

IL-5 and thromboxane A2 receptor gene polymorphisms are associated with decreased pulmonary function in Korean children with atopic asthma

Soo-Jong Hong, MD, PhD,^a So-Yeon Lee, MD,^a Hyo-Bin Kim, MD,^a Ja-Hyung Kim, MD,^a Bong-Seong Kim, MD,^a Soo-Ok Choi, BS,^b Seong-Gene Lee, PhD,^b Eun-Soon Shin, PhD,^c and Taek-Jong Hong, MD, PhD^d *Seoul and Pusan, Korea*

Background: Asthmatic airways undergo chronic inflammatory cell infiltration by T cells and eosinophils, which results in sustained airway hyperresponsiveness. IL-5 is important for eosinophil-induced airway inflammation and airway hyperresponsiveness. Thromboxane A2 and its receptor, TBXA2R, are involved in constriction of respiratory smooth muscles and may play a role in thickening and remodeling of airways, which contributes to the severity of asthma. The relationship between IL-5 and TBXA2R gene polymorphisms and pulmonary function in children with asthma has rarely been examined.

Objective: To determine whether IL-5 (T-746C) and TBXA2R (T924C) gene polymorphisms are associated with asthma phenotype and pulmonary function in Korean children with atopic and nonatopic asthma.

Methods: We conducted an association study between known polymorphisms of IL-5 (T-746C) and TBXA2R (T924C) and asthma phenotype and the parameters of atopy and pulmonary function in atopic and nonatopic Korean children with asthma. The subjects were 240 atopic children with asthma, 70 nonatopic children with asthma, and 106 nonatopic healthy children. Asthma phenotypes and bronchial responsiveness to methacholine were determined by a physician. IL-5 and TBXA2R gene polymorphisms were determined by genotyping by using PCR-RFLP assays.

Results: The genotype frequencies of IL-5 and TBXA2R polymorphisms did not differ between healthy controls and atopic or nonatopic children with asthma. A significant association was observed between the IL-5 polymorphism and forced expiratory flow at 25% to 75% of forced vital capacity (FEF_{25-75%}; %; $P = .002$), and between the TBXA2R polymorphism and FEV₁ (%; $P = .035$) and FEF_{25-75%}

(%; $P = .042$) in children with atopic asthma, whereas no such association between the polymorphisms and lung function was observed in nonatopic or control children. In atopic children with asthma, we identified a significant gene-gene interaction in that the combination of the IL-5 (T-746C) and TBXA2R (T924C) mutant alleles was shown to be associated with reduced pulmonary function as determined by FEF_{25-75%} (%) measurement.

Conclusion: The current study indicates that IL-5 (T-746C) and TBXA2R (T924C) polymorphisms alone are associated with spirometric markers of asthma severity, whereas they are not associated with presence of asthma per se. In addition, the data suggest that an interaction between IL-5 and TBXA2R genes may contribute to the severity of asthma, especially atopic asthma. These results suggest that IL-5 and TBXA2R genes may be disease-modifying genes in Korean children with atopic asthma. (*J Allergy Clin Immunol* 2005;115:758-63.)

Key words: Asthma, pulmonary function, IL-5, thromboxane A2 receptor, polymorphism

Asthma, one of the most common respiratory diseases, is a disorder with a strong hereditary component but complex inheritance. The characteristic features of asthma arise in part through the activity of eosinophil-derived proinflammatory mediators.^{1,2}

IL-5 levels have been shown to be elevated in the lungs, sputum, and blood of patients with asthma.³⁻⁵ IL-5 plays an important role in eosinophil growth and influx and airway hyperresponsiveness.^{6,7} In patients with asthma, FEV₁ is inversely correlated with IL-5 production.⁸⁻¹⁰ Although there is convincing evidence that IL-5 is important in asthma, no studies have proven the association between IL-5 polymorphism and atopy or lung function. In human beings, the IL-5 gene is located on chromosome 5q31, and some groups have reported 5q associations with asthma,¹¹ autosomal-dominant familial eosinophilia,¹² circulating eosinophil levels,¹³ and malignancies associated with eosinophilia.¹⁴

Allergic inflammation is also known to be induced by the products of the arachidonic acid cascade. Some inhibitors of this cascade are used for treating patients with asthma.¹⁵ Thromboxane A2 and its receptor (TBXA2R) are involved in constriction of respiratory smooth muscles

From ^athe Department of Pediatrics, University of Ulsan College of Medicine, Seoul; ^bthe Asan Institute for Life Sciences, University of Ulsan, Seoul; ^cthe Statistical Research Institute of Dongguk University, Pusan; and ^dthe Department of Internal Medicine, Pusan National University College of Medicine, Pusan.

