Gene–gene interaction between IL-13 and IL-13R α 1 is associated with total IgE in Korean children with atopic asthma

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Received: 13 July 2006 / Accepted: 24 August 2006 / Published online: 28 September 2006
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Abstract Interleukin (IL)-13, which is essential for IgE synthesis, mediates its effects by binding with a receptor composed of IL-4R α and IL-13R α 1. We investigated the effects of IL-13 and IL-13R α 1 polymorphisms in Korean children with asthma, and whether these have been associated with IgE production. We enrolled 358 atopic asthmatic, 111 non-atopic asthmatic, and 146 non-atopic healthy children. IL-13 and IL-13R α 1 genotypes were identified using the PCR-RFLP method. There was an association between

the asthma susceptibility and homozygosity for risk allele of IL-13 G+2044A. In children with atopic asthma, risk alleles in IL-13 (A–1512C and C–1112T) and IL-13R α 1 (A+1398G) showed increased total IgE ($P=0.012$, 0.015 and 0.017 , respectively). Three-loci haplotype analysis for IL-13 showed that the haplotype composed of –1512C, –1112T and +2044A was associated with higher total IgE than other tested haplotypes in children with atopic asthma ($P=0.003$). The gene–gene interaction between risk alleles of each IL-13 promoter polymorphism and IL-13R α 1 polymorphism was associated with higher total IgE in children with atopic asthma ($P=0.002$, 0.010). These findings indicate that the IL-13 G+2044A is associated with asthma development and the IL-13 and IL-13R α 1 polymorphisms may interact to enhance IgE production.

Hyo-Bin Kim, and Yong-Chul Lee contributed equally to this work. This work was supported by grants from the Asan Institute for Life Science (2005-091) and the National Research Laboratory Program, Korea Science and Engineering Foundation and by Korea Research Foundation Grants funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2005-201-E00014), Republic of Korea.

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Keywords Interleukin-13 · Interleukin-13 receptor alpha 1 · Asthma · IgE · Gene–gene interaction · Haplotype

Abbreviations

IL-13	Interleukin-13
IL-4R α	Interleukin-4 receptor alpha
IL-13R α 1	Interleukin-13 receptor alpha 1
IgE	Immunoglobulin E
STAT	Signal transducer and activator of transcription
OR	Odds ratio
CI	Confidence interval
Th1	T helper lymphocytes type 1
Th2	T helper lymphocytes type 2
LD	Linkage disequilibrium

Introduction

Atopy, a genetically determined hypersensitivity to common environmental allergens, is associated with increased generation of IgE (Kay 2001). Asthma is associated with atopy and with IgE-mediated inflammation of the airways, which occurs via production of interleukin (IL)-4 and IL-13, both of which have been implicated in the pathogenesis of asthma in multiple human and animal studies (Rankin et al. 1996; Zhu et al. 1999; Nakamura et al. 1996; Doucet et al. 1998).

IL-13 has been noted as a critical effector in the induction and maintenance of IgE production and IgE-mediated allergic airway responses (van der Pouw Kraan et al. 1998; Wills-Karp and Chiaramonte 2003; Cohn et al. 2004). However, analyses of the association between asthma phenotype and specific polymorphisms in the IL-13 gene have produced varied results. In a Dutch population, significant associations were reported between the IL-13/-1112T allele and asthma (Howard et al. 2001; van der Pouw Kraan et al. 1999), as well as bronchial hyperreactivity (BHR) (Howard et al. 2001), skin test responsiveness (Howard et al. 2001), altered regulation of IL-13 production (van der Pouw Kraan et al. 1999), and increased binding of nuclear proteins to this region (van der Pouw Kraan et al. 1999). In German populations, the IL-13/+2044A (Arg130Gln) variant showed a significant association with high IgE levels (Graves et al. 2000), whereas this allele was found to be associated with asthma, but not IgE levels in British and Japanese populations (Heinzmann et al. 2000). Thus, IL-13 polymorphisms may have varied and population-specific effects.

