

ORIGINAL PAPER

Combined effect of tumour necrosis factor- α and interleukin-13 polymorphisms on bronchial hyperresponsiveness in Korean children with asthma

H-B. Kim*, M-J. Kang[†], S-Y. Lee[‡], H-S. Jin[§], J-H. Kim[§], B-S. Kim[§], S-O. Jang[†], Y-C. Lee[¶], M-H. Sohn^{||}, K-E. Kim^{||} and S-J. Hong[§]

*Department of Pediatrics, Inje University Sanggye Paik Hospital, Seoul, Korea, [†]Asan Institute for Life Sciences, University of Ulsan College of Medicine, Seoul, Korea,

[‡]Department of Pediatrics, Hangeul Sacred Heart Hospital, Hallym University College of Medicine, Seoul, Korea, [§]Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, [¶]Department of Internal Medicine and Research Center for Allergic Immune Diseases, Chonbuk National University Medical School, Jeonju, Korea, and ^{||}Department of Pediatrics and Institute of Allergy, Severance Children's Hospital, Yonsei University College of Medicine, Seoul, Korea

Clinical and Experimental Allergy

Summary

Background TNF- α and IL-13, two pivotal pro-inflammatory cytokines, are increased in asthmatic airways and may be linked to asthma susceptibility and/or bronchial hyperresponsiveness (BHR).

Objective We investigated the association between the TNF- α -308G/A polymorphism and asthma susceptibility or asthma-related phenotypes in Korean children with asthma, and tested for a combined effect with IL-13 polymorphisms.

Methods Asthmatic children ($n = 719$) and non-atopic healthy control children ($n = 243$) were evaluated for asthma phenotypes including total serum IgE and BHR to methacholine. Genotypes were determined by PCR-restriction fragment length polymorphism analysis.

Results The allele frequency of TNF- α -308A in asthmatics (14.1%) was higher than that in control children [8.7%, odds ratio (OR) 1.72, 95% confidence interval (CI) 1.05–2.82].

Significantly lower PC₂₀ values were found in asthmatic children carrying one or two copies of the TNF- α risk allele (-308A) vs. those homozygous for the common allele ($P = 0.026$). Combined analysis revealed that atopic asthmatic children co-inherited the risk alleles of TNF- α -308G/A and IL-13 +2044G/A more frequently than control children (aOR 1.91, 95% CI 1.00–3.65), and asthmatic children co-inheriting both risk alleles had significantly lower PC₂₀ values vs. asthmatic children homozygous for the common alleles ($P = 0.024$).

Conclusion The TNF- α promoter polymorphism (-308G/A) may be associated with asthma susceptibility and BHR in Korean children with asthma. In addition, there appears to be a synergistic effect between the TNF- α promoter polymorphism and an IL-13 coding region polymorphism in terms of asthma susceptibility and BHR in this population.

Keywords asthma, bronchial hyperresponsiveness, combined effect, IL-13, TNF- α

Submitted 7 June 2007; revised 28 November 2007; accepted 10 January 2008

Correspondence:

Soo-Jong Hong, Department of Pediatrics, Division of Allergy and Respiratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, 388-1 Poongnap-dong Songpa-gu, Seoul 138-736, Korea.
E-mail: sjhong@amc.seoul.kr

Introduction

Asthma is a complex inflammatory disease involving the critical actions of several important cytokines. One such cytokine is TNF, a pro-inflammatory cytokine that is found in airways and is known to induce inflammatory responses and regulate immunity [1, 2]. On asthma pathogenesis, TNF- α has important biological effects on airway inflammation and remodelling, and plays a pivotal role in the induction and potentiation of airway hyperrespon-

siveness (AHR) [3–6]; thus, TNF- α can be suggested as a potential therapeutic target for treatment of symptomatic corticosteroid-dependent asthma [7].

Several T-helper type 2 (Th2) cytokines also play pivotal roles in the pathogenesis of allergic diseases. Among them, IL-13, which is a component of the IL-4 receptor signalling cascade essential for development of allergic diseases, has been identified as a critical effector in the induction and maintenance of IgE production and allergic airway responses [8]. Several studies have

suggested that IL-13 can induce AHR via direct effects on resident airway cells, such as airway smooth muscle or epithelium [9], and that this effect may be seen even in the absence of inflammatory cells [10–12]. However, the exact mechanisms by which IL-13 induces AHR are currently unknown.