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Reprint requests: Taek-Jong Hong, MD, PhD, Department of Internal Medicine, Pusan National University College of Medicine, 1-10 Ami-Dong Seo-Gu, Pusan 602-739, Korea. E-mail: sjhong@amc.seoul.kr. 0091-6749/\$30.00

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TABLE I. Clinical profiles of study subjects

Clinical parameter*	Controls	Atopic subjects with asthma	Nonatopic subjects with asthma
Number of subjects (male/female)	106 (53/53)	240 (150/90)	70 (44/26)
Age (y)	9.7 ± 3.0	9.8 ± 2.4	8.7 ± 2.5
FEV ₁ (%)†	95.5 ± 16.0	87.7 ± 15.1	91.8 ± 15.8
Log PC ₂₀ (mg/mL)†	1.39 ± 0.17	0.33 ± 0.43	0.52 ± 0.42
Log total IgE (IU/mL)†	1.64 ± 0.59	2.52 ± 0.45	1.68 ± 0.50
Log TEC (μL)†	2.15 ± 0.27	2.64 ± 0.29	2.32 ± 0.31
Log eosinophil (%)†	0.32 ± 0.27	0.77 ± 0.31	0.44 ± 0.28

*Mean ± SD.

†*P* < .01 for difference among the 3 groups according to ANOVA.

Abbreviations used

FEF_{25-75%}: Forced expiratory flow at 25% to 75% of forced vital capacity
SNP: Single nucleotide polymorphism
TBXA2R: Thromboxane A2 receptor
TEC: Total eosinophil count

and induce acetylcholine release and airway microvascular leakage.¹⁶ Therefore, they play an important role in the pathogenesis of bronchial inflammation and hyperresponsiveness.^{17,18}

The TBXA2R gene is located on chromosome 19p13.3, contains 4 exons with total length of 15 kb, and codes for a protein of 343 amino acids.¹⁶ Although TBXA2R (T924C) polymorphism was recently found to be associated with asthma,^{19,20} its relationship with pulmonary function in children with asthma has not been defined fully.

The aim of the current study was to determine whether IL-5 and TBXA2R gene polymorphisms were associated with asthma phenotype and clinical asthma parameters, especially pulmonary function, in Korean children with atopic and nonatopic asthma.

METHODS

Subjects

Two hundred forty atopic children with asthma, 70 nonatopic children with asthma, and 106 nonatopic healthy children were enrolled at the Asthma and Allergy Clinics of the Asan Medical Center. As shown in Table I, all patients with asthma and controls (healthy subjects) were appropriately matched for age and sex (*P* > .05). There were significant differences in FEV₁, PC₂₀, total IgE, total eosinophil counts (TECs), and eosinophil fraction among the 3 groups (*P* < .01; Table I).

Asthma phenotypes and bronchial responsiveness to methacholine were determined by a physician. Following the American Thoracic Society guidelines,²¹ asthma was confirmed on the basis of a history of dyspnea and wheezing during the previous 12 months, and a greater than 15% reversibility of FEV₁ spontaneously or after β₂-agonist inhalation, and/or a methacholine provocation test result with a PC₂₀ less than 16 mg/mL. Atopy was defined by a positive skin prick result (wheal diameter ≥3 mm) to at least 1 of the 27 common aeroallergens (Allergopharma, Germany), or elevated specific IgE. Nonatopic subjects were defined as those had a negative skin

response to 27 common aeroallergens. The healthy control subjects had no history of asthma or other allergic diseases and had negative skin prick results, normal total IgE (<100 IU/mL), normal lung function, and no airway hyperresponsiveness (PC₂₀ > 25 mg/mL).

