

Original article

Interleukin-10 haplotype associated with total serum IgE in atopic dermatitis patients

Background: The genetic background of atopic dermatitis (AD) is not clearly understood. Interleukin (IL)-10 is a powerful Th-2 cell cytokine produced by lymphoid cells that exerts its function by inhibiting macrophage/monocyte and T-cell lymphocyte replication and secretion of inflammatory cytokines [IL-1, tumour necrosis factor- α (TNFA), IL-6, IL-8 and IL-12].

Objective: In an effort to discover additional polymorphism(s) in genes whose variant(s) have been implicated in total immunoglobulin E (IgE) level in AD patients, we scrutinized the single nucleotide polymorphisms (SNPs) in the *IL10* gene as a potent candidate for contributing to the level of IgE in serum.

Methods: We recruited 334 AD patients and assayed their serum total IgE levels using the LIPA-200 system. Four SNPs in the *IL10* gene were genotyped using the single-base extension (SBE) method. Logistic regression analyses were performed with single polymorphisms and haplotypes (ht) to determine their association with the level of serum total IgE.

Results: Genetic association analysis of total serum IgE in AD patients revealed that one of the *IL10* ht, *IL10-ht2*, was associated with decreased serum total IgE in gene dose-dependent manner ($P = 0.02$ – 0.001).

Conclusions: It was predicted that the inhibition of innate immunity by increased IL-10 production in *IL10-ht2*-bearing individuals might be associated with decreased total serum IgE levels among AD patients. The greater effects of *IL10* ht on decreased total serum IgE levels suggest that the effect of IL-10 polymorphism might be the result of a combined genotype (ht) rather than single polymorphisms.

H. D. Shin¹, B. L. Park¹, L. H. Kim¹, J.-S. Kim², J.-W. Kim²

¹Department of Genetic Epidemiology, SNP Genetics, Inc., Seoul, Korea; ²Department of Dermatology, College of Medicine, The Catholic University of Korea, Seoul, Korea

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Dr Jin-Wou Kim
Department of Dermatology
College of Medicine
The Catholic University of Korea
620-56 Junnong 2 Dong
Dongdaemun-Gu
Seoul
Korea 130-709

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Atopic dermatitis (AD) is a common multifactorial disease that seems to be affected by both genetic and environmental factors. A strong genetic component determining the risk of AD has been established through twin study. The pairwise concordance rate in monozygotic twins was 0.72, compared with 0.23 in dizygotic twins. The magnitude of the concordance rates indicates that genetic factors are decisive in the development of AD (1). The risk of a child having AD is 50% if one parent has atopic disease (AD, asthma, or allergic rhinitis) and 75% if both parents are affected. Genetic factors are therefore thought to play a dominant role in predisposing to this condition. Atopy is understood to be an imbalance between the T_H2- and T_H1-type immune responses, with the cutaneous inflammatory response in AD being at least

partly mediated by T_H2 cytokines [interleukin (IL)-4, IL-13, and IL-5] (2, 3). However, rather than T_H1-type cytokines (interferon- γ , IFNG) down-regulating the allergic immune response, recently they have been found to exacerbate the inflammatory process (4). A third group of cytokines, which includes transforming growth factor- β_1 (TGFB) and IL-10, has been found to have general immunosuppressive activity, blocking both T_H1- and T_H2-type cytokine activity (5, 6). Although a number of candidate genes in various gene clusters (5q, 6p, 11q13, 12q15–24.1, and 14q), especially those which help determine the production or activity of T_H1 and T_H2 cytokines, have been suggested to predispose to atopic disease, the importance of these genetic factors is still unclear (7–9).

Interleukin-10 is a powerful T_H2-cell cytokine produced by lymphoid cells that exerts its function by inhibiting macrophage/monocyte and T-cell lymphocyte replication and secretion of inflammatory cytokines [IL-1, tumour necrosis factor- α (TNFA), IL-6, IL-8, and IL-12]

Abbreviations: AD, atopic dermatitis; IL-10, interleukin-10; IgE, immunoglobulin E; ht, haplotype; SNP, single nucleotide polymorphisms; IFNG, interferon- γ ; T_H2 cell, T-helper 2 cell; LD, linkage disequilibrium.

