Novel Variations in Human Interleukin-29 and Their Association

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Gene polymorphisms of cytokines and their receptors are attractive candidates as genetic factors in the pathogenesis of immune-mediated diseases and have been reported to be associated with disease susceptibility to autoimmune, inflammatory and infectious diseases. *IL-29* is one of important candidate genes for complex trait of genetic diseases but there is no published survey of single nucleotide polymorphisms (SNPs) in this gene. In this study, for the first time, we have examined the full genomic sequence of *IL-29* including the promoter regions to identify SNPs. We examined the frequencies of genotypes and alleles at the SNP site of *IL-29* in allergic rhinitis patients and non-allergic rhinitis controls using the direct sequencing method to determine whether this *IL-29* SNP is associated with allergic rhinitis in Korean population. We identified one novel SNP (1184C>A) in the intron 2 and one novel variation site (-1842_-1841dupGA) in the promoter region of human *IL-29* gene. The *P* values of SNP or variation site were not significant between the healthy controls and allergic rhinitis patients. Our results suggest that the 1184C>A polymorphism and -1842_-1841dupGA variation site in human *IL-29* gene were not associated to allergic rhinitis.

Key words – Interukin-29, polymorphisms, Allergic rhinitis, cytokine, variation

Cytokines are multifunctional proteins that mediate many 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 [4,9,14,15,17]. The polymorphisms within coding region of cytokine gene influence on protein structure and expression. Although the amino acid sequence is not changed, the silent mutations may influence protein expression by the alterative mRNA splicing, mRNA stability and levels of gene transcription. Polymorphisms within the regulatory region 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 [3].

The SNPs of many cytokines and their receptor genes, such as IL-10, IL-12, IFN γ, RANTES, CCR2 and CCR5, are identified [1,7,8,11] and indicate that the polymorphisms are associated with immune disorders [6,13]. Recently, the new cytokine family consisting of interleukin 28A (IL-28A), IL-28B and IL-29 and a component of their receptors, IL-28R α, are identified from the human genomic sequence. They are distantly related to type I interferons (IFNs) and the IL-10 family, and induced by viral infection and show antiviral activity [12,18]. 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-28R α. IL-29 expression is detected at small amounts in libraries from a wide range of tissue types, including blood, brain, lung, ovary, pancreas, pituitary, placenta, prostate and testis. *IL-29* genes are located on the chromosomal region 19q13.13 and have five exons. Although *IL-29* is one of important candidate genes for complex trait of genetic diseases, there is no published survey of single nucleotide polymorphisms (SNPs) in this gene.

In this study, for the first time, we have examined the full genomic sequence of *IL-29* including the promoter regions (~2.2 kb) to identify SNPs, which might be useful for understanding the genetic influences of this gene. The frequencies of genotype and allele at the SNP site of *IL-29* were analyzed in the genomic DNAs isolated from allergic rhinitis patients and non-allergic rhinitis controls using the direct sequencing method to determine whether this *IL-29* SNP might be associated with allergic rhinitis in Korean population.

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Materials and Methods

Subjects and DNA samples

Blood samples were obtained from 27 allergic rhinitis patients and 25 non- allergic rhinitis controls. Genomic DNA was extracted from leukocyte in peripheral blood by a standard phenol-chloroform method or by Invisorb® spin blood Maxi kit (Invitek, Germany) according to manufacturer's directions. The allergic rhinitis patients were recruited from our outpatient clinic at Wonkwang University Hospital. The non-asthmatic control subjects were recruited from the general population who took a comprehensive medical testing. All subjects in this study were Korean who was living in the same area.

PCR and sequencing analysis

Genomic regions of *IL-29* containing the promoter region were partially amplified and sequenced by using the 14 primers shown in Table 1. The polymerase chain reaction (PCR) was carried out in a 25 μl reaction volume containing 50 ng genomic DNA, 0.5 μM primers, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), and 1 U Ex Taq polymerase (TaKaRa, Japan). Amplification was carried out in a GeneAmp PCR system 9700 thermocycler (PE Applied Biosystems, USA) for 94℃ at 1 min, followed by 30 cycles at 98℃ for 10 seconds, 68℃ for 2 min, and 68℃ for 2 min and followed by a final extension at 72℃ for 10 min.

PCR products purified by PCR purification kit (Millipore, USA) were used as the template DNA for cycle sequencing. Purified PCR products were sequenced using the ABI Prism BigDye Terminator cycle sequencing system (PE Applied Biosystems, USA) on the ABI 3100 automatic sequencer (PE Applied Biosystems, USA). The reference sequence for *IL-29* was based on the sequence of human chromosome 19 clone CTC-246B18 (Human Genome Database).