None of the subjects with asthma had received inhaled or oral corticosteroids, antihistamines, or cysteinyl leukotriene receptor antagonists in the 3 months preceding the study. The ethics committee of Asan Medical Center Institutional Review Board approved the study, and consent was obtained from all parents after they were fully informed of the details.

Serum IgE, blood eosinophil measurement, and methacholine challenge test

Concentration of total IgE and specific IgE to Der f and Der p were measured with a COAT-A-COUNT Total IgE IRMB kit (Diagnostic Products Co, Los Angeles, Calif) and an AlaSTAT RIA Allergen-Specific IgE kit (Diagnostic Products Co), respectively. Blood eosinophils were counted by using an automatic blood cell counter (XE-2100; Sysmex Co, Tokyo, Japan). The methacholine challenge protocol used a dosimeter method with a concentration range of 0.625 mg/mL to 25 mg/mL, as previously described.²² FEV₁ was measured after each inhalation by using a spirometer (microspiro HI298; Chest Inc, Tokyo, Japan). The airway response was expressed as the concentration of methacholine that provoked a 20% fall in FEV₁ (PC₂₀). The airway response to methacholine was considered hyperresponsive at PC₂₀ <16 mg/mL.

Genotyping

The IL-5 (T-746C) and TBXA2R (T+924C) alleles were analyzed by using the PCR-RFLP method. The PCR primers for IL-5 (-746T/C) were 5'-GAAGGTATTGGCTCATAGTAC-3' and 5'-GCTCATGAACAGAATACGTA-3',²³ and primers for TBXA2R (+924 T/C) were 5'-CAGGTCTTCATTGCCAGAC-3' and 5'-GTCAACCCAAAACCCTGCT-3'.¹⁹ The restriction enzyme *RsaI* (MBI Fermentas, Hanover, Md) distinguished the IL-5 -746C allele as 124 and 9 bp digestion products and the TBXA2R C allele as 248 and 139 bp digestion products. PCR products were digested with 5 U restriction enzyme at 37°C for 4 hours and separated on 3% agarose gels (FMC BioProducts, Rockland, Me). To confirm the accuracy of RFLP analysis, 40 samples from the study subjects were randomly selected and genotypes determined by using DNA sequencing, and in each case, the sequencing data were compatible with the RFLP data.

Statistical analysis

All values of clinical parameters (including TEC, eosinophil fraction [%], total IgE, PC₂₀) were first converted to log₁₀ values to produce a normal distribution for statistical analysis and then analyzed by using ANOVA. For qualitative variables (sex, presence

TABLE II. Allele frequencies of IL-5 (T-746C) and TBXA2R (T924C) polymorphisms in atopic subjects with asthma, nonatopic subjects with asthma, and control subjects

	Control	Atopic asthma		Nonatopic asthma	
Genotype	n = 106 (%)	n = 240 (%)	aOR (95% CI)*	n = 70 (%)	aOR (95% CI)*
IL-5 (T-745C)					
TT	53 (50.0)	113 (47.1)	1.00	28 (40.0)	1.00
CT	39 (36.8)	100 (41.7)	1.20 (0.73-1.97)	31 (44.3)	1.50 (0.78-2.89)
CC	14 (13.2)	27 (11.2)	0.90 (0.44-1.83)	11 (15.7)	1.49 (0.60-3.71)
CT+CC	53 (50.0)	127 (52.9)	0.93 (0.71-1.76)	42 (60.0)	1.22 (0.81-2.76)
Frequency (T)	0.68	0.68		0.64	
TBXA2R (T924C)					
TT	67 (63.2)	156 (65.0)	1.00	41 (58.6)	1.00
CT	35 (33.0)	71 (29.6)	0.87 (0.53-1.43)	25 (35.7)	1.17 (0.61-2.23)
CC	4 (3.8)	13 (5.4)	1.40 (0.44-4.52)	4 (5.7)	1.63 (0.39-6.88)
CT+CC	39 (36.8)	84 (35.0)	0.93 (0.58-1.50)	29 (41.4)	1.22 (0.66-2.26)
Frequency (T)	0.80	0.80		0.76	

*Adjusted odds ratio (aOR) by age and sex. Logistic regression analysis was performed.