(10, 11). In an effort to discover additional polymorphism(s) in genes of those variant(s) that have been implicated in total immunoglobulin E (IgE) levels in AD patients, we scrutinized the single nucleotide polymorphisms (SNPs) in the *IL10* gene as a potent candidate gene for contributing to the level of IgE in serum.

Material and methods

Study subjects and serologic tests

All the patients ($n = 334$) enrolled in this study were from out-patient clinics of Catholic University Hospital in Seoul, Korea. The mean age of patients was $28.9 (\pm 16.6)$ and the sex ratio was 156 (M) : 178 (F). Informed consents were obtained from each patient and permission to conduct the genetic study was obtained from the Institutional Review Board of Catholic University Hospital. Total IgE was measured using the LIPA-200 system (Iatron Corp., Tokyo, Japan). The diagnosis of AD was based on the criteria published (12–14). Subjects were categorized into three groups: (i) low IgE group whose serum total IgE concentration was < 165 IU/ml, (ii) intermediate IgE group whose serum total IgE concentration was between 165 and 1500 IU/ml, and (iii) high IgE group whose serum total IgE concentration was higher than 1500 IU/ml (15).

Genotyping of IL-10 SNPs

Sequences of amplifying and extension primers for IL-10 SNP genotyping for *IL10* -1082 $A > G$, *IL10* -819 $T > C$, *IL10* -592 $A > C$ and *IL10* +117 $T > C$ are forward: 5'-ccaactggctcccttacctctac-3', reverse: 5'-caggattcatggaggctgg-3', extension: 5'-acttctcttacctatcctacttcccc-3' (*IL10* -1082 $A > G$); forward: 5'-gggtgaggaaaccaaattctcag-3', reverse: 5'-ggtagtgtcaccatgacccc-3', extension: 5'-gtaccttgtacaggtgatgtaa-3' (*IL10* -819 $T > C$); forward: 5'-ggtggaacatgtgcctgag-3', reverse: 5'-ctcaaagtccaagcagcc-3', extension: 5'-ttccatttttctccagagactggcttctacag-3' (*IL10* -592 $A > C$) and forward: 5'-atagctgaccagccctt-3', reverse: 5'-aaatcgttcacagagaagctcagt-3', extension: 5'-gctcagtaataatagaaatgggggttgaggatcagaggt aataaatattctat-3' (*IL10* +117 $T > C$), respectively. Polymerase chain reaction (PCR) was performed in the mixture of 1.25 pmol of each primer, 50 ng genomic DNA, 250 mM dNTPs, and 0.15 U *Taq* DNA Polymerase (Applied Biosystems, Foster City, CA, USA) in the buffer provided by the manufacturer. Amplification was performed in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) under touchdown conditions. To clean up the PCR for the primer extension reaction, 1 unit of shrimp alkaline phosphatase (SAP) (Amersham Life Sciences, Cleveland, OH, USA) and 2 units of *ExoI* (Amersham Life Sciences) were added to PCR products. The mixture was incubated at 37°C for 1 h, followed by 15 min at 72°C to inactivate the enzymes. Primer extension reactions were performed as described previously (16) using the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems).

Statistics

Logistic regression models were used for calculating P -values for each SNP site and haplotypes (ht) controlling age and sex as covariables. P -values of codominant, dominant, and recessive models are given. There were no ambiguous ht phases in the population because of complete and/or absolute linkage disequilibrium (LDs) between SNPs in *IL10*. Haplotype analyses were performed

only with *IL10*-ht2 and *IL10*-ht4 because *IL10*-ht1 and *IL10*-ht3 are equivalent with *IL10* -592 (*IL10* -819) and *IL10* +117, respectively (Fig. 1B).

