

Significant Association between *IL-17F* Promoter Region Polymorphism and Susceptibility to Asthma in a Korean Population

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Key Words

IL-17F • Asthma • Polymorphism • High-resolution melting

Abstract

Background: Individual differences in susceptibility to asthma would be expected because of common DNA variants of single nucleotide polymorphisms (SNPs) across populations. The pro-inflammatory cytokine *IL-17F* has homology with the *IL-17* motif and induces the expression of other inflammatory cytokines in airway epithelial cells. This study aimed to identify *IL-17F* gene polymorphisms and to determine a possible association between these polymorphisms and susceptibility to asthma through a case-control study in a Korean population. **Methods:** We identified SNPs in the *IL-17F* gene by sequencing. Genotyping was conducted using the high-resolution melting (HRM) method on 424 asthma patients and 548 healthy controls. **Results:** The genotype and allele frequencies of rs1889570 SNP were significantly different between asthma patients and healthy controls ($p = 0.001$ and 0.002 , respectively). The rs1889570 SNP genotype was also positively associated with the number of peripheral blood eosinophils in asthma patients ($p = 0.03$). The frequencies of haplotypes AA ($p = 0.01$), GG ($p = 0.01$) and AG ($p =$

0.006) were significantly different between asthma patients and healthy controls. **Conclusions:** In this study, we confirmed that the rs1889570 polymorphism of the *IL-17F* gene is associated with susceptibility to asthma in a Korean population.

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Introduction

Asthma is a common and complex disorder influenced by genetic and environmental factors and associated with T cell activation, production of Th1- and Th2-related cytokines as well as eosinophil accumulation [1, 2]. Several genome-wide linkage scans have been carried out to identify asthma susceptibility loci [3, 4]. Interleukin-17F (*IL-17F*, encoded by *IL-17F*, is 7,815 bp in length and contains 3 exons) was recently identified as an asthma susceptibility candidate because of its similarity to

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Table 1. Primer sequences used for *IL-17F* gene amplification, sequence analysis and genotyping

Application	Primer name	Primer sequence (5'→3')	Region
PCR analysis	IL-17F_PFI	TTCTTGAGCAGGCTGATGG	promoter
	IL-17F_PRI	GCCTCTGGGACTTCTGACTG	
	IL-17F_PFI	CAAACCTCAAGCGTTTGTGG	promoter and exon1
	IL-17F_PRI	TCCAAAAAGACCCCAATCAA	
	IL-17F_EFI	AGCCCTGATCCCATAAAAGG	exon 2
	IL-17F_ERI	GGACCATCAACCACAAAGGT	
	IL-17F_EFI	TCCCTGCAGGTGAACACTC	exon 3 and 3' UTR
	IL-17F_ERI	AGGATTTTCTTAACTCTCT	
DNA sequencing	IL-17F_SF1	GAAGGGTCCTCCCCTCTACC	promoter
	IL-17F_SR1	CCTTCCTTCTCTCTGGGTAG	promoter
	IL-17F_SF2	AGCACCAGTAGGAGTCTAACC	promoter
HRM analysis	IL-17F_HFI	CACCTTTTCTCCCCAAGACCA	rs1889570
	IL-17F_HRI	TTGGCCTCTCTGAGCAGCCTT	
	IL-17F_HFI	TCCAGTTGGAGAAGGTGCTG	rs763780
	IL-17F_HRI	TTGCAGAGCACTGGGTAAGG	

IL-17 and its overexpression in inflammatory cells such as activated CD4+, $\gamma\delta$ T cells, mast cells and basophils. IL-17F induces the production of IL-6, tumor growth factor- β (TGF- β) and IL-8 (involved in neutrophil recruitment, which subsequently triggers inflammatory responses) in primary bronchial epithelial cells of allergic asthma patients [5] and in the allergic asthma murine model [6, 7].