Statistical analysis

The allergic rhinitis patients and controls were compared using case-control association analyses. Allele carrier frequency was defined as the percentage of the individuals carrying the allele among the total number of the individuals. X^2 test from 2×3 or 2×6 contingency table was applied to analyze the comparison of the frequency of discrete variables between allergic rhinitis patients and healthy controls. A *P*-value less than 0.05 were considered to indicate statistical significance.

Results and Discussion

IL-29 is one of important candidate genes for complex trait of genetic diseases and is induced by viral infection. To determine the variation sites in the full genomic sequence of *IL-29* including the promoter regions, we scanned 27 allergic rhinitis patients and 25 non-allergic rhinitis controls using direct sequencing. We identified one novel SNP (1184C>A) in the intron 2 and one variation site (-1842_-1841dupGA) in the promoter region of human *IL-29* gene (Table 2). In 1184C>A polymorphism, the genotype CA was

Table 1. Primer sequences for amplifying and sequencing of the *IL-29* including promoter regions

Primer name	Primer sequence for PCR (5′→3′)[a]	Product size
IL29-LF1	GCGATGGGAATGGGAATTGGCA	3201 bp
IL29-LR1	GGAAGTGACAGGGACAGGTG	
IL29-LF2	GCTGCAGCTTGGACCGTGGT	2308 bp
IL29-LR2	ACACCCAGTCACAGACCCAC	
Primer name	**Primer sequence for sequencing (5′→3′)**	**Regions**
IL29-F1	CACTTTGCCTTCCTATGCCTCA	promoter
IL29-R1	GATGTGCAGCTGGGCATGGT	promoter
IL29-R2	CCTTAGAACTCCCTGGGC	promoter
IL29-F2	ACCAGTCAAGGTGACACC	promoter
IL29-R3	GCCAAGAAGGAGCTTCTGCC	promoter
IL29-R4	AGTTGGCCTGACAGCATGGG	exon1 and intron 1
IL29-F3	CACTCTCTTGGACCTCTCC	intron 2
IL29-R5	GCAGGTGAGGGAGAACAGGC	intron 2
IL29-F4	CTCCTTGACCATCCTGCC	exon3 and intron 3
IL29-R6	GGCTCCTATTGGCTCCCCAG	exon 4 and intron 4

[a]The primers for PCR were also used for sequencing analysis of exon 2 and exon 5.

Table 2. Genotype frequency of 1184C>A polymorphic site and allele frequency of -1842_-1841dupGA variation site in *IL-29* gene

Position[a] (combined variation sites)	Genotype / Allele	Control n (%)	Rhinitis n (%)	P^b
1184C>A	CC	1 (4.2)	2 (8.7)	0.835
	CA	22 (95.8)	21 (91.3)	
	AA	0	0	
-1842_-1841dupGA	14GA	0	1 (1.9)	0.245
	15GA	0	5 (9.3)	
	16GA	6 (12.0)	7 (13.0)	
	17GA	23 (46.0)	19 (35.1)	
	18GA	19 (38.0)	19 (35.1)	
	19GA	2 (4.0)	3 (5.6)	

[a]Calculated from the translation start site.
[b]Value was determined by X^2 test from 2×3 or 2×6 contingency table.

dominantly existed in a Korean population and the genotype CC was not detected in healthy controls (n = 23) as well as in allergic rhinitis patients (n = 23). The *P* value of this polymorphic site was not significantly different between the healthy controls and allergic rhinitis patients ($P = 0.835$, Table 2).

The -1842_-1841dupGA variation site of promoter region has many variations by GA duplication as shown in Fig. 1. The variations, -1842_-1841dupGA, -1844_-1841dupGAGA and -1846_-1841dupGAGAGA, resulting in by 17 GA, 18 GA and 19 GA repetitions respectively were existed in there. Moreover, -1842_-1841delGA and -1844_-1841delGAGA by deletions were also detected in promoter region of *IL-29* gene. The *P* value of this variation site was not significantly different between the healthy controls and allergic rhinitis patients ($P = 0.245$).

Allergic rhinitis is an inflammatory nasal disorder, in which a range of cells and mediators contribute, in a coordinated complex network [10,16]. Dendritic cells (DCs) increase in nasal mucosa of allergic rhinitis patients after allergen exposure [5] and may regulate the balance between Th1 and Th2 cells. Interactions between DCs and T cells from patients with allergic rhinitis result in release of Th2 cytokines [2]. Although it is not yet known whether these newly described variation site is associated with some functions and expression of IL-29 in allergic rhinitis or other allergic diseases, it will be important in future studies to determine whether or not this variation affects the levels of IL-29 production and functions. It would also be interesting to analyze the allele frequency of these variances with large sample size in patients with autoimmune and allergic diseases.

