



Association of *IL13* genetic polymorphisms with atopic dermatitis Fine mapping and haplotype analysis

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ARTICLE INFO

Article history:

Received for publication March 5, 2020.

Received in revised form April 16, 2020.

Accepted for publication April 22, 2020.

ABSTRACT

Background: Although previous studies had reported an important role of interleukin 13 (*IL13*) and its genetic polymorphisms in atopic dermatitis (AD), many of these previous reports focused on the missense variant rs20541 (Gln144Arg) without fine mapping of the gene region.

Objective: To analyze the potential associations of other *IL13* variants and their haplotypes with AD and assess total serum immunoglobulin E (IgE) levels.

Methods: We performed fine mapping of single-nucleotide polymorphisms (SNPs) within the *IL13* gene in a pilot study of 495 children with AD and 444 healthy controls. Then, we conducted a replication study of 757 children with AD and 1620 healthy controls to evaluate the association between the rs20541 variant of *IL13* and AD.

Results: In the pilot study, the rs20541 and rs1295685 SNPs in the 3'-untranslated region of *IL13* had significant associations with AD ($P < .001$ and $.01$, respectively). In addition, 2 haplotypes (BL2_ht1 and BL2_ht2), which harbored the significant rs20541 and rs1295685 SNPs, had an association with AD (minimum $P = .006$). BL2_ht1 and BL2_ht2 had nominal signals associated with the total serum IgE levels ($P < .05$) but not with the severity of AD ($P > .05$). In the replication study, rs20541 was associated with the total serum IgE levels but not with the severity of AD.

Conclusion: An additional *IL13* gene SNP, rs1295685, has a strong linkage disequilibrium with rs20541, and its haplotypes are associated with AD and the total serum IgE levels.

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Disclosures: The authors have no conflicts of interest to report.

Funding: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation funded by the Korean government (grant no.: 2017M3A9F3043834).

<https://doi.org/10.1016/j.anai.2020.04.023>

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Introduction

Atopic dermatitis (AD) is a chronic pruritic inflammatory skin disease that causes substantial socioeconomic burden. Skin barrier dysfunction and immune dysregulation are key pathophysiologies of AD.^{1–3} Interleukin 13 (IL13) is a key type 2 cytokine that drives inflammations in the periphery and functions in the recruitment of inflammatory cells and in inducing alterations to the skin microbiome.² With the production of other cytokines, IL13 also affects the expression levels of skin barrier proteins, such as filaggrin.⁴ The

associations between IL13 and AD might affect the clinical course of AD in that only early-onset AD with the persistent phenotype had considerably increased levels of IL13 in the serum than those in healthy controls.³

Previous studies had identified that single-nucleotide polymorphisms (SNPs) in the *IL13* gene, including rs1295686, and rs20541, are associated with AD,^{5,7} although no such associations had been identified in another report.⁸ The *IL13* promoter gene polymorphism –1112 C/T is known to affect total serum immunoglobulin E (IgE) levels and severity of AD,⁹ which suggested different roles of *IL13*, depending on the genetic background. However, there has been no fine mapping of *IL13* polymorphisms that were associated with AD. In addition, total serum IgE levels, which are closely linked to allergic diseases, are intricately affected by genetic factors, including polymorphisms of *IL13*.^{5–7} Previous linkage studies of *IL13* polymorphisms and total serum IgE levels were limited because only partial associations between them had been identified.^{10,11}

Genetic association studies had been performed to identify genetic markers, such as SNPs, by comparing specific diseases and healthy groups.¹² However, such association studies need to be performed at haplotype levels, which mean an evaluation of the combination of SNPs at a given locus, to properly define the genetic association. Although haplotypes and SNPs had been identified in diverse diseases, including cancer,¹³ such reports were lacking for AD.

In this study, we conducted fine mapping of *IL13* SNPs in children with AD and healthy children and identified other potential variants of *IL13* and their haplotypes that have associations with AD. We further analyzed the associations among total serum IgE levels, severity of AD, and SNPs of *IL13*.

Methods

Pilot Study

The study populations included 939 children, including 495 children with AD and 444 controls (Table 1). Children received a diagnosis of AD by pediatric allergists at the time of clinic visit based on the criteria of Hanifin and Rajka.¹⁴ The SCORing Atopic Dermatitis (SCORAD) index was calculated at the time of AD diagnosis with blood sampling for measurement of total serum IgE levels and genetic testing. This study was approved by the institutional review board at Asan Medical Center, and written informed consent was obtained from all parents.

