

Original article

IL-5 and IL-5 receptor alpha polymorphisms are associated with atopic dermatitis in Koreans

Background: Eosinophils are recruited into the affected tissue of asthma and atopic dermatitis (AD) patients. IL-5 and IL-5R are highly expressed in the AD skin lesions, yet the reported levels of IL-8 are controversial.

Method: We genotyped 17 singlenucleotide polymorphisms (SNPs) from five genes of the 1120 case-control samples (646 AD and 474 controls). We measured the serum IL-5 concentrations in 87 individuals [36 ADe (AD extrinsic), 18 ADi (AD intrinsic) and 33 controls] by ELISA, and compared the results among these groups.

Result: The rs2522411SNP and haplotype T-A in the IL-5 gene were significantly associated with the ADe. The serum IL-5 concentration was higher in the ADe than that in the ADi patients without any correlation with the rs2522411SNP. In the IL-5RA gene, the rs334809SNP showed a weak association with AD, and the rs6771148SNP and the haplotype T-C-T of the three adjacent tagged SNPs had an effect on the blood eosinophil counts and the serum ECP levels in the AD patients. However, we could not detect any relationship between AD and the SNPs in the IL-8 and IL-8R genes.

Conclusion: We found that the rs2522411SNP and the haplotype T-A in the IL-5 gene and the serum IL-5 levels were strongly associated with the allergic type of AD, but not with the nonallergic type of AD. The association of the rs6771148SNP and the haplotype T-C-T in the IL5RA gene with the blood eosinophil counts and the serum ECP levels indicates that the IL5RA gene has a role for controlling eosinophils in the peripheral blood.

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Atopic dermatitis (AD) is an inflammatory skin disease that is characterized by severe itching and a chronic relapsing clinical course with typical skin lesions (1, 2). The pathophysiology of AD involves a complex series of interactions between the resident and infiltrating cells that is orchestrated by proinflammatory cytokines and chemokines. The skin lesions of AD patients are infiltrated by activated T cells, eosinophils and antigen-presenting Langerhans cells that bind and present IgE complexed allergens on their surface (3, 4). In addition, the activation of peripheral blood T cells that preferentially secrete T helper (Th) 2 cytokines and that help B cells produce IgE has been reported to be associated with AD (5–9). Various cytokines that are derived from many cell types and their effect on T cells, monocytes and the resident skin cells, through autocrine or paracrine regulation, play crucial roles in the development and regulation of inflammatory responses, according to a complex network of cytokine interactions. It has been reported that the acute skin lesions of AD patients have increased the numbers of (interleukin) IL-4, IL-5 and IL-13 mRNA

expressing cells; however, chronic skin lesion are associated with increased GM-CSF and IL-12p40 subunit mRNAs (6, 10, 11).

The eosinophil count is the hallmark of the late-stage inflammatory reaction in asthma and other allergic inflammations (12). In asthma and AD patients, eosinophils are recruited into the affected tissue to a much higher degree than happens in healthy individuals (13). Prolonged eosinophil survival apparently plays a central role in the pathogenesis of both asthma (14) and AD (15). The eosinophil lifespan is prolonged mainly via GM-CSF and IL-5 (15, 16). The number of blood eosinophils appeared to be elevated in both types of AD without any significant difference being found between the extrinsic (ADe) and intrinsic (ADi) types (3, 9, 17). A higher number of eosinophils in the lesional skin from ADe patients, compared with ADi patients, was previously demonstrated in our studies, while the blood eosinophils did not show any difference between the two types of AD patients (18, 19). These different findings between the blood and lesional skin are rather interesting results, and

it is curious how they come about. The fact that the eosinophil lifespan is prolonged via IL-5 should be helpful for understanding this phenomenon (16).

Interleukin-5 is best known to be responsible not only for the early and late stages of eosinophil differentiation, but also for the prolongation of eosinophil survival through the activation of the IL-5 receptor (IL-5R). Interleukin-5 down-regulates the tumor necrosis factor-induced eotaxin mRNA expression in eosinophils (20), and when acting as an agonist with corticosteroid inhalant treatment, it reduces the serum IL-5 levels and the peripheral blood eosinophils in asthma patients (21). In AD skin lesions, the IL-5R alpha (IL-5RA) mRNA positive cells were increased compared with the uninvolved normal skin (22), and IL-5R antagonist inhibits eosinophil infiltration by preventing the binding of IL-5 to IL-5R (23).