IL-13 mediates its effect via a receptor composed of a heterodimer that includes IL-4R α and IL-13R α 1

(Miloux et al. 1997). IL-13R α 1 is widely expressed and has been identified on nearly every cell, except human T cells (Graber et al. 1998; Akaiwa et al. 2001). The gene encoding IL-13R α 1 has been mapped to the X chromosome, and in vitro expression studies of IL-13R α 1 revealed specific binding to IL-13 (Miloux et al. 1997; Hilton et al. 1996; Guo et al. 1997). By itself, IL-13R α 1 binds IL-13 with low affinity; when paired with IL-4R α , the heterodimer binds IL-13 with high affinity and forms a functional IL-13 receptor that is capable of signal transduction (Miloux et al. 1997). Polymorphisms in these receptor molecules contribute to the genetic influences on asthma susceptibility and atopy. For example, asthma susceptibility was significantly increased in Korean children by gene–gene interaction between IL-4 T-590C and IL-4R α Gln551Arg alleles (Lee et al. 2004) and between IL-13 C-1112T and IL-4R α Ser478Pro alleles in Dutch population (Howard et al. 2002). Furthermore, a noncoding polymorphism in IL-13R α 1 (A+1398G) was associated with increased IgE levels in British population (Heinzmann et al. 2000). These findings highlight the importance of studying gene–gene interactions in the complex biological pathways of IL-4/IL-13 and their receptors. However, no previous study has examined gene–gene interactions involving IL-13 and IL-13R α 1. Here, we examined the polymorphisms in IL-13 and IL-13R α 1, and assessed their independent and combined effects on the development of asthma or the elevation of total serum IgE in the Korean population.

Materials and methods

Subjects

The enrolled study subjects consisted of 615 Korean children who visited the Asthma and Allergy Clinics of the Asan Medical Center. They were classified into three groups (358 atopic asthmatics, 111 nonatopic

Table 1 Clinical profiles of study subjects

Clinical parameters ^a	Control	Atopic asthma	Non-atopic asthma
Number of subjects	146	358	111
Male/female	79/67	232/126	69/42
Age (years)	10.0 $\pm$ 2.6	9.8 $\pm$ 2.3	8.6 $\pm$ 2.5
Log total IgE (IU/ml)*	1.48 $\pm$ 0.50	2.50 $\pm$ 0.45	1.66 $\pm$ 0.50
Log PC ₂₀ (mg/ml)*	1.38 $\pm$ 0.12	0.33 $\pm$ 0.44	0.51 $\pm$ 0.54

^a Mean $\pm$ standard deviation

* $P < 0.01$ for difference among the three groups according to ANOVA

asthmatics, and 146 nonatopic controls without asthma) and adjusted according to age and sex (Table 1).

Asthma phenotypes and bronchial responsiveness were determined by a physician. Following the American Thoracic Society guidelines (American Thoracic Society 1987), asthma was confirmed by a history of dyspnea and wheezing during the previous 12 months, a greater than 12% reversibility of FEV₁ spontaneously or after β_2 -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 test (wheal diameter ≥ 3 mm) to at least one of 27 common aeroallergens in Korea (Lee et al. 2004) or by elevated allergen-specific IgE. Non-atopy was defined by negative specific IgE in response to *Dermatophagoides farinae* (*D.f.*) and *Dermatophagoides pteronyssinus* (*D.p.*), and negative skin prick test to the 27 common aeroallergens. The nonatopic control subjects had no history of asthma or other allergic diseases, negative skin prick tests, normal total IgE values (≤ 100 IU/ml), normal lung function tests, and no airway hyperresponsiveness (PC₂₀ > 16 mg/ml). The ethics committee of the Asan Medical Center Institutional Review Board approved the study, and written informed consents were obtained from the parents of all subjects.

Serum IgE, skin prick test and methacholine challenge test

The concentrations of total IgE and *D.f.*- and *D.p.*-specific IgE were measured by fluorescent enzyme immunoassay (AutoCAP System; Phadia, Uppsala, Sweden). Specific IgE was graded into classes from 1 to 6, according to the manufacturer's instructions, with a specific IgE concentration ≥ 0.35 kIU/l scored as positive.

Skin prick testing was performed with 27 common aeroallergens (Allegopharma, Germany) in Korea (Lee et al. 2004), which were considered positive if the maximum wheal diameter was ≥ 3 mm.