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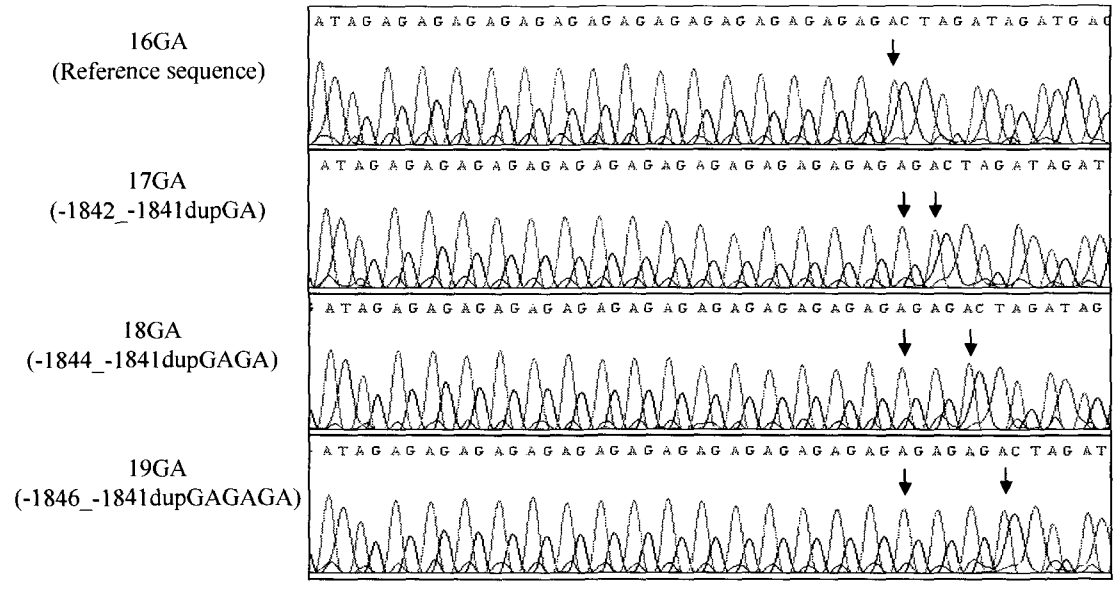

Fig. 1. DNA sequence chromatograms in variation site of *IL-29* promoter region. PCR products purified by PCR purification kit (Millipore, USA) were used as the template DNA for cycle sequencing. Sequence analysis was performed by BigDye Terminator cycle sequencing using an ABI 3100 Prism Automated DNA sequencer (PE Applied Biosystems, USA) according to the manufacturer's instruction. The arrows indicate variation sites.

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초록 : 사람의 Interleukin-29 유전자의 새로운 변이의 단리 및 그들의 연관

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사이토카인과 그들 수용체의 유전자 다형성은 면역작용에 의한 질병들의 발병원인에 있어서 유전적인 인자로 여겨지는 후보물질들로서, 자가면역질환 및 염증성 그리고 감염질환에 민감하게 연관되어 있다고 알려져 있다. 최근 새롭게 보고된 Interleukin-29 유전자는 유전학적 질병들의 복잡한 특성을 해결할 수 있는 중요한 후보유전자이지만 이 유전자에 대한 다형성에 대한 연구는 아직 보고된바 없다. 우리는 이 연구에서 처음으로 프로모터부분을 포함한 Interleukin-29 유전자의 전체 지놈 DNA에서 유전자의 다형성을 염기서열 분석 방법을 이용하여 탐색하였다. Interleukin-29 유전자의 다형성들이 한국인의 알레르기성 비염의 감염력과 관련되어 있는지를 알아보기 위하여 알레르기성 비염환자 및 알레르기성 비염이 걸리지 않은 정상인의 다형성을 유전자형과 대립유전자의 빈도를 비교분석 하였다. 우리는 이 연구에서 사람의 Interleukin-29 유전자의 한 개의 신규의 다형성 (1184C>A)을 intron 2에서 그리고 한 개의 신규의 변이부위 (-1842_-1841dupGA)를 프로모터에서 찾아냈다. 우리들의 연구결과는 이들 유전자 다형성 부위 및 변이부위가 알레르기성 비염과 연관은 없는 것으로 밝혀졌다.