

Leukotriene C₄ synthase promoter polymorphism in Japanese patients with aspirin-induced asthma

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Background: The A to C transversion in the promoter region of the gene encoding leukotriene C₄ synthase (*LTC₄S*) is proposed to be associated with the development of aspirin-induced asthma (AIA).

Objective: We investigated the frequency of the polymorphism in Japanese population and its association with clinical characteristics and cysteinyl leukotriene production.

Methods: Genotyping of *LTC₄S* gene promoter was performed on 60 patients with AIA, 100 patients with aspirin-tolerant asthma (ATA), and 110 control subjects. We assessed the basal levels of urinary LTE₄, the increment of urinary LTE₄ on venous aspirin challenge, and *LTC₄S* activity in peripheral blood eosinophils.

Results: The frequency of the variant C allele was significantly higher in patients with AIA (frequency of allele [q] = 0.192) than in patients with ATA (q = 0.110, *P* = .042). Variant C-allelic carriers experienced asthma at a significantly younger age (31.8 ± 2.9 years [mean ± SEM]) than wild-type A homozygotes (41.3 ± 2.2 years, *P* = .007). Basal levels of LTE₄ and the increment of urinary LTE₄ on venous aspirin challenge did not show a difference between wild-type A homozygotes and variant C-allelic carriers. There was no relationship between the polymorphism and the *LTC₄S* activity in eosinophils, although *LTC₄S* activities were significantly higher in patients with AIA than in patients with ATA.

Conclusion: Our findings reveal the lack of functionality of the polymorphism in the *LTC₄S* gene, whereas this polymorphism might have some effect on the development of AIA, probably in linkage disequilibrium with another causatively important mutation. (*J Allergy Clin Immunol* 2002;109:936-42.)

Key words: Aspirin-induced asthma, cysteinyl leukotrienes, leukotriene C₄ synthase, 5-lipoxygenase, polymorphism

Aspirin-induced asthma (AIA) is known as a syndrome with acute asthma attacks often accompanied by nasal symptoms to be precipitated by means of oral administration of aspirin and other nonsteroidal anti-

Abbreviations used

AIA: Aspirin-induced asthma
ATA: Aspirin-tolerant asthma
cysLT: Cysteinyl leukotriene
Egr-1: Early growth response protein 1
5-LO: 5-Lipoxygenase
LT: Leukotriene
LTC₄S: Leukotriene C₄ synthase
NSAID: Nonsteroidal anti-inflammatory drug
q: Frequency of allele

inflammatory drugs (NSAIDs).^{1,2} Although the exact pathogenetic mechanism of AIA is not fully understood, there is growing evidence that patients with AIA have a constitutive chronic overproduction of cysteinyl leukotrienes (cysLTs) that is further upregulated by aspirin administration.³⁻⁶ It is well known that cysLTs can mediate bronchial smooth muscle constriction and can increase mucous secretion, vascular permeability, and cellular infiltration.^{7,8} In this respect treatment of patients with AIA with specific leukotriene receptor antagonists have been shown to markedly attenuate their pathophysiologic responses to aspirin.^{9,10}

It has been postulated that the phenotype of AIA might stem from the inhibition of cyclooxygenase by NSAIDs, with resultant reduction of the PGE₂-dependent suppression of cysLT synthesis in the lung.^{7,11-13} CysLTs are synthesized through the 5-lipoxygenase (5-LO) pathway of arachidonic acid metabolism. The 5-LO pathway for synthesizing cysLTs has several steps, and distinct enzymes are involved in each step, namely cytosolic phospholipase A₂, 5-LO, 5-LO-activating protein, and leukotriene C₄ synthase (*LTC₄S*). It remains uncertain which step in the pathway is responsible for the overproduction of cysLTs in patients with AIA. Cowburn et al¹⁴ reported that the number of cells expressing *LTC₄S* was increased in the bronchial mucosa of patients with AIA compared with that of patients with aspirin-tolerant asthma (ATA) and that the amount of cysLTs in bronchoalveolar lavage fluid was strongly correlated with the number of *LTC₄S*-positive cells. Sanak et al¹⁵ revealed that the polymorphism (ie, A to C transversion at 444 nucleotides upstream of the ATG translation start site) in the promoter region of the *LTC₄S* gene is associated with the development of AIA in a study of a Polish population. In contrast, Van Sambeek et al¹⁶ reported that the C allele

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in the *LTC4S* gene was not correlated with the phenotype of AIA in the United States and that this polymorphism did not affect the transcriptional activity of the *LTC4S* gene. Furthermore, a series of naturally occurring mutations consisted of additions or deletions of the Sp1/early growth response protein 1 (Egr-1)-binding motifs were identified in the promoter region of the gene encoding 5-LO (*5-LO*), and these mutations were reported to be associated with the regulation of *5-LO* gene expression through the differences in Sp1 and Egr-1 transactivation.¹⁷ However, the association of the mutations of the *5-LO* gene with *cysLT* production has not been clearly elucidated.