Previous genetic studies have yielded inconsistent results with regard to linking single genes with asthma and its related phenotypes, suggesting that the presence of a single SNP in only one gene may not be sufficient to change asthma susceptibility or bronchial hyperresponsiveness (BHR). This indicates that it will be necessary to investigate interactions between genes in the complex immunologic responses underlying asthma. *TNF- α* and IL-13 are important cytokines working in asthma; therefore, herein, we investigated whether there is an association between the *TNF- α* (–308G/A) promoter polymorphism and its possible combined effects with three *IL-13* polymorphisms (–1512A/C, –1112C/T, +2044G/A) with regard to asthma susceptibility and BHR in Korean children with and without asthma.

Methods

Subjects

The enrolled study subjects consisted of 962 Korean children who visited the Asthma and Allergy Clinics of the Asan Medical Center and the Severance Children's Hospital, Seoul, Korea. The enrollees were classified as asthmatic ($n = 719$; 589 with atopic asthma) or normal controls ($n = 243$), and were adjusted according to age and sex (Table 1). Asthma phenotypes and BHR were physician diagnosed using the American Thoracic Society guidelines [13]. Asthma was diagnosed based on a history of dyspnea and wheezing during the previous 12 months, a $>12\%$ reversal of forced expiratory volume in 1 s (FEV₁) spontaneously or after β_2 -agonist inhalation, and/or a methacholine provocation test result with a PC₂₀ value of <16 mg/mL. Atopy was defined by a positive skin prick test (SPT) or by elevated allergen-specific IgE. The control subjects had no history of asthma or other

allergic diseases, negative SPTs, normal total IgE values (≤ 100 IU/mL), normal lung function tests, and no AHR (PC₂₀ >16 mg/mL). The Ethics Committees of the Asan Medical Center Institutional Review Board and the Severance Hospital Institutional Review Board approved the study, and written informed consents were obtained from the parents of all study subjects.

Serum immunoglobulin E and skin prick tests

Total serum IgE concentrations were measured by a fluorescent enzyme immunoassay using the AutoCAP System (Phadia AB, Uppsala, Sweden). SPTs were performed with 27 common Korean allergens (Allergopharma, Reinbek, Germany) and was considered to be positive if the maximum weal diameter was ≥ 3 mm.

Methacholine challenge test

The methacholine challenge test was performed as described previously [14], using a dosimeter with a concentration range of 0.625–25 mg/mL. A spirometer (Microspiro HI298, Tokyo, Japan) was used to measure FEV₁ after each inhalation. Airway responsiveness was expressed as the concentration of methacholine required to cause a 20% decline in FEV₁ (PC₂₀), and AHR to methacholine was defined as PC₂₀ <16 mg/mL.

Genotyping of the tumour necrosis factor- α and interleukin-13 polymorphisms

DNA was isolated from blood samples using standard procedures, including sodium dodecyl sulphate (SDS)-proteinase K-RNase digestion and phenol–chloroform extraction. *TNF- α* and *IL-13* polymorphisms were analysed by PCR, combined with restriction fragment length polymorphism (RFLP) analysis. Fragments were amplified in 15 μ L reaction mixtures containing 20 ng genomic DNA, 0.033 mm of each dNTP, 1 $\times$ PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.25 mM MgCl₂), 5 pmol of each primer, and 0.25 units AmpliTaq Gold Taq polymerase (Applied Biosystems, Foster City, CA, USA). Amplification reactions were performed using a GeneAmp PCR System 9700 (Applied Biosystems, Norwalk, CT, USA). The thermocycling conditions consisted of an initial denaturation at 95 °C for 12 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing for 2 min at 60 °C for *TNF- α* , 65 °C for *IL-13* –1512A/C, 54 °C for *IL-13* –1112C/T, and 55 °C for *IL-13* +2044G/A, and extension at 72 °C for 40 s followed by a final extension step at 72 °C for 5 min. Proper amplification was confirmed by 1.5–2.5% agarose gel electrophoresis of a 10 μ L aliquot from each reaction.

The utilized primers, restriction enzymes, and expected products are as follows: for *TNF- α* –308G/A, the primers

Table 1. Clinical characteristics of study subjects

	Control ($n = 243$)	Asthma ($n = 719$)	Atopic asthma ($n = 589$)
Clinical phenotypes*			
Sex (M/F)	132/111	473/246	395/194
Mean age (years)	10.20 $\pm$ 2.68	9.13 $\pm$ 2.65	9.30 $\pm$ 2.64
Log total IgE (IU/mL)	1.39 $\pm$ 0.09	2.32 $\pm$ 0.59	2.47 $\pm$ 0.49
Log PC ₂₀ (mg/mL)	1.52 $\pm$ 0.51	0.38 $\pm$ 0.52	0.34 $\pm$ 0.51

*Mean $\pm$ standard deviation.