Results

The *IL10* gene on chromosome 1q31–32 contains five exons with total size of approximately 10 kb. Four single nucleotide variants in the *IL10* gene (Fig. 1), three within the upstream of the promoter region (an A-G transition at position -1082, a T-C transition at position -819, and a A-C transversion at position -592 measured from the transcription start site) and one C-T transition at +117 in 5'-UTR calculated from the first nucleotide (+1) of 5'-UTR, were genotyped by single-base extension (SBE) method. Three SNPs in the IL-10 promoter region were genotyped. The frequencies of each SNP were 0.07 (*IL10* -1082 $A > G$), 0.31 (*IL10* -592 $A > C$), and 0.03 (*IL10* +117 $T > C$), respectively, in the Korean population ($n = 334$). All four SNPs were in complete ($|D'| = 1$ and $r^2 = 1$) or absolute LD ($|D'| = 1$ and $r^2 = 1$). As shown in Fig. 1, *IL10* -819 $T > C$ and *IL10* -592 $A > C$ were in absolute LD, and *IL10* -819 $T > C$ was not included in statistical analysis. Four ht were identified without any ambiguous phasing because of LDs among SNPs (Fig. 1B).

Logistic regression analysis controlling age and sex as covariables in all three analysis models (codominant, dominant, and recessive models for rare allele) were employed to show alternative effects of the variants. In the initial analysis, the rare allele (C allele) of *IL10* -592 $A > C$ showed a weak association with decreased total IgE among AD patients ($P = 0.11$ – 0.01), whereas the other two loci showed no significant associations. The frequency of C allele was highest in the low IgE group (0.34), lowest in the high IgE group (0.22), and intermediate in the intermediate group (0.32; Table 1).

In further ht analysis, one of the common ht (frequency = 0.23), *IL10*-ht2 [A - C - C - T] (abbreviated as *IL10*-ht2), which is also common in Caucasians (0.29, $n = 1813$) and African-Americans (0.41, $n = 858$; 17), revealed a dose-dependent effect on total serum IgE levels. The frequencies of *IL10*-ht2 containing genotypes decreased in order of IgE levels ($P = 0.001$), i.e. they were lowest in the high IgE group, highest in the low group, and intermediate in the middle group (Fig. 2), with significant effects in all three alternative analyzing models (codominant, dominant, and recessive models). Additionally, the frequency of *IL10*-ht2 among healthy controls (no asthma and no atopy) for asthma study in Korean population (18) was also analyzed. The frequency of associated *IL10*-ht2 among asthma control subjects was 0.488 ($n = 84$, data not shown), which was very similar with that among AD patients with low IgE levels (0.486). The patients with asthma and/or atopy were not