TABLE III. Relationship between clinical parameters and IL-5 (T-746C) genotypes in atopic subjects with asthma and controls*

Clinical parameters	Atopic subjects with asthma			Normal controls		
	TT	CT+CC	P value	TT	CT+CC	P value
Log TEC (μ L)	2.67 $\pm$ 0.25	2.61 $\pm$ 0.31	.115	2.15 $\pm$ 0.30	2.15 $\pm$ 0.24	.992
Log eosinophil (%)	0.80 $\pm$ 0.23	0.73 $\pm$ 0.36	.089	0.31 $\pm$ 0.27	0.33 $\pm$ 0.27	.776
Log IgE (IU/mL)	2.45 $\pm$ 0.44	2.58 $\pm$ 0.45	.022	1.60 $\pm$ 0.62	1.68 $\pm$ 0.57	.463
Log PC ₂₀ (mg/mL)	0.32 $\pm$ 0.41	0.33 $\pm$ 0.45	.798	1.38 $\pm$ 0.24	1.40 $\pm$ 0.05	.502
Forced vital capacity (%)	93.2 $\pm$ 12.5	92.7 $\pm$ 12.3	.733	92.6 $\pm$ 12.9	94.5 $\pm$ 12.4	.480
FEV ₁ (%)	89.3 $\pm$ 14.9	86.8 $\pm$ 14.5	.215	94.3 $\pm$ 15.8	96.8 $\pm$ 16.2	.457
FEF _{25-75%} (%)	85.3 $\pm$ 24.5	74.2 $\pm$ 21.6	.002	107.6 $\pm$ 25.8	103.2 $\pm$ 21.3	.439

*Data for each genotype are expressed as means $\pm$ SDs. The significance of clinical parameters according to genotype was analyzed by using *t* tests. The relationships between clinical parameters and IL-5 (T-746C) genotypes in nonatopic children with asthma were not significant (data not shown).

of an atopic disease, clinical diagnosis of asthma), statistical analyses were performed by using χ^2 analyses. These studies were performed to determine whether there was a disproportional presence of a given allele in affected individuals. To calculate *P* values for associations with the IL-5 or TBXA2R T/C polymorphisms, the genotype frequencies in the groups were analyzed with logistic regression adjusted for age and sex. The relationship between clinical parameters and genotypes was determined statistically by using *t* tests. No corrections were made for multiple comparisons. Because the intermediate phenotypes tested (TECs, IgE, PC₂₀, pulmonary function test) in the subjects of this study are strongly associated with each other, the statistical analyses do not represent independent tests. For the interaction analysis, *P* values were determined by multiple regression analyses by using Bonferroni correction after the adjustment with the age and sex. Statistical analyses were performed by using SPSS software for Windows (release 10.0.7; SPSS Inc, Chicago, Ill).

RESULTS

Genotyping of IL-5 (T-746C) and TBXA2R (T924C)

The association between asthma and IL-5 (T-746C) or TBXA2R (T924C) polymorphisms was investigated by comparing the occurrence of polymorphisms in children with asthma and healthy children. The frequencies of the

IL-5 CC homozygote (11.2%) and CT heterozygote (41.7%) in children with atopic asthma were not significantly different from the frequencies in healthy control children (13.2% and 36.8%, respectively; Table II). Similarly, the frequencies of the TBXA2R CC homozygote (5.4%) and CT heterozygote (29.6%) in children with atopic asthma were not significantly different from the frequencies in controls (3.8% and 33.0%, respectively; Table II). There was no difference between children with atopic asthma and control children in terms of the frequency of 1 or 2 copies of mutant alleles of IL-5 or TBXA2R (Table II). In addition, there was no difference in the frequency of IL-5 or TBXA2R polymorphism between nonatopic children with asthma and controls.