M, male; F, female.

were 5'-AGG CAA TAG GTT TTG AGG GCC AT-3' and 5'-TCC TCC CTG CTC CGA TTC CG-3', and the restriction enzyme was *NcoI* (New England BioLabs, Beverly, MA, USA); the -308G allele yielded a single 107 bp fragment and the -308A allele yielded 87 and 20 bp fragments [15]. For *IL-13* -1512A/C, the primers were 5'-CAA CCG CCG CGC CAG CGC CTT CTC-3' and 5'-CCG CTA CTT GGC CGT GTG ACC GC-3', and the restriction enzyme was *BstUI* (New England BioLabs); the -1512A allele yielded 216 and 29 bp fragments and the -1512C allele yielded 194, 29, and 22 bp fragments [16]. For *IL-13* -1112C/T, the primers were 5'-GGA ATC CAG CAT GCC TTG TGA GG-3' and 5'-GTC GCC TTT TCC TGC TCT TCC CGC-3', and the restriction enzyme was *BstUI*; the -1112C allele yielded 223 and 23 bp fragments and the -1112T allele yielded a single 246 bp band [16]. For *IL-13* +2044G/A, the primers were 5'-CTT CCG TGA GGA CTG AAT GAG ACG GTC-3' and 5'-GCA AAT AAT GAT GCT TTC GAA GTT TCA GTG GA-3', and the restriction enzyme was *Nla IV* (New England BioLabs); the +2044G allele yielded 178 and 32 bp fragments and the +2044A allele yielded a single 210 bp band [16]. Each PCR product was digested with 5 U of the listed restriction enzyme at 37 °C for 4 h, and the reactions were separated on 3% agarose gels (FMC BioProducts, Rockland, ME, USA). To confirm the accuracy of our RFLP analyses, we randomly selected 20% of the subjects for DNA sequence analysis of the tested polymorphisms. In each case, the DNA sequencing and RFLP data were consistent with one another.

Statistical analysis

Clinical parameters (e.g. total IgE and PC₂₀) were analysed as quantitative traits and log₁₀ transformed to produce a normal distribution for statistical analysis. Differences between groups were analysed by the Kruskal-Wallis test. Quality variables (e.g. sex and age) were analysed by multiple logistic regression to determine whether there was a disproportionate presence of a given allele among affected individuals. To analyse associations between genotypes and asthma, especially atopic asthma, we assumed a dominant model because only a small proportion of individuals were homozygous for the risk alleles. Unconditional logistic regression analysis adjusted for age and sex was used to calculate odds ratios (ORs), their 95% confidence intervals (CIs), and *P*-values. The Kruskal-Wallis test was used to determine the significance of clinical phenotypes (IgE or PC₂₀) according to genotype and the combined effect of the *TNF- α* and *IL-13* polymorphisms. Bonferroni's correction was applied to make the association more appropriate for the combined effect. All statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Genotype distributions of the tumour necrosis factor- α and interleukin-13 polymorphisms

To investigate possible associations of the *TNF- α* and *IL-13* polymorphisms with asthma susceptibility in Korean children, we first examined the occurrence of these genotypes in asthma and control groups, using subjects homozygous for each common allele as a reference group. In the control group, the allele frequency of the *TNF- α* -308G allele was 0.96 and those of the *IL-13* -1512A, *IL-13* -1112C, and *IL-13* +2044G alleles were 0.71, 0.81, and 0.66, respectively; these frequencies were concordant with Hardy-Weinberg equilibrium analysed by a χ^2 test with 1 degree of freedom (Table 2). In contrast, the *TNF- α* -308A allele showed significant associations with asthma susceptibility (OR = 1.72, 95% CI = 1.05–2.82), and atopic asthma susceptibility (OR = 1.85, 95% CI = 1.12–3.06; Table 2).

Clinical phenotypes of asthmatics harbouring the tumour necrosis factor- α and interleukin-13 risk alleles

Analysis of the association between total IgE levels and the selected *TNF- α* and *IL-13* polymorphisms in Korean children with asthma revealed that total IgE did not differ significantly between children homozygous for the common alleles and those harbouring one or two of the risk alleles for the *TNF- α* and *IL-13* polymorphisms (Table 3). However, analysis of possible associations between BHR and the risk alleles in asthmatic children revealed that the PC₂₀ values obtained by a methacholine challenge test were significantly lower in asthmatics harbouring one or two *TNF- α* risk alleles vs. those homozygous for the common allele (*P* = 0.026, Table 3). This association was not observed for the *IL-13* risk alleles, with asthmatic children harbouring these alleles showing no significant difference from children homozygous for the common allele in terms of PC₂₀ (Table 3).