Replication Study

A replication study for the most significant *IL13* SNP was performed in an independent, larger population of 2377 children from the Cohort for Childhood Origin of Asthma and allergic diseases birth cohort study¹⁵ and the Panel Study on Korean Children birth cohort study (Table 1).¹⁶ This study was approved by the Asan Medical Center Institutional Review Board, and written informed consent was again obtained from all parents.

Genotyping of Single-Nucleotide Polymorphisms

For SNP selection, *IL13* SNPs' genotype data of Asian populations (Chinese and Japanese) were downloaded from the 1000 Genomes Project (<https://www.internationalgenome.org/1000-genomes-browsers/>), within the range of the exon/intron and regulatory (2 kb upstream and 1 kb downstream) regions of the gene. Then, on the basis of the pairwise tagging, a number of tagged SNP blocks, from which 1 representative SNP was selected for each block, were constructed. When the representative SNPs were chosen from a tagging group, the following conditions were considered: (1) common SNPs with a minor allele frequency >0.05; (2) linkage disequilibrium

Table 1

Clinical Characteristics of the Study Populations

Characteristic	Children with AD	Unaffected controls	P value
Pilot study			
Sample size, No. (%)	495 (52.7)	444 (47.3)	NA
Age, mean (SD), y	7.0 (3.6)	11.9 (2.1)	<.001
Male sex, %	54.3	41.0	<.001
SCORAD index, mean (SD)	69.72 (18.33)	NA	NA
Total serum IgE, mean (SD), kU/L	684.7 (1441.7)	119.9 (225.1)	<.001
Blood eosinophils, mean (SD), %	6.3 ± 4.3	2.4 (1.6)	<.001
Replication study			
Sample size, No. (%)	757 (31.8)	1620 (68.2)	NA
Age, mean (SD), y	7.7 (2.4)	6.9 (2.5)	<.001
Male sex, %	438 (57.9)	826 (51.2)	.002
SCORAD index, mean (SD)	23.7 (61.7)	NA	NA
Total serum IgE, mean (SD), kU/L	223.3 (451.6)	106.8 (252.8)	<.001

Abbreviations: AD, atopic dermatitis; IgE, immunoglobulin E; NA, not applicable; SCORAD, SCORing Atopic Dermatitis.

(LD); (3) locations (SNPs in exons were preferred); and (4) amino acid changes (nonsynonymous SNPs were preferred). As a result, 6 common or nonsynonymous *IL13* SNPs were selected. Genomic DNAs in the peripheral blood were isolated using the WizPrep gDNA Mini Kit (Wizbio Solutions, Seongnam, South Korea). Genotyping was performed using the Fluidigm SNP Type assay (Fluidigm, South San Francisco, California) and the TaqMan assay (Applied Biosystems—Thermo Fisher Scientific, Waltham, Massachusetts) in accordance with the manufacturer's instructions.

Measurement of Total Serum Immunoglobulin Levels

Total serum IgE levels were measured using a fluorescent enzyme immunoassay in accordance with the manufacturer's instructions (ImmunoCAP system; Phadia AB, Uppsala, Sweden).¹⁷ The lower detection limit for total serum IgE was 2.0 kU/L (range, 2–5000 kU/L). If the levels of total serum IgE were >5000 kU/L, we verified the results after diluting the samples with a sample diluent at a 1:5 ratio. If the repeat value was still >5000 kU/L, the samples were further diluted to obtain an end point value of up to 50,000 kU/L.

In Silico Analysis

In silico analysis for the change of messenger RNA (mRNA) folding structure was performed using the mfold Web Server (<http://mfold.rna.albany.edu/?q=mfold>).