In the present investigation we evaluated the polymorphism of the *LTC4S* gene in Japanese patients with AIA and ATA and examined its association with clinical characteristics and *cysLT* production. Furthermore, we studied the mutations of the *5-LO* gene in the same subjects and the relation between these mutations and some of the clinical and laboratory indices.

METHODS

Subjects

All subjects were Japanese and were recruited from the outpatient clinics of National Sagami Hospital and Toyama Medical and Pharmaceutical University Hospital. The subjects consisted of 60 patients with AIA (age, 54.1 ± 1.6 years [mean $\pm$ SEM]; 20 men and 40 women), 100 patients with ATA (age, 50.0 ± 1.7 years; 48 men and 52 women), and 110 control subjects (age, 56.4 ± 1.9 years; 78 men and 32 women). All patients were given a diagnosis of asthma according to American Thoracic Society criteria. We excluded the patients taking leukotriene receptor antagonists or those who needed rescue use of systemic corticosteroids within 4 weeks before this study. The diagnosis of AIA was made on the basis of a positive result on an aspirin challenge test or an obvious history of asthma attack precipitated by NSAID administration. Patients with ATA and healthy control subjects all had a negative aspirin challenge test result or took aspirin or other NSAIDs without any adverse symptoms. Patients who had serum IgE levels of greater than 350 U/mL, at least one positive skin test reaction with 15 major allergens, or both were classified as atopic. Others were classified as nonatopic. The control subjects had no evidence of personal history of asthma and other allergic diseases. When the patients were in a clinically stable condition, we collected blood and urine samples and performed spirometry from 9 AM to 12 AM. All studies were approved by the local ethics committees, and all subjects involved provided written informed consent to participate in this study.

LTC4S genotyping

We studied genotyping of the A to C transversion in the promoter region of the *LTC4S* gene at 444 nucleotides upstream of the translation start site by means of PCR-restriction fragment length polymorphism. DNA was extracted from peripheral blood leukocytes. We used a previously described primer pair: 5'-TCC ATT CTG AAG CCA AAG GC-3' and 5'-GTG ACA GCA GCC AGT AGA GC-3'.¹⁵ Reaction mixtures were incubated in a Perkin-Elmer Cetus DNA thermal cycler for 30 cycles: denaturation for 1 minute at 96°C; annealing for 1 minute at 62°C; and extension for 1 minute at 72°C. The PCR products were digested with *MspI* for 2 hours at 37°C. Aliquots of digested samples were subjected to 10% polyacrylamide gel electrophoresis, and the gel pattern was visualized by using ethidium bromide staining.

5-LO genotyping

A family of mutations in the *5-LO* gene promoter were identified by using PCR-single-stranded conformational polymorphism, with some modifications of the protocol of In et al.¹⁷ Amplification of the region of the *5-LO* gene promoter required special PCR conditions because the sequence contains the repeat of highly G+C-rich transcription factor-binding motif. We used LA PCR Taq with GC buffer II (Takara Shuzo) to perform the PCR, with the 5' biotin-labeled primer pair: sense primer, 5'-AGG AAC AGA CAC CTC GCT GAG-3'; antisense primer, 5'-GAG CAG CGA GCG CCG GGA GCC TCG GC-3'. Reaction mixtures were incubated for 35 cycles: denaturation for 1 minute at 94°C; annealing for 1 minute at 59°C; and extension for 1 minute at 72°C. PCR products were diluted (1:14) in a stop solution (95% formamide with 20 mmol/L EDTA, 0.05% [wt/vol] xylene cyanol, and 0.05% [wt/vol] bromophenol blue). After denaturation, the samples were loaded on nondenaturing acrylamide gel. Electrophoresis was performed at room temperature and at 30W for 6 hours. The DNA on the gel was transferred to a nylon membrane (Hybond-N+, Amersham Pharmacia Biotech). The bands were detected by using a chemical luminescence method with a biotin-avidin system kit (Imaging high - Chemilumi-, Toyobo).

Measurement of urinary LTE₄

The free-radical scavenger 4-hydroxy-2,2,6,6-tetramethylpiperidyl-oxyl free radical (Wako Pure Chemical Industries, Ltd) was added to each urine sample at a final concentration of 1 mmol/L. The samples were immediately stored at -70°C and analyzed within 1 month after collection. Urinary LTE₄ was measured as described previously.¹⁸ Briefly, it was measured by using an Empore C18-HD cartridge (3M), an HPLC system (Shimadzu LC 6A) equipped with a C18 reverse-phase column (NOVAPAK, Waters), and an LTC₄/D₄/E₄ enzyme immunoassay system (Amersham). Authentic LTE₄ (Wako) was used as a standard for the assay. The analyzed concentrations ranged from 0.75 to 50 pg/mL. All samples were assayed in duplicate, and the intra-assay coefficient of variation was 8.2%.

