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Association of the single-nucleotide polymorphism and haplotype of the interleukin 18 gene with atopic dermatitis in Koreans

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Summary

Background IL-18 is a proinflammatory cytokine that plays an important role for T-helper type 1 (Th1) and Th2 cytokine in the presence/absence of IL-12. It has been recently shown that human IL-18 plays a role in atopic dermatitis (AD) by enhancing IL-4 and IL-13 production and by stimulating the synthesis of IgE.

Objective We wanted to evaluate the associations of single-nucleotide polymorphism (SNP) and the haplotype in the IL-18 gene, hence we performed genotyping for the SNPs in the IL-18 gene in AD patients and normal controls.

Method We genotyped three SNPs from the IL-18 gene for the 1120 case-control samples (646 AD patients and 474 normal controls). We measured the serum IL-18, IL-4 and IL-13 concentrations in 74 individuals (25 ADe, 25 ADi and 24 controls) by performing ELISA.

Result The rs795467 SNP and haplotype T-T-C were significantly associated with AD, and especially between the ADe and normal control groups ($P = 0.03$ and 0.01). The serum IL-18 concentration was higher in the AD group than in the normal controls without any correlation with the rs795467 polymorphism. We did not find any correlations between the serum IL-18 levels and the SCORing atopic dermatitis index, the blood eosinophil counts and the ECP, and there was no correlation between the serum IL-18 levels and the serum IL-4 and IL-13 levels.

Conclusion We found that the one SNP and the haplotype T-T-C were strongly associated with the allergic type of AD, but not with the non-allergic intrinsic type. These data support the hypothesis that IL-18 up-regulates IgE production, yet more experiments will be needed to prove the *in vivo* involvement of Th2 cytokine.

Keywords atopic dermatitis, haplotype, IL-18, SNP

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Introduction

IL-18 is a pro-inflammatory cytokine that is produced by a wide range of cells such as monocytes, macrophages and dendritic cells. IL-18 is synthesized as an inactive precursor (pro-IL-18), which is a 24 kDa polypeptide, and it is cleaved by caspase-1 into the active form. IL-18 is a member of the IL-1 family that was originally described as IFN- γ -inducing factor; it is generally considered to be involved in the T-helper type 1 (Th1)-mediated immune response and it inhibits IgE synthesis, usually by acting synergistically with IL-12. IL-18 is essential for the host's defense against bacteria, fungi, protozoa and viruses

through the induction of host-derived IFN- γ . On the other hand, IL-18 might also initiate Th2 responses with production of IgE via the stimulation of IL-4 and -13 synthesis in mast cells and basophils under certain conditions, as measured by Th2 cytokine production [1] (Fig. 1). The activity of IL-18 occurs by means of a signalling chain of the IL-18 receptor (IL-18R) complex. IL-18R is composed of α and β chains. IL-18R α represents the ligand-binding chain and this is selectively expressed on the surface of Th1 cells, but not on Th2 cells, and IL-18R β mediates intracellular signals [2, 3]. Keratinocytes from atopic dermatitis (AD) patients have recently been shown to have a higher expression of IL-18R α , as compared with those from the normal controls [4].

Single-nucleotide polymorphisms (SNPs) in the IL-18 gene have previously been reported for different chronic

¹The authors wish it to be known that in their opinion, the first two authors should be regarded as joint First Authors.

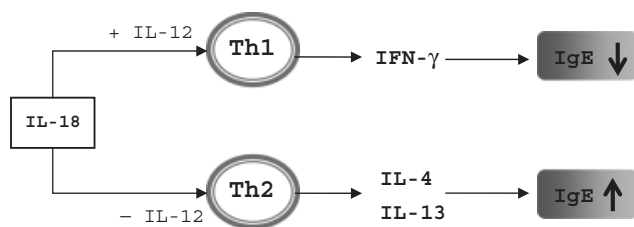


Fig. 1. Schematic diagram for the regulation of IgE levels through IL-18. Th1, T-helper type 1, Th2, T-helper type 2.