Statistical Analysis

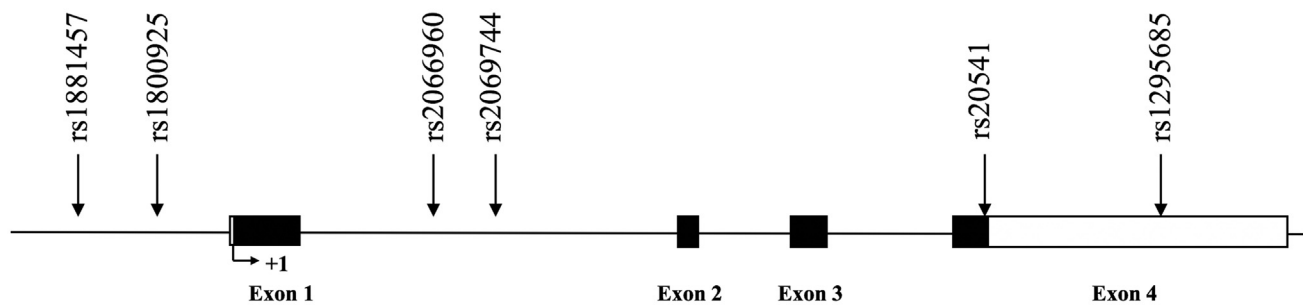
Logistic and linear regression analyses were performed to identify associations between *IL13* SNPs and haplotypes with AD and their related clinical features, including severity and total serum IgE levels. SAS software, version 9.4 (SAS Institute Inc, Cary, North Carolina) was used. For multivariate analysis, age and sex were adjusted. $P \leq .05$ was considered to indicate a significant LD between the SNPs using the Haploview software, version 4.2 (<http://www.broadinstitute.org/mpg/haploview>). Haplotypes were inferred using the PHASE software.¹⁸

Results

Characteristics of the Study Populations

The characteristics of the study populations are described in Table 1. In the pilot study, children with AD were significantly younger than the controls. The mean SCORAD index was 69.72, and mean (SD) total serum IgE levels were significantly increased in children with AD than those in children without AD (684.7 [1441.7] and 119.9 [225.1], respectively; $P < .01$). In the replication study, the

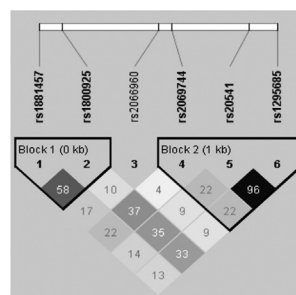
A Map of *IL-13* (interleukin 13) on chromosome 5q31.1 (2.94 kb) NM_002188.2



B Haplotypes of *IL-13*

BL1			BL2		
Haplotype	rs1881457	rs1800925	Haplotype	rs20669744	rs20541
BL1_ht1	A	C	BL2_ht1	C	C
BL1_ht2	C	T	BL2_ht2	C	T
BL1_ht3	C	C	BL2_ht3	T	T
Others	.	.	Others	.	.
Freq.	0.700	0.201	Freq.	0.647	0.227
	0.098	0.001		0.113	0.013

C LD plot



D Association between total serum IgE and rs20541

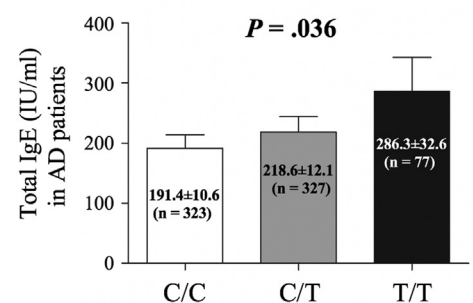


Figure 1. Position of the single-nucleotide polymorphisms analyzed in the (A) *IL13* gene variants and (B) their haplotypes. (C) Linkage disequilibrium (LD) of *IL13* SNPs. Values in the LD box represent coefficient r^2 value. (D) IgE and rs20541 association. AD, atopic dermatitis; IgE, immunoglobulin E; LD, linkage disequilibrium; SNPs, single-nucleotide polymorphisms.

mean total serum IgE levels and SCORAD index in children with AD were lower in the replication group than in the pilot study group (Table 1).

Single-Nucleotide Polymorphisms Selection and Genotyping

A total of 6 polymorphisms for the *IL13* gene were selected for evaluation (Fig 1A), and all SNPs were successfully genotyped in all the study participants. Using pairwise comparisons based on LD

coefficients ($|D'|$ and r^2), LD blocks were calculated; 3 and 2 common haplotypes (Fig 1B and C) with a frequency above 5% were inferred for each haplotype block BL1 and BL2.