Interleukin-8 is a member of the C-x-C family of proinflammatory cytokines and it is constitutively expressed by resting human eosinophils. Increased intracellular amounts of IL-8 protein in the eosinophils from asthma and AD patients have been reported (24). The cellular activities of IL-8 are mediated by two receptors, that is, CXCR1 (IL-8 receptor alpha; IL8RA) and CXCR2 (IL8RB) located on chromosomes 2q34–q35 (25, 26). The two receptors have 78% homology for their amino acid sequences and they bind to IL-8 with similar affinity (27). The CXCR2 C785T (L262L) polymorphism does not result in an amino acid substitution, yet it has the potential to alter mRNA processing, stability or translation (28). There have been controversial reports concerning the expression of IL-8 in AD patients. The IL-8 plasma concentration was significantly higher in severe AD patients than that in mild or moderate AD patients (29). Lippert et al. (30) have reported on increased IL-8 levels from the leukocytes of AD patients after stimulation with birch pollen. However, a GeneChip microarray (31) and an 8 K microarray from AD patients (32) showed a reduced IL-8 expression.

Based on the biological properties involved in AD, it is hypothesized that IL-5 and IL-8 and their receptors can play important roles in the development of AD. We performed extensive genotyping for determining the single nucleotide polymorphisms (SNP) of IL-5, IL-8 and their receptor genes to ascertain their genetic association with the risk of AD. We genotyped 18 tag SNPs from five genes of the Korean AD patients and their matched controls. We found that the SNPs in the IL-5 and IL-5RA genes are associated with AD.

Materials and methods

Subjects

One thousand one hundred and twenty case-control samples, 646 patients with AD and 474 normal control subjects (NR) were recruited for this study. The AD samples were collected from the nonasthmatic atopic patients who were examined at Samsung Medical Center, Seoul, Korea. The diagnosis of AD, the criteria for the classification of AD into ADe and ADi, and the blood tests and prick tests for the allergens were the same as our previous report (18). All the 646 AD patients (M : F = 357 : 289, age range: 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, such as the ADe, ADi and indeterminate types of AD (ADind) patients (33). The ADe and ADi patients were classified according to the previous papers, 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, age range: 15.70 ± 9.47 years) were classified as ADe and 213 (M : F = 100 : 113, age range: 9.26 ± 9.74 years) were classified as ADi. Those patients who had other atopic diseases, such as asthma and allergic rhinitis and the ADind type were not included in this study. The NR subjects were recruited from medical students and volunteers (M : F = 254 : 220, age range: 23.23 ± 2.24 years) who had no history of AD skin lesions. The demographic information of the study subjects is 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>; accessed 31 May 2007). Ninety-three SNPs were selected as polymorphic markers in the region between 5 kb upstream and 5 kb downstream of the following five genes: IL-5, IL-8, IL-5 R, IL-8RA, and RB. The SNPs were genotyped for 48 independent samples from the general Korean population (data not shown). Based on these genotype results, we chose SNPs whose minor allele frequencies were >0.1. Then, the tag SNPs among the chosen SNPs were determined through the LD (linkage disequilibrium) bin approach that was implemented in the Tagger program (<http://www.broad.mit.edu/mpg/tagger>; accessed 31 May 2007). The LD bin approach defines the bins of SNPs that are in very strong LD with a specified r^2 threshold, and then one SNP is selected that represents the remaining SNPs in each bin (34). We used an r^2 threshold of 0.8. A total of 18 SNPs from the five genes were selected as the markers for the association study (Fig. 1).

Table 1. Demographic features of the three groups. The number of subjects, the mean and standard deviation for age, the IgE level, the eosinophil count and the SCORAD index are shown