Association between clinical parameters and IL-5 (T-746C) polymorphism

We analyzed IL-5 (T/C) polymorphisms in relation to eosinophil fraction, IgE concentration, bronchial hyperresponsiveness, and pulmonary function. The log serum total IgE concentration was significantly higher in children with homozygous (CC) and heterozygous (CT) IL-5 mutant alleles (2.58 IU/mL $\pm$ 0.45 IU/mL) compared with those with wild-type (TT) alleles (2.45 IU/mL $\pm$ 0.44

TABLE IV. Relationship between clinical parameters and TBXA2R (T924C) genotypes in atopic subjects with asthma and controls*

Clinical parameters	Atopic subjects with asthma			Normal controls		
	TT	CT+CC	P value	TT	CT+CC	P value
Log TEC (μ L)	2.63 $\pm$ 0.30	2.66 $\pm$ 0.24	.503	2.12 $\pm$ 0.26	2.20 $\pm$ 0.28	.127
Log eosinophil (%)	0.76 $\pm$ 0.34	0.78 $\pm$ 0.23	.636	0.27 $\pm$ 0.28	0.39 $\pm$ 0.24	.033
Log IgE (IU/mL)	2.53 $\pm$ 0.43	2.51 $\pm$ 0.49	.747	1.54 $\pm$ 0.56	1.82 $\pm$ 0.60	.020
Log PC ₂₀ (mg/mL)	0.34 $\pm$ 0.42	0.30 $\pm$ 0.45	.568	1.41 $\pm$ 0.03	1.36 $\pm$ 0.27	.210
Forced vital capacity (%)	93.0 $\pm$ 13.0	91.4 $\pm$ 12.4	.389	92.4 $\pm$ 12.9	94.7 $\pm$ 11.7	.409
FEV ₁ (%)	89.0 $\pm$ 14.7	84.6 $\pm$ 14.6	.035	93.4 $\pm$ 14.0	98.1 $\pm$ 18.1	.184
FEF _{25-75%} (%)	81.6 $\pm$ 22.8	74.2 $\pm$ 23.4	.042	104.4 $\pm$ 23.6	106.2 $\pm$ 22.6	.744

*Data for each genotype are expressed as means $\pm$ SDs. The significance of clinical parameters according to genotype was analyzed by using *t* tests. The relationships between clinical parameters and IL-5 (T-746C) genotypes in nonatopic children with asthma were not significant (data not shown).

TABLE V. Effect of combined IL-5 and TBXA2R polymorphisms on FEV₁ (%) and FEF_{25-75%} (%) in atopic children with asthma*

Group	Number of cases (N = 210)	IL-5(T-746C)	TBXA2R(T924C)	FEV ₁ (%) (mean $\pm$ SD)	FEF _{25-75%} (%) (mean $\pm$ SD)
I	70	TT	TT	91.3 $\pm$ 13.9	90.7 $\pm$ 22.7
II	43	TT	CT+CC	85.4 $\pm$ 16.0	76.3 $\pm$ 24.5 [†]
III	85	CT+CC	TT	87.8 $\pm$ 14.1	74.7 $\pm$ 20.0 [‡]
IV	42	CT+CC	CT+CC	83.8 $\pm$ 13.1 [§]	72.0 $\pm$ 22.2 [‡]

*The nonrisk genotype for each gene was used as the reference value. Group was defined by the combination of IL-5 and TBXA2R polymorphisms.

[†]*P* < .05; [‡]*P* < .005; [§]*P* = .06 after Bonferroni correction compared with group I (IL-5 [TT] and TBXA2R [TT]).

IU/mL), especially in atopic children with asthma (*P* = .022). In contrast, TECs, the percentage of eosinophil fraction and PC₂₀ did not differ according to the genotype, even in atopic children with asthma and controls (Table III).

In addition, atopic children with asthma with CC homozygote and CT heterozygote had lower forced expiratory flow at 25% to 75% of forced vital capacity (FEF_{25-75%}; %) than those with the TT homozygote (74.2% $\pm$ 21.6% vs 85.3% $\pm$ 24.5%; *P* = .002; Table III). In nonatopic children with asthma and control children, different IL-5 polymorphisms were not associated with any differences in FEV₁ (%) and FEF_{25-75%} (%). These results suggest that IL-5 CC homozygote and CT heterozygote polymorphisms may be linked to lower pulmonary function in children with atopic asthma.