Associations of Interleukin 13 Single-Nucleotide Polymorphisms and Haplotypes With Atopic Dermatitis

In the pilot study, the allele frequencies of the SNPs between children with AD and controls are listed in Table 2. Among the

Table 2
Associations of *IL13* Single-Nucleotide Polymorphisms and Their Haplotypes With Atopic Dermatitis in the Pilot Study

SNP	Position	Minor allele	MAF		Genetic model								
			AD cases (n = 495)	Controls (n = 444)	Additive			Dominant			Recessive		
					aOR ^a (95% CI)	P	P _{cor}	aOR ^a (95% CI)	P	P _{cor}	aOR ^a (95% CI)	P	P _{cor}
rs1881457	Promoter	C	0.305	0.293	1.07 (0.83–1.39)	.61	NA	1.13 (0.80–1.59)	.50	NA	0.99 (0.56–1.78)	.99	NA
rs1800925	Promoter	T	0.215	0.188	1.27 (0.94–1.72)	.12	NA	1.43 (0.99–2.04)	.05	NA	0.92 (0.39–2.20)	.85	NA
rs2066960	Intron	A	0.340	0.323	1.00 (0.78–1.29)	.99	NA	1.05 (0.74–1.48)	.81	NA	0.90 (0.53–1.53)	.71	NA
rs2069744	Intron	T	0.123	0.109	1.26 (0.86–1.84)	.23	NA	1.33 (0.88–2.00)	.17	NA	0.73 (0.13–4.08)	.72	NA
rs20541	Exon	T	0.369	0.322	1.40 (1.09–1.80)	.008	.048	1.57 (1.11–2.22)	.01	NS	1.56 (0.94–2.61)	.09	NA
rs1295685	3'UTR	A	0.365	0.321	1.39 (1.08–1.79)	.01	NS	1.52 (1.07–2.15)	.02	NS	1.60 (0.95–2.68)	.08	NA
Frequency			Additive			Dominant			Recessive				
			aOR ^a (95% CI)	P	P _{cor}	aOR ^a (95% CI)	P	P _{cor}	aOR ^a (95% CI)	P	P _{cor}		
BL1_ht1	0.307	0.293	1.09 (0.84–1.41)	.53	NA	1.16 (0.82–1.63)	.41	NA	1.00 (0.56–1.78)	.99	NA		
BL1_ht2	0.213	0.188	1.25 (0.93–1.69)	.14	NA	1.39 (0.97–2.00)	.07	NA	0.92 (0.39–2.20)	.85	NA		
BL1_ht3	0.092	0.105	0.79 (0.52–1.19)	.26	-	0.76 (0.49–1.18)	.22	NA	1.23 (0.17–9.14)	.84	NA		
BL2_ht1	0.374	0.329	1.38 (1.07–1.77)	.01	NS	1.49 (1.05–2.11)	.03	NS	1.61 (0.97–2.67)	.06	-		
BL2_ht2	0.244	0.208	1.41 (1.05–1.88)	.02	NS	1.44 (1.01–2.05)	.047	NS	2.03 (0.94–4.35)	.07	NA		
BL2_ht3	0.119	0.107	1.25 (0.85–1.84)	.25	NA	1.35 (0.90–2.04)	.15	NA	0.38 (0.05–2.80)	.34	NA		

Abbreviations: aOR, adjusted odds ratio; MAF, minor allele frequency; NA, not applicable; NS, not significant; P_{cor}, P value after the Bonferroni correction; SNP, single-nucleotide polymorphism; UTR, untranslated region.

^aAdjusted for age and sex as covariates.

Table 3Comparisons of Total Serum IgE Levels According to *IL13* Single-Nucleotide Polymorphisms and Their Haplotypes in the Pilot Study

SNP	Position	Minor allele	Total serum IgE, mean (SD), IU/L			P values for genetic models ^a		
			M/M	M/m	m/m	Additive	Dominant	Recessive
rs1881457	Promoter	C	601.4 (40.7) (n = 218)	633.6 (46.3) (n = 187)	622.1 (92.7) (n = 45)	.79	.74	.97
rs1800925	Promoter	T	572.6 (34.6) (n = 274)	698.7 (55.8) (n = 157)	578.2 (132.6) (n = 19)	.44	.34	.89
rs2066960	Intron	A	690.3 (48.7) (n = 201)	511.6 (36.6) (n = 195)	723.3 (98.4) (n = 54)	.66	.23	.37
rs2069744	Intron	T	614.0 (33.2) (n = 342)	638.6 (62.3) (n = 105)	186.1 (107.4) (n = 3)	.72	.78	.59
rs20541	Exon	T	525.2 (38.9) (n = 182)	732.4 (51.8) (n = 200)	522.3 (63.3) (n = 68)	.73	.19	.27
rs1295685	3'-UTR	A	528.7 (38.9) (n = 185)	754.7 (53.6) (n = 198)	452.8 (55.3) (n = 67)	.99	.22	.09
BL1_ht1			366.8 (17.8) (n = 425)	402.7 (21.8) (n = 341)	372.9 (39.7) (n = 88)	.77	.65	.94
BL1_ht2			348.3 (14.8) (n = 551)	456.1 (28.2) (n = 262)	356.8 (55.7) (n = 41)	.22	.13	.92
BL1_ht3			398.3 (15.2) (n = 691)	315.6 (25.4) (n = 155)	235.6 (83.3) (n = 8)	.21	.23	.54
BL2_ht1			301.8 (15.7) (n = 368)	465.0 (24.3) (n = 366)	373.0 (34.0) (n = 120)	.09	.01	.91
BL2_ht2			330.5 (14.6) (n = 515)	494.9 (29.4) (n = 284)	277.8 (37.5) (n = 55)	.13	.02	.30
BL2_ht3			376.9 (14.5) (n = 672)	413.0 (31.4) (n = 173)	140.0 (46.7) (n = 9)	.98	.85	.37