For the methacholine challenge test, we used a dosimeter with a concentration range of 0.625–25 mg/ml, as previously described (Chai et al. 1975). FEV₁ was measured after each inhalation with a spirometer (Microspiro HI298; Tokyo, Japan). Airway responsiveness was expressed as the concentration of methacholine that provoked a 20% fall in FEV₁ (PC₂₀), and airway hyperresponsiveness to methacholine was defined as PC₂₀ < 16 mg/ml.

Genotyping of IL-13 and IL-13R $\alpha 1$ polymorphisms

IL-13 and IL-13R $\alpha 1$ polymorphisms were analyzed by polymerase chain reaction (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, Calif.). 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 65°C for IL-13 –1512A/C, 54°C for IL-13 –1112C/T, 55°C for IL-13 +2044G/A or 45°C for IL-13R $\alpha 1$ +1398A/G, and extension at 72°C for 40 s, followed by a final extension step at 72°C for 5 min, using a Gene-Amp PCR System 9700 (Applied Biosystems, Norwalk, Conn.).

The tested polymorphisms of IL-13 (Graves et al. 2000) and IL-13R $\alpha 1$ (Heinzmann et al. 2000) were amplified with gene-specific PCR primers and digested with specific restriction endonucleases. For –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 *Bst*UI (New England BioLabs, Beverly, Mass., USA), which digested the –1512A allele into 216 and 29 bp fragments and the –1512C allele into 194, 29, and 22 bp fragments. For –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 *Bst*UI, which digested the –1112C into 223 and 23 bp fragments and yielded a single 246 bp band for the –1112T allele. For +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), which digested the +2044G into 178 and 32 bp fragments and yielded a single 210 bp band for the +2044A allele. For +1398A/G, the primers were 5'-TCA GTG ATG GAG ATA ATT TA-3' and 5'-TGA GCT GCC TGT TTA TAA AT-3', and the restriction enzyme was *Mse*I (New England BioLabs), which digested the +1398A allele into 85 and 45 bp fragments and yielded a single 130 bp band for the +1398G allele. The PCR products were digested with 5 U of the listed restriction enzyme at 37°C for 4 h, and separated on 3% agarose gels (FMC BioProducts, Rockland, Me., USA). To confirm the accuracy of these RFLP analyses, 20% of the subjects were randomly selected for DNA sequencing.

Statistical analysis

Clinical phenotypes, including total IgE and PC₂₀, were analyzed as a quantitative trait. Differences of clinical

phenotypes between groups were analyzed by the Kruskal–Wallis and Mann–Whitney tests. Quality variables, including sex and age, were analyzed by multiple logistic regression to determine whether there was a disproportionate presence of a given allele in affected individuals. To analyze associations between genotypes and atopic or non-atopic asthma, a dominant model was assumed because there were only small proportions of homozygotes 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. For the three-loci haplotype frequency analyses between the case and control groups, we used an Expectation–Maximization algorithm to model the combined IL-13 polymorphisms of each patient. Five haplotypes (ACG, CTA, ACA, CCG and CTG) were predicted, and accounted for 99% of all those seen; the other options, having frequencies smaller than 1%, were excluded because of possible genotyping errors. Chi-square tests were used to determine the difference of clinical phenotypes between the IL-13 risk haplotype (C-T-A) and the other tested haplotypes. Chi-square tests were used to determine the association among total IgE levels and the gene–gene interaction between IL-13 and IL-13R α 1 polymorphisms. Bonferroni cor-

rection was also applied to make the association more appropriate with the gene–gene interaction. All statistical analyses were performed using SAS program (Version 8e; SAS Institute, Cary, N.C.).

Results

Genotype distributions of the IL-13 and IL-13R α 1 polymorphisms

Medline searches allowed us to identify asthma development- or asthma-related phenotype-associated polymorphisms in the promoter and coding regions of the IL-13 gene on chromosome 5q31 (Howard et al. 2001; van der Pouw Kraan et al. 1999; Graves et al. 2000; Heinzmann et al. 2000; Vercelli et al. 2005). The –1512A, –1112C and +2044G IL-13 polymorphisms in control groups had allele frequencies of 0.70, 0.81 and 0.67, respectively, and were concordant with Hardy–Weinberg equilibrium (Table 2).