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

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

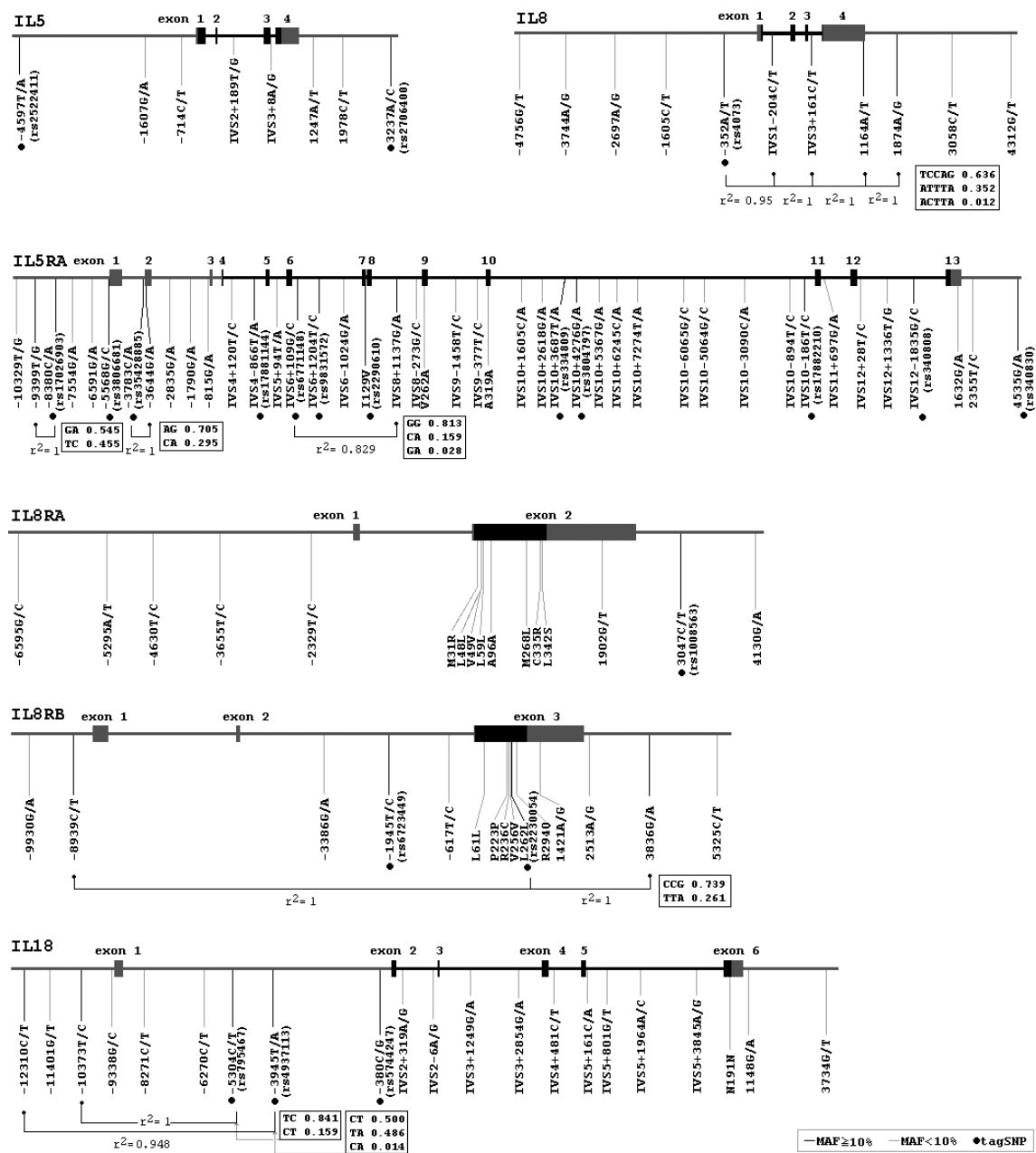


Figure 1. Map of IL-5, IL-8, IL-5RA, IL-8RA and RB on chromosome 5q31.1, 3p26–24, 4q13–21, 2q35 and 2q35, respectively. The coding exons are marked by black blocks, and the 5' and 3' UTRs are marked by gray blocks. The first base of the translation start site is denoted as nucleotide +1. The bound black dots in each gene indicate the polymorphisms that were genotyped in a large population ($n = 1120$).

Genotyping with fluorescence polarization detection

Genomic DNA was extracted from 5 ml of the whole blood by using a DNA isolation kit (Gentra Genomic DNA purification kit, Minneapolis, MN, USA) according to the protocol.

Genotype identification was performed with the GenomeLab SNPstream system [Ultra-high throughput; UHT system (35)]; this

uses multiplexed PCR in conjunction with tag array single base extension genotyping technology (Beckman Coulter, Fullerton, CA, USA) and its accompanying SNPstream software, as was previously described by Demomme and Van Oene (36). Using the 48 Korean individuals' genotype data that were used to select the tag SNPs, we compared the haplotype structure within the IL5RA and IL8RB genes that had more than three SNPs commonly genotyped for our Korean samples and also for Europeans,

Japanese, Chinese and Africans, whose genotypes are deposited in the hapmap project website (<http://hapmap.org>; accessed 31 May 2007). The LD among the SNPs in the L5RA gene in the Koreans showed a similar pattern with that of the Japanese and Chinese, and the SNPs in the IL8RB gene showed a very similar LD pattern with all the three populations except the African population (data not shown).