inflammatory diseases such as Crohn's disease [5], diabetes mellitus [6] and multiple sclerosis [7]. The human IL-18 gene is located on chromosome 11q22, and it has been linked to atopy-related traits. The levels of IL-18 and other cytokines have been measured and SNP studies of the IL-18 gene have been conducted for Th2-mediated diseases. The increased IL-18 and eosinophilic cationic protein (ECP) levels in the nasal secretions from allergic rhinitis (AR) patients were significantly higher than those of the controls [8], and the SNPs of the IL-18 gene were reported to be associated with AR [9]. The serum levels of IL-18 were reported by several papers [10–12] to be increased in bronchial asthma (BA) patients and the serum levels of IFN- γ did not show any significant change as compared with the controls [10, 12], but the serum levels of IL-13 were reported to be increased [11] or unchanged [10]. However, a careful search of the SNPs in the promoter regions of the IL-18 gene did not find any association between the SNPs and BA [13]. For hayfever patients, the -606AA homozygote of the IL-18 gene was reported to increase the risk of elevated IgE levels against inhaled allergens [14]. The serum levels of IL-18 have been reported to be increased in AD patients [15–17], and the serum levels of IFN- γ were decreased [16]; the serum levels of IL-12 were decreased or there were no significant changes compared with the normal controls [15, 16]. Novak et al. [15] have recently reported that AD patients are associated with SNPs of IL-18 and AD patients have increased IL-18 serum levels after stimulation by bacterial endotoxin, along with lower levels of serum IL-12. That paper supports other evidence that IL-18 with a reduced serum IL-12 level can initiate the Th2 response.

On the basis of the previous data, we hypothesized that IL-18 can play an important role in the development of AD. We performed extensive genotyping of the SNPs of the IL-18 gene to determine their genetic association with the risk of AD. Herein, we genotyped three tagging SNPs from the IL-18 gene of Korean AD patients and their matched controls. We found that one SNP and haplotype in the IL-18 gene was associated with the AD phenotype. As was previously mentioned, IL-18 might initiate Th2 responses through stimulation of IL-4 and IL-13 synthesis in mast cells and basophils, and the serum IL-12 and IFN- γ levels were decreased in AD patients, hence we tried to determine

the serum levels of IL-18, -4 and -13 in AD patients and the controls according to the SNP genotype in the IL-18 gene.

Methods and materials

Subjects

Six hundred and forty-six patients with AD and 474 normal control subjects (NR) were included in this study. The AD samples were collected from the non-asthmatic atopic patients who were examined at Samsung Medical Center, Seoul, Korea. All 646 patients (M:F = 357:289, mean age: 13.58 ± 10.02 years) meet the criteria of our previous reports [18, 19]. To select good patient samples, we divided the AD patients into three different subgroups as the extrinsic (ADe), intrinsic (ADi) and indeterminate types of AD (ADind) patients [20]. The ADe and ADi patients were classified according to the criteria of our previous paper, and the ADind patients were defined as the patients who had typical AD skin lesions and they had either a total IgE level over 200 U/mL with negative-specific IgE responses or a total IgE level < 200 U/mL with positive-specific IgE responses. Among the 646 AD patients, 433 (M:F = 257:176, mean age: 15.70 ± 9.47 years) were classified as ADe and 213 (M:F = 100:113, mean age: 9.26 ± 9.74 years) were classified as ADi according to their history, their total and specific IgE levels and their responses to skin prick tests. The patients who have other atopic diseases such as asthma and AR and the ADind-type patients were not included in this study. The control group subjects were recruited from medical students and volunteers (M:F = 254:220, age: 23.23 ± 2.24 years) who had no history of AD skin lesions. The age, serum total IgE levels, blood eosinophil counts and SCORing atopic dermatitis (SCORAD) index of the ADe and ADi patients and the controls are summarized in Table 1. This study was conducted according to the Declaration of Helsinki Principles and written informed consents were obtained from all the participants. The Samsung Medical Center Ethics Committee approved this study.