Abbreviations: BL, haplotype block; ht, haplotype; IgE, immunoglobulin E; M/M, homozygote of the major allele; M/m, heterozygote of the major allele; m/m, homozygote of the minor allele; SNP, single-nucleotide polymorphism; UTR, untranslated region.

^aAdjusted for age and sex as covariates.

several *IL13* SNPs with a minor allele frequency above 0.05, significant associations were identified between AD and rs20541 in the exon and rs1295685 in the 3'-untranslated region (UTR) (under additive models, $P = .008$ for rs20541 and $P = .01$ for rs1295685). Then, we performed haplotype analysis to identify genetic associations with AD. The haplotypes BL2_ht1 and BL2_ht2 were associated with AD ($P = .01$ and $P = .02$, both under an additive model, respectively).

Comparisons of Total Serum Immunoglobulin E Levels and SCORing Atopic Dermatitis Index according to *IL13* Single-Nucleotide Polymorphisms and Their Haplotypes

In linear regression analysis adjusted for age and sex as covariates, BL2_ht1 and BL2_ht2 had statistically significant signals in terms of an association with the total serum IgE levels under the dominant model ($P = .01$ and $P = .02$, respectively; Table 3). However, when the severity of AD was compared, as reflected by the SCORAD index, no significant differences were found between the *IL13* SNPs and their haplotypes (Table 4).

Association Between *IL13* rs20541 and Atopic Dermatitis in the Replication and Combined Study

We identified that *IL13* rs20541 had the most significant association with AD in our current analyses. To validate this result, we performed replication analysis using another independent cohort and combined analysis of the association of *IL13* rs20541 and AD. Even in the replication and combined analysis, *IL13* rs20541 had

consistent and significant associations with AD (adjusted odds ratio, 1.20; 95% confidence interval, 1.05–1.37; $P = .007$; and adjusted odds ratio, 1.25; 95% confidence interval, 1.12–1.39; $P < .001$, respectively; Table 5).

Comparisons of Total Serum Immunoglobulin E Levels and SCORing Atopic Dermatitis Index in Association With *IL13* rs20541 in the Replication Study

In the participants of the replication study, there were significant differences in total serum IgE levels in association with *IL13* rs20541 polymorphisms but not in the SCORAD index (Fig 2).

Discussion

We had identified that the rs20541 SNP in the exon and the rs1295685 variant in the 3'-UTR of the *IL13* gene are significantly associated with AD. In addition, the 2 haplotypes of *IL13*, BL2_ht1 and BL2_ht2, were also associated with AD. In further linear regression analysis, BL2_ht1 and BL2_ht2 had nominal signals in association with total serum IgE levels but not with the severity of AD. Analysis of the replication study and combined analyses from pilot and replication studies revealed that rs20541 has consistent associations with AD. Although further functional evaluations will be needed, our analyses had identified for the first time that the *IL13* SNP rs1295685 is also potentially associated with AD because it showed a strong LD with rs20541. Moreover, the corresponding haplotypes are associated with AD and total serum IgE levels that play a pivotal role in the pathophysiology of AD. Our study had thus

Table 4Comparisons of SCORing Atopic Dermatitis Index Scores According to *IL13* Single-Nucleotide Polymorphisms and Their Haplotypes in the Pilot Study

