Analysis of the variations in *IL-28RA* gene and their association with allergic rhinitis

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Abbreviations: AR, allergic rhinitis; PCR, polymerase chain reaction; SBE, single-base extension; SNP, single nucleotide polymorphism

Abstract

IL-28RA is one of the important candidate genes for complex trait of genetic diseases, but there is no published information of the genetic variation in this gene. We scanned the seven exons and their boundary introns sequence of *IL-28RA* including the promoter regions to analyze genetic variation sites, and identified eighteen single nucleotide polymorphisms (SNPs) and two variation sites. We chose seven SNPs (*g.-1193 A > C*, *g.-30 C > T*, *g.17654 C > T*, *g.27798 A > G*, *g.31265 C > T*, *g.31911 C > T* and *g.32349 G > A*) of them for large sample size genotyping, and assessed the association of genotype and allele frequencies of these SNPs between allergic rhinitis patients and non-allergic rhinitis controls. We also compared the genotype frequencies between Korean controls and Han Chinese control or Korean Chinese control. We investigated the frequencies of haplotype con-

structed by these SNPs between allergic rhinitis patients and non-allergic rhinitis controls. Our results suggested that the *g.32349 G > A* polymorphism of *IL-28RA* might be associated with susceptibility to allergic rhinitis ($P = 0.032$), but seems to have no relationship with serum total IgE levels. The haplotype frequencies by these SNPs also show significant association between controls and allergic rhinitis patients.

Keywords: cytokines; haplotypes; interleukins; polymorphism, rhinitis, allergic; single nucleotide

Introduction

Cytokines are multifunctional proteins that mediate many of responses of innate and adaptive immunities. They are produced in response to microbes, antigens, and other cytokines and stimulate diverse responses of cells involved in immunity and inflammation. They act on target cells by binding to specific cytokine receptor, initiating signal transduction and second signal pathways within the target cell (Carpenter *et al.*, 1998; Hibi and Hirano, 1998; Onishi *et al.*, 1998; Rubinstein *et al.*, 1998; O'Shea *et al.*, 2002). The polymorphisms within coding region of cytokine gene have influence on protein structure and expression. Although the amino acid sequence is not changed, the silent mutations may have influence on protein expressions by the alternative mRNA splicing, mRNA stabilities and levels of gene transcription. Polymorphisms within the regulatory regions or introns of genes may have a significant effect on transcription, since they may alter the structure of transcription factor binding sites within gene promoters or the structure of enhancers and silencers within introns (Bidwell *et al.*, 1999).

The SNPs of many cytokines and their receptor genes, such as *IL-10*, *IL-12*, *IFN γ* , *RANTES*, *CCR2* and *CCR5*, are identified (Hall *et al.*, 2000; Azzawi *et al.*, 2001; Gibson *et al.*, 2001; Iwasaki *et al.*, 2001) and indicate that the polymorphisms are associated with immune disorders (Fryer *et al.*, 2000; Nakao *et al.*, 2001). Allergic rhinitis is an inflammatory disorder characterized by the symptoms of nasal itch, sneeze, anterior nasal secretions, and swelling of the nasal mucosa. The several numbers of cytokines have been reported for analyzing the association of SNPs with allergic rhinitis. The polymorphisms of

IL-13 were associated with allergic rhinitis susceptibility and might responsible for the high IgE level in allergic rhinitis patients (Wang *et al.*, 2003). The genotypes of *TNF- α* and *TGF- β 1* polymorphisms and the haplotype block containing CXCL9, CXCL10, CXCL11, and SDAD1 may be associated in the pathogenesis of allergic rhinitis (Gentile *et al.*, 2004; Zhang *et al.*, 2005). We previously reported that the polymorphisms of *eotaxin-2*, *eotaxin-3*, T cell immunoglobulin and mucin domain-3 (*TIM-3*), and *FOXJ1* are associated with allergic rhinitis in Korean population (Chae *et al.*, 2004b; Chae *et al.*, 2005b; Li *et al.*, 2006).