Statistical analysis

Chi-square tests were used to determine whether individual variants were in the Hardy-Weinberg equilibrium (HWE) at each locus in the samples. The allelic (additive allelic effect) and the genotypic effects of the individual SNPs were tested by using a logistic regression model where the gender and age were used as the adjusting covariates. The *P*-values < 0.05 were considered to be significant. The odds ratios were also estimated from the logistic regression model. Haplotype associations were tested with using the method developed by Schaid et al., which is implemented in *R* (<http://www.r-project.org>), and the method is named as haplo.glm (37). Haplo.glm provides estimates and the significance of the relative effects of the haplotypes on the trait as compared to the effect of the baseline haplotype. In this study, a haplotype that consisted of the combination of major alleles from each locus was used as a baseline. The total IgE levels were categorized into five ordered groups (<40, 40–200, 200–500, 500–2000 and ≥2000 U/ml). The cumulative logistic regression analysis was then conducted to examine the association between the genotypes and the total IgE levels. The genetic effect on the blood eosinophil counts and ECP (Eosinophilic Cationic Protein) levels in the AD subjects was tested with using a linear regression model. The log-transformed eosinophil counts and ECP levels were used in the regression analysis. Age, gender and the scoring of atopic dermatitis (SCORAD) index were used in the regression analysis model as the adjusted covariates. We performed the Kruskal-Wallis test to compare the concentrations of serum IL-5, which is not normally distributed. Statistical analysis was performed with using sas 9.1 (SAS Institute Inc., Cary, NC, USA) and *R* statistical language (<http://www.r-project.org>; accessed 31 May 2007).

Protein assay using ELISA

To test for the effect of genetic variation on the concentration of IL-5 protein in peripheral blood, we measured the IL-5 protein level in the serum by performing ELISA. We collected the serum samples from our patients and control subjects for each genotype. We selected a total of 87 available samples: 4–16 samples from each genotype in the ADe, ADi and control subjects. The experiments were performed according to the manufacturer's protocol, using the IL-5 ELISA kit (Quantikine Human IL-5 ELISA; R&D Systems; Minneapolis, MN, USA).

Result

The 18 SNPs from the five genes (IL-5, IL-8, IL-5 R, IL-8RA, and RB) were genotyped for the 1120 case-control samples (Fig. 1). The marker information, including the genomic function, chromosomal position, dbSNP id and the minor allele frequency are displayed in Table 2. As one SNP, rs2230054 on IL-8RB, was not successfully genotyped because of some technical problems, the genotype data were obtained from 17 SNPs and further analyzed. All the 17 SNPs were in HWE and the average genotyping success rate for the 17 SNPs was 98.96%.

We compared the genotype distribution of the SNPs between the AD and NR groups. Additionally, we tested the association of the SNPs with the serum IgE levels, the blood eosinophil counts and the serum ECP levels in the AD subjects.

Difference in the allelic distribution of the IL-5 polymorphism and the serum IL-5 concentrations between the AD subtypes

Two SNPs (–4597T/A; rs2522411, 3237A/C; rs2706400) were genotyped from the IL-5 gene. From the association

Table 2. The SNP markers genotyped for the case-control samples (dbSNP build 126)

Gene	ID	RS number	Chr	Position	Function	MAF*	HWE*
IL-5	–4597T/A	2522411	Chr5	131911666	Promoter	0.17	1.00
IL-5	3237A/C	2706400	Chr5	131902570	3'UTR	0.14	0.56
IL-5RA	–8380C/A	17026903	Chr3	3130047	Promoter	0.36	0.92
IL-5RA	–5568 G/C	3806681	Chr3	3127235	Promoter	0.47	0.65
IL-5RA	–3783C/A	35428885	Chr3	3125450	Promoter (intron 1)	0.25	0.38
IL-5RA	IVS4–866T/A	17881144	Chr3	3120370	Intron 4	0.10	0.43
IL-5RA	IVS6 + 109 G/C	6771148	Chr3	3118267	Intron 6	0.22	0.69
IL-5RA	IVS6 + 1204T/C	9831572	Chr3	3117172	Intron 6	0.32	0.53
IL-5RA	I129 V	2290610	Chr3	3114957	Exon 7	0.36	0.32
IL-5RA	IVS10 + 3687T/A	334809	Chr3	3105220	Intron 10	0.40	0.30
IL-5RA	IVS10 + 4276 G/A	3804797	Chr3	3104631	Intron 10	0.34	0.92
IL-5RA	IVS10–186T/C	17882210	Chr3	3093497	Intron 10	0.31	0.59
IL-5RA	IVS12–1835 G/C	340808	Chr3	3088830	Intron 12	0.38	0.28
IL-5RA	4535G/A	340830	Chr3	3083637	3'UTR	0.48	0.78
IL-8	–352A/T	4073	Chr4	74971059	Promoter	0.37	0.84
IL-8RA	3047C/T	1008563	Chr2	218852394	3'UTR	0.24	0.89
IL-8RB	–1945T/C	6723449	Chr2	218823086	Promoter (intron 2)	0.37	1.00
IL-8RB	L262L	2230054	Chr2	218825816	Exon 3	Fail	Fail

