

Association between interleukin-18 gene polymorphism 105A/C and asthma

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Summary

Background IL-18 has been shown to exert anti-allergic or allergy-promoting activities, but the existence of genetic polymorphisms in the coding regions of IL-18 gene has not been demonstrated. **Objective** The aim of this study was to investigate whether polymorphism is present in the coding regions of the IL-18 gene and, if so, to further analyse the association between polymorphism and asthma in a case-control study.

Methods We screened the coding regions of the IL-18 gene for polymorphisms by using PCRsingle-stranded conformation polymorphism and direct sequencing of PCR products, followed by analysis of the association between polymorphism and asthma.

Results We identified one polymorphism (105A/C) in the coding regions. The frequency of the 105A allele was significantly higher in asthmatic patients than in controls ($P < 0.01$; odds ratio (OR) = 1.83 (1.37–2.26)). Significant linkage disequilibrium was observed between the 105A/C and –137G/C polymorphisms in the 5' flanking region of the IL-18 gene ($D = 0.58$, $P < 0.0001$). However, in asthmatic patients the 105A allele was not associated with either total serum IgE or IL-18 levels.

Conclusion The 105A/C polymorphism of the IL-18 gene may be associated with the pathogenesis of asthma.

Keywords asthma, IL-18, polymorphism

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Introduction

The IFN- γ -inducing cytokine IL-18 has been shown to exert innate as well as cellular immunity through both NK activation and Th1 induction by acting on the responding cell population [1–4]. It also has an inhibitory effect on IgE synthesis by directly acting on B cells when stimulated with IL-12 through the induction of endogenous IFN- γ [5]. In contrast, it was found that IL-18 directly stimulates IL-4 and histamine release from basophils [6], IL-13 production by NK and T cells [7], induces naïve T cells to differentiate into Th2 cells [8] and enhances IL-8 production by eosinophils [9]. These findings indicate that IL-18 has both anti-allergic and allergy-promoting effects [10].

Excess of IL-18 production was recently found in patients with allergic diseases, including asthma and atopic dermatitis [11–15]. Since IL-18 also increased in patients with other diseases in which a predominance of Th1 cells may play an important role [16–20], it remains to be clarified whether IL-18 has a positive or negative function at the onset or development of allergic diseases. In order to identify the

genetic determinants for asthma, we examined whether or not polymorphisms occur in the coding regions of the IL-18 gene and, if so, what the relation is between polymorphism and asthma. In fact, one silent polymorphism (A/C) located at the 105th nucleotide initiating from ATG was identified in the coding regions of the IL-18 gene. The frequency of this polymorphism in controls significantly differed from that in patients with asthma, indicating an association of IL-18-105A/C with asthma.

Materials and methods

Subjects

A total of 221 asthmatic children 1–18 years of age, 276 asthmatic adults 18–78 years old and 85 adult controls without any past or current allergic diseases, including asthma, atopic dermatitis and pollen's diseases, were enrolled in the study. Asthma was diagnosed on the basis of the American Thoracic Society guidelines. Classification of asthma severity was judged by global strategy for asthma management and prevention. Four hundred and seven asthmatic patients showed total IgE concentrations above 200 IU/mL or specific sensitization to major allergens such as

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house dust mite, dog and cat, and classified into atopic type of asthma. A full verbal and written explanation of the study was given to the parents of the asthmatic children or asthmatic adults and written informed consent was obtained. The study was approved by the relevant ethical committees in accordance with the guidelines for the management of genetic studies.

Screening for polymorphisms in the coding regions of the IL-18 gene

Total RNA was extracted from peripheral blood mononuclear cells by using RNeasy (Qiagen, Crawley, UK) according to the manufacturer's instructions. The extracted RNA was then dissolved in DEPC water and its concentration was measured with a spectrometer. The coding region of the IL-18 gene spanning exons 3–7 was amplified by RT-PCR according to the manufacturer's protocol (Perkin-Elmer Cetus Co., Norwalk, CT, USA). The cDNA was amplified by PCR in the presence of 180 kBq (α - ^{32} P)-dCTP (Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, UK). PCR conditions were as follows: 94 °C, 1 min; 63 °C, 1 min; and 72 °C, 1 min for 36 cycles. The primers used for amplification of the coding region of IL-18 spanning exons 3–7 were: sense, ATG-GCT-GCT-GAA-CCA-GTA-GA; anti-sense, AGC-TAG-TCT-TCG-TTT-TGA-ACA-G. The product was then digested with the *MspI* restriction enzyme (Toyobo Co., Osaka, Japan) at 37 °C for 4 h and examined for the presence of polymorphisms by single-stranded conformation polymorphism (SSCP) analysis [21].