The single nucleotide polymorphism (SNP) allelic frequencies among Caucasians and African Americans for *IL-17* have been established. This study aimed to identify whether *IL-17F* polymorphisms are associated with asthma or asthma-related phenotypes (such as total IgE levels and peripheral blood eosinophil numbers) in a Korean population.

Materials and Methods

Patients and Genomic DNA

After institutional review board approval was granted and informed consent was collected, blood samples and records were obtained from 548 healthy controls (208 females, 340 males) without asthma and 424 asthma patients (214 females, 210 males). The mean age of the healthy controls and asthma patients was 40.4 and 54.8 years, respectively. Genomic DNA was extracted from peripheral blood leukocytes by a standard phenol-chloroform method or by using a Genomic DNA Extraction Kit (iNtRON Biotechnology, Taejon, Korea) according to the manufacturer's instructions. The asthma patients were recruited from the outpatient clinic at Chonbuk National University Hospital. Asthma was

diagnosed according to American Thoracic Society criteria [8]. Each healthy control had visited the Wonkwang University medical center for annual physical examinations and had no history of asthma. All subjects in this study were Korean. Blood eosinophil counts and total serum IgE levels in asthma patients were measured at the Department of Hematology of Chonbuk National University Hospital by using a Coulter GenSTM Hematology Analyzer (Coulter, Miami, Fla., USA) and a Roche Cobas-Core II (Roche Diagnostics, Basel, Switzerland), respectively.

Polymerase Chain Reaction and DNA Sequencing

The full-length *IL-17F* gene, including the ~2.0 kb promoter region, was partially amplified. Polymerase chain reaction (PCR) was performed using 50 ng of genomic DNA, Taq DNA polymerase (EF Taq; SolGent, Korea) and 0.5 μ M of each primer (table 1) under the following conditions: 30 cycles of PCR consisting of denaturation for 10 s at 98°C, annealing for 30 s at 65°C, extension for 1 min at 72°C and a final extension for 10 min at 72°C in a thermocycler (Gene Amp PCR system 9700; Applied Biosystems, Foster, Calif., USA). After the PCR products were purified using a PCR purification kit (Millipore, Bedford, Mass., USA), they were used as a template DNA for sequencing analysis using the ABI Prism BigDye Terminator Cycle Sequencing Kit (ABI 3100 automatic sequencer; Applied Biosystems). The PCR primers and additional 3 primers were used for DNA sequencing (table 1). The SNPs of *IL-17F* were detected by sequence analysis based on the reference sequence of human chromosome 6 clone RP1-108C2 (AL034343).

Genotyping

Genotyping was performed by high-resolution melting (HRM) curve analysis. PCR was carried out in a total volume of 10 μ l containing 50 ng of genomic DNA, 100 nM of each primer (table 1), 1 $\times$ QuantiTect Probe PCR Kit (Qiagen, Valencia, Calif., USA), and 1 $\times$ EvaGreen solution (Biotium, Hayward, Calif.,

Table 2. Genotype and allele frequencies in SNPs of the *IL-17F* gene between healthy controls and asthma patients

SNP ID	Genotype/allele	Control	Asthma	Odds ratio ^a	p ^b
rs1889570	GG	197 (35.9)	134 (31.6)	1.00	0.001
	GA	274 (50.0)	190 (44.8)	1.02 (0.77–1.36)	
	AA	77 (14.1)	100 (23.6)	1.91 (1.32–2.76)	
	G	668 (60.9)	458 (54.0)	1.00	0.002
	A	428 (39.1)	390 (46.0)	1.33 (1.11–1.59)	
rs763780	AA	428 (78.1)	346 (81.6)	1.00	0.088
	AG	112 (20.4)	77 (18.2)	0.85 (0.62–1.18)	
	GG	8 (1.5)	1 (0.2)	0.16 (0.02–1.24)	
	A	968 (88.3)	769 (90.7)	1.00	0.103
	G	128 (11.7)	79 (9.3)	0.78 (0.58–1.04)	

Figures in parentheses are percentages unless indicated otherwise.

^a Logistic regression analysis was used to estimate odds ratio (95% CI are given in parentheses).