*MAF: minor allele frequency; HWE: *P*-values from the chi-square tests for Hardy-Weinberg equilibrium. MAF and HWE are calculated from the 474 normal control samples.

Table 3. (a) Comparison of the allelic and genotype frequencies of the rs2522411 SNP in the IL-5 gene and the results of testing for the association of the two loci haplotypes (rs2522411 and rs2706400) in the IL5 gene with using halpo.glm (37), while adjusting for gender and age

Group	Allele		OR (95% CI)	<i>P</i> -value†
	A	T		
Normal	779 (0.83)	165 (0.17)		
AD	991 (0.78)	277 (0.22)	1.38 (1.07, 1.78)	0.01*
ADe	646 (0.77)	198 (0.23)	1.36 (1.04, 1.78)	0.04§*
ADi	345 (0.81)	79 (0.19)	1.42 (0.91, 2.23)	0.26§

Group	Genotype			OR (95% CI)		<i>P</i> -value†‡
	AA	AT	TT	AT vs AA	TT vs AA	
Normal	390 (0.62)	211 (0.33)	33 (0.05)			
AD	321 (0.68)	137 (0.29)	14 (0.03)	1.26 (0.93, 5.36)	2.53 (1.19, 5.36)	0.03*
ADe	249 (0.59)	148 (0.35)	25 (0.06)	1.25 (0.89, 5.11)	2.36 (1.09, 5.11)	0.02§*
ADi	141 (0.67)	63 (0.3)	8 (0.04)	1.4 (0.81, 7.87)	2.12 (0.57, 7.87)	1§

Haplotype	AD vs NR			ADe vs NR			ADi vs NR		
	Frequency	<i>P</i> -value	OR (95% CI)	Frequency	<i>P</i> -value	OR (95% CI)	Frequency	<i>P</i> -value	OR (95% CI)
A-C	0.142	0.77	1.05 (0.78, 1.41)	0.144	0.8‡	1.15 (0.83, 1.58)	0.136	1‡	0.85 (0.49, 1.47)
T-A	0.198	0.01*	1.4 (1.08, 1.81)	0.200	0.03*‡	1.4 (1.06, 1.84)	0.178	0.3‡	1.4 (0.88, 2.21)
T-C	0.002	0*	1.1 (1.1, 1.1)	0.003	1‡	1.14 (0.03, 41.77)			
A-A	0.659			0.653			0.686		

*Significant at the 0.05 level.

†*P*-values were obtained from the logistic regression analysis while age and gender was used as controlling covariates.

‡*P*-values of the type III effect of the genotypes.

§Adjusted *P*-values for the multiple testing in the subgroup analysis by Bonferroni's method.

test with using the logistic regression model, the rs2522411 SNP showed a significant effect on AD, both in the allelic and genotypic models with *P*-values of 0.01 and 0.03, respectively. When we compared the allelic and genotypic distributions between the two AD subgroups (ADe and ADi) and the normal controls, the rs2522411 SNP showed a significant result on the test between the ADe patients and the normal controls, with *P*-values of 0.04 and 0.02 for the allelic and genotypic effect, respectively (Table 3a). The odds ratio of the T allele was 1.36 (95% CI: 1.04–1.78) and the odds ratios of the genotypes were 1.25 (95% CI: 0.89 and 5.11) for AT and 2.36 (95% CI: 1.09 and 5.11) for TT. However, the rs2522411 SNP did not show any significant association with ADi. This result shows that IL-5 polymorphism is only associated with the allergic type of AD. Additionally, we tested for the haplotype association with the two loci of IL5 (rs2522411, rs2706400), and the T-A haplotype showed significant associations with both AD and ADe (adjusted *P* = 0.01 and 0.03, respectively; the *P*-values were corrected for multiple testing on the subgroup analysis by using Bonferroni's method). The odds ratio of the T-A haplotype (frequency: 0.2) to the reference haplotype was 1.4 (95% CI: 1.06, 1.84). The heterogeneity of the genetic background of IL-5 among AD patients was recently reported (38). This result is consistent with ours where the genetic distributions between the ADe and ADi subgroups were different.

