Association of Interleukin 18 (IL-18) /-133(C/G) Polymorphism with Specific IgE Levels to Der p Allergen in Thai Allergic Rhinitis Patients

ความสัมพันธ์ในความแตกต่างของเบสที่ตำแหน่ง -133 ของยีนอินเตอร์ลิวคิน 18 กับระดับ specific IgE ต่อสารก่อภูมิแพ้ไรฝุ่นชนิด Der p ในผู้ป่วยโรคจมูกอักเสบภูมิแพ้

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ABSTRACT

Allergic rhinitis (AR) is one of the most common allergic diseases which develops by the interaction of genetic and environmental factors. IL-18 is a cytokine that plays an important role in allergic inflammatory reactions by upregulated IgE production. We investigated possible association of -133(C/G) polymorphism in *IL-18* gene with AR in Thai patients. Blood samples were obtained from 150 AR patients and 50 control individuals. Genotyping of IL-18/-133(C/G) polymorphism was analyzed by PCR-RFLP. We found that IL-18/-133 polymorphism was significantly associated with specific IgE levels to Der p allergen (P=0.025) but there was no significant differences in the genotype distribution of AR patients when compared with control group. The G allele in the IL-18/-133 gene promoter region may relate with increasing level of specific IgE to Der p allergen in AR patients.

บทคัดย่อ

โรคจมูกอักเสบภูมิแพ้จัดเป็นโรคภูมิแพ้ที่พบมากที่สุด ซึ่งเกิดจากปัจจัยทางพันธุกรรมและสิ่งแวดล้อม อินเตอร์ลิวคิน 18 เป็นไซโตไคน์ที่สำคัญกับกระบวนการอักเสบภูมิแพ้โดยควบคุมการเพิ่มการสร้างอิมมูโนโกลบูลินอี เราได้ทำการศึกษาหาความสัมพันธ์ของความหลากหลายทางพันธุกรรมในยีนอินเตอร์ลิวคิน 18 ที่ตำแหน่ง -133 กับโรคจมูกอักเสบภูมิแพ้ในผู้ป่วยคนไทย โดยการเก็บตัวอย่างเลือดจากกลุ่มผู้ป่วย 150 รายและกลุ่มควบคุม 50 ราย ทำการศึกษายีนอินเตอร์ลิวคิน 18 ที่ตำแหน่ง -133 ด้วยวิธี PCR-RFLP พบว่าความหลากหลายของยีนอินเตอร์ลิวคิน 18 ที่ตำแหน่ง -133 มีความสัมพันธ์อย่างมีนัยสำคัญกับระดับ specific IgE ต่อสารก่อภูมิแพ้ไรฝุ่นชนิด Der p (P=0.025) แต่การกระจายตัวของยีนอินเตอร์ลิวคิน 18 ในผู้ป่วยโรคจมูกอักเสบภูมิแพ้ไม่มีความแตกต่างกับกลุ่มควบคุม แอลลีลจีที่ตำแหน่ง -133 ซึ่งอยู่ในบริเวณ promoter ของยีนอินเตอร์ลิวคิน 18 อาจมีความสัมพันธ์ต่อการเพิ่มระดับของ specific IgE ต่อสารก่อภูมิแพ้ไรฝุ่นชนิด Der p ในผู้ป่วยโรคจมูกอักเสบภูมิแพ้

Key Words: Allergic rhinitis, IL-18, Polymorphism

คำสำคัญ: โรคจมูกอักเสบภูมิแพ้ อินเตอร์ลิวคิน 18 ความหลากหลายทางพันธุกรรม

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Introduction

Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa having clinical manifestation of itching, sneezing, rhinorrhea (runny nose) and nasal congestion. AR is the most common allergic diseases which is currently affecting approximately 10-20% in the general population (Bousquet *et al*., 2008; Wallace *et al*., 2008) with an increasing proportion of people worldwide. In addition, AR is strongly associated with the presence of asthma and accounted to be a serious public health problem. Allergy is type I hypersensitivity which develops by the interaction of genetic and environmental basis. The reaction occurs when the sensitized person re-exposures to specific allergen which leads to activate the allergen-specific T helper2 (T_H2) cells and IgE synthesis.

Inflammatory reactions in allergic diseases are regulated by many cytokines. Recently, interleukin (IL)-18 is much of interest as this cytokine is capable to be both anti-allergic and allergy-promoting effect (Nakanishi *et al*., 2001). IL-18 is a member of the IL-1 superfamily of cytokines that is mainly produced by activated macrophages monocytes and dendritic cells. It was originally discovered as an important factor in the T_H1 response that induces interferon-gamma production in T-cells, B-cells and NK cells in the presence of IL-12 (Okamura *et al*., 1998). IL-18 has the potential to induce the production of various T_H2 cytokines including IL-4, IL-5 and IL-13 (Yoshimoto *et al*., 2000). IL-18 can regulate both innate and acquired immunity. Therefore, IL-18 plays an important role in allergic inflammatory reactions by upregulated IgE production.