SNP	Position	Minor allele	SCORAD index score, mean (SD)			P values for genetic models ^a		
			M/M	M/m	m/m	Additive	Dominant	Recessive
rs1881457	Promoter	C	68.62 (7.31) (n = 88)	70.10 (7.74) (n = 82)	72.53 (16.64) (n = 19)	.62	.63	.77
rs1800925	Promoter	T	69.31 (6.52) (n = 113)	68.43 (8.49) (n = 65)	80.46 (24.26) (n = 11)	.53	.93	.14
rs2066960	Intron	A	70.10 (7.47) (n = 88)	68.55 (7.92) (n = 75)	71.35 (13.99) (n = 26)	.75	.97	.49
rs2069744	Intron	T	69.48 (5.75) (n = 146)	70.88 (11.07) (n = 41)	57.80 (40.87) (n = 2)	.81	.97	.35
rs20541	Exon	T	69.71 (7.84) (n = 79)	68.00 (7.75) (n = 77)	73.39 (12.78) (n = 33)	.71	.86	.34
rs1295685	3'-UTR	A	69.56 (7.73) (n = 81)	68.91 (7.90) (n = 76)	71.68 (12.67) (n = 32)	.91	.94	.75
BL1_ht1			69.08 (7.41) (n = 87)	69.60 (7.64) (n = 189)	72.73 (16.64) (n = 19)	.79	.87	.77
BL1_ht2			68.95 (6.46) (n = 114)	69.05 (8.63) (n = 64)	80.46 (24.26) (n = 11)	.37	.69	.14
BL1_ht3			69.98 (5.58) (n = 157)	68.05 (12.42) (n = 30)	68.45 (48.40) (n = 2)	.61	.62	.84
BL2_ht1			69.96 (7.92) (n = 78)	68.03 (7.75) (n = 77)	72.65 (12.46) (n = 34)	.89	.73	.47
BL2_ht2			69.21 (6.57) (n = 111)	70.11 (8.98) (n = 61)	70.93 (17.20) (n = 17)	.77	.79	.83
BL2_ht3			69.34 (5.72) (n = 147)	71.40 (11.29) (n = 40)	57.80 (40.87) (n = 2)	.95	.86	.35

Abbreviations: BL, haplotype block; ht, haplotype; M/M, homozygote of the major allele; M/m, heterozygote of the major allele; m/m, homozygote of the minor allele; SNP, single-nucleotide polymorphism; UTR, untranslated region.

^aAdjusted for age and sex as covariates.

Table 5
Replication and Combined Analysis of the Most Significant *IL13* Single-Nucleotide Polymorphism rs20541 in Association With Atopic Dermatitis

SNP	Position (amino acid change)	Variation	Study stage	MAF		aOR ^a (95% CI)	P value
				AD cases	Controls		
rs20541	Missense (Gln144Arg)	C>T	First stage	0.369 (n = 495)	0.322 (n = 443)	1.40 (1.09-1.80)	.008
			Second stage (replication)	0.329 (n = 757)	0.294 (n = 1620)	1.20 (1.05-1.37)	.007
			Combined	0.346 (n = 1252)	0.300 (n = 2063)	1.25 (1.12-1.39)	<.001

Abbreviations: AD, atopic dermatitis; aOR, adjusted odds ratio; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

^aAdjusted for age and sex as covariates.

made important inroads into our understanding of the AD association with *IL13* using fine mapping and haplotype analysis of polymorphisms.

AD is a heterogeneous disorder with diverse clinical courses ranging from very mild forms of dryness to severe symptoms, unresponsive to treatment. These outcomes can be predicted by identifying the underlying pathophysiologic mechanism of AD. Previous studies on the association between *IL13* genetic polymorphisms and AD had found that these associations can be affected by ethnicity.^{8,9,19} In addition, these previous reports had enrolled individuals across significantly different age groups, and most did not consider clinical phenotypes, including severity of AD, which may differ according to genetic background. In this study, we included children with relatively severe and persistent AD (ie, a mean SCORAD index of 69.72 and a mean age of 7.0 years at the time of enrollment).²⁰ Notably, approximately 11.3% of children with AD have AD symptoms at 6 years of age as early persistent or later AD phenotypes. Hence, the results of this study indicated a positive association between the 2 significant *IL13* SNPs rs20541 and rs1295685, the haplotypes BL2_ht1 and BL2_ht2, AD, and total serum IgE levels in Korean children with severe and persistent AD phenotypes.