Lysin-aspirin challenge test

FEV₁ was measured with a spirometer. Three recordings of FEV₁ were made at each time point, and the highest value was used. At the beginning of the study, urine was collected, and a pulmonary function test was performed at about 9 AM. We intravenously administered increasing doses (12.5, 25, 50, 100, and 200 mg equivalent to aspirin) of lysin-aspirin. FEV₁ was recorded every 10 minutes after the administration, and the time interval between each administration of increasing doses was 30 minutes. Provocation was stopped on the appearance of a positive reaction, which was defined as a decrease in FEV₁ by 20% or more from the baseline. Urine was collected for the measurement of LTE₄ immediately before and 3, 6, 9, and 24 hours after the provocation.

Analysis of LTC₄S activity in peripheral eosinophils

Purified peripheral blood eosinophils, which were obtained by means of negative immunomagnetic selection according to the methods of Hansel et al,¹⁹ were used to analyze LTC₄S activity. We analyzed LTC₄S activity on the basis of the methods of Boyce et al.²⁰ Purified eosinophils were washed into 250 μ L of 50 mmol/L HEPES buffer, pH 7.6, containing 10 mmol/L MgCl₂. The suspended eosinophils of 1×10^5 cells/250 μ L were sonicated on ice with a Branson sonicator 3 times for 5 seconds each. Reduced glutathione and LTA₄ methyl ester (Cayman Chemical) were added to the sonicates to final concentrations of 10 mmol/L and 20 μ mol/L, respec-

TABLE I. Genotype frequency of the *LTC4S* promoter in each group

Genotype	A/A	A/C	C/C	Frequency of allele	P value*	Odds ratio† (95% CI)
Patients with AIA (n = 60)	40	17	3	0.192	.042	1.88 (0.91-3.87)
Patients with ATA (n = 100)	79	20	1	0.110	—	1
Control subjects (n = 110)	74	28	8	0.200	.011	—

*C allele in patients with AIA and control subjects were compared with that in patients with ATA by means of the χ^2 test.

†Odds ratio of patients with AIA was compared with that of patients with ATA.

TABLE II. Clinical characteristics of patients with AIA

Genotype	A/A	A/C, C/C
No.	40	20
Age (y)	56.3 ± 1.9	49.4 ± 2.7*
Sex (M/F)	13/27	7/13
Type (atopic/nonatopic)	14/26	12/8
Duration of asthma (y)	15.1 ± 1.8	18.2 ± 2.3
Age of asthma onset (y)	41.3 ± 2.2	31.8 ± 2.9†
FEV ₁ (% predicted)	81.9 ± 4.8	74.6 ± 4.6
No. (%) of steroid-naïve patients	3 (7.5)	1 (5.0)
No. (%) of patients receiving beclomethasone dipropionate		
≤800 µg/d	12 (30.0)	8 (40.0)
>800 µg/d	18 (45.0)	5 (25.0)
Mean dose of beclomethasone dipropionate (µg/d)	1050 ± 107	860 ± 169
No. (%) of patients receiving oral steroid	7 (17.5)	6 (30.0)

*P < .05 compared with A/A genotype.

†P < .01 compared with A/A genotype.

tively. The samples were incubated at room temperature for 10 minutes, and the reactions were terminated by the addition of 2 volumes of cold methanol containing 400 ng/mL PGB₂ (Wako). After centrifugation of the sonicates, the supernatants were removed and applied to a 5-µm C18 ultrasphere column. After injection of the sample, the column was eluted at a flow rate of 1 mL/min methanol/acetone/nitrile/water/acetic acid (27:168:305:5 vol/vol, pH 5.6). UV absorbance at 280 nm and UV spectra were recorded simultaneously. The retention times for PGB₂ and LTC₄ methyl ester were 8.5 and 16.0 minutes, respectively. LTC₄ methyl ester was quantified by calculating the ratio of its peak area to that of the PGB₂ internal standard. We confirmed that the level of activity was proportional to the incubation time and cell number of eosinophils (data not shown).

Statistical analysis

To calculate P values for associations with the C allele, the allele frequency in each group was analyzed by using the χ^2 test. Differences among groups were evaluated with the Mann-Whitney U test. The difference was considered to be significant when the P value was less than .05.

RESULTS

Genotype distribution of *LTC4S* promoter in each group

Table I shows the frequency of the C allele in each group. The genotype distribution fulfills the Hardy-Weinberg equilibrium in all groups. The frequencies of the C allele in patients with AIA (frequency of allele [q] = 0.192) and control subjects (q = 0.200) were similar, whereas the frequency in the ATA group was decreased to half of those of the 2 groups (q = 0.110). The frequencies of the C variant allele in both the AIA (P = .042) and

control (P = .011) groups were significantly higher than that in the ATA group. The odds ratio of AIA to ATA associated with the C allele was 1.88.