Association between clinical parameters and TBXA2R (T924C) polymorphism

We also examined the association between TBXA2R (T/C) polymorphism and clinical parameters. TECs, the percentage of eosinophil fraction (%), serum total IgE concentration, and PC₂₀ did not differ according to genotype, even in atopic children with asthma and controls, except that normal children with TBXA2R CC homozygote and CT heterozygote polymorphisms showed a higher percentage of log eosinophil fraction (0.39% $\pm$ 0.24%; *P* = .033) and log serum total IgE concentration (1.82 IU/mL $\pm$ 0.60 IU/mL; *P* = 0.020) than those with the TT homozygote (0.27% $\pm$ 0.28% and 1.54 IU/mL $\pm$ 0.56 IU/mL, respectively; Table IV). In contrast,

in atopic children with asthma, FEV₁ (%) was significantly lower in those carrying 1 or 2 copies of the mutant allele compared with those with the TT homozygote (84.6% $\pm$ 14.6% vs 89.0% $\pm$ 14.7%; *P* = .035; Table IV). In addition, atopic children with asthma with CC homozygote and CT heterozygote showed lower FEF_{25-75%} (%) than those with the TT homozygote (74.2% $\pm$ 23.4% vs 81.6% $\pm$ 22.8%; *P* = .042; Table IV). In nonatopic children with asthma and control children, FEV₁ (%) and FEF_{25-75%} (%) did not differ according to TBXA2R gene polymorphism. These results suggest that the CC homozygote and CT heterozygote of TBXA2R gene polymorphism may be linked with lower pulmonary function in children with atopic asthma.

Interaction between IL-5 and TBXA2R genes

These data show that individually, each polymorphism can significantly influence pulmonary function. To investigate an interaction between IL-5 and TBXA2R genes, we analyzed pulmonary function in relation to various combinations of genotypes. In atopic children with asthma, we found that the combination of IL-5 (T-746C) and TBXA2R (T924C) polymorphisms affected pulmonary function (FEF_{25-75%} [%]; Table V). This effect was most obvious in atopic children with asthma with the combination of mutant alleles (CT or CC) for IL-5 and TBXA2R polymorphism compared with those carrying the wild-type allele (TT; 72.0% $\pm$ 22.2% vs 90.7% $\pm$ 22.7%; *P* = .0018; Table V). Although it also appeared that this combination of polymorphisms was associated with a lower level of FEV₁ (%), the difference was found

to be of borderline significance ($P = .06$; Table V). These results suggest that a gene-gene interaction may influence the severity of asthma.

DISCUSSION

Although IL-5 is important in allergic inflammation, no studies have proven the association between IL-5 polymorphism and asthma per se or the clinical parameters of asthma. IL-5 polymorphism was not associated with development of asthma per se in this study. However, FEF_{25-75%} (%) was lower in atopic children with asthma carrying homozygous and heterozygous mutant alleles of the IL-5 gene compared with those carrying the homozygous wild-type allele. These findings suggest a possibility of IL-5 polymorphism being associated with the severity of asthma but not with the development of asthma. Therefore, the current study is the first to show a significant association between IL-5 gene polymorphism and lung function as measured by spirometry in atopic asthma. Our results are supported by findings that IL-5 production in atopic patients with asthma is inversely correlated with several objective measures of asthma severity, such as baseline FEV₁ and peak expiratory flow rate,⁸⁻¹⁰ and that anti-IL-5 treatment improves lung function.^{24,25}

In atopic children with asthma, serum IgE concentration was significantly higher in those with homozygous (CC) and heterozygous (CT) mutant IL-5 alleles compared with those with wild-type (TT) alleles, whereas these allelic effects on IgE were not observed in healthy children. These data are consistent with those showing that IL-5 polymorphism is associated with lung function more in children with atopic asthma than in children with nonatopic asthma. In the future, functional assays for IL-5 gene polymorphisms will be needed.