To investigate whether the rs2522411 SNP is associated with the serum IL-5 levels, we measured the IL-5 protein levels for the 87 available samples: 4–16 subjects with each genotype of the rs2522411 SNP from the ADe, ADi and control groups. From the statistical analysis, we did not detect any significant association of the rs2522411 SNP with the serum IL-5 concentration (Data not shown). However, we found that the serum IL-5 level of the ADe group was significantly different from that of the ADi group (*P*-value = 0.03). The mean level of the serum IL-5 protein of the ADe group (mean = 3.71, SD = 2.96, *n* = 36) was higher than that of the ADi group (mean = 2.02, SD = 1.23 *n* = 19) (Table 4). Together with the previously mentioned genetic heterogeneity of the IL-5 polymorphism, this result indicates that the classification of AD according to the presence/absence of allergy can be associated with IL-5.

In addition, we tested for the association of the SNPs in the IL-5 gene with the total serum IgE levels, the blood eosinophil counts and the serum ECP levels via performing regression analysis. However, we did not find any significant results.

Association of the IL5RA polymorphisms with the eosinophil count and the ECP level

We genotyped 12 SNPs in the IL-5RA gene and we found that two SNPs (rs334809; IVS10 + 3687T/A, and

Table 3. (b) Comparison of the allelic and genotype frequencies of the rs334809 SNP in the IL5RA gene and the results of testing for the association of the three loci haplotypes (rs334809, rs3804797 and rs17882210) in the IL5RA gene with using halpo.glm (37), while adjusting for gender and age

Group	Allele		OR (95% CI)	P-value†
	A	T		
Normal	571 (0.6)	375 (0.4)		
AD	825 (0.65)	451 (0.35)	0.81 (0.66,1)	0.05
ADe	548 (0.64)	302 (0.36)	0.83 (0.66,1.04)	0.2§
ADi	277 (0.65)	149 (0.35)	0.74 (0.51,1.08)	0.24§

Group	Genotype			OR (95% CI)		P-value†‡
	AA	AT	TT	AT vs. AA	TT vs AA	
Normal	167 (0.35)	237 (0.5)	69 (0.15)			
AD	276 (0.43)	273 (0.43)	89 (0.14)	0.68 (0.5 ,1.16)	0.75 (0.48 ,1.16)	0.02*
ADe	184 (0.43)	180 (0.42)	61 (0.14)	0.71 (0.51 ,1.23)	0.77 (0.48 ,1.23)	0.06§
ADi	92 (0.43)	93 (0.44)	28 (0.13)	0.61 (0.35 ,1.4)	0.64 (0.3 ,1.4)	0.24§

Haplotype	AD vs NR			ADe vs NR			ADi vs NR		
	Frequency	P-value	OR (95% CI)	Frequency	P-value	OR (95% CI)	Frequency	P-value	OR (95% CI)
A-A-C	0.099	0.07	0.64 (0.4, 1.03)	0.100	0.08	0.59 (0.35, 0.98)	0.104	0.94	0.75 (0.35, 1.61)
A-G-T	0.196	0.36	0.84 (0.58, 1.22)	0.197	0.58	0.81 (0.55, 1.19)	0.197	1	0.95 (0.52, 1.77)
A-G-C	0.089	0.04*	0.62 (0.39, 0.98)	0.088	0.04*	0.56 (0.34, 0.92)	0.092	1	0.86 (0.4, 1.82)
T-A-T	0.247	0.01*	0.63 (0.44, 0.91)	0.247	0.02*	0.59 (0.4, 0.87)	0.260	0.68	0.76 (0.42, 1.34)
T-A-C	0.085	0.6	0.87 (0.52, 1.46)	0.081	1	1 (0.58, 1.73)	0.074	1	0.49 (0.2, 1.23)
T-G-T	0.030	0.94	1.03 (0.44, 2.41)	0.029	1	1.05 (0.43, 2.54)	0.024	1	0.95 (0.21, 4.27)
T-G-C	0.016	0*	0.19 (0.06, 0.59)	0.016	0.02*	0.14 (0.03, 0.61)	0.025	0.44	0.37 (0.08, 1.8)
A-A-T	0.240			0.243			0.224		

*Significant at the 0.05 level.