Association of genotypes with characteristics of patients

Next we assessed the clinical characteristics of patients with AIA in relation to genotypes (Table II). The patients with variant alleles (A/C and C/C) were significantly younger than the patients with the wild-type allele (A/A, P = .037). The duration of asthma was longer in variant-type patients than in wild-type patients, although there was no statistical difference between the 2 groups (P = .186). These findings lead to the consequence that the age of asthma onset in variant-type patients (31.8 ± 2.9 years) is significantly lower than that in wild-type patients (41.3 ± 2.2 years, P = .007). The ratio of atopy tended to be higher in variant-type patients than in wild-type patients (P = .065). There was no significant difference between the 2 groups in terms of sex, percent predicted FEV₁, dose of inhaled steroid, and rate of patients receiving oral steroid.

In regard to patients with ATA, we also evaluated the clinical characteristics in relation to genotypes. The duration of asthma was significantly longer in variant-type patients (27.4 ± 5.1 years) than in wild-type patients (14.3 ± 1.6 years, P = .015). The age of onset showed a tendency to be lower in variant-type patients (24.4 ± 5.4 years) than in wild-type patients (36.0 ± 2.4 years, P = .053). There was no significant difference in the other features between the 2 groups (data not shown).

Association of genotypes with basal levels of urinary LTE₄

Basal levels of urinary LTE₄ in the AIA, ATA, and control groups are shown in Fig 1. Although there was no significant difference in basal urinary LTE₄ levels between patients with wild-type (A/A) and those with variant-type (A/C and C/C) alleles in each group, the urinary LTE₄ levels in patients with AIA (328.2 ± 46.3 pg/mg creatinine, n = 48) were significantly higher than those in patients with ATA (124.0 ± 86.4 pg/mg creatinine, n = 51, P < .0001) and those in control subjects (82.8 ± 59.6 pg/mg creatinine, n = 34, P < .0001), as previously reported.⁶ It has been reported that the basal urinary LTE₄ levels in patients with ATA were not significantly different from those in control subjects.²¹ However, in our study the urinary LTE₄ levels in patients with ATA were significantly higher than those in control subjects (P = .009).

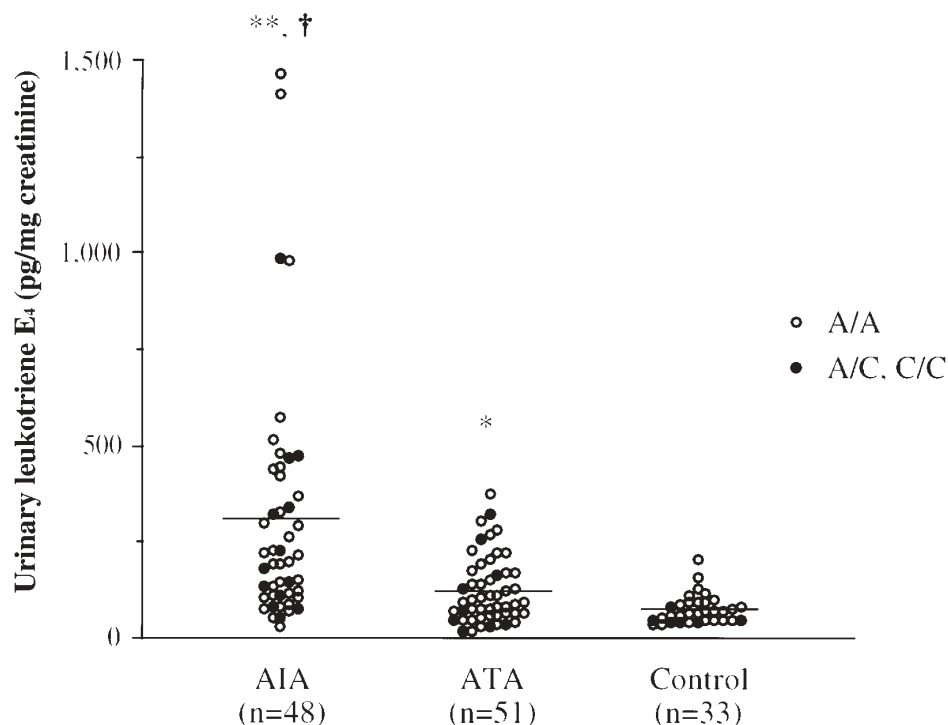


FIG 1. Basal levels of urinary LTE₄ in each group. Horizontal bars indicate geometric means. Patients with wild-type and variant-type alleles are expressed as open and filled circles, respectively. * $P < .001$ compared with the control group; ** $P < .0001$ compared with the control group; † $P < .0001$ compared with the ATA group.