^b Values were determined by Fisher's exact test or the χ^2 test from a 2×3 contingency table.

USA). PCR cycling and HRM analysis were carried out using the Rotor-Gene Thermal Cycler RG6000 (Corbett Research, Mortlake, N.S.W., Australia). The amplicons were produced according to the following conditions: 1 cycle at 95°C for 15 min, and 45 cycles at 95°C for 15 s, 68°C for 10 s and 72°C for 30 s. Optical measurements in the green channel (excitation at 470 nm and detection at 519 nm) were recorded during the extension step. After completion of 45 cycles, melting curve data were generated by increasing the temperature from 77°C to 95°C at 0.1°C per second and recording fluorescence. HRM curve analysis was performed using the software Rotor-Gene 1.7.40 with in-built HRM algorithm.

Statistical Analysis

χ^2 tests were used to estimate the Hardy-Weinberg equilibrium. Linkage disequilibrium (LD) analyses by pair-wise comparison of biallelic loci and the haplotype frequencies of *IL-17F* for multiple loci were estimated using the expectation maximization algorithm with SNPalyze software (Dynacom, Mobara, Japan). Analysis of variance was applied to analyze differences between each genotype and IgE levels or the number of peripheral blood eosinophils in asthma patients. A p value of less than 0.05 was considered statistically significant.

Results

Polymorphisms of the *IL-17F* gene in this Korean population were identified through sequencing of all 3 *IL-17F* exons (2.12-kb promoter region, 0.254-kb 3' UTR region) from 24 asthma patients with reference to 24 healthy controls. We identified 2 SNPs at 6p12, rs1889570 in the promoter region and rs763780 in exon 3. The nonsynony-

mous change of alleles at rs763780 SNP in exon 3 resulted in amino acid substitution from histidine to arginine (p.His161Arg). LD coefficients ($|D'|$) were calculated between rs1889570 and rs763780 ($|D'| = 0.82$ and $r^2 = 0.06$). They were not in absolute LD ($|D'| = 1$ and $r^2 = 1$; data not shown). On the basis of LD frequencies, large-sample genotyping was performed for these 2 SNPs.

To determine whether the *IL-17F* gene variations were associated with asthma susceptibility, we analyzed the genotypes and allele frequencies of these polymorphisms between asthma patients and healthy controls. Two polymorphisms had minor allele frequencies >9% (table 2). The genotype frequencies of all loci were in Hardy-Weinberg equilibrium in both the asthma patients and healthy controls ($p > 0.05$; data not shown), but the genotype and allele frequencies of rs1889570 SNP of *IL-17F* were significantly different between the asthma patients and healthy controls ($p = 0.001$ and $p = 0.002$, respectively). Frequencies of the rs763780 SNP did not differ between the 2 groups (table 2).

The possible correlation between the genotype of each SNP in the *IL-17F* gene and total serum IgE levels (or the number of peripheral blood eosinophils) in asthma patients was defined (table 3). The eosinophil level was highest in asthma patients with the G/A genotype (6.62%) and lowest in asthma patients with the A/A genotype (4.57%). The genotype of the rs1889570 SNP was positively associated with the number of peripheral blood eosinophils in asthma patients ($p = 0.03$).

Table 3. Analysis of serum total IgE levels and peripheral blood eosinophil counts among genotypes of each SNP of the *IL-17F* gene in asthma patients

SNP ID	Genotype	IgE, IU/ml			p ^b	Eosinophil, % ^a			p ^b
		n	mean	SD		n	mean	SD	
rs1889570	GG	85	328.07	400	0.06	86	4.84	5.48	0.03
	GA	132	237.24	212		142	6.62	7.10	
	AA	68	284.96	204		72	4.57	5.47	
rs763780	AA	237	266.22	215	0.21	241	4.97	5.50	0.88
	AG	48	322.59	495		45	5.10	5.52	
	GG	–	–	–		–	–	–	

^a Values were determined by the number of eosinophil cells per total cell number in a cubic millimeter volume.