†P-values were obtained from the logistic regression analysis while age and gender was used as controlling covariates.

‡P-values of the type III effect of the genotypes.

§Adjusted P-values for the multiple testing in the subgroup analysis by Bonferroni's method.

Table 4. The mean concentrations of the serum IL-5 levels among each group and the rs2522411 genotype

Group	Genotype	Number of subjects	Mean	Standard deviation	P-value
ADe	AA	13	4.50	3.59	0.03*
	AT	17	7.21	14.09	
	TT	6	2.30	1.74	
	Total	36	3.71	2.96	
ADi	AA	5	2.66	1.17	
	AT	7	1.32	0.69	
	TT	6	2.01	1.50	
	Total	18	2.02	1.23	
NR	AA	15	2.24	1.54	
	AT	14	3.38	1.55	
	TT	4	3.64	1.04	
	Total	33	2.90	1.58	

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

*P-values from the Kruskal–Wallis test for comparison of means between ADe and ADi.

rs6771148; IVS6 + 109G/C) could be associated with AD. When comparing the genetic distribution of the 12 SNPs between the AD and normal groups, the rs334809

genotype showed a significant association with AD ($P = 0.02$, Table 3b). However, it did not show a significant result on the subgroup analysis. The association of the haplotypes in the IL5RA gene was also tested with the use of 2-, 3-, and 4-locus haplotype windows. The T-A-T haplotype (frequency: 0.25) of the three locus rs334809, rs3804797, and rs17882210 resulted in the most significant association with ADe (adjusted $P = 0.02$). The odds ratio of the haplotype was 0.59 (95% CI: 0.40–0.87), relative to the reference haplotype.

Tests for the association of the IL-5RA SNPs with the three variables (the total serum IgE levels, the blood eosinophil counts and the serum ECP levels) were also conducted for the atopic subjects. The total IgE level did not show any significant result. However, the allele of the rs6771148 SNP had a significant effect on the log-transformed blood eosinophil counts ($P = 0.003$, GG: 5.83 ± 0.92 , $n = 399$; GC: 6.01 ± 0.85 , $n = 196$; CC: 6.1 ± 0.71 , $n = 35$) (Table 5). To control for the effect of the various courses of disease, the SCORAD index, age and gender were used as the adjusting covariates in the regression model. The ECP also resulted in a significant association with the rs6771148 SNP ($P = 0.039$), as was expected from the fact that ECP is secreted from

Table 5. Regression analysis of the blood eosinophil counts and the serum ECP levels with the genotypes of the rs6771148 SNP in the IL-5RA gene of AD patients. The number of objects, the mean of the log-eosinophil counts and the log-ECP for each genotype of the rs6771148 SNP are shown. The results of the tests for the association of the three loci haplotypes (rs17881144, rs6771148 and rs9831572) in the IL5RA gene are also shown. The haplotype association was tested for by using *haplo.glm* (37) while adjusting for gender and age

Response variable	Genotype			Estimate*	SE†	P-value‡
	GG	GC	CC			
Eosinophil	399 (5.83 ± 0.92)	196 (6.01 ± 0.85)	35 (6.1 ± 0.71)	0.16	0.05	0.003*
ECP	390 (3.61 ± 0.94)	190 (3.64 ± 1.00)	33 (3.95 ± 0.76)	0.12	0.06	0.039*
Haplotype	Frequency	Coefficient		SE	P-value	
T-G-T	0.005	-0.04		0.33	0.91	
T-C-T	0.211	0.15		0.06	0.01*	
A-G-C	0.003	0.33		0.40	0.41	
A-G-T	0.120	-0.08		0.07	0.27	
T-G-C	0.660					

*Significant at the 0.05 level.

†The *P*-value, the estimate of the effect and the standard error (SE) of the estimate were obtained from the regression analysis of the log eosinophil counts and the number of minor alleles (allelic effect), while adjusting for age, gender and the SCORAD indices.

activated eosinophils. The ECP concentration in the serum is sometimes used as an indirect measurement of the peripheral eosinophils, and Spearman's rank correlation coefficient between the ECP and the eosinophils shows the relationship (Spearman's rank correlation coefficient = 0.602, $n = 603$). In the haplotype analysis, the T-C-T haplotype (frequency: 0.21) of rs17881144, rs6771148, and rs9831572 was significantly associated with the log of the eosinophil count in the AD patient group ($P = 0.009$), while gender, age and the SCORAD index were adjusted for. The T-C-T haplotype was noted to have an effect; it increased the eosinophil count by about 16% comparing to the reference haplotype.