Marker selection

The SNP information was retrieved from the dbSNP (build 119, <http://www.ncbi.nlm.nih.gov/SNP>). A total of 21 SNPs were selected as polymorphic markers in the region between 5 kb upstream and 5 kb downstream of the IL-18 gene. The SNPs were genotyped in 48 independent samples from Koreans of the general population (data not shown). On the basis of the genotype results, we selected three SNPs for further genotyping of the 1120 case-control samples. The SNP selection was performed using the Tagger program (<http://www.broad.mit.edu/mpg/tagger>), and the linkage disequilibrium (LD) bin approach was applied, which is a method based on the pairwise r^2 of the LD measurement [21]. Using this method, bins of the

Table 1. Demographic features of three groups

Group	Number of subjects (F/M)	Age	IgE	Eosinophil	SCORAD
ADe	433 (176/257)	15.7 ($\pm$ 9.47)	1933.43 ($\pm$ 3315.06)	583.84 ($\pm$ 641.08)	33.35 ($\pm$ 20.06)
ADi	213 (113/100)	9.26 ($\pm$ 9.74)	52.08 ($\pm$ 46.22)	380.54 ($\pm$ 364.41)	22.3 ($\pm$ 16.38)
NR	474 (220/254)	23.23 ($\pm$ 2.24)	240.53 ($\pm$ 416.01)		

Number of subjects, mean and standard deviation for age, the IgE level, the eosinophil count and the SCORAD index are shown.

AD, atopic dermatitis; ADe, extrinsic type of AD; ADi, intrinsic type of AD; NR, normal control subjects; SCORAD, SCORing atopic dermatitis; M, male; F, female.

common SNPs that are in very strong LD with a specified r^2 threshold are identified and then one SNP is selected to represent the remaining SNPs in each bin. We used an r^2 threshold of 0.8 and a minimum allele frequency of 0.1.

Genotyping with fluorescence polarization detection

Genomic DNA was extracted from 5 mL of whole blood using a commercially available DNA isolation kit (Gentra Genomic DNA purification kit, MN, USA) according to the manufacturer's protocol.

Genotype identification was performed with the GenomeLab SNPstream (Ultra-high throughput; UHT system [22]), which uses multiplexed polymerase chain reaction (PCR) in conjunction with tag array, single-base extension genotyping technology (Beckman Coulter, Fullerton, CA, USA) and its accompanying SNPstream software package, as described by Denomme and Van Oene [23].

Statistical analysis

χ^2 tests were used to determine whether individual variants were in Hardy–Weinberg equilibrium (HWE) at each locus in the samples. The allelic frequency and genotypic distribution of the SNPs among the groups were compared; statistical significance was determined by the P -values obtained from the logistic regression analysis, while age and gender were used as adjusting covariates. The adjusted P -value for multiple testing was obtained by Bonferroni's method in which the P -values are multiplied by the number of tests in each gene. The odds ratios were estimated using a logistic regression procedure in the SAS program. A haplotype association test was performed using the *haplo.score* function in the R package *haplo.stats* and the effects of age and gender were used as adjusting covariates. The *haplo.score* method was proposed by Schaid et al. and it provides score tests for the effect of each haplotype on a trait conditioned on covariates [24]. The total IgE levels were categorized into five ordered groups (<40 , 40–200, 200–500, 500–2000 and ≥ 2000 U/mL). Cumulative logistic regression analysis was then conducted to examine the association between the genotypes and the total IgE levels. The genetic effects on the blood eosinophil counts and ECP levels from the peripheral blood of AD subjects were tested for using a linear regression model. The log-transformed eosinophil

counts and ECP levels were used in the regression analysis. Age, gender and the SCORAD index, which is a measure of the severity of AD, were used in the regression analysis model as the adjusting covariates. Empirical P -values were obtained from 10 000 permuted samples and the corrected P -values for multiple testing were calculated by Westfall and Young's step-down procedure [25]. To compare the concentrations of serum IL-18, we used the Kruskal–Wallis test because the data did not satisfy the normality assumption. Statistical analysis was performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA) and R statistical language (<http://www.r-project.org>).