The possible effects of TBXA2R gene T924C polymorphism on asthma severity or atopy have been rarely investigated. We showed that a variant of the TBXA2R gene was associated with FEV₁ (%) and FEF_{25-75%} (%) in children with atopic asthma, whereas there was no association with the diagnosis of asthma. Also, our data show a gene-dose effect of the mutant allele (C) on FEV₁ (%) and FEF_{25-75%} (%) in atopic children with asthma (data not shown). These findings suggest that this variant may play a role as a disease-modifying gene in Korean children with atopic asthma. However, because the associated polymorphism is a synonymous substitution, it is unlikely to influence the function of the receptor, although it could possibly affect the efficiency of its transcription or translation. Our results may be supported by findings that TBXA2R antagonists reduce inflammation, eosinophil infiltration, chemokine expression, and bronchial hyperresponsiveness in patients with asthma.²⁶⁻²⁸ However, the data presented in the current study differ from recent studies that have revealed an association between the TBXA2R (T924C) polymorphism and diagnosis of asthma in adults¹⁹ and children.²⁰ The calculated power from this study was not significantly strong

because the differences of allele and genotype frequencies between the study groups were very low. Therefore, we could not rule out the possibility of this result being false-negative and of the existence of an additional polymorphism related to asthma susceptibility in Korean children. Until recently, the allele frequency of the TBXA2R gene has rarely been studied in populations, especially in white subjects. Further studies are required for a better understanding of the association between the TBXA2R gene polymorphism and asthma.

In healthy control subjects, we found that the eosinophil fraction (%) and total serum IgE concentrations were higher in those with 1 or 2 copies of the TBXA2R mutant allele compared with those with 2 wild-type alleles, whereas this was not found in atopic subjects with asthma. This polymorphism may be associated with the Cat-specific IgE level in patients with the mutant allele,²⁰ which also suggests that this TBXA2R gene may be associated with atopy. Even though the controls of this study are not atopic, they may have some capacity to produce high IgE and high eosinophils. If this TBXA2R polymorphism has some minor influence on T_H2 tendency or atopy, it might be possible to detect a difference in patients with low IgE or low eosinophils. However, in this study, the eosinophil fraction (%) and IgE levels in the atopic children with asthma were probably too high to differentiate. Therefore, further studies will be needed to confirm its association with atopy or to understand the function of this single nucleotide polymorphism (SNP).

In the current study, we showed that both the IL-5 and the TBXA2R polymorphisms were associated with decreased lung function in atopic subjects with asthma. These findings prompted us to investigate a possible gene-gene interaction that may affect asthma severity in terms of combinations of IL-5 and TBXA2R polymorphisms. We identified a significant gene-gene interaction effect on pulmonary function in that FEF_{25-75%} (%) was affected, particularly in children with atopic asthma carrying the combination of mutant alleles for both IL-5 and TBXA2R. These findings suggest the presence of an interaction that may influence the severity of asthma in Korean children with atopic asthma, and suggest that there may be a genetic and functional interaction between IL-5 and TBXA2R genes. A possibility remains open that variants in some other functional polymorphism could be tightly linked to this tested polymorphism, or that some nearby genes could influence the severity of asthma. Further SNP screening of both genes will be necessary to detect true functional variants. To our knowledge, this study is the first report that shows a potential gene-gene interaction between IL-5 polymorphisms and TBXA2R polymorphisms. Recent studies have demonstrated interactive genetic and functional effects between SNP in IL-4 receptor and the IL-13 gene^{29,30} or IL-4 gene.³¹ The combined effect on function may underlie the gene-gene interaction that contributes to risk and clinical phenotype of asthma. Gene-environment interactions also play a critical role in the pathogenesis of asthma.³² The functional significance of gene-gene interaction and gene-environment interaction in combina-

tion for the risk and clinical phenotypes of asthma will need to be investigated.

In summary, the current study indicates that IL-5 and TBXA2R polymorphisms may play a role in the pathogenesis of asthma. In addition, the study provides evidence of an interaction between IL-5 and TBXA2R polymorphisms that contributes to the severity of asthma. These results suggest that IL-5 and TBXA2R genes may have roles as disease-modifying genes in Korean children with atopic asthma.

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