Association between asthma susceptibility and the co-inheritance of tumour necrosis factor - α and interleukin-13 risk alleles in children with asthma

Because genetic changes do not function in isolation, we next evaluated the effect of co-inheritance of the *TNF- α* and *IL-13* polymorphisms in our study population. Children who co-inherited one or two risk alleles for *TNF- α* -308G/A along with those for *IL-13* -1512A/C or -1112C/T did not show increased susceptibility to asthma or atopic asthma vs. those inheriting risk alleles at only one of these SNPs. In contrast, children co-inheriting one or two risk alleles at both *TNF- α* -308G/A and *IL-13* +2044G/A showed higher susceptibility for

Table 2. Allele frequencies of the *TNF- α* –308G/A and *IL-13* –1512A/C, –1112C/T and +2044G/A polymorphisms in asthmatic children and non-asthmatic controls

	Control	Asthma		Atopic asthma	
Genotype	<i>n</i> = 240 (%)	<i>n</i> = 715 (%)	aOR (95% CI)	<i>n</i> = 585 (%)	aOR (95% CI)
<i>TNF-α -308G/A</i>					
GG	219 (91.3)	614 (85.9)	1.00	497 (85.0)	1.00
GA	21 (8.7)	95 (13.3)	1.61 (0.98–2.65)	83 (14.2)	1.74 (1.05–2.88)
AA	0 (0)	6 (0.8)	–	5 (0.8)	–
GA+AA	21 (8.7)	101 (14.1)	1.72 (1.05–2.82)	88 (15.0)	1.85 (1.12–3.06)
Frequency (G)	0.96	0.93		0.92	
<i>IL-13 -1512 A/C</i>					
AA	119 (49.2)	369 (51.4)	1.00	307 (52.3)	1.00
AC	107 (44.2)	291 (40.6)	0.88 (0.65–1.19)	232 (39.5)	0.84 (0.61–1.15)
CC	16 (6.6)	57 (8.0)	1.15 (0.64–2.08)	48 (8.2)	1.16 (0.63–2.12)
AC+CC	123 (50.8)	348 (48.6)	0.91 (0.68–1.22)	280 (47.7)	0.88 (0.65–1.19)
Frequency (A)	0.71	0.72		0.72	
<i>IL-13 -1112C/T</i>					
CC	155 (64.3)	455 (63.5)	1.00	376 (64.1)	1.00
CT	80 (33.2)	236 (33.0)	1.09 (0.74–1.61)	189 (32.2)	0.97 (0.70–1.34)
TT	6 (2.5)	25 (3.5)	2.07 (0.61–7.02)	22 (3.7)	1.51 (0.60–3.80)
CT+TT	86 (35.7)	261 (36.5)	1.15 (0.79–1.68)	211 (35.9)	1.01 (0.74–1.38)
Frequency (C)	0.81	0.80		0.80	
<i>IL-13 +2044G/A</i>					
GG	99 (43.6)	301 (42.4)	1.00	248 (42.8)	1.00
GA	100 (44.1)	318 (44.9)	1.05 (0.76–1.45)	257 (44.4)	1.03 (0.74–1.43)
AA	28 (12.3)	90 (12.7)	1.06 (0.66–1.71)	74 (12.8)	1.06 (0.65–1.74)
GA+AA	128 (56.4)	408 (57.6)	1.05 (0.78–1.42)	331 (57.2)	1.03 (0.76–1.40)
Frequency (G)	0.66	0.65		0.65	

*Adjusted odds ratio (aOR) by age and sex. Logistic regression analysis was performed.
OR, odds ratio; CI, confidence interval.

Table 3. Association between the *TNF- α* –308G/A and *IL-13* –1512A/C, –1112C/T and +2044G/A polymorphisms and clinical phenotypes in children with asthma

Loci	Log total IgE (IU/mL)	Log PC ₂₀ (mg/mL)
<i>TNF-α</i> –308G/A		
GG (<i>n</i> = 554)	2.31 ± 0.59	0.40 ± 0.53
GA+AA (<i>n</i> = 165)	2.34 ± 0.57	0.27 ± 0.45*
<i>IL-13</i> –1512A/C		
AA (<i>n</i> = 369)	2.31 ± 0.58	0.38 ± 0.49
AC+CC (<i>n</i> = 348)	2.33 ± 0.60	0.37 ± 0.54
<i>IL-13</i> –1112C/T		
CC (<i>n</i> = 455)	2.30 ± 0.58	0.39 ± 0.52
CT+TT (<i>n</i> = 261)	2.35 ± 0.61	0.36 ± 0.52
<i>IL-13</i> +2044G/A		
GG (<i>n</i> = 301)	2.29 ± 0.58	0.41 ± 0.50
GA+AA (<i>n</i> = 408)	2.34 ± 0.60	0.36 ± 0.53