Recently, the new cytokine family consisting of interleukin 28A (IL-28A; also designated as IFN- γ 2), IL-28B (IFN- γ 3) and IL-29 (IFN- γ 1) and a component of their receptors, IL-28RA (IFN- γ R1), are identified from the human genomic sequence (Dumoutier *et al.*, 2003; Kotenko *et al.*, 2003; Sheppard *et al.*, 2003). They are distantly related to type I interferons (IFNs) and the IL-10 family, and induced by viral infection and show antiviral activity (Kotenko *et al.*, 2003; Sheppard *et al.*, 2003). However, IL-28 and IL-29 interact with a heterodimeric class II cytokine receptor that consist of IL-10 receptor β (IL-10R β) and an orphan class II receptor chain, designated IL-28RA. More recently, Dumoutier and co-workers suggested that IL-29 induced STAT2 tyrosine phosphorylation is mediated through tyrosines 343 and 517 of the IL-28RA and these two tyrosines are also responsible for antiviral and anti-proliferative activities of IL-29. In contrast, STAT4 phosphorylation is independent from IL-28RA tyrosine residues (Dumoutier *et al.*, 2004). *IL-28RA* genes are located on the chromosomal region 1p36.11 and have seven exons.

Although, *IL-28RA* is one of the crucial candidate genes for the infection and inflammation disease, a correlation between SNPs of *IL-28RA* and individual susceptibility of inflammation disease such as allergic rhinitis was poorly demonstrated. In an attempt to better understand the genetic influences of *IL-28RA* on allergic rhinitis, we have identified the possible variation sites and SNPs through the seven exons and their boundary introns sequence of *IL-28RA* including the promoter regions (~2.0 kb). The genotype frequencies of the SNPs identified in *IL-28RA* were analyzed in the genomic DNAs isolated from allergic rhinitis patients and healthy controls to determine whether these *IL-28RA* SNPs are associated with allergic rhinitis. We further investigated the relationships among these polymorphisms to IgE levels and peripheral blood eosinophil counts in allergic rhinitis patients.

Materials and Methods

Subjects and DNA samples

Blood samples were obtained from 267 Korean patients with allergic rhinitis (169 males and 98 females), 559 Korean non-allergic rhinitis controls (346 males and 213 females), 276 Han Chinese (131 males and 145 females) and 148 Korean Chinese (64 males and 84 females). The mean ages of non-allergic rhinitis controls, allergic rhinitis patients, Han Chinese and Korean Chinese were 40.8 years, 24.0 years, 21.4 years and 20.8 years, respectively. To escape the change of eosinophil count and serum IgE level in patient's blood by medication, the blood samples from allergic rhinitis patients were obtained before medication of anti-allergic drug such as antihistamine and topical or oral steroid. Genomic DNA was extracted from leukocytes in peripheral blood, by a standard phenol-chloroform method, or by using the Puregene[®] DNA Purification kit (Gentra) according to the manufacturer's instruction. The allergic rhinitis patients were recruited from our outpatient clinic at Wonkwang University Hospital. The diagnosis of allergic rhinitis was based on symptoms of sneezing, watery rhinorrhea, and nasal obstruction, and the positive results of a skin test. The skin test was performed with six common aeroallergens, such as house dust mites, house dust, grass mix, tree pollens, animal dander, and molds (Torii, Tokyo, Japan). All patients with allergic rhinitis had histories of all salient symptoms, and had a positive skin test. The controls without allergic rhinitis were recruited from the general population, and had received comprehensive medical testing at the Wonkwang University Hospital. Blood eosinophil counts and total serum IgE levels in allergic rhinitis patients were also determined in a routine laboratory at the Wonkwang University Hospital using the SE9000 (Sysmex Co, Kobe, Japan) and the electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany), respectively.