This association is likely to be more helpful for developing future mechanistic or precision therapies for AD compared with a consideration of only *IL13* polymorphisms. The *IL13* rs20541 polymorphism had been reported to be associated with AD in both white and Asian populations.^{5,21} However, a previous Japanese study in young girls reported no association between rs20541 polymorphisms and AD.²² Some of the previous reports on the positive association between *IL13* rs20541 polymorphisms and AD had suggested that an elevated total serum IgE level may play a key role in the susceptibility to AD.⁵ However, some debate remains in the association between *IL13* rs20541 polymorphisms and total serum IgE levels because not all reports had identified a positive association.^{23,24} This inconsistency may be partially attributable to variations in the total serum IgE levels according to age, even in the

same person.²⁵ In this study, we could not confirm the associations between *IL13* rs20541 polymorphisms and total serum IgE levels, although the BL2_ht1 and BL2_ht2 haplotypes had a positive association with AD and total serum IgE levels in our analysis. We surmised that the sample size, characteristics of the study populations, and allergic disease phenotypes might affect these associations. According to the evidence from previous studies and the present investigation, *IL13* rs20541 polymorphisms are involved in the susceptibility to AD, which is partially mediated through the IgE pathway. Future studies of these associations need to be combined with phenotype classifications of AD.

With regard to *IL13* rs1295685 polymorphisms, most previous reports had identified associations of these variants with respiratory allergic diseases, including asthma, and with lung function or chronic obstructive pulmonary diseases.^{26,27} Another previous study had identified a possible association between rs1295685 polymorphism and psoriasis.²⁸ The *IL13* rs1295685 SNP is associated with elevated levels of cord blood IgE and total serum IgE in adults.^{19,27} However, studies on the *IL13* rs1295685 polymorphism and AD are still lacking. We identified a possible association between rs1295685 and AD that may be linked to an increased total serum IgE level. Structures of 3'-UTRs and/or its genetic variants are important for stability and expression of mRNAs (eg, rs3480 at 3'UTR of *FNDC5*).^{29,30} Therefore, to investigate the possible role in mRNA structure for the significant rs1295685 in 3'-UTR region of *IL13*, in silico analysis was performed. Interestingly, the possible changes depending on major G allele and minor A allele were predicted (Fig 3), suggesting that changes from major allele to minor allele of rs1295685 might influence mRNA folding and stability.

There were some limitations of this study. First, total serum IgE levels and SCORAD index were measured only at the time of enrollment, and this single value restricted our ability to identify the association with *IL13* polymorphisms. The significant results in both the more severe AD group in the pilot study, which likely maximized the differences among genetic factors underlying the

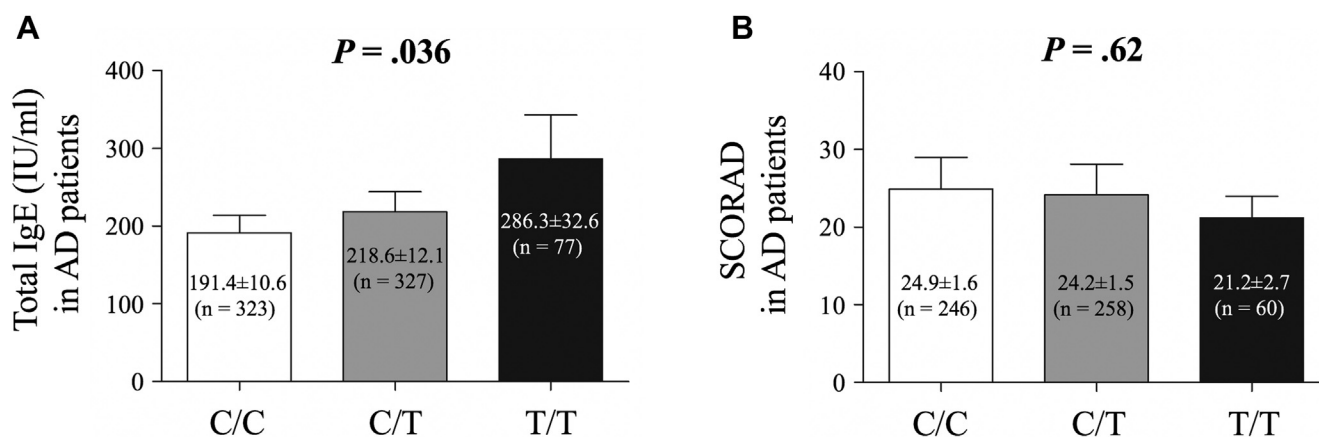


Figure 2. Association between the total serum IgE levels (A), SCORing Atopic Dermatitis (SCORAD) index (B), and rs20541 single-nucleotide polymorphism in the replication study. AD, atopic dermatitis; SCORAD, SCORing atopic dermatitis; SNP, single-nucleotide polymorphism.

