A > G) at positions −634, −475, −336 from the transcription start site which was determined by 5′-RACE (data not shown). The gene map of CysLTR1 is shown in Fig. 1; the three SNPs are in strong LD with each other. Allele and genotype frequencies of the three SNPs within the phenotypic groups are described in Table 2. Male AIA patients were found to have significantly higher frequencies of the minor alleles (T, C, G) of the CysLTR1 promoter SNPs than male control subjects ($P = 0.03$ for AIA vs. NC; $P = 0.02$ for AIA vs. ATA). In contrast, there were no significant differences in allele and genotype frequencies among the three groups within female subjects ($P > 0.05$) (Table 2).

Three-SNP CysLTR1 haplotypes were constructed using the Haploview program (Table 2), and three common haplotypes were analyzed. The three CysLTR1 SNPs were in perfect LD with each other within male subjects; the three-SNP haplotype frequency within male subjects was therefore the same as the allele frequency of each of the three individual SNPs (Fig. 1B). The ht2 haplotype was therefore associated with increased AIA risk vs. both the NC (odds ratio (OR) = 2.71, 95% confidence interval (CI) = 1.10–6.68, $P = 0.03$) and ATA (OR = 2.89, 95% CI = 1.14–7.28, $P = 0.02$) groups. In contrast, significant differences in the frequency of haplotypes ht1 and ht2 between cases and controls were not found in female subjects by three alternative analysis models (additive, dominant and recessive models).



(b)

r^2 \1D′1	–634 C>T	–475 A>C	–336 A>G
–634 C>T		1	1
–475 A>C	0.94		1
–336 A>G	1	0.94	

Fig 1. Gene Map and linkage disequilibrium (LD) coefficients in cysteinyl leukotrienes (CysLTR1). (a) Gene Map and single nucleotide polymorphisms (SNPs) in CysLTR1 on chromosome Xq13–q21. Coding exons are marked by black blocks and untranslated regions by white blocks. Transcription start site was denoted as nucleotide +1. Positions of three SNPs, − 634C > T, − 475A > C, − 336A > G were denoted by asterisk. (b) LD coefficient (lD'l) of CysLTR1 SNPs.

Association analysis of aspirin-intolerant asthma - associated quantitative phenotypes

The AIA-related phenotypes of serum total IgE levels, initial baseline FEV_1 % predicted value, and the PC_{20} to methacholine were evaluated for association with the CysLTR1 − 634C > T SNP. As the CysLTR1 − 634C > T SNP is a tagging SNP for the three promoter SNPs, this was the only single-SNP analysis necessary (Table 3). Female AIA patients with minor alleles of the CysLTR1 promoter polymorphisms showed higher total IgE levels ($P = 0.003$) and atopy rate ($P = 0.03$), while no significant associations were found in male subjects. No significant associations were found between CysLTR1 polymorphisms and other phenotypes such as age, airway hyper-responsiveness to methacholine and pulmonary function in either sex.

The association between CysLTR1 haplotype and transcription

To examine whether the three CysLTR1 SNPs are associated with altered promoter activity, a construct composed of promoter sequence and luciferase reporter gene was transfected into human Jurkat cells. The reporter activities were compared between two constructs containing either ht1 [C–A–A] or ht2 [T–C–G] at − 634, − 475, − 336 bp in the CysLTR1 gene promoter region (Fig. 2). Significantly enhanced luciferase activity was associated with the haplotype ht2 [T–C–G] construct (150% increases, $P = 0.04$) compared with the haplotype ht1 [C–A–A] construct (Fig. 2a). Additional transfection experiments were performed in another cell line, A549 cells in which significantly higher luciferase activity was noted with ht2 [T–C–G] construct than that with ht1 [C–A–A] construct ($P < 0.001$, Fig. 2b).

To investigate whether the genetic variants create a transcription factor-binding site, sequences containing three SNPs of CYSLTR1 were submitted to the 'Signal Scan' online program (www-bimas.cit.nih.gov/molbio/

Table 2. Case–control analysis of allele, genotype and hapotype frequencies of three SNPs in CysLTR1 promoter