Polymerase chain reaction (PCR) and sequence analysis

The entire coding regions of *IL-28RA* including ~2.0 kb promoter regions were partially amplified by PCR using the six primer pairs (Table 1). PCR reactions were prepared by previously described condition (Chae *et al.*, 2004a). Amplification was carried out in a GeneAmp PCR system 9700 thermocycler (PE Applied Biosystem) at 94°C for 2 min, followed by 10 cycles at 94°C for 10 seconds, 68°C for 45 seconds (2 min for promoter and exon 1 region, and 4 min for exon 7 and 3' UTR region), and 68°C for 10 min.

Table 1. Primer sequences for amplification and genotyping of the *IL-28RA* gene.

Application	Primer sequence (5' → 3')		Regions
	Forward	Reverse	
PCR amplification	ATCCTCCTCCACCCTGCGCCT	AAGCAAACCTGGGGGCGCGT	Promoter and exon 1
	ACCTGCTTGCCCTTCGTGCT	ATCTTCCCCACCTCCAGACC	Exon 2
	GCCTGTCTGGACGGATGGAC	TGCTGGTCCCACACCCCAT	Exon 3
	CAGGGTCAGTGCAAGGCTGA	CTTCCTGGAGGAGGGGTGC	Exon 4
	CCTACCATGTCTGCCAACCCC	CATAGCTCCAGCCCCACTCC	Exon 5 and 6
	CCCCAGTCCCATCTGGTGTG	CAGCCTGGGCAACATAGGAGA	Exon 7 and 3'-UTR
Single base extension	TCATCACCCAAAGACAACAACCAT		g.-1193A > C
	GCGGGAGGCGGGGACCTGGG		g.-30C > T
	CTCTTCCACTTCGCGCCACCGTCTACGGGT		g.17654C > T
	TTGCTGGAGGTCCCAGGTGGGTATCAAGTGGTGCAGAA		g.27798A > G
	CAGTTTGATGGCACGTTAGACTCTTGGTTGAC		g.31265C > T
	CACATCCCCAAGGGTAAGAGCTT		g.31911C > T
	TCTTAAACCTTACCATTTTGTTTTGTTTTGTGAGTC		g.32349G > A

Then 20 cycles at 94°C for 10 seconds, 68°C for 45 seconds and 68°C for 10 min (with 10 seconds incremental increases per cycle) were followed by a final extension at 68°C for 7 min. PCR products purified by PCR purification kit (Millipore) were used as the template DNA for sequencing analysis. Purified PCR products were sequenced using the ABI Prism BigDye Terminator cycle sequencing system (PE Applied Biosystems) on the ABI 3100 automatic sequencer (PE Applied Biosystem). Both sense and antisense strands of PCR products were directly sequenced using the same primers used for the PCR amplification, and eleven primers (IL28R-R1: GCGCCACTGCACTCCAGCCT, IL28R-R2: AGG-CACACGCCACGACGCGCA, IL28R-R3: TCAGTAA-ATCCGTGCACGGG, IL28R-F1: GCAAGAGCACCA-AGGCTCCAG, IL28R-F2: GGAGGAAGAGGAGGC-GAGG, IL28R-F3: GTGAGGTCAGGTCACTGCGG, IL28R-F4: GTGGTGAGGGTTCTAGGGGAT, IL28R-F5: TCCAGTTTGGATGGCACGT, IL28R-F6: CCA-CTTTCACCAGAGCAGCCT, IL28R-F7: GTCCAGA-TGATTGAGCCCAGGA, IL28R-F8: GGCACCCGC-CACCACAC) were additionally used to sequence the promoter and 3' UTR region. The SNPs and variation sites were detected by direct sequence analysis. The reference sequence for *IL-28RA* was based on the sequence of human chromosome 1 clone RP11-10N16 (AL590683).