Only the A+1398G polymorphism in the noncoding region of IL-13R α 1 gene was relatively common (Heinzmann et al. 2000; Guo et al. 1997), with the +1398A allele having a frequency of 0.51 in control group (Table 2). Since male subjects are hemizygous at

Table 2 Allele frequencies of the IL-13 A–1512, C–1112T, and G+2044A and IL-13R α 1 A+1398G polymorphisms in asthmatic, atopic asthmatic and control subjects

Genotype	Control <i>n</i> =146 (%)	Asthma		Atopic asthma	
		<i>n</i> =469 (%)	aOR (95% CI)	<i>n</i> =358 (%)	aOR (95% CI)
IL-13 A–1512C					
AA	68 (46.9)	245 (52.2)	1.00	190 (53.1)	1.00
AC	66 (45.5)	189 (40.3)	0.79 (0.54–1.16)	140 (39.1)	0.76 (0.51–1.14)
CC	11 (7.6)	35 (7.5)	0.88 (0.42–1.82)	28 (7.8)	0.91 (0.43–1.93)
AC+CC	77 (53.1)	244 (47.8)	0.81 (0.56–1.18)	168 (47.9)	0.78 (0.53–1.15)
Frequency (A)	0.70	0.72		0.73	
IL-13 C–1112T					
CC	94 (64.4)	292 (63.1)	1.00	223 (63.4)	1.00
CT	49 (33.6)	160 (34.5)	1.05 (0.71–1.56)	121 (34.4)	1.04 (0.69–1.54)
TT	3 (2.1)	11 (2.4)	1.18 (0.32–4.32)	8 (2.3)	1.12 (0.29–4.31)
CT+TT	52 (35.6)	171 (36.9)	1.06 (0.72–1.56)	129 (36.4)	1.05 (0.70–1.57)
Frequency (C)	0.81	0.80		0.81	
IL-13 G+2044A					
GG	65 (45.8)	171 (36.5)	1.00	158 (44.1)	1.00
GA	60 (42.2)	210 (44.9)	1.33 (0.89–1.99)	159 (44.4)	1.09 (0.72–1.65)
AA	17 (12.0)	87 (18.6)	1.95 (1.08–3.53)	41 (11.5)	0.99 (0.52–1.87)
GA+AA	77 (54.2)	297 (63.5)	1.47 (1.01–2.15)	200 (55.9)	1.07 (0.72–1.58)
Frequency (G)	0.67	0.59		0.66	
IL-13R α 1 A+1398G					
AA	61 (43.3)	205 (44.4)	1.00	152 (43.2)	1.00
AG	23 (16.3)	79 (17.1)	1.02 (0.59–1.76)	62 (17.6)	1.08 (0.61–1.90)
GG	57 (40.4)	178 (38.5)	0.93 (0.62–1.41)	138 (39.2)	0.97 (0.63–1.49)
AG+GG	80 (56.7)	257 (55.6)	0.96 (0.66–1.40)	200 (56.8)	1.00 (0.67–1.48)
Frequency (A)	0.51	0.53		0.52	

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

the IL-13R α 1 gene, we adjusted the odds ratio (OR) by sex. However, asthma did not differ significantly in males (data not shown).

To investigate the associations of the IL-13 and IL-13R α 1 polymorphisms with asthma susceptibility, we determined the occurrence of these genotypes in asthmatic and control groups, using subjects homozygous for the two common alleles as a reference group. We found an association between the susceptibility of asthma and homozygosity for the risk alleles of IL-13 G+2044A (OR=1.95, 95% CI=1.08–3.53; Table 3), but not with the susceptibility of atopic (OR=0.99, 95% CI=0.52–1.87) or non-atopic asthma (data not shown). Other IL-13 promoter polymorphisms and IL-13R α 1 polymorphism were not associated with the susceptibility of asthma or atopic asthma (Table 2).