**P* = 0.026.

Data for each genotype are expressed as mean ± standard deviation. The significance of clinical parameters according to genotype was analyzed using the Kruskal–Wallis test.

atopic asthma than those inheriting the risk alleles at only one of these SNPs (aOR = 1.91, 95% CI = 1.00–3.65) (Table 4).

Combined effect of tumour necrosis factor- α and interleukin-13 polymorphisms on bronchial hyperresponsiveness in asthma

Because the *TNF- α* –308G/A risk allele was associated with BHR to metacholine and co-inheritance of risk alleles at both *TNF- α* –308G/A and *IL-13* +2044G/A was associated with susceptibility to atopic asthma, we investigated whether there might be an association between BHR and co-inheritance of the *TNF- α* risk allele along with one of the *IL-13* risk alleles. In children with asthma, those harbouring one or two copies of the *TNF- α* –308A allele along with one or two copies of the *IL-13* +2044A allele had significantly lower PC₂₀ values than those homozygous for the *TNF- α* –308G and *IL-13* +2044G alleles (log PC₂₀, 0.22 ± 0.47 vs 0.42 ± 0.51 mg/mL; *P* = 0.024 after Bonferroni's correction; Table 5). The other *IL-13* polymorphisms showed a similar trend, in that the PC₂₀ values were lower in asthmatic children harbouring one or more risk alleles at both *TNF- α* –308A/G and the *IL-13* polymorphisms (–1512C and –1112T) compared with children homozygous for their common alleles (Table 5), but these trends did not reach statistical significance after Bonferroni's correction.

Table 4. Allele frequencies of combined inheritance of the *TNF- α* – 308G/A and *IL-13* polymorphisms in asthmatic children and non-asthmatic controls

Genotype	Control	Asthma		Atopic asthma	
	<i>n</i> (%)	<i>n</i> (%)	aOR (95% CI)	<i>n</i> (%)	aOR (95% CI)
<i>TNF-α – 308G/A and IL-13 – 1512A/C</i>					
Group I	105 (43.9)	313 (43.9)	1.00	260 (44.6)	1.00
Group II	113 (47.3)	299 (41.9)	0.89 (0.65–1.21)	235 (40.3)	0.84 (0.61–1.16)
Group III	12 (5.0)	53 (7.4)	1.48 (0.76–2.88)	44 (7.6)	1.48 (0.75–2.91)
Group IV	9 (3.8)	48 (6.7)	1.79 (0.85–3.77)	44 (7.6)	1.97 (0.93–4.18)
<i>TNF-α – 308G/A and IL-13 – 1112C/T</i>					
Group I	139 (58.4)	386 (54.2)	1.00	318 (54.6)	1.00
Group II	78 (32.8)	225 (31.6)	1.04 (0.75–1.44)	177 (30.4)	0.99 (0.71–1.38)
Group III	13 (5.5)	65 (9.1)	1.80 (0.96–3.37)	54 (9.3)	1.82 (0.96–3.44)
Group IV	8 (3.4)	36 (5.1)	1.62 (0.39–1.92)	34 (5.8)	1.86 (0.84–4.12)
<i>TNF-α – 308G/A and IL-13 + 2044G/A</i>					
Group I	93 (41.5)	262 (37.2)	1.00	217 (37.7)	1.00
Group II	114 (50.9)	343 (48.7)	1.07 (0.78–1.47)	271 (47.1)	1.02 (0.74–1.41)
Group III	4 (1.8)	37 (5.3)	3.28 (1.14–9.45)	29 (3.1)	3.11 (1.06–9.10)
Group IV	13 (5.8)	63 (8.9)	1.72 (0.90–3.27)	58 (10.9)	1.91 (1.00–3.65)

The groups were defined by the combination of *TNF- α* and *IL-13* polymorphisms.

Patients in Group I had *TNF- α* – 308GG and *IL-13* – 1512AA, – 1112CC, or + 2044GG (used as the reference value).

Patients in Group II had *TNF- α* – 308GG and *IL-13* – 1512(AC+CC), – 1112(CT+TT), or + 2044(GA+AA).

Patients in Group III had *TNF- α* – 308(GA+AA) and *IL-13* – 1512AA, – 1112CC, or + 2044GG.