Protein levels as determined by enzyme linked immunosorbent assay

To test for the effect of genetic variation on the concentrations of IL-18 in peripheral blood, we measured the protein levels of IL-18, IL-4 and IL-13 in the individual serum samples using ELISA. We collected the serum samples from our patients and the control pools for each genotype. We then selected a total of 74 available samples: 2–13 samples from each genotype in the ADe and ADi patients and the controls. One hundred microlitres of serum was used in the IL-18 ELISA (Biosource, California, CA, USA), the IL-4 ELISA (Quantikine Human IL-4 ELISA; R&D Systems, Minneapolis, MN, USA) and the IL-13 ELISA (Quantikine Human IL-13 ELISA; R&D Systems). The experiments were performed according to the manufacturer's protocol.

Results

Heterogeneity of the interleukin-18 polymorphism frequency between the atopic dermatitis patients and the controls

Three SNPs (rs795467, rs4937113 and rs5744247) in the IL-18 gene were genotyped for 1120 case–control samples. The gene structure and position of the 21 SNPs in the IL-18 gene are shown in Fig. 2a and the marker information, including the genomic function, the chromosomal position, the dbSNP id and the minor allele frequency, are shown in Fig. 2b. All the three SNPs were in HWE, and the genotype success rate of the three tagging SNPs was over 99% (0.9946, 0.9911 and 0.9929, respectively).

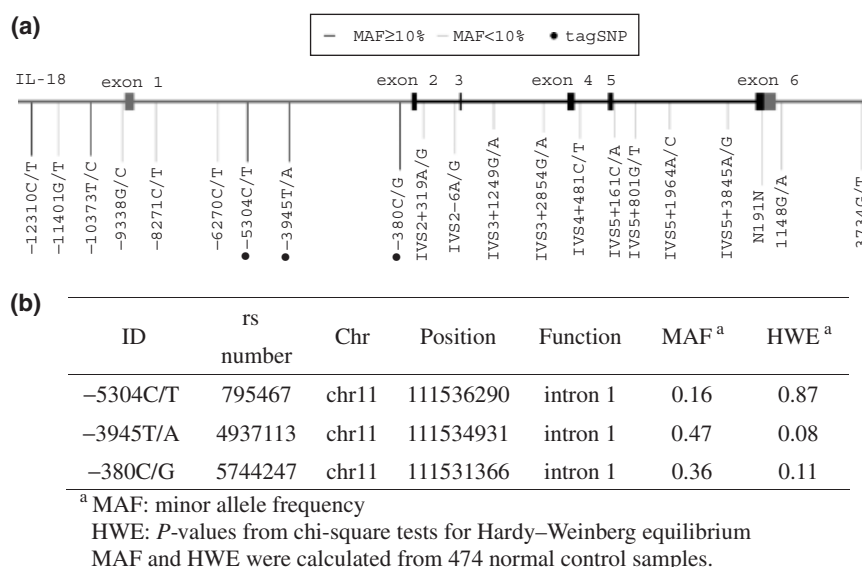


Fig. 2. (a) Map of the IL-18 gene on chromosome 11q22.2-q22.3 (26.9 kb). Coding exons are marked by black blocks, and the 5' and 3' UTRs are marked by grey blocks. The first base of the translation start site is denoted as nucleotide + 1. The genotyped polymorphisms in the IL-18 gene are marked. The minor allele frequencies of the polymorphism in 48 samples are distinguished as over 10% or less. Dots indicate the polymorphisms genotyped in a larger population ($n = 1120$). (b) The single-nucleotide polymorphism (SNP) markers genotyped for the case-control samples (dbSNP build 126).

Table 2. Comparison of the allelic and genotypic frequencies of the three SNPs on the IL-18 gene among the groups