Comparison of reaction to aspirin challenge between the patients with wild-type and variant-type alleles

We examined the increment of urinary LTE₄ after aspirin challenge in 21 patients with AIA (17 wild-type and 4 variant-type patients, Fig 2). We measured urinary LTE₄ every 3 hours after aspirin provocation. There was no significant difference in the provocative dose causing a 20% decrease in FEV₁ (PD₂₀) between patients with wild-type (68.7 ± 15.3 mg) and variant-type (65.3 ± 46.5 mg) alleles, and both genotypes showed the significant increment of urinary LTE₄ after aspirin challenge. However, the ratio of increase in urinary LTE₄ after challenge in wild-type patients showed a tendency to be more prominent than those of variant-type patients. At 6 hours after challenge, the ratio of increase in urinary LTE₄ was significantly higher in wild-type patients than in variant-type patients ($P = .032$).

LTC₄S activity in peripheral blood eosinophils

We assessed the basal levels of LTC₄S activity in peripheral blood eosinophils in the AIA, ATA, and control groups. The levels of LTC₄S activity in patients with AIA (15.37 ± 2.13 nmol/L per 10⁶ cells per hour, $n = 19$) were significantly higher than those in patients with ATA (9.01 ± 1.08 nmol/L per 10⁶ cells per hour, $n = 17$, $P = .017$). However, there was no statistically significant dif-

ference in LTC₄S activity between the AIA group and the control group (11.25 ± 2.25 nmol/L per 10⁶ cells per hour, $n = 7$, $P = .285$).

With regard to the polymorphism of the *LTC4S* gene, we evaluated the basal levels of LTC₄S activity in each group. The average levels in wild-type and variant-type patients were 16.98 ± 2.87 ($n = 13$) and 11.88 ± 2.66 nmol/L per 10⁶ cells per hour ($n = 6$) in patients with AIA and 9.97 ± 1.23 ($n = 13$) and 5.91 ± 1.78 nmol/L per 10⁶ cells per hour ($n = 4$) in patients with ATA, respectively. Although the average activity was higher in wild-type than in variant-type patients, there was no significant difference between wild-type and variant-type patients ($P = .293$ in patients with AIA, $P = .113$ in patients with ATA).

Allele distribution of 5-LO promoter in each group

We investigated the allele distribution of 5-LO promoter in 55 patients with AIA, 63 patients with ATA, and 53 control subjects. The wild-type allele has a series of 5 binding motifs for the transcription factors Sp1 and Egr-1 (ie, GGGCGG), whereas variant alleles consist of the addition of one or deletion of one or two Sp1/Egr-1-binding motifs. There was a close resemblance in the allele distribution among the 3 groups (Table III). In regard to the genotype, approximately 60% of subjects were wild-type patients (5/5), 30% were heterozygous (5/V), and the others had no wild-type alleles (V/V) in each group (data not shown). We also studied the relation

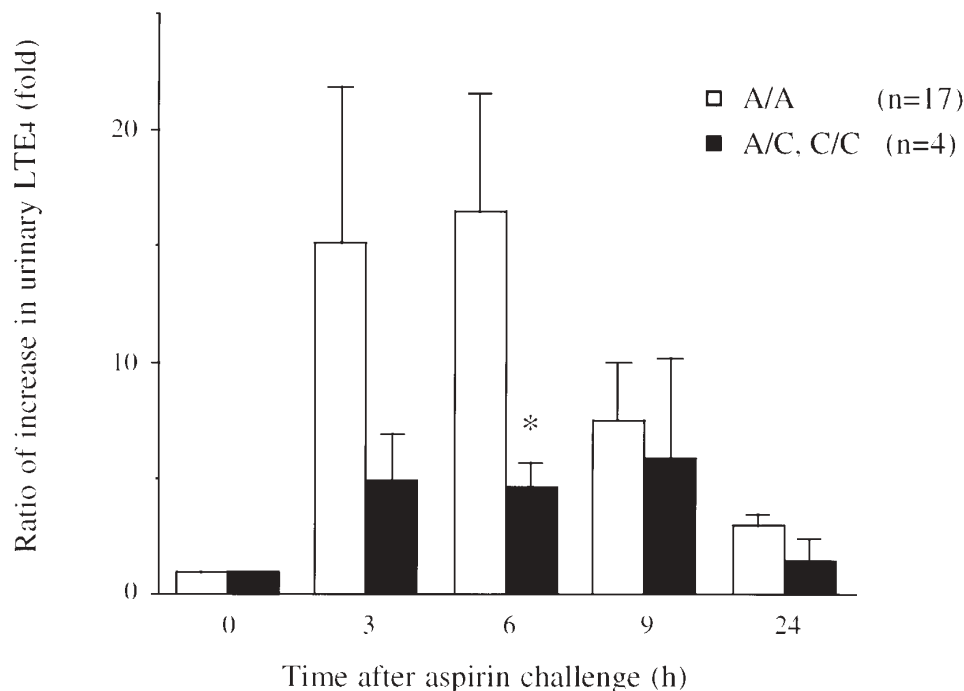


FIG 2. Ratio of increase in urinary LTE₄ after aspirin challenge. The data are presented as means $\pm$ SEM of the percent increase over the prechallenge basal levels. Patients with wild-type and variant-type alleles are expressed as *open* and *filled bars*, respectively. * $P < .05$ compared with wild-type patients at 6 hours after aspirin challenge.