Single-base extension (SBE)

Genotyping for g.-1193 A>C, g.-30 C>T, g.17654 C>T, g.27798 A>G, g.31265 C>T, g.31911 C>T and g.32349 G>A in the *IL-28RA* gene was performed by single-base extension (SBE) method

(Park *et al.*, 2005) using the ABI Prism® SNaPshot™ Multiplex kit (Applied Biosystems). The PCR products purified by PCR purification kit (Millipore) were used as the template DNA for genotyping. Seven SBE primers were used in this study are shown in Table 1. The SBE reaction mix was prepared according to previously described method (Chae *et al.*, 2005a). The primer extension reaction was performed at 96°C for 1 min, followed by 25 cycles at 96°C for 10 seconds, 55°C for 40 seconds, and 60°C for 30 seconds. To clean up the primer extension reaction, 1.5 u of CIP (New England Bio-Labs) was added to the reaction mixture, and the mixture was incubated at 37°C for 90 min, followed by 15 min at 72°C, for purposes of enzyme inactivation. The purified extension products were added to Hi-Di formamide (Applied Biosystems) according to the recommendations of the manufacturer. The mixture was incubated at 95°C for 5 min, followed by 5 min on ice, and then electrophoresis was performed, using the ABI Prism 3100 Genetic Analyzer. The results were analyzed using the ABI Prism GeneScan and Genotyper software (Applied Biosystems).

Statistic analysis

The allergic rhinitis patients and non-allergic rhinitis controls were compared using case-control association analyses. The χ^2 tests were used to estimate the Hardy-Weinberg equilibrium (HWE). Logistic regression analyses were used to calculate odds ratios (95% confidence interval) for SNP sites. Linkage Disequilibrium (LD) analyses by pair-wise comparison of biallelic loci and the haplotype

Figure 1. Locations of each single nucleotide polymorphisms (SNPs) and variation sites in *IL-28RA* and their rare frequencies. Coding exons are marked by black blocks and 5'- and 3'-UTR by white blocks. The positions of SNPs were calculated from the translation start site. Putative transcription factor sites were searched at www.cbrc.jp/research/db/TFSEARCH.html. The reference sequence is based on the sequence of human chromosome 1 clone RP11-10N16 (AL590683).

Table 2. The variation sites of *IL-28RA* gene identified in this study, and rare allele frequencies in a Korean ($n = 64$ or 559), a Korean Chinese ($n = 148$) and a Han Chinese ($n = 276$) populations.

Position ^a	Region	Rare allele frequencies		
		Korean	Korean Chinese	Han Chinese
g.-1193A > C	Promoter	0.090	0.072	0.058
g.-1140(AC)15-25	Promoter	-	-	-
g.-191_190insTCCCC	Promoter	0.067	-	-
g.-30C > T (rs 4090311)	5'-UTR	0.385	0.397	0.397
g.17654C > T	Exon 3	0.090	0.045	0.034
g.27798A > G (rs 4649195)	Intron 5	0.423	0.390	0.393
g.27926A > G (rs 4649194)	Intron 5	0.008	-	-
g.30747G > C (rs 7531447)	3'-UTR	0.375	-	-
g.31155G > T	3'-UTR	0.009	-	-
g.31265C > T (rs 11249006)	3'-UTR	0.375	0.334	0.349
g.31680G > A (rs 11249002)	3'-UTR	0.364	-	-
g.31735C > T (rs 12028945)	3'-UTR	0.178	-	-
g.31799C > T (rs 10903035)	3'-UTR	0.466	-	-
g.31911C > T	3'-UTR	0.181	0.190	0.230
g.32349G > A (rs 7525481)	3'-UTR	0.367	0.338	0.352
g.32363T > G (rs 7529826)	3'-UTR	0.367	-	-
g.32388A > G (rs 7532647)	3'-UTR	0.367	-	-
g.32390A > G (rs 7532646)	3'-UTR	0.367	-	-
g.32517C > T (rs 7516266)	3'-UTR	0.367	-	-
g.32834G > A (rs 10903034)	3'-UTR	0.367	-	-

^aCalculated from the translation start site.

Table 3. The haplotype frequencies of *IL-28RA* polymorphisms between a Korean (Kor.) and a Korean Chinese (Kor. Chin.) or a Han Chinese (Han Chin.) populations.