rs number	Group	Allele		<i>P</i> -value	Genotype			<i>P</i> -value [†]
		C	T		C/C	C/T	T/T	
795467	NR	789 (0.84)	153 (0.16)		331 (0.7)	127 (0.27)	13 (0.03)	
	AD	1141 (0.89)	145 (0.11)	0.007**	504 (0.78)	133 (0.21)	6 (0.01)	0.02*
	ADe	770 (0.9)	90 (0.1)	0.009**	343 (0.8)	84 (0.2)	3 (0.01)	0.03*
	ADi	371 (0.87)	55 (0.13)	0.14	161 (0.76)	49 (0.23)	3 (0.01)	0.34
4937113		A	T		A/A	A/T	T/T	
	NR	444 (0.47)	492 (0.53)		115 (0.25)	214 (0.46)	139 (0.3)	
	AD	647 (0.5)	637 (0.5)	0.25	154 (0.24)	339 (0.53)	149 (0.23)	0.16
	ADe	442 (0.51)	418 (0.49)	0.23	105 (0.24)	232 (0.54)	93 (0.22)	0.19
5744247		C	G		C/C	C/G	G/G	
	NR	604 (0.64)	342 (0.36)		201 (0.42)	202 (0.43)	70 (0.15)	
	AD	796 (0.62)	482 (0.38)	0.71	239 (0.37)	318 (0.5)	82 (0.13)	0.27
	ADe	535 (0.63)	319 (0.37)	0.86	161 (0.38)	213 (0.5)	53 (0.12)	0.27
	ADi	261 (0.62)	163 (0.38)	0.65	78 (0.37)	105 (0.5)	29 (0.14)	0.86

[†]*P*-value for the type III effect of the genotype.

*Significance at the 0.05 level.

**Significance at the 0.01 level.

Bold numbers mean the statistically significant ones.

SNPs, single nucleotide polymorphism; AD, atopic dermatitis; ADe, extrinsic type of AD; ADi, intrinsic type of AD; NR, normal control subjects.

We compared the genotype frequencies of the three SNPs between the AD group and the normal control group. The allelic and genotypic distributions of rs795467 were significantly different between the AD and control groups; the *P*-values from the logistic regression analysis were 0.007 and 0.02, respectively. When we

compared the allelic and genotypic frequencies between the AD subtypes and the controls, rs795467 showed a significant difference in allelic distribution between the ADe group and the normal control group with a *P*-value of 0.009 (Table 2). This significance was preserved after correction for multiple comparisons ($P = 0.027$), and the

odds ratio (OR) was 1.53 [95% confidence interval (CI): 1.11–2.10]. There was no significant difference between the ADi group and the normal control group.

We constructed the haplotypes with the three SNPs in the IL-18 gene and we tested their effects. One of the haplotypes (T–T–C) had a significant effect on AD ($P = 0.007$), and it was also associated with ADe ($P = 0.01$), but not with ADi ($P = 0.14$) (Table 3). These results show that the IL-18 polymorphism and the haplotype are associated with only the allergic type of AD, and IL-18 is associated with high IgE production. Moreover, this indicates that the classification of AD into the extrinsic and intrinsic types has meaning for the genetic aspect.

Serum concentrations of interleukins-18, -4 and -13 between the atopic dermatitis patients and the controls

To see whether the polymorphism of rs795467 is related with the serum IL-18 levels, we measured the IL-18

Table 3. Haplotype association test between the AD or AD subtypes and the NR group while gender and age were the adjusting covariates, with using the method developed by Schaid et al. (*haplo.score*)

Compared groups	Haplotype	Frequency	Haplo score	P-value
AD vs. NR ($n = 646$, $n = 474$)	T–T–C	0.134	– 2.701	0.007**
	C–T–C	0.003	0.045	0.964
	C–T–G	0.372	0.544	0.587
	C–A–C	0.491	1.329	0.184
ADe vs. NR ($n = 433$, $n = 474$)	T–T–C	0.135	– 2.591	0.010*
	C–T–C	0.003	0.249	0.803
	C–T–G	0.369	0.378	0.706
	C–A–C	0.493	1.401	0.161
ADi vs. NR ($n = 213$, $n = 474$)	T–T–C	0.152	– 1.474	0.141
	C–T–C	0.003	– 0.423	0.672
	C–T–G	0.369	0.450	0.652
	C–A–C	0.476	0.638	0.524

*Significance at the 0.05 level.

**Significance at the 0.01 level.

Bold numbers mean the statistically significant ones.