^b Values were analyzed by ANOVA.

The haplotype frequencies of rs1889570 and rs763780 SNPs were compared between asthma patients and healthy controls (table 4). More than 5% of 2 common haplotypes were identified, and >98.5 and >99.7% distribution was revealed in the asthma patients and healthy controls, respectively. The frequency of the GA haplotype of *IL-17F* was the highest, but no difference in frequency was observed between the asthma patients and healthy controls ($p = 0.130$). The distribution rate of the AA, GG and AG haplotypes differed significantly between the asthma patients and healthy controls ($p = 0.01$, 0.01 and 0.006 , respectively). These results suggest that *IL-17F* polymorphisms may be one of the most important genetic factors in asthma susceptibility.

Discussion

A number of polymorphisms in the *IL-17F* gene have recently been identified in an African American group, a European American group and a Japanese group [9, 10]. Furthermore, association studies between *IL-17F* polymorphisms and asthma have been conducted in these groups. In our study, 2 polymorphisms (in the promoter region and in exon 3 of the *IL-17F* gene) were identified in a Korean population. Genetic association studies to define polymorphisms in the *IL-17F* gene have been performed for several immune disorders such as asthma, atopic dermatitis, psoriasis vulgaris, ulcerative colitis and Behçet's disease [11–14]. Most of these studies demonstrated an association between SNPs in exon 3 (especially rs763780) and disease susceptibility. A positive associa-

Table 4. Haplotype frequencies between healthy controls and asthma patients in *IL-17F* polymorphisms

Haplotype		Frequency ^a		p ^b
rs1889570	rs763780	control	asthma	
G	A	0.496	0.461	0.130
A	A	0.387	0.445	0.010
G	G	0.114	0.079	0.011
A	G	0.003	0.015	0.006

^a Values were constructed by the expectation maximization algorithm with genotyped single nucleotide polymorphisms.

^b Values were analyzed by permutation test.

tion between rs2397084 SNP in exon 3 of the *IL-17F* gene and Behçet's disease was reported in a Korean population [11]. A significant association between the rs763780 SNP in exon 3 of the *IL-17F* gene and ulcerative colitis was observed in both Japanese and Chinese populations [12, 13]. A negative association between the rs763780 SNP and atopic dermatitis or psoriasis vulgaris was reported in a Japanese population [14]. Ramsey et al. [9] failed to find any association between polymorphisms of the *IL-17F* gene and asthma. In contrast, however, Kawaguchi et al. [10] found a positive association between the rs763780 SNP in exon 3 and asthma. Interestingly, unlike their results, our novel findings showed a significant association between the rs1889570 SNP in the promoter region and asthma but not between the rs763780 SNP in exon 3 and asthma (table 2). This finding suggests that the rs1889570

polymorphism in the promoter region of the *IL-17F* gene might be strongly associated with asthma susceptibility. Additionally, we observed a positive association between the genotype of the rs1889570 SNP and peripheral blood eosinophil counts in asthma patients (table 3). The eosinophil counts were highest in asthma patients with the G/A genotype and lowest in patients with the A/A genotype of the rs1889570 SNP. A double dose of allele A may negatively influence eosinophil recruitment in blood. The heterozygous genotypes of rs1889570 might influence eosinophil recruitment in blood. Our study results showed a significant difference in distribution rate of the AA, GG and AG haplotypes of *IL-17F* SNPs between asthma patients and healthy controls (table 4). This result demonstrates that a person who has the AA or AG haplotype is more susceptible to asthma than a person with the GG haplotype.

In conclusion, our findings strongly suggest that the promoter SNP at rs1889570 of the *IL-17F* gene is significantly associated with asthma susceptibility.

However, we need to conduct further functional studies on the promoter region to define whether rs1889570 polymorphism of the *IL-17F* gene influences *IL-17F* function and how the *IL-17F* gene contributes (in the context of dimerization with IL-17A or IL-17F) to the development of asthma.

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