Patients in Group IV had *TNF- α* – 308(GA+AA) and *IL-13* – 1512(AC+CC), – 1112(CT+TT), or + 2044(GA+AA).

OR, odds ratio; CI, confidence interval.

Table 5. Combined effect of the *TNF- α* – 308G/A and *IL-13* polymorphisms on PC₂₀ levels in children with asthma

Group	<i>TNF-α</i> – 308G/A and <i>IL-13</i> – 1512A/C		<i>TNF-α</i> – 308G/A and <i>IL-13</i> – 1112C/T		<i>TNF-α</i> – 308G/A and <i>IL-13</i> + 2044G/A	
	Number of cases (<i>n</i> = 671)	Log PC ₂₀ (mg/mL) (Mean $\pm$ SD)	Number of cases (<i>n</i> = 670)	Log PC ₂₀ (mg/mL) (Mean $\pm$ SD)	Number of cases (<i>n</i> = 666)	Log PC ₂₀ (mg/mL) (Mean $\pm$ SD)
I	295	0.40 $\pm$ 0.51	359	0.40 $\pm$ 0.54	248	0.42 $\pm$ 0.51
II	280	0.40 $\pm$ 0.55	215	0.39 $\pm$ 0.51	323	0.38 $\pm$ 0.54
III	50	0.31 $\pm$ 0.42	62	0.32 $\pm$ 0.42	34	0.34 $\pm$ 0.42
IV	46	0.22 $\pm$ 0.49	34	0.17 $\pm$ 0.50 [†]	61	0.22 $\pm$ 0.47 [*]

**P* = 0.024 (after Bonferroni's correction compared to group I), [†]*P* = 0.051.

The groups were defined by the combination of *TNF- α* and *IL-13* polymorphisms.

Patients in Group I had *TNF- α* – 308GG and *IL-13* – 1512AA, – 1112CC, or + 2044GG (used as the reference value).

Patients in Group II had *TNF- α* – 308GG and *IL-13* – 1512(AC+CC), – 1112(CT+TT), or + 2044(GA+AA).

Patients in Group III had *TNF- α* – 308(GA+AA) and *IL-13* – 1512AA, – 1112CC, or + 2044GG.

Patients in Group IV had *TNF- α* – 308(GA+AA) and *IL-13* – 1512(AC+CC), – 1112(CT+TT), or + 2044(GA+AA).

In addition, we investigated associations between total IgE levels and co-inheritance of the *TNF- α* – 308A and *IL-13* + 2044A risk alleles in children with asthma, but we did not find any significant difference between children co-inheriting the common and risk alleles (data not shown).

Discussion

Herein we report that the *TNF- α* – 308G/A polymorphism is associated with asthma susceptibility and BHR (PC₂₀) in a population of Korean children. The *TNF- α* gene is

located in the human major histocompatibility complex on chromosome 6p. This region was linked previously to asthma susceptibility [17, 18], and previous genetic studies have identified associations between *TNF- α* gene polymorphisms and BHR [19] and asthma [20]. The *TNF- α* – 308G/A promoter polymorphism is particularly interesting because it has been associated with increased *in vitro* transcription of TNF [21] and elevated plasma TNF levels pre and post-stimulation both *in vivo* and *ex vivo* [22]. Intriguingly, while some studies have reported association of the *TNF- α* – 308A allele with asthma [15, 20, 23–25] or increased BHR [19], other studies found no

association between asthma and this allele [26, 27], or even identified an association between asthma and the wild-type allele [28, 29]. Thus, our present findings and the previous reports show a lack of agreement regarding the association of the *TNF- α* –308A allele with asthma and/or BHR, strongly suggesting that a large, population-based study or a functional study is needed to fully examine whether and how the *TNF- α* –308G/A polymorphism is associated with asthma susceptibility and BHR.

Our present results further show that *IL-13* polymorphisms are involved in the apparent association between the *TNF- α* –308G/A polymorphism and asthma. Although previous reports have shown that genetic variants in the *IL-13* promoter and coding regions were involved in the pathogenesis of asthma and atopy [16, 30–32], we did not find any evidence that *IL-13* polymorphisms were associated with asthma susceptibility or atopy in our study population. These different results may be dependent on the study design and the characteristics of the enrolled subjects, such as ethics and the circumstantial environment. In addition, gene-to-gene interaction and gene-to-environment interaction could be involved in these differences. However, we found that co-inheritance of the risk allele of the *TNF- α* promoter polymorphism along with the risk allele of the *IL-13* +2044G/A coding polymorphism was associated with increased BHR. To begin examining where *TNF- α* and *IL-13* changes affected asthma pathogenesis, we examined whether co-inheritance of the risk alleles for the *TNF- α* –308G/A and *IL-13* gene polymorphisms (–1512C, –1112T and +2044A) was associated with changes in BHR or IgE production. Our results revealed that asthmatic children, especially atopic asthmatic children, co-inherited the *TNF- α* –308A and *IL-13* +2044A risk alleles more frequently than normal controls. These results suggest that the combined effects of the *TNF- α* and *IL-13* risk alleles appear to be associated with susceptibility to asthma and atopic asthma, perhaps suggesting some physiological mechanisms underlying asthma and its related phenotypes.