Total IgE levels in asthmatics with IL-13 and IL-13R α 1 polymorphisms

We found that total IgE was significantly higher in children with atopic asthma with hetero- or homozy-

gous for the risk alleles in the promoter region of IL-13 (A–1512C and C–1112T) and IL-13R α 1 (A+1398G) versus those with homozygous for common alleles ($P=0.012$, 0.015 , and 0.017 , Table 3). A similar result was observed in the asthmatic group ($P=0.049$, 0.024 , and 0.034 ; Table 3), but not in the control group.

Three-loci haplotype analysis of IL-13 polymorphisms

There was no significant association between the haplotype with risk alleles (C-T-A) and asthma susceptibility in Korean children with asthma or atopic asthma (data not shown). However, children with atopic asthma with the C-T-A haplotype showed significantly higher levels of total IgE versus those with the other haplotypes ($P=0.006$; Table 4). Similarly, total IgE levels were significantly higher in children with asthma with the C-T-A haplotype versus those with the other haplotypes ($P=0.019$; Table 4). Conversely, this association was not observed in the control group.

Association between total IgE and the combination of IL-13 and IL-13R α 1 polymorphisms

Because each of the tested IL-13 promoter polymorphisms and IL-13R α 1 polymorphism were associated with total IgE levels, we investigated whether there might be an association between total IgE levels and a combination of the IL-13 risk allele along with one of the IL-13 risk alleles. We observed that children with atopic asthma, who had a combination of hetero- or homozygous for –1512C or –1112T in IL-13 and +1398G in IL-13R α 1, showed higher total serum IgE levels than those with atopic asthma, who had a combination of homozygous for –1512A or –1112C in IL-13 and +1398A in IL-13R α 1 ($P=0.002$, 0.010 after Bonferroni correction, respectively; Table 5). However, the combination of hetero- or homozygous for +2044A in IL-13 and +1398G in IL-13R α 1 tended to have higher

Table 3 Relationship between log total IgE (IU/ml) and IL-13 and IL-13R α 1 polymorphisms

Loci	Asthma ($n=469$)	Atopic asthma ($n=358$)	Control ($n=146$)
IL-13 A–1512C			
AA	2.26 $\pm$ 0.58	2.45 $\pm$ 0.46	1.47 $\pm$ 0.49
AC+CC	2.35 $\pm$ 0.58* ¹	2.56 $\pm$ 0.43* ²	1.56 $\pm$ 0.47
IL-13 C–1112T			
CC	2.26 $\pm$ 0.58	2.47 $\pm$ 0.45	1.53 $\pm$ 0.47
CT+TT	2.37 $\pm$ 0.58* ³	2.57 $\pm$ 0.43* ⁴	1.51 $\pm$ 0.50
IL-13 G+2044A			
GG	2.31 $\pm$ 0.58	2.48 $\pm$ 0.46	1.52 $\pm$ 0.50
GA+AA	2.35 $\pm$ 0.57	2.52 $\pm$ 0.44	1.52 $\pm$ 0.46
IL-13R α 1 A+1398G			
AA	2.25 $\pm$ 0.59	2.45 $\pm$ 0.48	1.49 $\pm$ 0.49
AG+GG	2.34 $\pm$ 0.58* ⁵	2.55 $\pm$ 0.42* ⁶	1.54 $\pm$ 0.47

*¹ $P=0.049$, *² $P=0.012$, *³ $P=0.024$, *⁴ $P=0.015$, *⁵ $P=0.034$, *⁶ $P=0.017$

Table 4 Association between risk allele haplotypes of each IL-13 polymorphism (–1512C, –1112T and +2044A) and log total IgE among Korean children with asthma, especially atopic asthma

Haplotypes	Asthma		Atopic asthma		Control	
	n	Log total IgE (IU/ml) ^c	n	Log total IgE (IU/ml) ^c	n	Log total IgE (IU/ml) ^c
C-T-A ^a	154	2.38 $\pm$ 0.58	101	2.62 $\pm$ 0.42	47	1.49 $\pm$ 0.54
Others ^b	312	2.26 $\pm$ 0.58	219	2.47 $\pm$ 0.45	93	1.48 $\pm$ 0.49
P value		0.019		0.006		0.585