The human *IL-18* gene is located on chromosome 11q22.2-22.3 where it is found to be associated with allergic diseases (Koppelman *et al*., 2002) and sensitization to mite allergens (Kurz *et al*., 2000). Recent studies have shown that single nucleotide polymorphisms (SNPs) in the *IL-18* gene are related to AR (Kruse *et al*., 2003; Lee *et al*., 2006). These SNPs may exert an effect on its expression and are suspected to have direct consequence on the pathogenesis of AR. However, a heterogeneity of allelic frequencies at certain loci among different populations may be generating discordant outcomes. To date, the relationship of *IL-18* gene polymorphisms and AR in Thai population has not been studied. Therefore, we have chosen the IL-18/-133(C/G) polymorphism and formerly reported its correlation with atopy in other populations for the present study.

Objectives of the study

This study aimed to investigate the possible association of -133(C/G) polymorphism in *IL-18* gene with AR in Thai patients as well as their specific IgE levels to Der p allergen.

Methodology

Study population

One hundred fifty AR patients (61 male, 89 female) and 50 healthy controls (14 male, 36 female) of Thai nationality were recruited from Allergy Clinic of the Departments of Pediatrics and Oto-rhino-laryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand during June 2012 – June 2013. The subjects presented with a clinical diagnosis of allergic rhinitis (sneezing, rhinorrhea, and/or nasal congestion on most days) and had a positive skin prick test to *Dermatophagoides pteronyssinus* (Der p) allergen were grouped as AR patients. The control group was healthy volunteers who had a negative skin prick test to Der p allergen and without atopic symptom and any chronic diseases. All plasma samples were measured for specific IgE levels to Der p allergen using immunoCAP system (ImmunoCAP® 100E Automate, Uppsala, Sweden). In

clinical practice, 0.35 KUA/L is used as a cut-off. This research study was approved by the Human Ethical Committee, Faculty of Medicine Siriraj Hospital, Mahidol University.

PCR-RFLP

Genomic DNA was extracted from the peripheral leukocytes in EDTA-treated blood sample using the Flexi gene DNA kit (Qiagen, Hilden, Germany). Genotyping of IL18/-133 polymorphism was performed with mutated primers (forward; 5'-GTATT CATAAGCTGAAACTCCCGG-3' and reverse; 5'-TGTTCTATGGCATTAGCCTTAC-3'). PCR was performed in 25 µl volumes consisting of DNA 150 ng, dNTP 200 µM, $MgCl_2$ 1.5 mM, *Taq* DNA polymerase (Thermo Fisher Scientific Inc., San Diego, CA) 1.5 U, and 0.32 µM of each primer. After denaturation for 5 minutes at 95 ⁰C, 30 cycles were carried out, with each cycle consisting of 1 minute at 95 ⁰C, 45 seconds at 51 ⁰C (annealing temperature), and 45 seconds at 72 ⁰C. The last synthesis step was extended to 6 minutes. The amplified product was digested with *Sma*I (Thermo Fisher Scientific Inc., San Diego, CA) and analyzed on a 4% agarose gel and visualized, stained with ethidium bromide.

Statistical analysis

Analysis was performed using SPSS for Windows Version 16.0 (SPSS Inc., Chicago, IL, USA). SNPs were tested for conformation with Hardy-Weinberg expectations (HWE) using Michael H. Court's (2005-2008) online calculator. The Kruskal-Wallis Test was used to compare genotypes frequencies to specific IgE levels to Der p allergen. Comparison of allelic and genotypes frequencies and association of polymorphisms with AR patients and control groups were examined for statistical significance using standard Pearson X^2-test or Fisher's exact test as appropriate. A value of $P< 0.05$ was considered statistically significant.

Results

IL-18/-133(C/G) polymorphism analysis by PCR-RFLP

The 265 bp DNA segment of PCR was digested using *Sma*I. DNA from CC homozygous individuals were shown 265 bp fragments, whereas DNA from GG homozygous individuals showed 243 and 22 bp fragments. Moreover, DNA from CG heterozygous individuals showed the expected fragments at 265, 243, and 22 bp fragments (Fig 1).

Table 1 The association of specific IgE levels to Der p allergen with genotype IL-18/-133 polymorphism

Variant	Genotype	Mean Rank	$\chi 2$	*P* value
-133 (C/G)				
	CC	95.15		
	CG	121.45	7.357	0.025
	GG	144.00		

The Kruskal-Wallis Test calculated using to compare three genotype to specific IgE levels

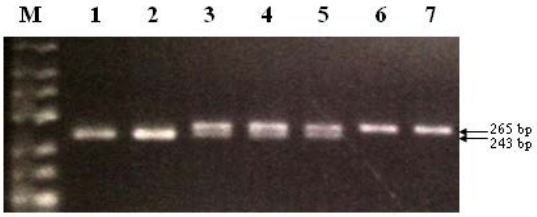

Fig 1 Genotyping of -133(C/G) polymorphism by PCR-RFLP. Size markers are shown in lane M; GG homozygous are in lanes 1 and 2; CG heterozygous in lanes 3, 4, and 5; and CC homozygous in lanes 6 and 7.