TABLE III. Allele distribution of 5-LO promoter in each group

No. of tandem repeats	3	4	5	6
Patients with AIA (n = 55)	0 (0%)	24 (21.8%)	79 (71.8%)	7 (6.4%)
Patients with ATA (n = 63)	0 (0%)	24 (19.1%)	98 (77.8%)	4 (3.2%)
Control subjects (n = 53)	0 (0%)	22 (20.8%)	77 (72.6%)	7 (6.6%)

of the 5-LO genotype to characteristics of patients, urinary LTE₄, response to aspirin challenge, and LTC₄S activity. We did not find any association between the genotype and these parameters (data not shown).

DISCUSSION

Polymorphisms in a number of candidate genes have been identified and associated with asthma. Studies of the chromosome 5q region, in which the *LTC4S* gene is located,²² revealed linkage to atopy and bronchial hyper-responsiveness. Other candidate genes for asthma are located in the 5q region, including the IL-4 cytokine cluster (IL-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF) and the β_2 -adrenergic receptor. The *LTC4S* gene was previously screened for mutations, and a polymorphism consisting of an adenine (A) to cytosine (C) transversion 444 nucleotides upstream of the ATG translation start site was identified and associated with an increased risk of AIA in a Polish population.¹⁵ The recent study on the US population did not replicate this association.¹⁶ Therefore to further assess the relationship between the polymor-

phism of the *LTC4S* gene and the phenotype of AIA, we performed the genotyping of the *LTC4S* gene on patients with AIA in a Japanese population and evaluated its clinical relevance to AIA.

In line with the report of Sanak et al,¹⁵ we demonstrated that the frequency of the variant C allele was significantly higher in patients with AIA than in patients with ATA. However, the frequency of the variant C allele in patients with AIA was similar to that in control subjects, and the frequency of the variant C allele in the current study tended to be lower than those in the previous studies investigating white populations.^{15,16} Considering that an ethnic restriction has been reported for mutations in several genes for asthma, such as the platelet-activating factor acetyl hydrolase gene,²³ these findings suggest that the polymorphism of the *LTC4S* gene is not related to the phenotype of AIA and might represent a population-specific polymorphism within patients of Eastern European descent.

Next we assessed the relationship between the polymorphism and the clinical characteristics in patients with AIA. The mean age of asthma onset was approximately

10 years less in patients with variant-type AIA than in those with wild-type alleles. The recent study of a total of 500 patients with AIA by Szczeklik et al²⁴ has shown that aspirin intolerance appeared approximately 3 years after the commencement of asthma in an average patient with AIA. Atopic patients were reported to experience symptoms of asthma 7 years earlier than nonatopic patients; however, the mean age at which aspirin intolerance emerged did not differ between the 2 groups. In our study the percentage of atopy was approximately double in variant C-allelic patients (60%) compared with in wild-type patients (35%, $P > .05$), although the mean age of asthma onset in atopic patients with AIA (37.2 ± 2.6 years) was not significantly different from that in nonatopic patients with AIA (38.6 ± 2.5 years). Taken together, in our study the mean age at first aspirin intolerance symptoms seems likely to be younger in variant C-allelic patients than in wild-type patients, irrespective of the rate of atopy in each genotype group. The A to C transversion in the promoter of the *LTC4S* gene might therefore contribute to the early commencement of asthma symptoms followed by the early onset of aspirin intolerance in patients with AIA. In patients with ATA, variant C-allelic carriers also appear to be younger in asthma onset than those with wild-type A homozygotes, although the difference is not statistically significant. It is generally believed that AIA is qualitatively different from ATA and that bronchial asthma commonly occurs at all ages but predominantly in early life, whereas AIA tends to occur after the third decade of life. In our study the symptoms of asthma commenced by 15 years of age in 15 of 100 patients with ATA. In contrast, only 2 of 60 patients with AIA manifested asthma before 15 years of age. Therefore it does not seem appropriate that the commencement of asthma symptoms in patients with ATA is compared with that in patients with AIA. However, the possibility that a variant C allele might be related to the early onset of asthma, irrespective of aspirin sensitivity, is not altogether denied. We found no significant difference in respiratory function and dose of steroid between patients with wild-type A homozygotes and variant C-allelic patients, indicating that the polymorphism of the *LTC4S* gene promoter has no association with the severity of AIA.

