Allergy 2005: 60: 900–906

ALLERGY
DOI: 10.1111/j.1398-9995.2005.00619.x

Original article

Association of interleukin 18 (IL18) polymorphisms with specific IgE levels to mite allergens among asthmatic patients

Background: Allergy is regarded as a multifactorial condition. Its onset and severity are influenced by both genetic and environmental factors. Identification of genetic factors involved in asthma development and related phenotypes is a major task in understanding the genetic background of asthma. The possible involvement of IL18 polymorphisms in asthma was examined in a Korean asthma cohort.
Methods: Direct sequencing was performed to discover single-nucleotide polymorphisms (SNPs) in the IL18 gene. Single-base extension (SBE) method was employed for genotyping. Genotypic influence of IL18 was analysed using logistic and multiple-regression models.
Results: Although no polymorphisms in the IL18 gene showed significant association with the risk of asthma development, analyses of the association with specific serum IgE levels to *Dermatophagoides farinae* (D.f.) and *D. pteronyssinus* (D.p.) among asthmatic patients revealed significant associations with two completely linked SNPs, i.e. −148G > C and +13925A > C(Ser35Ser) ($P = 0.01$–0.11 for D.f. and $P = 0.005$–0.11 for D.p.). Both C allele of −148G > C and C allele of +13925A > C showed gene dose-dependent effects on the levels of specific IgE. The lowest IgE levels in homozygotes of minor alleles (1.13 and 1.22 of D.f.; 1.38 and 1.33 of D.p., respectively), intermediate IgE levels in heterozygotes (1.60 and 1.70 of D.f.; 1.84 and 1.92 of D.p., respectively), and the highest levels in homozygotes for major allele (1.93 and 1.93 of D.f.; 2.24 and 2.24 of D.p., respectively), were found.
Conclusion: The genetic relevance of IL18 to specific IgE might offer an important step in understanding the genetic background of allergic diseases.

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Key words: allergy; asthma; IL18; single-nucleotide polymorphism; specific IgE.

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Accepted for publication 17 April 2004

Asthma and related phenotypes are thought to be complex traits caused by an interaction of multiple disease susceptibility genes and environmental factors. Asthma is recognized as a T-helper type 2 (Th2) disease with a particular profile of cytokine release, including interleukin 4 (IL4) and interleukin 5 (IL5). However, increasing evidence indicates that other cytokines, which were classically considered to belong to Th1-type profiles, are also associated with the inflammatory response that characterizes human asthma. The prevalence of atopic diseases such as atopic dermatitis and asthma has increased over the last decades, making allergies a very serious public health problem. Allergy is regarded as a multifactorial condition, in that its onset and severity are influenced by both genetic and environmental factors (1).

The innate function of T-cell activation is one of the outstanding properties of IL18. Moreover, administration of IL18 to normal BALB/c or C57BL/6 mice induces polyclonal IgE production (2). Increased IL18 production has been found in patients with allergic diseases, including asthma, atopic dermatitis and allergic rhinitis, in which a predominance of Th1 cells is significant (3–6). In addition, it also induces naive T cells to differentiate into Th2 cells (7) and enhances IL8 production by eosinophils (8). All of these findings suggest that IL18 plays important roles in both anti-allergic and allergy-promoting effects (2).

Three previous genome-wide studies revealed that the chromosome region around IL18 (chr. 11q22.2-q22.3) belonged to the candidate segment for association with specific IgE of common aeroallergens and the skin test for the house dust mite (9–11). Moreover, two independent studies revealed genetic associations of IL18 polymorphisms with asthma and related phenotypes (12, 13). In the current study, extensive efforts to search for additional SNPs in IL18, along with an examination of genetic association, were performed in a Korean asthma cohort.