^a Individuals having one or two copies of the C-T-A haplotype (–1512C, –1112T and +2044A in order)

^b Individuals lacking the C-T-A haplotype

^c Mean $\pm$ SD

Table 5 Combination effect of IL-13 and IL-13R α 1 polymorphisms on total IgE levels in atopic asthmatic children

Group	IL-13 A-1512C and IL-13R α 1 A+1398G		IL-13 C-1112T and IL-13R α 1 A+1398G		IL-13 G+2044A and IL-13R α 1 A+1398G	
	Number of cases (n=350)	Log total IgE (IU/ml) (Mean $\pm$ SD)	Number of cases (n=345)	Log total IgE (IU/ml) (Mean $\pm$ SD)	Number of cases (n=350)	Log total IgE (IU/ml) (Mean $\pm$ SD)
I	82	2.37 $\pm$ 0.47	103	2.40 $\pm$ 0.48	74	2.44 $\pm$ 0.51
II	70	2.54 $\pm$ 0.47 ^{*1}	47	2.58 $\pm$ 0.46 ^{*2}	78	2.46 $\pm$ 0.45
III	103	2.53 $\pm$ 0.43 ^{*3}	115	2.54 $\pm$ 0.41 ^{*3}	80	2.53 $\pm$ 0.39
IV	95	2.57 $\pm$ 0.40 ^{*5}	80	2.56 $\pm$ 0.41 ^{*6}	118	2.56 $\pm$ 0.43

^{*1} $P=0.044$, ^{*2} $P=0.045$, ^{*3} $P=0.023$, ^{*4} $P=0.026$, ^{*5} $P=0.002$, ^{*6} $P=0.010$ (after Bonferroni correction compared to group I). Statistical significance was analyzed by Kruskal–Wallis and Mann–Whitney tests

The groups were defined by the combination of IL-13 and IL-13R α 1 polymorphisms: patients in Group I had the common alleles at each gene (used as the reference value); patients in Group II had at least one risk allele for the IL-13 polymorphism and were homozygous for the common allele of the IL-13R α 1 polymorphism; patients in Group III were homozygous for the common allele of the IL-13 polymorphism and had at least one risk allele at the IL-13R α 1 polymorphism; patients in Group IV had at least one risk allele of both IL-13 and IL-13R α 1 polymorphisms

total serum IgE levels versus those homozygous for +2044G in IL-13 and +1398G in IL-13R α 1, but not to a significant degree ($P=0.099$ after Bonferroni correction; Table 5).

Similarly, we investigated associations between total IgE levels and the combination of IL-13 and IL-13R α 1 risk alleles in children with asthma. Children with asthma with the combination of hetero- or homozygous for either IL-13 -1512A or -1112C and hetero- or homozygous for IL-13R α 1 +1398G showed significantly higher total IgE levels compared to those homozygous for the common alleles ($P=0.012$ and $P=0.011$ after Bonferroni correction, respectively; data not shown). Children with asthma with the combination of hetero- or homozygous for IL-13 +2044G and IL-13R α 1 +1398G tended to have higher IgE levels (Log IgE, 2.38 $\pm$ 0.58 mg/ml) versus those with the combination of homozygous for the common alleles (Log IgE, 2.29 $\pm$ 0.60 mg/ml), but not to a statistically significant degree ($P=0.249$ after Bonferroni correction; data not shown).

Discussion

In this study, we found that two IL-13 promoter polymorphisms (A-1512C and C-1112T) and one IL-13R α 1 polymorphism (A+1398G) were significantly associated with increased total IgE levels in Korean children with asthma and atopic asthma. We further found that children with atopic asthma with a combination of hetero- or homozygous for each of the IL-13 polymorphism and the IL-13R α 1 polymorphism showed significantly higher total IgE levels compared to those with homozygous for the common alleles at both loci. To our knowledge, this is the first report showing that

IgE phenotypes are associated with a gene–gene interaction between polymorphic variants of IL-13 and IL-13R α 1. We did not observe any association between combinations of IL-13 and IL-13R α 1 polymorphisms and other clinical phenotypes, such as eosinophil and BHR phenotypes (data not shown).