We further found that the *TNF- α* –308A and *IL-13* +2044A risk alleles appeared to be associated with increased BHR. We showed previously that the *TNF- α* –308G/A polymorphism is in near complete linkage disequilibrium with the tightly linked *TNF- α* –1031T/C, –863C/A, –857C/T, +252A/G and +318G/C polymorphisms in a Korean population [28]. Because only the –308G/A polymorphism showed a significant association with decreased atopy in our previous study [28], we chose to examine this polymorphism in the present study. However, we did not specifically confirm the haplotypes of the *TNF- α* gene and the *TNF- α* -related genes (e.g. lymphotoxin- α and MHC) in our population of asthmatic and non-asthmatic Korean children. Thus, we

cannot rule out the possibility that the tested polymorphism may be in linkage disequilibrium with others in the study population. In addition, our previous study in a Korean adult population showed that the *TNF* –308G/A polymorphism was associated with decreased atopy among asthmatics [28], whereas the present study found no association with atopy in Korean children. Even though our study has some limitations, this study has some positive findings and some strengths such as a large group of children with asthma.

In sum, herein we report a large-scale study of the *TNF- α* –308G/A and *IL-13* (–1512A/C, –1112C/T, and +2044G/A) polymorphisms in Korean children with asthma. Our results revealed that the *TNF* –308G/A polymorphism was associated with asthma susceptibility and BHR. In addition, we identified a synergistic effect between the *TNF- α* –308G/A and *IL-13* +2044G/A polymorphisms in terms of BHR. These findings suggest that there might be a significant synergistic gene-gene interaction between *TNF- α* and *IL-13* polymorphisms, indicating that these genes may co-mediate asthma susceptibility and BHR in Korean children with asthma.

Acknowledgements

We would like to thank all the study participants, as well as Hee Jeong Seo (Nurse, Pediatric Asthma and Allergy Clinic, Asan Medical Center, Seoul, Korea) for her assistance with patient recruitment.

This work was supported by a grant from the Asan Institute for Life Science (2006–091) and by Korea Research Foundation Grants funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2005-201-E00014).

Clinical impact: There appears to be a synergistic effect between the *TNF- α* promoter polymorphism and an *IL-13* coding region polymorphism in terms of asthma susceptibility and BHR in this population.

References

- 1 Wajant H, Henkler F, Scheurich P. The *TNF*-receptor-associated factor family: scaffold molecules for cytokine receptors, kinases and their regulators. *Cell Signal* 2001; 13:389–400.
- 2 Aggarwal BB. Signalling pathways of the *TNF* superfamily: a double-edged sword. *Nat Rev Immunol* 2003; 3:745–56.
- 3 Amrani Y, Chen H, Panettieri RA Jr. Activation of tumor necrosis factor receptor 1 in airway smooth muscle: a potential pathway that modulates bronchial hyper-responsiveness in asthma? *Respir Res* 2000; 1:49–53.
- 4 Kips JC, Tavernier J, Pauwels RA. Tumor necrosis factor causes bronchial hyper-responsiveness in rats. *Am Rev Respir Dis* 1992; 145:332–6.
- 5 Thomas PS, Yates DH, Barnes PJ. Tumor necrosis factor alpha increases airway responsiveness and sputum neutrophilia in