Materials and methods

Subjects

Subjects were recruited from the Asthma Genome Research Center that consists of four tertiary hospitals in Korea (Soonchunhyang University Hospital, Ajuo University Hospital, Ulsan University Hospital, and Choong-Ang University Hospital). Ethical approvals were obtained from the institutional review board of each hospital. All patients had the clinical symptoms and the physical examinations compatible with asthma. Each patient showed airway reversibility as documented by an inhalant bronchodilator-induced improvement of $>15\%$ of forced expiratory volume in one second (FEV1), and/or an airway hyperreactivity of <10 mg/ml of methacholine. Normal subjects were recruited from spouses of the patients and the general population who answered negatively to a screening questionnaire for respiratory symptoms and had FEV1 $>75\%$ predicted, the provocation concentration causing a fall in the FEV1 of 20% (PC_{20}) by methacholine >10 mg/ml; and normal findings on a simple chest radiogram. Total IgE and specific IgE to *Dermatophagoides farinae* (D.f.) and *D. pteronyssinus* (D.p.) were measured using the UniCAP system (Pharmacia Diagnostics, Uppsala, Sweden). UniCap-specific IgE calibrators were used for determination of specific IgE antibodies. The concentrations were measured in kU/l and semi-quantitatively expressed as class 0–6. Atopy was defined as having wheal reaction by allergen extract equal to or greater than that by histamine (1 mg/ml) or 3 mm in diameter. The clinical parameters are summarized in Table 1.

Sequencing analysis of the human IL18 gene

We sequenced all exons, including exon–intron boundaries and the promoter region (approximately 1.5 kb), to discover single-nucleotide polymorphisms (SNPs) in 24 Korean DNA samples using the ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Twenty-four primer sets for the amplification and sequencing analysis were designed based on GenBank sequences (Ref. Genome seq. NT_033899 released on October 17, 2003). Information regarding primers is available on our website (http://www.snp-genetics.com/user/news_content.asp?board_idx = 176). Sequence variants were verified by chromatograms. In order to compare allele frequencies of IL18 SNPs identified in the Korean population with other major ethnic groups, we also genotyped 50 Caucasian and 50 African–American DNAs obtained from the Human Genetic Cell Repository (http://www.locus.umdnj.edu/nigms/).

Table 1. Clinical profiles of the study subjects

Clinical profiles	Normal controls	Asthmatics
No. of subjects	140	435
Age [mean (range)]	28.5 (7–75)	35.0 (7–80)
Sex (male/female)	69/71	201/247
Current smoker	30.9%	19.4%*
FVC1%, predicted (±SD)	89.2 (±1.7)	87.7 (±0.7)
FEV1%, predicted (±SD)	93.5 (±1.2)	83.2 (±0.9*)
PC_{20}, methacholine [mg/ml (±SD)]	24.1 (±1.4)	2.8 (±0.7*)
Total IgE [IU/ml (±SD)]	212.2 (±41.7)	537.3 (±52.4*)
Specific IgE (D.f.) (±SD)	0.86 (±1.31)	1.30 (±1.61)
Specific IgE (D.p.) (±SD)	0.99 (±1.39)	1.50 (±1.64)
Positive rates on skin test (%)	26 (18.6)	247 (56.8)*

*P-value < 0.001 for difference between asthmatics and normal controls.

Genotyping by single-base extension and electrophoresis

For genotyping of polymorphic sites in our asthma study, amplifying and extension primers were designed for single-base extension (SBE) (14). Primer extension reactions were performed with the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems). Information regarding the primers is available on our website (http://www.snp-genetics.com/user/news_list.asp). To clean up the primer extension reaction, one unit of SAP was added to the reaction mixture, and the mixture was incubated at 37°C for 1 h, followed by 15 min at 72°C for enzyme inactivation. The DNA samples, containing extension products, and Genescan 120 Liz size standard solution were added to Hi-Di formamide (Applied Biosystems) according to the recommendation of the manufacturer. The mixture was incubated at 95°C for 5 min, followed by 5 min on ice, and then electrophoresis was performed using the ABI Prism 3100 Genetic Analyzer. The results were analysed using ABI Prism GeneScan and Genotyper programs (Applied Biosystems).

Statistical analysis

We examined a widely used measure of linkage disequilibrium between all pairs of biallelic loci, Lewontin's D′ (|D′|) (15). Haplotypes of each individual were inferred using the algorithm developed by Stephens et al. (16) (PHASE; http://www.stat.washington.edu/stephens/software.html; accessed 28 April 2005), which uses a Bayesian approach incorporating *a priori* expectations of haplotypic structure from population genetic and coalescent theory. Genetic effects of inferred haplotypes were analysed in the same way as SNPs. Phase probabilities of each site were calculated for each individual using this software. Genotype distribution of IL18 SNPs and haplotypes among bronchial asthmatics and normal subjects was analysed with logistic regression models controlling age (continuous value), sex (male = 0, female = 1), and smoking status (nonsmoker = 0, ex-smoker = 1, smoker = 2) as covariates.