Haplotype				Frequency ^a			<i>P</i> ^b	
g.-30C>T	g.17654C>T	g.27798A>G	g.32349G>A	Kor.	Kor. Chin.	Han Chin.	vs. Kor. Chin.	Han Chin.
T	C	G	G	0.203	0.208	0.236	0.866	0.187
T	C	A	G	0.171	0.196	0.173	0.410	0.899
T	C	G	A	0.158	0.153	0.114	0.866	0.072
C	C	G	A	0.114	0.155	0.111	0.167	0.900
C	C	A	G	0.111	0.133	0.102	0.427	0.684
C	C	G	G	0.085	0.087	0.110	0.930	0.234
T	C	A	A	0.050	0.014	0.054	0.052	0.869
C	T	A	G	0.031	0.013	1.2E-56	0.204	0.009
T	T	A	G	0.021	0.025	4.4E-31	0.747	0.055
C	C	A	A	0.020	0.009	0.065	0.522	0.019
Others				0.036	0.007	0.035	-	-

^aValues were calculated by EM algorithm with genotyped SNPs. ^bValues were analyzed by permutation test.

frequencies of *IL-28RA* for multiple loci were estimated using the expectation maximization (EM) algorithm with SNPalyze software (DYNACOM, Japan). The haplotype analyses of *IL-28RA* SNPs among Korean control, Korean Chinese and Han Chinese populations, and between allergic rhinitis patients and control were performed by Permutation test. A *P*-value of less than 0.05 was considered to indicate statistical significance.

Results and Discussion

IL-28RA is recently identified from the human genomic sequence as a component of their receptors for the new cytokine consisting of *IL-28A*, *IL-28B* and *IL-29* (Kotenko *et al.*, 2003; Sheppard *et al.*, 2003). Although *IL-28RA* is one of the important candidate genes for complex trait of genetic diseases, there are only a few published results for this gene. To investigate the association between SNPs of human *IL-28RA* and allergic rhinitis, we scanned the entire coding regions of *IL-28RA* including promoter regions by direct sequencing method from 32 individuals, and identified eighteen SNPs and two variation sites. The locations and rare allele frequencies of identified variation sites are shown in Figure 1 and Table 2. Linkage disequilibrium coefficients ($|D'|$) between all SNP pairs were calculated. Among thirteen SNPs identified (*g.30747 G>C*, *g.31155 G>T*, *g.31265 C>T*, *g.31680 G>A*, *g.31735 C>T*, *g.31799 C>T*, *g.31911 C>T*, *g.32349 G>A*, *g.32363 T>G*, *g.32388 A>G*, *g.32390 A>G*, *g.32517 C>T* and *g.32834 G>A*) in 3'-UTR, the all sets of SNPs were in absolute LD ($|D'| = 1$

and $\gamma^2 = 1$) except *g.31265 C>T* and *g.31911 C>T* (data not shown). Among identified polymorphisms, seven SNPs (*g.-1193 A>C*, *g.-30 C>T*, *g.17654 C>T*, *g.27798 A>G*, *g.31265 C>T*, *g.31911 C>T* and *g.32349 G>A*) were selected for large samples genotyping based on locations, LDs and frequencies. The genotyping analysis was carried out by the SEB method using genomic DNA samples isolated from 267 Korean patients with allergic rhinitis, 559 Korean non-allergic rhinitis controls, 276 Han Chinese and 148 Korean Chinese, who lived in Yianbian located in the northeast of China. We analyzed the haplotype frequencies in four SNPs (*g.-30 C>T*, *g.17654 C>T*, *g.27798 A>G* and *g.32349 G>A*) between Korean control and Han Chinese or Korean Chinese to confirm the perpetuity of genetic heredity by racial migration. A Korean Chinese are immigrant nationality, whose ancestors immigrated to China from Korean peninsula during the middle 19th century to the early 20th century. There is no significant difference, when compared among the haplotype frequencies of Korean, Korean Chinese and Han Chinese (Table 3).