AD, atopic dermatitis; NR, normal control subjects; ADe, extrinsic type of AD; ADi, intrinsic type of AD.

protein levels of each genotype by performing ELISA. A total of 74 serum samples from each genotype in the ADe ($n = 25$), ADi ($n = 25$) and normal groups ($n = 24$) were evaluated. In all three groups, the rs795467 genotype had no effect on the serum IL-18 concentration (data not shown). However, the serum IL-18 concentration of the AD patients ($n = 50$, 43.8–908.9 pg/mL, mean: 259.15) was higher than that of the normal controls ($n = 24$, 86.2–1112.6 pg/mL, mean: 213.5, $P = 0.05$) (Table 4). When we tested for the correlation among the serum IL-18 levels, the blood eosinophil counts, the ECP levels and the SCORAD index for 50 AD patients, we could not find any correlation between the IL-18 level and the other three parameters, but we found a significant relationship between the SCORAD index and both the eosinophil counts and the ECP levels (Table 5). A haplotype effect on the blood eosinophil counts was also tested for the ADe group while controlling for the SCORAD index. However, we could not find any significant results (data not shown).

As IL-18 stimulates IL-4 and IL-13 production in basophils [1], we measured the serum IL-4 and IL-13 levels in all 74 case–control samples. We could not detect serum IL-4 in all the test samples, but we could detect serum IL-13 in 21 individuals (8/25 in the ADe group, 6/25 in the ADi group and 7/24 in the control group). The mean serum IL-13 levels in the AD and control groups were 25.9 and 20.9 pg/mL, respectively, and this was without any statistical difference (Table 4). When we compared the serum IL-18 levels between the detectable serum IL-13 group and the undetectable group, the mean value of the IL-18 was higher (237.37 ± 21.31 pg/mL) in the detectable IL-13 group than that in the undetectable group

Table 4. The average serum levels of IL-18 and IL-13

	NR ($n = 24$)	AD ($n = 50$)	P-value*
IL-18	213.5 (± 41.4)	259.2 (± 24.1)	0.05
IL-13	20.9 (± 6.9)	25.9 (± 6.2)	0.75

*P-values obtained from the test for the difference in means between the AD and normal groups by the Kruskal–Wallis two-sample rank-sum test. AD, atopic dermatitis; NR, normal control subjects.

Table 5. Correlation among the serum IL-18 level, the eosinophil counts, the ECP and the SCORAD index

	Serum IL-18 concentration	Eosinophil counts	ECP	SCORAD
Serum IL-18 concentration	1	0.228	0.142	0.01
Eosinophil Counts	0.11 ($n = 50$)	1	0.693	0.353
ECP	0.33 ($n = 49$)	< 0.0001 ($n = 49$)	1	0.401
SCORAD	0.49 ($n = 50$)	0.01 ($n = 50$)	0.004 ($n = 49$)	1

Spearman's correlation coefficients (δ) are shown in the upper diagonal. The P-values from the test for $H_0: \delta = 0$ and the sample sizes are shown in the lower diagonal.

SCORAD, SCORing atopic dermatitis; ECP, eosinophilic cationic protein.

(262.10 ± 32.86 pg/mL); however, there was no statistical significance (Kruskal–Wallis P -value = 0.66).

Discussion

IL-18 is a proinflammatory cytokine that plays an important role for Th1 and Th2 cytokine in the presence/absence of IL-12. IL-18 acts synergistically with IL-12 to promote immune responses of the Th1 cell to produce IFN- γ and to inhibit IgE production; however, in the absence of IL-12, IL-18 induces the production of IgE and promotes immune reactions of the Th2 type (Fig. 1). The serum level of IL-18 has been reported to be increased in AD patients [15–17] and the serum level of IL-12 was reported to be decreased in AD patients [15]. Those reports mean that the functional dysregulation of IL-18 production can contribute to the development of the AD phenotype.