Results

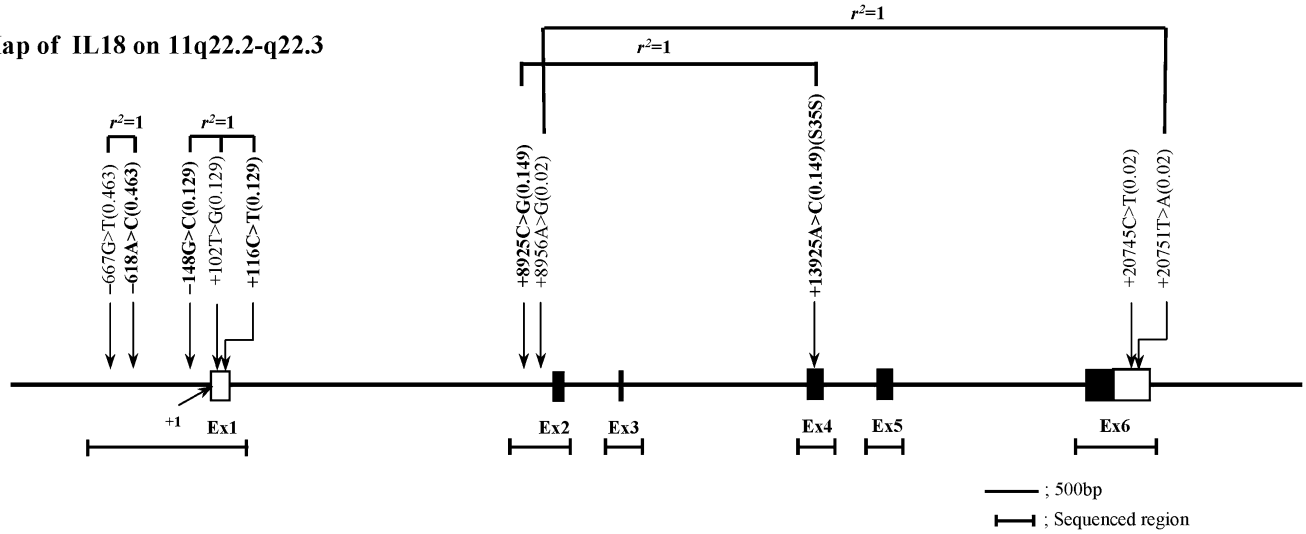
Through direct sequencing of all exons of the IL18 gene, including the −1500 bp 5′ flanking region and exon–intron boundaries, three additional SNPs were identified ($+20751T>A$, $+20745C>T$, and $+8956A>G$; Table 2, Fig. 1A). Three SNPs [$+8138T>C$, $+8926A>G$, and $+20351C>T$(Phe137Phe)] reported in Caucasians (12) were not identified in the Korean population. SNPs in IL18 were renamed by calculating from the transcriptional start site, to avoid confusion caused by alternative nomenclature in previous studies and the revised human genome sequence (Ref. Genome seq. NT_033899, released in Oct. 2003). The comparisons with previous names are listed in Table 2. Pair-wise comparisons among SNPs revealed four sets of absolute LDs ($|D'| = 1$ and $r^2 = 1$) ($-667G>T : -618A>C; -148G>C : +102T>G : +116C>T; +8925C>G : +13925A>C$(Ser35Ser); and $+8956A>G : +20751T>A$). Several complete LDs ($|D'| = 1$ and $r^2 = 1$) were also found (Fig. 1C). Five SNPs were selected for larger scale genotyping based on LDs, frequencies, and haplotype tagging status. Six haplotypes in IL18 genes were constructed in three ethnic