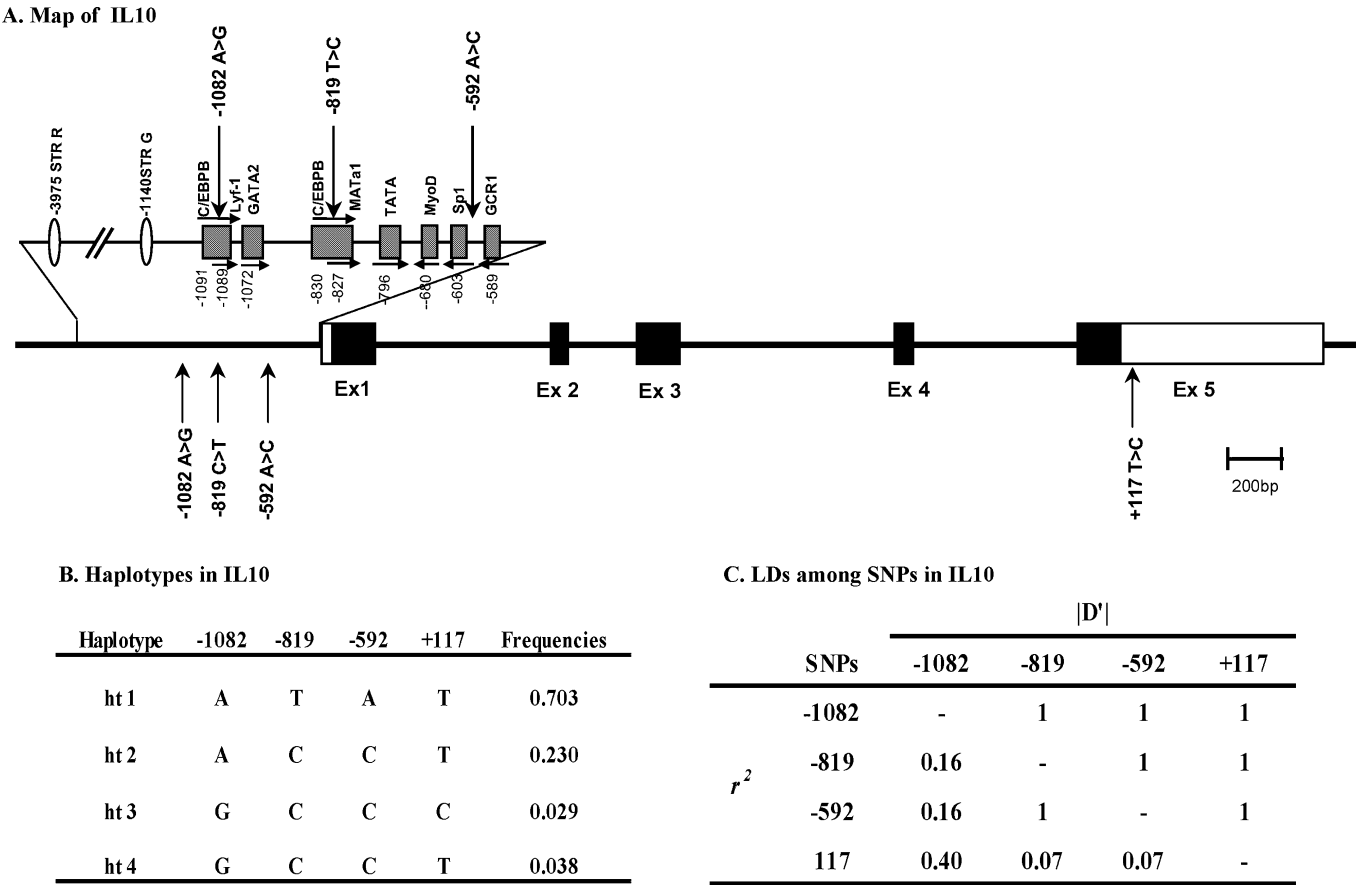


Figure 1. Map and haplotype of *IL10* gene on chromosome 1q31–32. (A) Coding exons are marked by shaded blocks and 5′- and 3′-UTR by white blocks. Putative transcription factor sites are indicated (<http://molsun1.cbrc.aist.go.jp/research/db/TFSEARCH.html>, putative score > 0.9). The positions were calculated from the transcriptional start site (*IL10* −1082, *IL10* −819 and *IL10* −592) and from the first nucleotide (+1) of 5′-UTR (*IL10* +117 T>C). The STR markers in 5′-flanking region were also shown (−3975 STR-R, and −1145 STR-G; 35). (B) IL-10 haplotypes constructed by four single nucleotide polymorphisms (SNPs) and frequencies in the Korean population (*n* = 344). Genotype distributions in all loci were in Hardy–Weinberg equilibrium (*P* > 0.05). There was no ambiguous haplotype phasing due to absolute linkage disequilibrium (LD; |D'| = 1 and *r*² = 1) and complete LD (|D'| = 1 and *r*² = 1) among SNPs. Haplotypes 1 and 3 are equivalent with *IL10* −592 and *IL10* +117, respectively. The IL-10 haplotypes could be constructed simply by eye due to absolute LD and/or complete LD. Those haplotypes constructed by eye were identical with those from the expectation maximization (EM) algorithm (arlequin, <http://anthro.unige.ch/arlequin/>). (C) Analysis of linkage disequilibrium; |D'| and *r*²-values among SNPs in *IL10*.

analyzed because it would be complicated to interpret when asthma/atopy involved in the analysis.