We analyzed the genotype and allele frequencies between allergic rhinitis patients and non-allergic rhinitis controls. The genotype frequencies of all loci were in HWE ($P > 0.05$, data not shown). The genotype and allele frequencies of *g.32349 G>A* in allergic rhinitis patients were significantly different (Table 4) from those in the controls group ($P = 0.032$ and 0.01, respectively). This result suggests the *g.32349 G>A* polymorphism of *IL-28RA* might be associated with susceptibility to allergic rhinitis. We also calculated the haplotype frequencies among *g.-30 C>T*, *g.17654 C>T*, *g.27798 A>G* and *g.32349*

Table 4. Genotype and allele analyses of the polymorphisms of *IL-28RA* gene in allergic rhinitis (AR) patients and controls.

Position ^a	Genotype/ Allele	Control n (%)	AR n (%)	Odds ratio ^b (95% CI)	P
g.-1193A > C	AA	462 (82.7)	221 (82.8)	1.00	0.377
	AC	93 (16.6)	46 (17.2)	1.03 (0.70-1.52)	
	CC	4 (0.7)	0 (0.0)	0.01 (0.00-6456.4)	
	A	1,017 (91.0)	488 (91.4)	1.00	
	C	101 (9.0)	46 (8.6)	0.95 (0.66-1.37)	
g.-30C > T	CC	88 (15.8)	43 (16.2)	1.00	0.159
	CT	274 (49.0)	112 (42.3)	0.84 (0.55-1.28)	
	TT	197 (35.2)	110 (41.5)	1.14 (0.74-1.76)	
	C	430 (38.5)	198 (37.4)	1.00	
	T	688 (61.5)	332 (62.6)	1.05 (0.85-1.30)	
g.17654C > T	CC	453 (82.5)	221 (83.1)	1.00	0.482
	CT	93 (16.9)	45 (16.9)	0.99 (0.67-1.47)	
	TT	3 (0.6)	0 (0.0)	0.01 (0.00-48996.7)	
	C	999 (91.0)	487 (91.5)	1.00	
	T	99 (9.0)	45 (8.5)	0.93 (0.65-1.35)	
g.27798A > G	AA	99 (17.8)	58 (21.9)	1.00	0.208
	AG	272 (49.0)	133 (50.2)	0.84 (0.57-1.23)	
	GG	184 (33.2)	74 (27.9)	0.69 (0.45-1.05)	
	A	470 (42.3)	249 (47.0)	1.00	
	G	640 (57.7)	281 (53.0)	0.83 (0.67-1.02)	
g.31265C > T	CC	49 (9.5)	28 (10.7)	1.00	0.202
	CT	232 (44.9)	100 (38.2)	0.75 (0.45-1.27)	
	TT	236 (45.6)	134 (51.1)	0.99 (0.60-1.66)	
	C	330 (31.9)	156 (29.8)	1.00	
	T	704 (68.1)	368 (70.2)	1.11 (0.88-1.39)	
g.31911C > T	CC	348 (65.8)	183 (69.8)	1.00	0.494
	CT	170 (32.1)	75 (28.6)	0.84 (0.61-1.16)	
	TT	11 (2.1)	4 (1.5)	0.69 (0.22-2.20)	
	C	866 (81.9)	441 (84.2)	1.00	
	T	192 (18.1)	83 (15.8)	0.85 (0.64-1.13)	
g.32349G > A	GG	216 (40.8)	132 (50.4)	1.00	0.032
	GA	241 (45.5)	103 (39.3)	0.70 (0.51-0.96)	
	AA	73 (13.8)	27 (10.3)	0.61 (0.37-0.99)	
	G	673 (63.5)	367 (70.0)	1.00	
	A	387 (36.5)	157 (30.0)	0.74 (0.59-0.93)	

^aCalculated from the translation start site. ^bLogistic regression analyses were used for calculating OR (95% CI; confidence interval).

G>A in both non-allergic rhinitis controls and allergic rhinitis patients. While six major (more than 5%) haplotypes explaining more than 88.0% of distributions were identified in allergic rhinitis patients, seven major haplotypes (~89.2%) in controls were identified out of sixteen possible haplotypes (Table 5). While the major haplotype in control group is TCGG (20.3%), the major haplotype in allergic rhinitis patients is TCAG (28.0%). The distribution of haplotype TCAG and TCGA were significantly di-

fferent between the non-allergic rhinitis controls and allergic rhinitis patients ($P < 0.0000007$ and $P = 0.019$, respectively). These results suggested that the *IL-28RA* polymorphisms might be one of the most important genetic factors in allergic rhinitis susceptibility.