Table 2. Allele frequencies of IL18 polymorphisms in different ethnic groups

Locus*	Kruse et al.	Higa et al.	Position	Frequencies				
				Korean†	Caucasian†	Afr. Amer.†	Caucasian‡	Japanese§
−667G>T	−656G	–	Promoter	0.463	–	–	0.357	–
−618A>C	−607C	−607C/A	Promoter	0.463	0.300	0.378	0.403	0.428
−148G>C	−137C	−137G/C	Promoter	0.129	0.293	0.275	0.283	0.185
+102T>G	113G	–	Exon1	0.129			0.274	–
+116C>T	127T	–	Exon1	0.129	0.240	0.240	0.259	–
+8138T>C	−920C	–	Intron1	–			0.103	–
+8925C>G	−133G	–	Intron1	0.149	0.250	0.240	0.303	–
+8926A>G	−132G	–	Intron1	–	–	–	0.282	–
+8956A>G	–	–	Intron1	0.02	–	–	–	–
+13925A>C(Ser35Ser)	179C>A(Ser35Ser)	105A/C	Exon4	0.149	0.250	0.224	–	0.120
+20351C>T(Phe137Phe)	486C>T(Phe137Phe)	–	Exon6	–	–	–	–	–
+20745C>T	–	–	3′ UTR	0.02	–	–	–	–
+20751T>A	–	–	3′ UTR	0.02	–	–	–	–

*Loci genotyped in larger population of this study are marked in bold.
†This study.
‡Kruse et al. (12).
§Higa et al. (13).

A Map of IL18 on 11q22.2-q22.3



B Haplotypes in IL18

Haplotype	−618	−148	+116	+8925	+13925	Frequency		
						Korean	African American	Caucasian
ht1	C	G	C	C	A	0.509	0.63	0.69
ht2	A	G	C	C	A	0.360	0.13	0.06
ht3	A	C	T	G	C	0.121	0.22	0.24
ht4	A	G	C	G	C	0.009	0	0
ht5	A	C	T	G	A	0	0.02	0
ht6	C	G	C	G	C	0	0	0.01

C LDs(|D'| and r^2) among SNPs in IL18

	SNPs	|D'|				
		−618	−148	+116	+8925	+13925
r^2	−618	–	1	1	1	1
	−148	0.143		1	1	1
	+116	0.143	1	–	1	1
	+8925	0.156	0.918	0.918	–	1
	+13925	0.156	0.918	0.918	1	–

Figure 1. Gene map, haplotypes, and LD coefficients in IL18: (A) Gene map and SNPs in IL18 on chromosome 11q22.2–22.3. Coding exons are marked by black blocks, and 5′ and 3′ UTR by white blocks. The first base of transcriptional site is denoted as nucleotide + 1 (Ref. Genome seq. NT_033899, released on October 17, 2003); (B) Haplotypes of IL18; (C) Linkage disequilibrium coefficient (|D′| and r^2) among IL18 SNPs.

Table 3. Genotype distributions of IL18 polymorphisms in bronchial asthmatics (BA) and normal subjects (NC) in the Korean population

Locus	Group	Genotype			Frequency*	HWE†	*P*-value‡		
							Co-dominant	Dominant	Recessive
		AA	AC	CC					
IL18-618C>A	BA	114 (29)	185 (47.1)	94 (23.9)	0.47	0.54	0.87	0.27	0.43
	NC	30 (24.2)	70 (56.5)	24 (19.4)	0.48	0.34			
		GG	CG	CC					
−148G>C	BA	337 (76.9)	93 (21.2)	8 (1.8)	0.12	0.87	0.46	0.59	0.36
	NC	114 (76.5)	34 (22.8)	1 (0.7)	0.12	0.66			
		AA	AC	CC					
+13925A>C(Ser35Ser)	BA	307 (74)	99 (23.9)	9 (2.2)	0.14	0.95	0.66	0.85	0.34
	NC	101 (73.2)	36 (26.1)	1 (0.7)	0.14	0.51			
		−/−	−/ht2	ht2/ht2					
ht2	BA	128 (36.4)	179 (50.9)	45 (12.8)	0.38	0.35	0.40	0.72	0.26
	NC	32 (33.7)	48 (50.5)	15 (15.8)	0.41	0.91			

The values in parentheses are percentages.
*Minor allele frequencies.
†*P*-values of deviation from Hardy–Weinberg equilibrium.
‡*P*-values for logistic regression analyses controlling smoking, age, and sex as covariates.
Haplotypes and their frequencies were inferred using the algorithm developed by Stephens et al. (16). Among four haplotypes constructed in the Korean population, IL18-ht1[CGCCA] and ht3[CATCTGG] were not analysed because they are equivalent with −618A>C and +116C>T, respectively. IL18-ht4[AGCGC] was not analysed either, because of low frequency (freq. = 0.009) (Fig. 1B).

groups, using the PHASE software (16). There were significant differences found among the three ethnic groups (Fig. 1B). Among four haplotypes constructed in the Korean population, IL18-ht1[CGCCA] and ht3[CATCTGG] were not analysed because they are equivalent with $-618A>C$ and $+116C>T$, respectively. IL18-ht4[AGCGC] was not analysed either, because of low frequency (freq. = 0.009).