Discussion

Interleukin-10, produced by macrophages, T cells, dendritic cells, etc. is a potent immunosuppressive cytokine that down-regulates the expression of T_H1 cytokines and costimulatory molecules (19). The two SNPs, *IL10* −1082 T>C and −592 A>C, contributing to *IL10*-ht2 are particularly interesting, because several previous reports have demonstrated quantitative differences in IL-10 transcription and/or expression mediated by alternative alleles or ht. Differences in nuclear-binding activity and

IL-10 production mediated by the *IL10* −592 A>C polymorphism (17, 20), by the *IL10* −1082 A>G polymorphism (21, 22) and by the IL-10 promoter ht (23–25; *IL10*-ht2 was associated with increased production of IL-10) have been reported. Positive associations with the following diseases have been detected: AIDS progression (17), HCV infection and resistance to antiviral therapy (24, 26, 27), advanced alcoholic liver disease(28), Epstein–Barr virus (EBV) infection (29), gastric cancer (30), cervical cancer (31), multiple myeloma (32), hepatocellular carcinoma (33) and cutaneous malignant melanoma (34). The role of IL-10 in these diseases and serum IgE level in AD patients in this study suggest that IL-10 polymorphisms play a critical role in immunity and in inflammation progress.

Table 1. Logistic regression analyses for defined categories of total immunoglobulin E (IgE) level with three single nucleotide polymorphisms (SNPs) and two haplotypes among atopic dermatitis patients

Locus	Genotype	Defined categories of IgE level			P-values for each analyzing model		
		Low (%)	Intermediate (%)	High (%)	Codominant	Dominant	Recessive
<i>IL10 -1082 A>G</i>	A	136 (91.9)	101 (80.8)	54 (88.5)	0.21	0.19	0.99
	AG	12 (8.1)	23 (18.4)	7 (11.5)			
	G	0 (0)	1 (0.8)	0 (0)			
<i>IL10 -592 A>C (IL10-ht1)</i>	A	68 (46.3)	55 (44)	37 (60.7)	0.02	0.11	0.012
	AC	58 (39.5)	59 (47.2)	21 (34.4)			
	C	21 (14.3)	11 (8.8)	3 (4.9)			
<i>IL10 +117 T>C (IL10-ht3)</i>	T	143 (97.3)	113 (90.4)	56 (93.3)	0.18	0.18	—
	TC	4 (2.7)	12 (9.6)	4 (6.7)			
	C	0 (0)	0 (0)	0 (0)			
<i>IL10-ht2</i>	—/—	75 (51.4)	74 (59.2)	43 (71.7)	0.001	0.005	0.02
	—/ht2	55 (37.7)	46 (36.8)	14 (23.3)			
	ht2/ht2	16 (11)	5 (4)	3 (5)			
<i>IL10-ht4</i>	—/—	138 (94.5)	112 (89.6)	57 (95)	0.65	0.65	—
	—/ht4	8 (5.5)	13 (10.4)	3 (5)			
	ht4/ht4	0 (0)	0 (0)	0 (0)			

Genotype, haplotype distributions, and P-values for logistic regression analyses (age- and sex-adjusted as covariables) using three alternative models (codominant, dominant, and recessive) are shown. No statistical analyses were performed with *IL10 -819 T > C* because of absolute linkage disequilibrium (LD; $ID'1 = 1$ and $r^2 = 1$) with *IL10 -592 A > C*. Haplotypes 1 and 3 were not analyzed because these haplotypes were equivalent models with *IL10 -592 A > C* and *IL10 +117 T > C*, respectively.