By genotyping the IL-18 gene, we found that one SNP (rs795467) located in intron 1 and the haplotype T–T–C were associated with the AD phenotype. Both the SNP and the haplotype T–T–C of IL-18 had strong association with the allergic type of AD (ADe); on the other hand, there was no correlation between these parameters and the non-allergic, intrinsic type of AD (ADi). As IL-18 induces the production of IgE and so promotes immune mechanisms of the Th2 type in the absence of IL-12 [1], our genotyping data associated with ADe indicated that the classification of AD into the extrinsic and intrinsic types had meaning for the genetic aspect. As the rs795467 SNP had stronger correlation than the other SNPs, we selected 74 case–control samples from each genotype in each group to look for the genetic effect of this SNP on the IL-18 production, and we checked the serum IL-18 levels. Although we could not find any correlations between the IL-18 protein levels and the genotypic variations, we found that the serum IL-18 level in the AD group was higher than that of the control group ($P < 0.05$). This result was compatible with the results of previous papers [15–17]. When we compared the serum IL-18 levels with the blood eosinophil counts, the ECP and the SCORAD index in 50 AD patients, we could not find any correlations between the IL-18 levels and the other three parameters; however, we found correlations between the SCORAD index and the blood eosinophil counts and the ECP levels. The serum IL-18 concentrations were previously shown to be correlated with the ECP level for AR patients [8], with the blood eosinophil counts for AD patients [16], and with the SCORAD index for young children suffering with AD [17]. As these previous papers included relatively small sample sizes as compared with ours, and our data are different from those studies, more experiments are needed with larger sized samples. We previously reported the correlations between the clinical severity of AD patients and the blood eosinophil counts and the serum ECP levels

[20]. This suggests that eosinophils play an important role in the pathogenesis of AD and that ECP in the skin may induce inflammation and eczematous change, which reflect the current activity of skin inflammation.

As IL-18 enhances Th2 cytokine production and it up-regulates IgE production, and our data showed an association between the SNP and the haplotype of IL-18 and the allergic type of AD, we also checked the serum IL-4 and IL-13 levels from the same 74 case–control serum samples. Unfortunately, we could not detect any serum levels of IL-4 in all the samples and we could detect serum IL-13 levels in only 21 serum samples; there were no significant differences of these protein levels between the AD and control groups. We also did not find any correlations between the serum IL-18 concentrations and the serum IL-4 and IL-13 levels. Either the cytokine levels in the serum measured directly by ELISA or the cytokine production by peripheral blood mononuclear cells (PBMCs) from the AD patients showed conflicting results. Although IL-4 and IL-13 in allergic lesions have commonly been found to induce IgE production by B cells, the peak time for detecting IL-4 protein was 4–6 h after cell stimulation; on the other hand, the peak time for detecting IL-13 was 18–20 h after stimulation. Our experimental data might be explained by either the short action of IL-4 or the different clinical stage of our case–controls.

Yoshimoto et al. [1] reported that IL-18 caused bone marrow-derived basophils and mast cells that express IL-18R chains to release large amounts of IL-4 and IL-13 in the presence of IL-3, both *in vivo* and *in vitro*. However, the plasma IL-18 levels were reported to be increased in allergic asthma patients [10, 11], but the plasma IL-13 levels were increased [11] or there were no changes [10]. For AD, the plasma IL-18 was increased, but IL-13 showed no significant changes in the PBMCs, which were cultured with stimulants, from among the asthma, AD and control groups [10]. Although our data are compatible with that of El-Mezzein et al.'s study [10], there have been discrepancies reported between the serum cytokine levels and the tissue levels. We previously reported on increased IL-13 mRNA levels in the skin samples from AD patients, as was determined by semiquantitative RT-PCR [18].

In conclusion, we found that one SNP and the T–T–C haplotype were strongly associated with the allergic type of AD, but not with the non-allergic ADi type. This datum supports the hypothesis that IL-18 up-regulates IgE production; however, more experiments will be needed to prove the involvement of Th2 cytokine that is associated with IL-18 *in vivo*.