Using logistic regression analysis for association with the risk of asthma controlling for age, sex, and smoking status as covariates, no polymorphisms in the IL18 showed significant association (Table 3). Genetic effects of IL18 polymorphisms on total serum IgE and specific IgE levels were also tested. Although no significant associations were detected with total serum IgE (data not shown), analyses of the association with specific serum IgE levels (D.f. and D.p.) among asthmatic patients revealed significant associations with two completely linked SNPs, in all subjects (All) and bronchial asthmatics (BA) in three alternative analysing models (i.e. IL18-$148G>C$ and $+13925A>C$(Ser35Ser) ($P = 0.01$–0.11 with D.f. and $P = 0.005$–0.11 for D.p.) (Table 4). The genotype effects of two SNPs both on specific IgE to D.f. and D.p. were similar, e.g. both C allele of $-192G>C$ and C allele of $+13881A>C$ showed gene dose-dependent effects on the levels of specific IgE. The lowest IgE levels were found in homozygotes of minor alleles (1.13 and 1.22 of D.f.; 1.38 and 1.33 of D.p., respectively), intermediate IgE levels were found in heterozygotes (1.60 and 1.70 of D.f.; 1.84 and 1.92 of D.p., respectively), and the highest levels were found in homozygotes for major alleles (1.93 and 1.93 of D.f.; 2.24 and 2.24 of D.p., respectively). Although the associations of IL18 polymorphisms were not apparent in normal controls (NC), the genetic effects on the levels of specific IgE to D.f. and D.p. were clear in BA (Table 4) in subgroup analyses.

Discussion

As the close relationship between the T helper (Th) lymphocyte subset and its cytokines has been described (17), it has been generally accepted that atopy and asthma are Th2 diseases and that a relative increase in Th2 responses drives the allergic phenotype (18). IL18 not only induces Th1 cytokines such as INFG but also generates Th2 cell responses under certain conditions, as measured by the production of Th2 cytokines and initiation of allergic manifestation. Those findings suggest that IL18 is a potent pro-inflammatory cytokine that has patho-physiological roles in several inflammatory conditions. In principle, IL18 enhances Th1 immune responses, but it can also stimulate Th2 immune responses such as allergic inflammation (2).

Previous reports indicate that IL18 directly stimulates IL4 production and histamine release from basophils (19), enhances IL4 and IL13 production from both NK and T cells in synergy with IL2 (20), strongly co-induces Th1 or Th2 cytokines (21) increases IgE production by B

Table 4. Regression analyses for age, sex, and smoking-adjusted specific IgE level (*Dermatophagoides farinae* (D.f.) and *D. pteronyssinus* (D.p.)) with IL18 polymorphisms among bronchial asthma patients