						P value*	
Gender	Locus	Genotype/haplotype	AIA (*N* = 105)	NC (*N* = 125)	ATA (*N* = 110)	AIA vs. NC	AIA vs. ATA
Male	− 634C > T	T	20 (51.3%)	13 (28.3%)	14 (27.5%)	**0.03**	**0.02**
	− 475A > C	C	20 (51.3%)	13 (28.3%)	14 (27.5%)	**0.03**	**0.02**
	− 336A > G	G	20 (51.3%)	13 (28.3%)	14 (27.5%)	**0.03**	**0.02**
	ht2	[T–C–G]	0.513	0.283	0.275	**0.03**	**0.02**
Female	− 634C > T	CC	23 (34.8%)	37 (46.8%)	21 (35.0%)	0.34	0.97
		CT	31 (47.0%)	30 (38.0%)	29 (48.3%)	0.63	0.82
		TT	12 (18.2%)	12 (15.2%)	10 (16.7%)	0.14	0.99
		Allele frequency (q)†	0.417	0.342	0.408	0.19	0.89
	− 475A > C	AA	23 (34.8%)	33 (41.8%)	19 (31.7%)	0.68	0.87
		AC	31 (47.0%)	34 (43.0%)	31 (51.7%)	0.63	0.82
		CC	12 (18.2%)	12 (15.2%)	10 (16.7%)	0.39	0.7
		Allele frequency (q)	0.417	0.367	0.425	0.39	0.89
	− 336A > G	AA	23 (34.8%)	37 (46.8%)	21 (35.0%)	0.34	0.97
		AG	31 (47.0%)	30 (38.0%)	29 (48.3%)	0.63	0.82
		GG	12 (18.2%)	12 (15.2%)	10 (16.7%)	0.14	0.99
		Allele frequency (q)	0.417	0.342	0.408	0.19	0.89
	ht1	[C–A–A]	0.583	0.633	0.575	0.39	0.89
	ht2	[T–C–G]	0.417	0.342	0.408	0.19	0.89
	ht3	[C–C–A]	0	0.025	0.017	0.99	0.99

*Each P value of genotype frequency was calculated with additive, dominant and recessive models.
†q; Minor allele frequency. ‡AIA, ASA-intolerant asthma; NC, normal controls; ATA, ASA-tolerant asthma; *N*, number of patients; NS, not significant. Values in bold indicate significant *P* value.

Table 3. Association of $-634C > T^*$ in CysLTR1 promoter with continuous outcomes

Gender	Genotype	Age†	Log total IgE†	Asthma duration (years)†	PC_{20} to methacholine (mg/mL)†	FEV_1 (%)†
Male	C	43.16 ± 12.9	2.26 ± 0.63	8.4 ± 6.1	4.56 ± 8.78	84.8 ± 25.1
	T	36.1 ± 12.2	2.34 ± 0.57	5.5 ± 4.2	8.81 ± 15.3	76.8 ± 23.8
	p	0.09	0.67	0.1	0.27	0.31
Female	CC+CT	47.7 ± 12.9	**2.08 ± 0.47**	6.5 ± 6.1	4.01 ± 7.05	86.7 ± 20.2
	TT	42.6 ± 13.7	**2.56 ± 0.55**	7.2 ± 5.6	3.76 ± 4.83	77.3 ± 28.5
	p	0.23	**0.003**	0.72	0.93	0.18

* $-634C > T$ is a tagging SNP of the three SNPs in CysLTR1 promoter. Significant associations ($P < 0.01$) are printed in bold.
†Values are genotype-specific means ± SD.

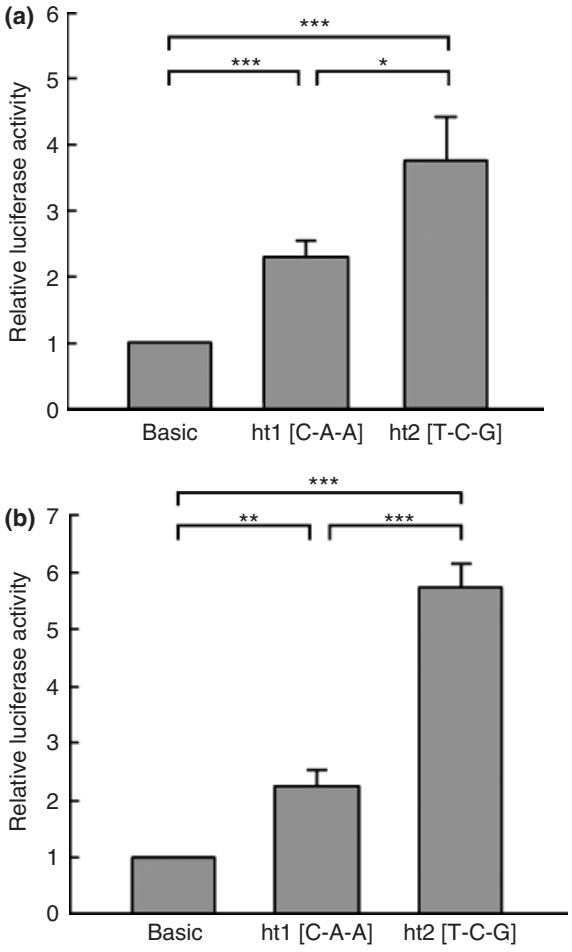


Fig. 2. Promoter activity assay of human cysteinyl leukotrienes gene promoter constructs. Data are the mean values of independent experiments, plus or minus the SEM. (a) Transfection into Jurkat cell. Luciferase activity assay was performed in nine times experiments (in total, $n = 27$). (b) Transfection into A549 cell (human lung carcinoma, $n = 9$). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

signal) and compared with mammalian transcription factor binding sequences in the transcription factors (TRANSFAC) database. No transcription factor-binding site matched with the three SNPs was observed.