In the present study the baseline levels of urinary LTE_4 concentration in patients with AIA with the variant C allele were not significantly different from those in wild-type patients with AIA. Moreover, the ratio of increase in urinary LTE_4 after venous aspirin challenge tended to be lower in variant-type patients than in wild-type patients. In contrast, Sanak et al²⁵ have shown that the increase in urinary output of LTE_4 after inhaled aspirin challenge was more intense in variant C-allelic carriers than in patients with wild-type A homozygotes. They also have reported that the PD_{20} aspirin dose in variant C-allelic carriers was approximately 4.5-fold higher than that in patients with wild-type A homozygotes. This also differs from our finding that the PD_{20} values of both genotypes in patients with AIA were very similar. These data of

Sanak et al suggest that carriers with the variant C allele in Eastern Europe might be *cysLT* resistant. In other words, *cysLT* receptors, their downstream intracellular signals, or both are relatively insensitive to *cysLT*s. Therefore there might be some significant disequilibrium between this single-nucleotide polymorphism in the *LTC4S* gene and the genes relating to *cysLT*s resistant around the q35 region of the fifth chromosome in a Polish population.

There has been no study on the relationship between the polymorphism of the *LTC4S* gene and its activity in eosinophils, which are the predominant source of *cysLT*s in the airways in patients with AIA.¹⁴ To clarify this, we examined the activity of LTC_4S in peripheral blood eosinophils obtained from patients with AIA with both genotypes. Our finding demonstrated that there was no obvious association between the polymorphism and the activity of LTC_4S in eosinophils. This result is consistent with the finding of Sanak et al²⁵ that patients with AIA with the variant C allele had similar *LTC4S* mRNAs in peripheral blood eosinophils compared with patients with wild-type A homozygotes not only at baseline but also after IL-5 incubation. These results support the notion that the polymorphism does not affect the expression and activity of LTC_4S . Furthermore, we found that activity of LTC_4S in peripheral blood eosinophils was increased in patients with AIA than in patients with ATA. This result is also consistent with the recent report that *LTC4S* mRNA in peripheral blood eosinophils was increased in patients with AIA compared with that in patients with ATA.²⁵ It has been reported that the number of eosinophils expressing LTC_4S in the bronchial mucosa was increased in patients with AIA compared with that in patients with ATA.¹⁴ These findings imply that the *LTC4S* gene in peripheral blood eosinophils in patients with AIA is upregulated by factors distinct from the A to C transversion of the promoter, such as circulating inflammatory mediators.

There have been no detailed investigations on the mutations in the Sp1/Egr-1-binding region in the *5-LO* gene promoter in patients with AIA, except one study examining only 6 subjects.¹⁷ We therefore evaluated these mutations in the *5-LO* gene in patients with AIA and their association with clinical characteristics and *cysLT* production. Our results have shown that there was a substantial similarity in allele frequencies and distribution of genotypes among the AIA, ATA, and control groups and that no relationship was recognized between the mutations and the clinical and laboratory indices in patients with AIA. Although it has been reported that the mutations in the *5-LO* gene promoter modify transcription factor binding and reporter gene transcription and alter the clinical response to the treatment with *5-LO* inhibitor in asthmatic patients,²⁶ our findings imply that these mutations might not be causatively related to AIA.

In conclusion, unlike the studies of patients with AIA in Poland and the United States,^{15,16,24} the frequencies of the C allele in patients with AIA and nonasthmatic control subjects were significantly higher than that in

patients with ATA. As opposed to the report of Sanak et al,²⁵ the A to C transversion in the *LTC4S* gene has no association with the upregulation of the cysLT pathway in Japanese patients with AIA. However, patients with AIA with the variant C allele had asthma 10 years earlier than wild-type patients with AIA. Several genes might be involved in AIA susceptibility, and their interactions might be so varied and context dependent that there is a unique profile of the disease alleles for each population. Consequently, identification of AIA-susceptible genes in one population might be hard to reproduce in another population, and false-positive associations will be difficult to resolve if effects are population stratified. Our findings, combined with those of the previous 2 studies on the polymorphism of the *LTC4S* gene, indicate that this polymorphism should be interpreted in the context of linkage disequilibrium with other markers and other known and unknown genes in the terminal region of the long arm of the chromosome 5. Future large-scale investigation will be necessary to confirm the significance of the polymorphism of the *LTC4S* gene in patients with AIA, along with the study of genotyping of the gene cluster affecting asthma on the fifth chromosome.