The association of specific IgE levels to Der p allergen with genotypes frequencies of -133(C/G) polymorphism in *IL-18* gene

The association for all the three genotypes to specific IgE levels are shown in Table 1. The median of specific IgE levels to Der p allergen were significant difference in CC, CG, and GG genotypes (P=0.025).

Comparison of genotype and allele frequencies distributions of -133(C/G) polymorphisms in *IL-18* gene between AR and control groups

The genotype of the AR and control groups are shown in Table 2. The frequencies of the CC, CG, and GG genotypes in AR patients were no significantly when compared with the control group (P=0.64).

Discussion and Conclusions

In this study, we investigated the genotype and allele frequencies distributions of IL-18/-133(C/G) polymorphisms in Thai AR patients. Kurz et al. had exhibited that the location of *IL-18* on chromosome 11q22.2-22.3, a region where had influence on atopy-related phenotype with mite sensitization. Hence, our investigation mainly focused with mite sensitive subjects because this allergen is the most common allergen in this country. We discovered that the median of specific IgE levels to mite allergen namely Der p allergen showed significant differences from the genotype distribution of IL-18/-133(C/G) polymorphism. Our finding, which was performed in perennial AR subjects who are sensitive to mite allergen is correlated with the result previously reported by Kruse *et al*. They found that -133G SNP was highly significantly associated with high IgE levels and specific sensitization to common allergens in seasonal AR patients (Kruse *et al*., 2003). Comparing of the sensitized allergens in these 2 studies might imply that this SNP is not only associated with the indoor allergen derived from house dust mite but also the outdoor allergen like pollens. In fact, the associations between polymorphisms with

Table 2 Comparison of genotype distributions of IL-18/-133 polymorphisms between AR and control groups

Variant	Genotype	AR group (%) n = 200	Control group (%) n = 50	*P* value
-133 (C/G)				
	CC	119 (79.3)	42 (84)	
	CG	29 (19.3)	8 (16)	0.64
	GG	2 (1.3)	0	

P value calculated using Chi-Square test of association for all the three genotypes (P<0.05)

atopy among ethnic differences may appears diverse results. We performed our study in Thai patients considered as an Asian population whereas that of Kruse et al. was carried out with German subjects regarded as a white population. Several studies have indicated that increased IL-18 cytokine concentrations in nasal secretions, serum levels, and plasma are associated with atopic diseases, such as AR, AD, and allergic asthma (Verhaeghe *et al*., 2002; Yoshizawa *et al*., 2002; Wong *et al*., 2001). These might be the function of IL-18 which is known to influence the balance of T_H1/ T_H2 immune response (Izakovicova *et al*., 2003), allergic inflammatory reactions by upregulation of IgE production as well as inducing IL-4 and IL-13 secretion. According to these results, we suggest that IL-18/-133(C/G) polymorphism might be a common SNP which may effect either IL-18 concentrations or some kinds of atopic disorders.

Promoter is a part of DNA that initiates transcription of a particular gene, where either direct mutations of its sequence or mutation in a transcription factor or transcription co-activator is believed to have direct involvement with hundreds of diseases including atopy. Mutation in promoter regions would effect IL-18 production which may lead to the final impact on IgE production. Nevertheless, Kalina *et al.* proposed that human IL-18 may be regulated by promoter 1 only, but not for promoter 2 (Kalina *et al*., 2000). The -133(C/G) polymorphism is located in promoter 2 region. Both from our outcome and the mentioned publications it is indicated the linkages effect the -133(C/G) polymorphism in promoter 2 with allergy, which contrasts with the finding of Kalina *et al*. The C to G substitution at this position may affect to potential binding site for cytosolic proteins STAT that are involved in inflammation. Those proteins may alter *IL-18* transcription and result in their consecutive expression (Puthothu *et al*., 2007).

However, we found that the SNP -133G in promoter 2 was not associated with allergic rhinitis phenotype. The negative association reported in our study was also observed between AR patients in Czech and Egyptian populations (Sebelova *et al*., 2007; Ibrahim *et al*., 2012). A definitive effect of the -133G polymorphism and allergic diseases had been observed in asthma among the Korean subjects (Shin *et al*., 2005) and atopic dermatitis in Egyptian patients with regards to disease severity (Ibrahim *et al*., 2012) but not AR. This may suggest that the -133G polymorphism would has influence on allergic phenotypes for some diseases like atopic asthma and dermatitis but appear to achieve only specific IgE levels in AR. Hence, further study should be performed to find out the possible association of -133G polymorphisms in Thai population with other allergic diseases such as atopic dermatitis and asthma. On the other hand, the potential involvement of AR in Thai residents and other SNPs in *IL-18* gene should be explored. Moreover, it would be interesting to investigate the potential effect of the -133G polymorphism on the expression of *IL-18* gene and the cytokine function of IL-18 as well as its serum levels.

Our study was the initial investigation of the relations of polymorphisms and AR in Thai population. This study indicated that the G allele in the IL-18/-133 gene promoter region may be related to increase specific IgE levels to Der p allergen. It may be the foundation of basic knowledge of a polymorphisms of candidate gene in AR and make it possible to predict the susceptibility of a particular individual at risk of allergic diseases.

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