To precisely determine whether these SNPs of *IL-28RA* gene were related to total serum IgE levels and peripheral blood eosinophil counts in allergic rhinitis patients, we analyzed their association and

Table 5. The haplotype frequencies of *IL-28RA* polymorphisms in allergic rhinitis (AR) patients and controls.

Haplotype				Frequency ^a		<i>P</i> ^b
g.-30C > T	g.17654C > T	g.27798A > G	g.32349G > A	Control	AR	
T	C	G	G	0.203	0.186	0.492
T	C	A	G	0.171	0.280	0.0000007
T	C	G	A	0.158	0.105	0.019
C	C	G	A	0.114	0.161	0.069
C	C	A	G	0.111	0.084	0.268
C	C	G	G	0.085	0.064	0.282
T	C	A	A	0.050	0.014	0.003
C	T	A	G	0.031	0.035	0.730
T	T	A	G	0.021	0.039	0.109
C	C	A	A	0.020	0.019	0.935
Others				0.036	0.013	-

^aValues were calculated by EM algorithm with genotyped SNPs. ^bValues were analyzed by permutation test.

Table 6. The levels of IgE and eosinophil counts among the genotypes of polymorphisms of *IL-28RA* gene in allergic rhinitis patients.

Position ^a	Genotype	IgE (IU/ml)			<i>P</i> ^b	Eosinophil (10 ³ /μl)			<i>P</i> ^b
		n	Mean	SD		n	Mean	SD	
g.-30C > T	CC	11	476	612	0.48	34	0.55	0.42	0.02
	CT	37	708	689		91	0.39	0.28	
	TT	22	545	600		81	0.36	0.35	
g.17654C > T	CC	54	637	678	0.70	170	0.42	0.35	0.28
	CT	16	564	552		35	0.35	0.25	
	TT	0	-	-		0	-	-	
g.27798A > G	AA	12	593	466	0.23	41	0.34	0.27	0.34
	AG	38	528	612		102	0.41	0.35	
	GG	19	839	797		61	0.44	0.37	
g.32349G > A	GG	31	681	708	0.64	102	0.39	0.36	0.14
	GA	28	570	625		75	0.39	0.26	
	AA	6	831	627		22	0.54	0.47	

^aCalculated from the translation start site. ^bValues were analyzed by ANOVA.

found that these SNPs in allergic rhinitis patients have no significant association with total serum IgE levels, while a polymorphism *g.-30 C > T* showed significant association with peripheral blood eosinophil counts ($P = 0.02$) in allergic rhinitis patients (Table 6). These results suggest that these polymorphisms of *IL-28RA* might not affect the IgE production in allergic rhinitis patients, but it is possible to think that *g.-30 C > T* might affect eosinophil recruitments in allergic rhinitis patients.

Allergic rhinitis is a multi-complex disorder influenced by genetic predisposition and environmental factors. The several numbers of studies have been reported for analyzing the association of SNPs with

allergic rhinitis. Cheng and coworkers reported that the polymorphisms of disintegrin and metalloprotease domain 33 (*ADAM33*) gene are associated with susceptibility to allergic rhinitis in Japanese population (Cheng *et al.*, 2004). We previously reported that the polymorphisms of *eotaxin-2*, *eotaxin-3* and *FOXJ1* are associated with allergic rhinitis in Korean population (Chae *et al.*, 2005b; Li *et al.*, 2006). In this study, our results strongly suggest that the polymorphisms of *g.32349 G > A* in *IL-28RA* gene might be associated with susceptibility to allergic rhinitis, but not with IgE production. Our study is the first analysis about *IL-28RA* SNPs, and may contribute to the future study on the *IL-28RA*

functions and association studies with infectious disease such as hepatitis.

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