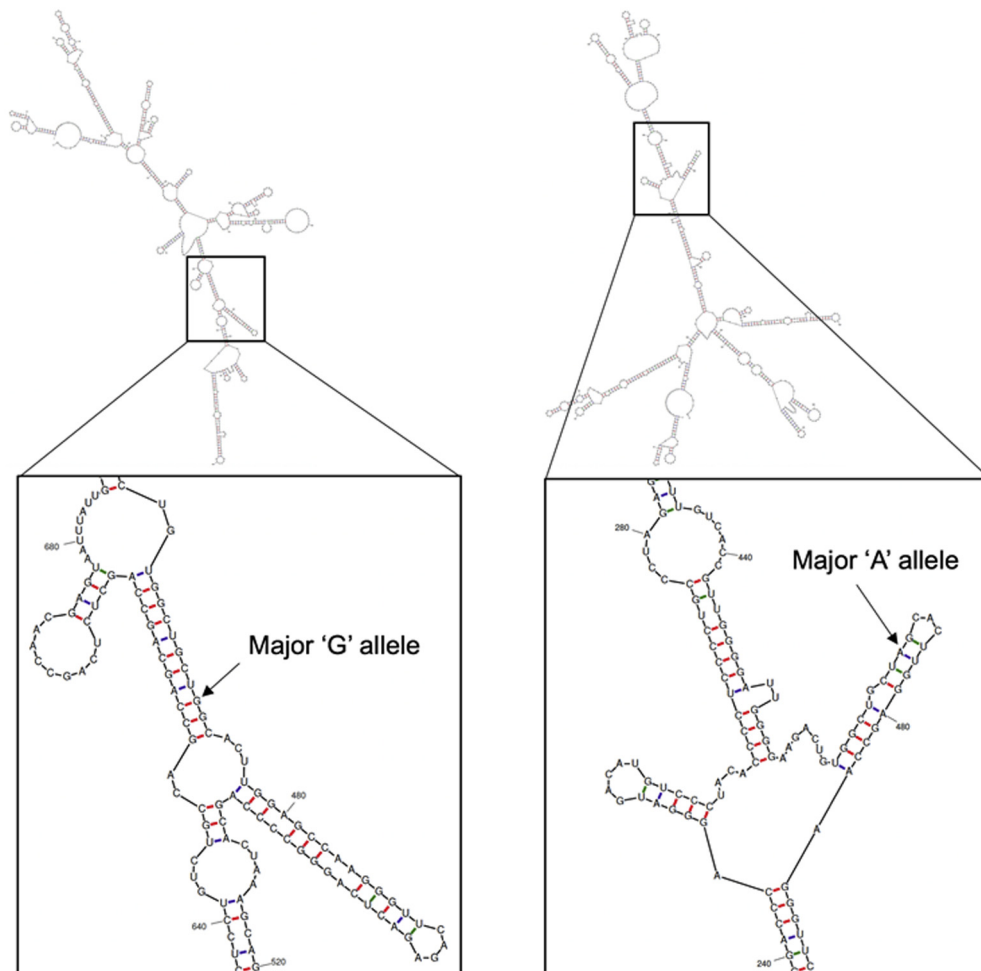


Figure 3. Comparison of messenger RNA folding structures between major G allele and minor A allele of rs1295685. Each modeled structure for major G allele and minor A allele of rs1295685 in the 3'-untranslated region (UTR) of *IL13* was predicted by mfold (<http://mfold.rna.albany.edu/?q=mfold>). The 800 nucleotide sequences from 3'-UTR region, including rs1295685, were used for predicting mRNA folding structure. (A) Major G allele of rs1295685. (B) Minor A allele of rs1295685.

pathophysiologic mechanisms of AD, and those with diverse ranges of AD severity in the replication cohort were likely to have increased the power of the data. Although no significant association between *IL13* SNPs and AD severity was identified in children with relatively severe AD in the pilot study, this finding was verified in the replication study that covered children with diverse AD severity (ranging from mild to severe). Therefore, the results of this study may be applied to children with diverse AD severity. Further functional studies on the newly identified *IL13* SNP rs1295685 are needed to elucidate its role in the pathophysiologic mechanisms of AD. In addition, replication studies in other ethnic populations are also warranted.

In conclusion, rs1295685 is an additional *IL13* SNP that showed a strong LD with rs20541, and its haplotypes are associated with AD and total serum IgE levels. These findings may be helpful in the future development of precision medicine or targeted therapies for the management of AD.

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