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 (Howard et al. 2001; van der Pouw Kraan et al. 1999; Graves et al. 2000; Heinzmann et al. 2000; He et al. 2003; Leung et al. 2001; Arima et al. 2002), we found that G+2044A of IL-13 was associated with asthma susceptibility and that A-1512C and C-1112T of IL-13 were also significantly associated with total IgE levels in Korean children with asthma. When we investigated the effect of the risk allele haplotypes of three IL-13 gene polymorphisms (-1512C, -1112T and +2044A) in Korean children with asthma, especially with atopic asthma, we found that the C-T-A haplotype was associated with significantly higher total IgE levels compared to those found harboring all other haplotypes. The combination of the IL-13 G+2044A and IL-13R α 1 A+1398G risk alleles was associated with increased IgE levels, but not to a statistically significant degree, while the combination of either IL-13 promoter polymorphism (A-1512C and C-1112T) risk allele with +1398G of IL-13R α 1 was associated with significantly increased total IgE levels in Korean children with atopic asthma. This seems to indicate that variations in the promoter region of IL-13 affect their amount of signaling, and that there could be a gene–gene effect with the tested polymorphism in IL-13R α 1.

IL-13 mediates its effect through its receptor, a heterodimer composed of IL-4R α and IL-13R α 1. IL-13 signaling uses the JAK-signal transducer and activator

of transcription (STAT) pathway, specifically STAT6 (Takeda et al. 1996). This is thought to occur through IL-4R α , because stimulation of the complex by both IL-4 and IL-13 results in activation of signaling intermediates characteristic of IL-4 responses, including phosphorylation of IL-4R α , insulin receptor substrate 2 (IRS-2), JAK1, and Tyk2 (Wills-Karp 2001; Welham et al. 1995; Hershey 2003). The functional role of IL-13R α 1 and its variants remain unknown, although a previous study showed that a variant of IL-13R α 1 (+1398A) was associated with IgE levels, but not with asthma in British male subjects (Heinzmann et al. 2000). Here, we found that hetero- or homozygosity for the risk allele of IL-13R α 1 A+1398G was significantly associated with increased total IgE levels in children with asthma, especially atopic asthma. This may suggest that this non-coding polymorphism of IL-13R α 1 has a functional consequence for the binding of IL-13, or alternatively that the IL-13R α 1 A+1398G polymorphism is in linkage disequilibrium with as yet unidentified polymorphisms in the regulatory or coding regions of the gene encoding IL-13R α 1. Yet another possibility is that IL-13R α 1 could have additional but as yet unknown signaling functions that are impacted by this polymorphism.

Structurally, the extracellular portions of IL-13R α 1 are composed of three fibronectin type III domains, designated D1, D2, and D3 (Arima et al. 2005). Mutagenesis analyses of IL-13 and IL-13R α have shown that mutation of Leu319 and Tyr321 in the D2 and D3 domains of IL-13R α 1, respectively, led to significant impairment of the IL-13 response (Arima et al. 2005). A truncated murine IL-13R α 1 lacking the intracellular domain was not able to mediate IL-13-induced signals or responses, supporting the possibility that IL-13R α 1 is required for signaling (Orchansky et al. 1997). Analysis of molecular interactions showed that helices A and C of human IL-13 interacted with IL-4R α , while helix D of human IL-13 was responsible for the interaction with IL-13R α 1 (Zuegg et al. 2001). Furthermore, mutation of arginine 110 in the helix D region to a glutamine (G+2044A) resulted in enhanced IL-13 signaling (Heinzmann et al. 2000). Collectively, these studies indicate that IL-13R α 1 is likely to play a critical role not only in binding but also in signaling of IL-13.

Future work will be required to identify gene–gene and gene–environment interactions on a genome-wide level, with the aim of fully elucidating the genetic risk factors for asthma and atopy, understanding the pathogenesis of these common diseases, and perhaps devising new treatment strategies aimed at modulating IL-13 signaling via IL-4R α /IL-13R α 1.

Acknowledgments We thank all the study participants. We are also grateful to nurse Hee-Jung Seo (Asthma and Allergy Clinic, Asan Medical Center, Seoul, Korea) for her work in patient recruitment.

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