		Specific IgE (D.f.)						Specific IgE (D.p.)					
		Genotype			Analysing model			Genotype			Analysing model		
Loci	Group	C/C*	C/R*	R/R*	Co-dominant	Dominant	Recessive	C/C*	C/R*	R/R*	Co-dominant	Dominant	Recessive
IL18-618C>A	All	85 (1.79 ± 1.6)	151 (1.97 ± 1.57)	87 (1.63 ± 1.6)	0.48	0.63	0.10	85 (2.18 ± 1.51)	151 (2.17 ± 1.56)	87 (1.93 ± 1.53)	0.27	0.73	0.15
−148G>C		285 (1.93 ± 1.59)	73 (1.6 ± 1.55)	8 (1.13 ± 1.46)	**0.02**	**0.04**	**0.03**	285 (2.24 ± 1.51)	73 (1.84 ± 1.52)	8 (1.38 ± 1.41)	**0.007**	**0.02**	**0.04**
+13925A>C(Ser35Ser)		258 (1.93 ± 1.6)	77 (1.7 ± 1.56)	9 (1.22 ± 1.39)	**0.01**	**0.03**	**0.05**	258 (2.23 ± 1.51)	77 (1.92 ± 1.53)	9 (1.33 ± 1.32)	**0.005**	**0.01**	**0.03**
ht2		109 (1.73 ± 1.61)	140 (2.1 ± 1.54)	35 (1.89 ± 1.69)	0.39	0.49	0.47	109 (1.97 ± 1.59)	140 (2.34 ± 1.48)	35 (2.14 ± 1.63)	0.30	0.33	0.51
IL18-618C>A	BA	72 (1.85 ± 1.64)	124 (1.99 ± 1.58)	73 (1.6 ± 1.63)	0.32	0.92	0.08	72 (2.22 ± 1.52)	124 (2.23 ± 1.56)	73 (1.88 ± 1.56)	0.14	0.55	0.06
−148G>C		230 (2 ± 1.63)	63 (1.59 ± 1.54)	7 (1.29 ± 1.5)	**0.01**	**0.03**	0.07	230 (2.3 ± 1.56)	63 (1.83 ± 1.48)	7 (1.57 ± 1.4)	**0.008**	**0.01**	0.11
+13925A>C(Ser35Ser)		209 (1.99 ± 1.64)	66 (1.7 ± 1.57)	8 (1.38 ± 1.41)	**0.01**	**0.02**	0.11	209 (2.28 ± 1.56)	66 (1.92 ± 1.5)	8 (1.5 ± 1.31)	**0.006**	**0.010**	0.08
ht2		96 (1.76 ± 1.63)	118 (2.04 ± 1.58)	29 (1.76 ± 1.79)	0.55	0.64	0.61	96 (1.99 ± 1.59)	118 (2.31 ± 1.52)	29 (2.03 ± 1.7)	0.44	0.42	0.74
IL18-618C>A	NC	13 (1.46 ± 1.39)	27 (1.85 ± 1.51)	14 (1.79 ± 1.48)	0.55	0.28	0.98	13 (1.92 ± 1.44)	27 (1.89 ± 1.55)	14 (2.21 ± 1.37)	0.49	0.59	0.55
−148G>C		55 (1.67 ± 1.38)	10 (1.7 ± 1.7)	1 (0)	0.73	0.91	0.14	55 (2 ± 1.28)	10 (1.9 ± 1.85)	1 (0)	0.53	0.87	0.10
+13925A>C(Ser35Ser)		49 (1.71 ± 1.4)	11 (1.73 ± 1.62)	1 (0)	0.58	0.91	0.12	49 (2.02 ± 1.3)	11 (1.91 ± 1.76)	1 (0)	0.41	0.72	0.08
ht2		13 (1.54 ± 1.51)	22 (2.41 ± 1.3)	6 (2.5 ± 1.05)	0.40	0.46	0.57	13 (1.85 ± 1.63)	22 (2.45 ± 1.3)	6 (2.67 ± 1.21)	0.57	0.75	0.55

Genotype and haplotype distributions, mean values, standard deviations (SD) of specific IgE level (specific IgE to D.f. and D.p.) and *P*-values of three alternative models are shown.
*C/C, C/R, and R/R represent homozygotes for common allele, and heterozygotes and homozygotes for rare allele, respectively.

cells, and induces naive CD4+ T cells to develop into IL4-producing cells *in vitro* (7). In addition, studies have demonstrated the functions of IL18 in induction of allergic sensitization in mice (22, 23). IL18 has been shown to dramatically increase Th2 cytokine production, IgE levels, eosinophil recruitment in the airways, and airway mucus. Recently, several independent studies have been published concerning the functions of IL18 expression and serum levels in allergic diseases. Elevated serum IL18 levels were detected in patients with acute asthma (24) and allergic asthma (3), atopic dermatitis (25) and allergic rhinitis (6).

In a genome-wide linkage study, chromosomal region 11q22-q23 was shown to be linked with atopy (10) and sensitization to mite allergens (11). In addition, the same chromosomal region to which IL18 belongs also revealed an association with the specific IgE of D.p. through genome-wide multipoint linkage analysis (26).