Discussion

This is the first investigation of the association of CysLTR1 genetic polymorphism with asthma phenotypes and AIA. We hypothesized that human CysLTR1 may play an important role in AIA development, and therefore investigated CysLTR1 gene promoter polymorphism in patients with AIA compared to ATA or normal healthy controls. This study has shown that three CysLTR1 promoter SNPs were associated with AIA risk in males; males with AIA had significantly higher frequencies of the minor alleles (T, C, G) at the three SNPs than male control subjects. Moreover, the two common three-SNP haplotypes were also associated with AIA risk in males. The ht1 [C–A–A] haplotype was associated with decreased disease risk and the ht2 [T–C–G] haplotype with increased disease risk. Within the AIA group, male patients were younger than female patients. We therefore speculate that male AIA patients carrying ht2 [T–C–G] may be at greater risk of developing AIA at an earlier age. In female subjects, there were no significant associations of CysLTR1 genotypes or three-SNP haplotypes with AIA risk. The different association found between the sexes is consistent with a previous report that male subjects more often showed higher levels of CysLTR1 than female subjects in colorectal adenocarcinomas [27]. Although a preliminary finding, expression level of CysLTR1 mRNA was higher in male AIA patients than in female after the ASA challenges (data not shown). The observed differences in association between males and females may be related to allele-dependent dysregulation of the CysLTR1 gene in AIA patients.

To investigate the association of the three promoter polymorphisms with CysLTR1 expression, we conducted functional studies of the two common three-SNP haplotypes. First of all, we screened several cell lines such as HEK-293T cell (No. CRL-1573, ATCC), HUVEC (No. CRL-1730, ATCC), HL-60 cell (No. CCL-240, ATCC), Jurkat cell (No. 40152, KCLB) and A549 (No. 10185, KCLB) by RT-PCR using a pair of primer located in exon 3. Among them, endogenous expression of CysLTR1 was detected in HL-60, Jurkat and A549 cells (data not shown). The transfection efficiency of HL-60 was too low to detect the

difference of our reporter constructs, so we conducted transfection experiment in Jurkat cells. Constructs with ht2 [T–C–G] showed significantly higher promoter activity in human Jurkat cell than construct with ht1 [C–A–A] which was replicated in another cell line, A549, suggesting that the ht2 [T–C–G] haplotype may be associated with increased expression of the CysLTR1 gene.

To investigate whether the genetic variants create a transcription factor binding site, gel shift assay was tried using HeLaScribe Nuclear extract (Promega Corp.) and SYBR green I nucleic acid gel stain kit (Invitrogen, Carlsbad, CA, USA). Among six probes, −634C, −634T, −475A, −475C, −366A, −366G, a shifted band was noted on −475C probe (data not shown). Although there was no transcription factor binding site matched on 'Signal Scan' online program and further studies such as competition assay may be needed to identify the transcription factor bound to −475C probe, it may suggest that nucleotide substitutions at the three SNPs may create an unknown transcription factor binding sequence, which induces to increase the CysLTR1 protein and enhance pro-inflammatory effects of CysLTs.

CysLTs is known to promote the generation of Th2 cytokines and increase the expression of other mediators such as tumour necrosis factor (TNF)-α, endothelin-1 [28], metalloproteinases [29], reactive oxygen intermediates [30], and histamine receptor [31]. CysLT is also known to induce nuclear factor κB activation, leading to RANTES via a CysLTR1-mediated mechanism [32]. In addition, Th2 cytokines and other mediators can enhance the generation of CysLT and the expression of CysLTR1. In fact, CysLTR1 expression can be up-regulated in a Th2 cytokine-rich environment, promoting the pro-inflammatory effects of CysLTs [16–18]. In this study, CysLTR1-634C > T_TT genotype in females was associated with higher serum total IgE levels. An increased total IgE level is not a characteristic of AIA, and has not been associated with an increased risk of AIA [33]. Although we do not have enough supporting biological data, we speculate that in patients with the CysLTR1 −634 TT genotype, Th2 cytokine production was enhanced, which may lead to IgE production.

This study has several potential limitations. Our sample, although intensively phenotyped, was of moderate size. Our limited power to detect modest effects may at least partially explain our modest evidence of association to CysLTR1 SNPs. While the minimum *P*-values we observed were modest, this is consistent with our expectations regarding common, complex conditions such as asthma and subtypes such as AIA. The generalizability of association results in our Korean sample to AIA in other populations with different exposures is undetermined. We have included known covariates of asthma, such as age and sex, as potential covariates in our analyses, but we have not formally tested for genotype-by-environment interactions.

In conclusion, we have shown that novel genetic variants of the CysLTR1 promoter are associated with AIA risk in male patients. Our studies suggest that CysLTR1 is a potentially important gene in AIA susceptibility in the Korean population, and that CysLTR1 polymorphism may contribute to the development of AIA by increasing the expression level of CysLTR1 in the asthmatic airway. Further studies will be needed to replicate these associations in a larger asthma cohort or non-Korean populations and to evaluate whether these genetic variants of CysLTR1 can contribute to the individual response to CysLTR1 antagonist.

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