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References

- Yoshimoto T, Tsutsui H, Tominaga K *et al.* IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. *Pro Natl Acad Sci USA* 1999; **96**:13962–6.
- Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H Interleukin-18s a unique cytokine regulates both Th1 and Th2 response. *Ann Rev Immunol* 2001; **19**:423–74.
- Dinarello CA. IL-18: a Th1-inducing, proinflammatory cytokine and new member of IL-1 family. *J Allergy Clin Immunol* 1999; **103**:11–24.
- Wittman M, Purwar R, Hartmann C, Gutzmer R, Werfel T. Human keratinocytes respond to interleukin-18: implication for the course of chronic inflammatory skin diseases. *J Invest Dermatol* 2005; **124**:1225–33.
- Tamura K, Fukuda Y, Sashio H *et al.* IL18 polymorphism is associated with an increased risk of Crohn's disease. *J Gastroenterol* 2002; **37**:111–6.
- Kretowski A, Mironczuk K, Karpinska A *et al.* Interleukin-18 promoter polymorphisms in type 1 diabetes. *Diabetes* 2002; **51**:3347–9.
- Giedraitis V, He B, Huang W-X, Hillert J. Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. *J Neuroimmunol* 2001; **112**:146–52.
- Verhaeghe B, Gevaert P, Holtappels G *et al.* Up-regulation of IL-18 in allergic rhinitis. *Allergy* 2002; **57**:825–30.
- Kruse S, Kuehr J, Moseler M *et al.* Polymorphisms in the IL18 gene are associated with specific sensitization to common allergens and allergic rhinitis. *J Allergy Clin Immunol* 2003; **111**:117–22.
- El-Mezzein REH, Matsumoto T, Nomiya H, Miike T. Increased secretion of IL-18 in vitro by peripheral blood mononuclear cells of patients with bronchial asthma and atopic dermatitis. *Clin Exp Immunol* 2001; **126**:193–8.
- Wong CK, Ho CY, Ko FWS *et al.* Proinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFN- γ , IL-4, IL-10 and IL-13) in patients with allergic asthma. *Clin Exp Immunol* 2001; **125**:177–83.
- Tanaka H, Miyazaki N, Oashi K *et al.* IL-18 might reflect disease activity in mild and moderate asthma exacerbation. *J Allergy Clin Immunol* 2001; **107**:331–6.
- Heinzmann A, Gerhold K, Ganter K *et al.* Association study of polymorphisms within interleukin-18 in juvenile idiopathic arthritis and bronchial asthma. *Allergy* 2004; **59**:845–9.
- Nieters A, Linseisen J, Becker N. Association of polymorphism in Th1, Th2 cytokine genes with hayfever and atopy in a subsample of EPIC-Heidelberg. *Clin Exp Allergy* 2004; **34**:346–53.
- Novak N, Kruske S, Potreck J *et al.* Single nucleotide polymorphisms of the IL-18 gene are associated with atopic eczema. *J Allergy Clin Immunol* 2005; **115**:828–33.
- Yoshizawa Y, Nomauguchi H, Izaki S, Kitamura K. Serum cytokine levels in atopic dermatitis. *Exp Dermatol* 2002; **27**:225–9.
- Hon KLE, Leung TF, Ma KC, Wong CK, Wan H, Lam CWK. Serum concentration of IL-18 correlates with disease extent in young children with atopic dermatitis. *Pediatr Dermatol* 2004; **21**:619–22.
- Jung C-W, Ahn K-S, Rho N-K *et al.* Differential in vivo cytokine mRNA expression in lesional skin of intrinsic versus extrinsic atopic dermatitis patients using semiquantitative RT-PCR. *Clin Exp Allergy* 2003; **33**:1717–24.
- Rho N-K, Kim W-S, Lee D-Y, Lee J-H, Lee E-S, Yang J-M. Immunophenotyping of Inflammatory Cells in the Lesional Skin of Extrinsic and Intrinsic Type of Atopic Dermatitis. *Br J Dermatol* 2004; **151**:119–25.
- Park J-H, Choi Y-L, Namkung J-H *et al.* Characteristics of extrinsic vs. intrinsic atopic dermatitis in infancy: correlations with laboratory variables. *Br J Dermatol* 2006; **155**:778–83.
- Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am J Hum Genet* 2004; **74**:106–20.
- Bell PA, Chaturvedi S, Gelfand CA *et al.* SNPstream UHT: ultra-high throughput SNP genotyping for pharmacogenomics and drug discovery. *Biotechniques* 2002; **57**:2, 74:76–7.
- Denomme G, Van Oene M. High-throughput multiplex single-nucleotide polymorphism analysis for red cell and platelet antigen genotypes. *Transfusion* 2005; **45**:660–6.
- Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 2002; **70**:425–34.
- Westfall PH, Young SS. *Resampling-based multiple testing*. New York: Wiley, 1993.