- normal human subjects. *Am J Respir Crit Care Med* 1995; 152:76–80.
- 6 Thomas PS, Heywood G. Effects of inhaled tumor necrosis factor alpha in subjects with mild asthma. *Thorax* 2002; 57:774–8.
 - 7 Howarth PH, Babu KS, Arshad HS *et al.* Tumor necrosis factor (TNF- α) as a novel therapeutic target in symptomatic corticosteroid-dependent asthma. *Thorax* 2005; 60:1012–8.
 - 8 Wills-Karp M, Chiaramonte M. Interleukin-13 in asthma. *Curr Opin Pulm Med* 2003; 9:21–7.
 - 9 Kuperman DA, Huang X, Koth LL *et al.* Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat Med* 2002; 8:885–9.
 - 10 Wills-Karp M, Luyimbazi J, Xu X *et al.* Interleukin-13: central mediator of allergic asthma. *Science* 1998; 282:2258–61.
 - 11 Grunig G, Warnock M, Wakil AE *et al.* Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998; 282:2261–2.
 - 12 Venkayya R, Lam M, Willkom M, Grunig G, Corry DB, Erle DJ. The Th2 lymphocyte products IL-4 and IL-13 rapidly induce airway hyperresponsiveness through direct effects on resident airway cells. *Am J Respir Cell Mol Biol* 2002; 26:202–8.
 - 13 American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987; 136:225–44.
 - 14 Chai H, Farr RS, Froehlich LA *et al.* Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975; 56:323–7.
 - 15 Wang TN, Chen WY, Wang TH, Chen CJ, Huang LY, Ko YC. Gene–gene synergistic effect on atopic asthma: tumour necrosis factor- α -308 and lymphotoxin- α -Ncol in Taiwan's children. *Clin Exp Allergy* 2004; 34:184–8.
 - 16 Graves PE, Kabesch M, Halonen M *et al.* A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000; 105:506–13.
 - 17 Daniels SE, Bhattacharrya S, James A *et al.* A genome-wide search for quantitative trait loci underlying asthma. *Nature* 1996; 383:247–50.
 - 18 Xu J, Meyers DA, Ober C *et al.* Genomewide screen and identification of gene–gene interactions for asthma-susceptibility loci in three US populations: collaborative study on the genetics of asthma. *Am J Hum Genet* 2001; 68:1437–46.
 - 19 Li Kam Wa TC, Mansur AH, Britton J *et al.* Association between –308 tumour necrosis factor promoter polymorphism and bronchial hyperreactivity in asthma. *Clin Exp Allergy* 1999; 29:1204–8.
 - 20 Noguchi E, Yokouchi Y, Shibasaki M *et al.* Association between TNFA polymorphism and the development of asthma in the Japanese population. *Am J Respir Crit Care Med* 2002; 166:43–6.
 - 21 Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA* 1997; 94:3195–9.
 - 22 Louis E, Franchimont D, Piron A *et al.* Tumor necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin Exp Immunol* 1998; 113:401–6.
 - 23 Moffatt MF, Cookson WO. Tumour necrosis factor haplotypes and asthma. *Hum Mol Genet* 1997; 6:551–4.
 - 24 Moffatt MF, James A, Ryan G, Musk AW, Cookson WO. Extended tumour necrosis factor/HLA-DR haplotypes and asthma in an Australian population sample. *Thorax* 1999; 54:757–61.
 - 25 Witte JS, Palmer LJ, O'Connor RD, Hopkins PJ, Hall JM. Relation between tumor necrosis factor polymorphism TNF alpha -308 and risk of asthma. *Eur J Hum Genet* 2002; 10: 82–5.
 - 26 Buckova D, Holla LI, Vasku A, Znojil V, Vacha J. Lack of association between atopic asthma and the tumor necrosis factor alpha-308 gene polymorphism in Czech population. *J Investig Allerg Clin Immunol* 2002; 12:192–7.
 - 27 El Bahlawan L, Christensen M, Binaei S, Murphy C, Zhang Q, Quasney M. Lack of association between the tumor necrosis factor-alpha regulatory region genetic polymorphisms associated with elevated tumor necrosis factor-alpha levels and children with asthma. *Chest* 2003; 123:374S–5S.
 - 28 Shin HD, Park BL, Kim LH *et al.* Association of tumor necrosis factor polymorphisms with asthma and serum total IgE. *Hum Mol Genet* 2004; 13:397–403.
 - 29 Randolph AG, Lange C, Silverman EK, Lazarus R, Weiss ST. Extended haplotype in the TNF gene cluster is associated with asthma and asthma related phenotypes. *Am J Respir Crit Care Med* 2005; 172:687–92.
 - 30 Howard TD, Whittaker PA, Zaiman AL *et al.* Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch population. *Am J Respir Cell Mol Biol* 2001; 25:377–84.
 - 31 Heinzmann A, Mao XQ, Akaiwa M *et al.* Genetic variants of IL-13 signaling and human asthma and atopy. *Hum Mol Genet* 2000; 9:549–59.
 - 32 Kim HB, Lee YC, Lee SY *et al.* Gene–gene interaction between IL-13 and IL-13R α 1 is associated with total IgE in Korean children with atopic asthma. *J Hum Genet* 2006; 51:1055–62.