Two important studies about polymorphisms in IL18 and associations with risk of asthma and allergy were recently performed in the Japanese and Caucasians (12, 13) Four promoter SNPs of IL18 (−148G > C, +102T > G, +116C > T, and +8925C > G) were reported to be associated with higher serum IgE levels, specific sensitization to common allergens (including grass pollens, mite allergens, cat dander, and birch pollen), and the presence of seasonal allergic rhinitis in the German population (12). Moreover, a silent variant in exon 4, +13925A > C(Ser35Ser), was reported to be associated with reduced risk of asthma in the Japanese population (497 cases and 85 controls). There was also an increase of IL18 levels in patients with allergic asthma compared with the control group, but no significant relation was detected between the IL18 levels and genotypes of +13925A > C(Ser35Ser) polymorphism.

In addition, although most of previous studies had focused on only two promoters [−607C > A and −137G > C (−618A > C and −148G > C, respectively, in this study)] and one exonic (+13925A > C(Ser35Ser) in exon 4) polymorphisms, several disease associations of IL18 polymorphisms had been were identified, e.g. −607C > A and −137G > C with the elevated IgE (27), −137G > C (28), −607C > A and one haplotype (−607C > A : −137G > C) with type 1 diabetes (29), IL18 haplotype (−607C > A : −137G > C) with the development of post-injury sepsis (30), −607C > A with sarcoidosis (31), −607C > A with necrotising enterocolitis (32) and +13925A > C(Ser35Ser) with Crohn's disease (33).

In this study, the positive association of +13925A > C(Ser35Ser) (in absolute linkage disequilibrium (LD) with +8925C > G) with risk of asthma development in the Japanese population (13), was not reconfirmed in the Korean population (435 cases and 140 controls). The frequency of minor allele [C allele of +13925A > C(Ser35Ser)] in this study was 0.14 in both asthmatics and controls (Table 3), which is about midway between frequencies in asthmatics (freq. = 0.109;

$n = 497$) and controls freq. = 0.182; $n = 85$) in the previous study (12, 13). Although it is hard to account for the discrepancies between the two studies of the effect of this important IL18 variant on asthma, the low sample size of the control group in the previous study could be a plausible explanations, i.e. only 86 controls, which was much smaller than the number of cases studied ($n = 497$). However, the genetic associations of three absolutely linked SNPs (−148G > C, +102T > G, and +116C > T, which are all in absolute LD) and the exonic variant (+13925A > C(Ser35Ser), which is in absolute LD with +8925C > G) with specific sensitization to the common allergens (12) were reconfirmed in this study (Table 4; Fig. 1). When considering absolute LDs among SNPs, five SNPs were significantly associated with the levels of specific IgE (Fig. 1; Table 4).

The production of specific IgE is initiated by Th2 cells that release IL4 and IL13 in response to antigen presentation. Mite-specific clones from donors with high specific IgE produced significantly more IL4 and less IFN-gamma than mite-specific clones from donors with low levels of specific IgE (34). In addition, the production of IL4 showed a close positive correlation with specific IgE production in response to the house dust mite (35). These results suggest that circulating allergen-specific T cells may be skewed towards a TH2 profile. In the process of Th2 differentiation, IL18 and T-cell receptor-mediated stimulation with allergens such as house dust mites could induce naive CD4+ T cells to develop into IL 4-producing cells *in vitro*. In addition, IL18 increases specific IgE production in CD4+ T cells, IL4, CD40 ligand (CD154), and STAT6 (7).

In summary, three additional SNPs were identified in the IL18 gene, although these were not analysed because of low frequencies (freq. = 0.02) in the Korean population. Genetic information about IL18 genes was clearly established by linkage analysis among SNPs. Using statistical analyses, the genetic linkage of IL18 polymorphisms with specific IgE to mite allergens was revalidated, although the genetic effect of +13925A > C(Ser35Ser) on the risk of asthma development was not reproduced. Current study of the genetic influence of the IL18 gene variant suggests IL18 as a possible marker for disease susceptibility, and the genetic relevance of IL18 to the specific IgE might offer an important piece in understanding the genetic background of allergic diseases.

Acknowledgments

This work was supported by a grant from National Research Lab. Program as part of National Research and Development Program from the Ministry of Science and Technology of Korea; Contract grant number: 1-0302-00-0073.

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