REFERENCES

- Samter M, Biers RF Jr. Intolerance to aspirin: clinical studies and consideration of its pathogenesis. *Ann Intern Med* 1968;68:975-83.
- Lee TH. Mechanism of aspirin sensitivity. *Am Rev Respir Dis* 1992;145:S34-6.
- Christie PE, Tagari P, Ford-Hutchinson AW, Charlesson S, Chee P, Arm JP, et al. Urinary LTE_4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. *Am Rev Respir Dis* 1991;143:1025-9.
- Kumlin M, Dahlén B, Björck T, Zetterström O, Granström E, Dahlén S-E. Urinary excretion of leukotriene E_4 and 11-dehydro-thromboxane B_2 in response to bronchial provocations with allergen, aspirin, leukotriene D_4 and histamine in asthmatics. *Am Rev Respir Dis* 1992;146:96-103.
- Israel E, Fischer AR, Rosenberg MA, Lilly CM, Callery JC, Shapiro J, et al. The pivotal role of 5-lipoxygenase products in the reaction of aspirin-sensitive asthmatics to aspirin. *Am Rev Respir Dis* 1993;148:1447-51.
- Oosaki R, Mizushima Y, Mita H, Shida T, Akiyama K, Kobayashi M. Urinary leukotriene E_4 and 11-dehydrothromboxane B_2 in patients with aspirin-sensitive asthma. *Allergy* 1997;52:470-3.
- Lewis RA, Austen KF, Soberman RJ. Leukotrienes and other products of the 5-lipoxygenase pathway. *N Engl J Med* 1990;323:645-55.
- Henderson WR Jr. The role of leukotrienes in inflammation. *Ann Intern Med* 1994;121:684-97.
- Christie PE, Smith CM, Lee TH. The potent and selective sulfidopeptide leukotriene antagonist, SK&F 104353, inhibits aspirin-induced asthma. *Am Rev Respir Dis* 1991;144:957-68.
- Yamamoto H, Nagata M, Kuramitsu K, Tabe K, Kiuchi H, Sakamoto Y, et al. Inhibition of analgesic-induced asthma by leukotriene receptor antagonist ONO-1078. *Am J Respir Crit Care Med* 1994;150:254-7.
- Sestini P, Armetti L, Gambaro G, Pieroni MG, Refini RM, Sala A, et al. Inhaled PGE_2 prevents aspirin-induced bronchoconstriction and urinary LTE_4 excretion in aspirin-sensitive asthma. *Am J Respir Crit Care Med* 1996;153:572-5.
- Sanak M, Sampson AP. Biosynthesis of cysteinyl-leukotrienes in aspirin-intolerant asthma. *Clin Exp Allergy* 1999;29:306-13.
- Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis and management. *J Allergy Clin Immunol* 1999;104:5-13.
- Cowburn AS, Sladek K, Soja J, Adamek L, Nizankowska E, Szczeklik A, et al. Overexpression of leukotriene C_4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J Clin Invest* 1998;101:834-46.
- Sanak M, Simon H-U, Szczeklik A. Leukotriene C_4 synthase promoter polymorphism and risk of aspirin-induced asthma. *Lancet* 1997;350:1599-600.
- Van Sambeek R, Stevenson DD, Baldasaro M, Lam BK, Zhao J, Yoshida S, et al. 5' Flanking region polymorphism of the gene encoding leukotriene C_4 synthase does not correlate with the aspirin-intolerant asthma phenotype in the United States. *J Allergy Clin Immunol* 2000;106:72-6.
- In KH, Asano K, Beier D, Grobholz J, Finn PW, Silverman EK, et al. Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. *J Clin Invest* 1997;99:1130-7.
- Oosaki R, Mizushima Y, Kawasaki A, Mita H, Akiyama K, Kobayashi M. Correlation among urinary eosinophil protein X, leukotriene E_4 , and 11-dehydrothromboxane B_2 in patients with spontaneous asthmatic attack. *Clin Exp Allergy* 1998;28:1138-44.
- Hansel TT, Pound JD, Pilling D, Kitas GD, Salmon M, Gentle TA, et al. Purification of human blood eosinophils by negative selection using immunomagnetic beads. *J Immunol Methods* 1989;122:97-103.
- Boyce JA, Lam BK, Penrose JF, Friend DS, Parsons S, Owen WF, et al. Expression of LTC_4 synthase during the development of eosinophils in vitro from cord blood progenitors. *Blood* 1996;88:4338-47.
- Smith CM, Hawksworth RJ, Thien FC, Christie PE, Lee TH. Urinary leukotriene E_4 in bronchial asthma. *Eur Respir J* 1992;5:693-9.
- Penrose JF, Spector J, Baldasaro M, Xu K, Boyce J, Arm JP, et al. Molecular cloning of the gene for human leukotriene C_4 synthase: organization, nucleotide sequence, and chromosomal localization to 5q35. *J Biol Chem* 1996;271:11356-61.
- Stafforini DM, Numao T, Tsodikov A, Vaitkus D, Fukuda T, Watanabe N, et al. Deficiency of platelet-activating factor acetylhydrolase is a severity factor for asthma. *J Clin Invest* 1999;103:989-97.
- Szczeklik A, Nizankowska E, Duplaga M. Natural history of aspirin-induced asthma. *Eur Respir J* 2000;16:432-6.
- Sanak M, Pierzchalska M, Bazan-Socha S, Szczeklik A. Enhanced expression of the leukotriene C_4 synthase due to overactive transcription of an allelic variant associated with aspirin-intolerant asthma. *Am J Respir Cell Mol Biol* 2000;23:290-6.
- Drazen JM, Yandava CN, Dubé L, Szczerback N, Hippensteel R, Pillari A, et al. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. *Nat Genet* 1999;22:168-70.