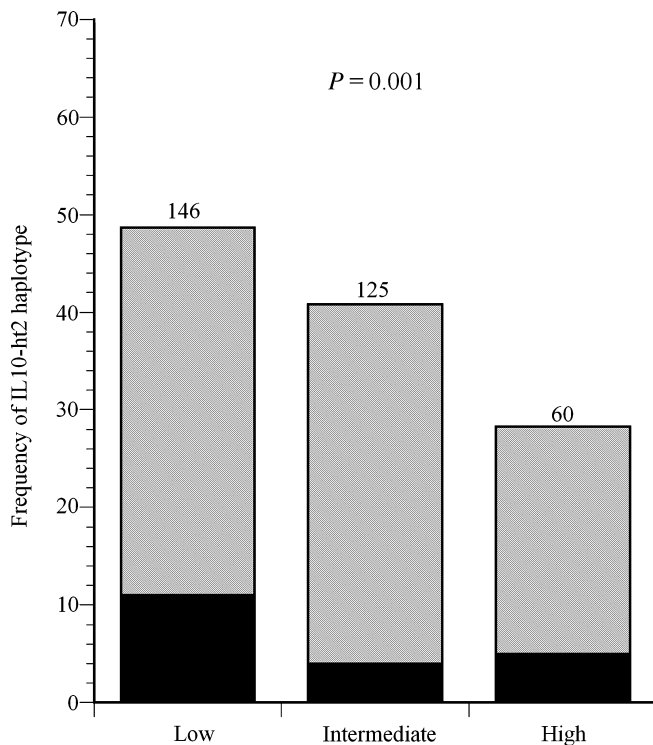


Figure 2. Frequency of *IL10-ht2* containing genotypes in three defined categories of total serum immunoglobulin E (IgE) in atopic dermatitis (AD) patients. The result of *IL10-ht2* in Table 1 was visualized. The P-value was derived from codominant models, and the number of patients in each category is indicated above bars. Low: IgE ≤ 165 IU/ml, intermediate: $165 < \text{IgE} \leq 1500$ IU/ml, and high: IgE > 1500 IU/ml. The black and grey bars indicate homozygotes (ht2/ht2) and heterozygotes (—/ht2) for *IL10-ht2*, respectively.

Recently, a new piece of evidence supporting the role of IL-10 polymorphisms in dermatitis, has come to light. The IL-10 promoter microsatellite, IL10G, which is also located in promoter (–1140; Fig. 1) with strong linkage to the nearby IL-10 promoter SNPs (17), was found to be associated with the development and onset age of psoriasis (35). Concordant evidence for IL-10 function in dermatitis has also been published: psoriasis patients treated with recombinant IL-10 showed a marked reduction of clinical disease activity. The clinical response was associated with a significant decrease in cutaneous T-cell infiltration and the lesional expression of type 1 cytokines, IFN- γ and tumour necrosis factor- α (36). The corresponding results described above, along with the strong associations discovered in this current study, suggest that increased IL-10 production mediated by *IL10-ht2* suppresses the production of IgE in AD patients. The different roles of IL-10 in IgE production were reported; IL-10 decreases IgE production early stage of peripheral blood mononuclear cell (PBMC) culture *in vitro*, and increases IgE production, in contrast, if added later on B cells that are already IgE switched (37). Although we could not performed subgroup analysis according to allergic status, our results suggest that increased IL-10 production in *IL10-ht2*-bearing individuals avoids the development of an enhanced IgE synthesis or maybe protects this group of patients from enhanced IgE production instead of down-regulating the production of IgE in AD patients. The very similar frequencies of *IL10-ht2*-bearing genotypes among controls for asthma study (no asthma and no atopy) and AD patients with low IgE levels, in part, support our result

that lower total serum IgE levels are associated with *IL10-ht2*.

In conclusion, *IL10-ht2* shows a strong association with total serum IgE level in gene dose-dependent manner among AD patients. Therefore, it could be inferred that increased IL-10 production in *IL10-ht2*-bearing individuals might down-regulate the production of IgE in AD patients. The greater effects of IL-10 ht on total serum IgE levels suggest that the alternative differences of the

IL-10 polymorphism might be the result of a combined genotype (ht) rather than single polymorphisms.

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