To sum up with the above results, IL-5RA may be related with AD via increasing the number of eosinophils.

IL8, IL8RA and IL8RB

Four SNPs from the IL8 gene and its receptor genes were selected to genotype for the case-control samples. rs4073 in the IL8 gene represents four adjacent SNPs that are in strong LD by $r^2 = 0.95-1$. rs2230054 in the IL8RB gene represents two other SNPs that are in strong LD by $r^2 = 1$, but this was not successfully genotyped. As a result, the three sets of polymorphism data from the IL8, IL8RA and IL8RB genes were analyzed. The association test of the SNPs with the AD or AD subtypes did not show significant results. The total serum IgE level, blood eosinophil count and the serum ECP level were not significantly correlated with the SNPs either.

Discussion

Through genotyping the five genes, the rs2522411 polymorphism (-4597T/A) and the haplotype T-A in the IL-5

gene showed significant differences between the ADe group and the control group. We checked the serum IL-5 levels for each genotype in each group to see whether the T/A genotype of the rs2522411 polymorphism affects the IL-5 protein levels. We could not find any statistical differences of the serum IL-5 levels for each genotype in the AD and control groups. Nevertheless, the serum IL-5 levels in the ADe group showed a significant difference (1.9-fold higher) from that of the ADi group (Table 4). We previously reported on the differences of the mRNA expression levels of IL-5 in skin samples from ADe and ADi patients by performing semiquantitative RT-PCR, and the IL-5 levels were higher in the order of ADe > ADi > control group (18). We also previously reported that the differences of dermal infiltration of eosinophils and the ECP levels among the ADe, ADi and control groups showed a similar result, as compared with the mRNA levels of IL-5 (19). Our genotyping data and the serum IL-5 levels showed the association between ADe and the IL-5 gene, which was similar to the results of our previous data. Yamamoto et al. (38) reported that the IL-5 gene might play a role in the blood eosinophilia that is associated with AD; however, we could not find any correlation between the SNP of the IL-5 gene and the serum IgE levels, the blood eosinophil counts and the serum ECP levels.

By genotyping the 12 tag SNPs in the IL-5RA gene, we found that two SNPs could be associated with AD. The rs334809 SNP showed relatively weak associations; however, the rs6771148 SNP showed significant associations with the blood eosinophilia and the serum ECP levels in the AD patient. The IL-5RA mRNA positive cells were increased in both the acute and chronic AD skin lesions and they were increased much more for the chronic AD stage than for the acute skin lesions (22). A previous study showed that the T-C exchange of 12 nucleotides

upstream of the initiation codon of the IL-5RA gene in a mouse caused differences in the amounts of gene products on a reporter gene assay, and the authors suggested the importance of the IL-5RA chain as a strong candidate in the genetics of atopy (39). However, we could not find any reports on the SNP of the IL-5RA gene for AD. Although the rs6771148 SNP and the haplotype T-C-T showed significant associations with the blood eosinophilia and the serum ECP levels in AD patients, it is hard to explain the effect of an SNP located in the intron sequences of the gene. It can be explained that an intronic SNP might not sufficiently alter the IL-5RA signaling pathway as this might require additional regulatory elements, or the significant association might be derived from the LD between the tagged SNPs that were scored and the other functionally important SNPs, which were not scored in this study (40).

As was mentioned, there is controversy concerning the expression of IL-8 in AD patients (29–32). Although we tried to verify the genetic effects of the IL-8 gene and its receptors through genotyping between the AD patients and the control, we could not find any correlations of the SNPs of the IL-8, IL-8RA, and IL-8RB genes in this study. This will require further genotyping of larger samples or the use of other genetic methods with AD patients.

In conclusion, by genotyping 17 SNPs from five genes in 1120 case-control samples, we found that the rs2522411

SNP and the haplotype T-A in the IL-5 gene were significantly associated with the susceptibility to ADe, and the serum IL-5 concentrations were higher in the ADe subjects than those in the ADi subjects without any correlation with the IL-5 polymorphism. These data suggest that the classification of AD into ADe and ADi can be associated with the IL-5 gene. We also found that the rs334809 polymorphism in the IL-5RA gene showed a weak association with AD, and the rs6771148 polymorphism and the haplotype T-C-T had an effect on the blood eosinophil counts and the serum ECP levels in the AD patient. These results indicate that the IL-5RA gene has a role for controlling the eosinophils in the peripheral blood. However, we could not detect any relationship between the polymorphisms in the IL8, IL8RA and IL8RB genes and AD.

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