DNA sequencing

PCR products were purified from the gel and DNA sequencing was performed according to the protocol for the dye terminator cycle sequencing FS ready reaction kit (Applied Biosystems, Osaka, Japan). Briefly, the amplification of the coding region of IL-18 cDNA for sequencing was performed in 25 cycles of denaturation for 10 s at 96 °C, annealing for 5 s at 50 °C and extension for 4 min at 60 °C by using the dye terminator cycle sequencing FS ready reaction kit (ABI) and the 2400 Thermal cycle and 377 sequencer (Perkin-Elmer Cetus).

Genotyping

Genomic DNA was extracted from peripheral blood leucocytes by means of the QIA prep Spin (Qiagen GmbH, Hilden, Germany). For the PCR, 100 ng of DNA was used, and for the detection of IL-18-105A/C polymorphism, primers (sense, TGT-TTA-TTG-TAG-AAA-ACC-TGG-AAT-T; anti-sense, CCT-CTA-CAG-TCA-GAA-TCA-GT) were designed. The partial coding region of the IL-18 gene was then amplified by PCR according to the manufacturer's protocol. PCR conditions were as follows: 94 °C, 1 min; 50 °C, 1 min; and 72 °C, 1 min for 40 cycles. The PCR product was digested with *TaqI* restriction enzyme (Toyobo) at 65 °C for 2 h and electrophoresed on the PAGE gel (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). The gel was then stained with ethidium bromide and photographed. In order to examine the promoter regions, –607C/A and –137G/C for polymorphisms, previously reported methods and primers were

employed [22]. The genotype of β 2-adrenergic receptor (β 2-ADR)-16 Arg/Gly was determined by using primers (sense, CGG-GAA-CGG-CAG-AGC-CTT-CTT-GCT-GGC-ACC-CAC-T; anti-sense, CCG-TCT-GCA-GAC-GCT-CTA-AC), and the PCR conditions were as follows: 94 °C, 1 min; 60 °C, 1 min; and 72 °C, 1 min for 40 cycles. The PCR product was digested with *BbvI* (Toyobo) at 37 °C for 2 h.

Measurement of IL-18

Serum concentrations of IL-18 were measured by means of ELISA assays (IL-18 ELISA kit, MBL, Nagoya, Japan).

Statistical analysis

The frequencies of alleles were calculated by counting. Data were analysed with MedCalc and Arlequin software and the Hardy–Weinberg equilibrium was determined by means of the χ^2 goodness-of-fit test. Fisher's exact test was used to detect differences in allele distribution between the groups, and the exact-test-of-population differentiation was used to determine whether the genotypic composition of the groups was significantly heterogeneous. The odds ratio (OR) was calculated by means of logistic regression, and the confidence interval (CI) was calculated at the 95% level. The maximum likelihood estimates of relative linkage disequilibria between 105A/C and –607C/A or –137G/C were calculated according to the methods of Thompson et al. [23]. Statistical significance was assumed for *P*-values less than 0.05.

Results

A novel polymorphism, 105A/C, in the coding regions of the IL-18 gene

It has been recently shown that IL-18 concentrations in the sera from patients with asthma and atopic dermatitis are elevated [11–15]. As shown in Fig. 1, we could confirm that the serum IL-18 levels for the patients with asthma were higher than for controls. Specifically, the average level of

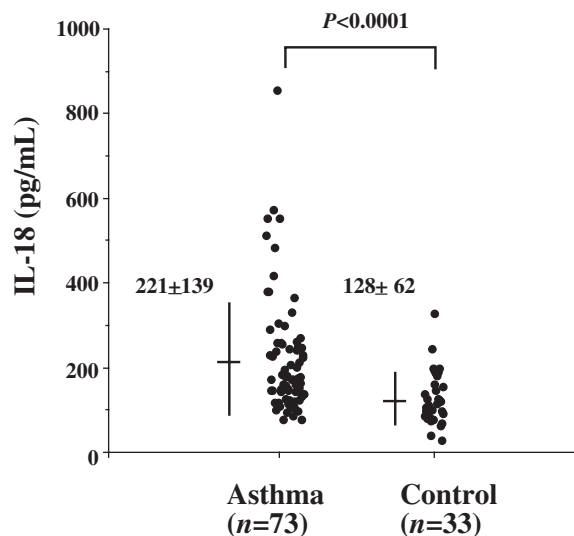


Fig. 1. Increased serum levels of IL-18 in patients with asthma.

serum IL-18 for patients with asthma was 221 pg/mL, which was significantly higher than for the controls (128 pg/mL) ($P < 0.0001$). In order to determine whether this up-regulation of IL-18 levels in asthmatic patients was associated with genetic polymorphisms or whether IL-18 might play a positive or negative role at the onset of asthma, we examined the coding regions of the IL-18 gene for the existence of polymorphisms. The translational coding region spanning exons 3–7 was amplified through RT-PCR of 30 different samples. The existence of polymorphisms was then verified by SSCP analysis. Fig. 2 shows the results. A predominant band was obtained from most of the samples, as well as another, more slowly migrating band from some of them. The sequence analysis of the PCR products identified a novel polymorphism (A/C) at the 105th site counting from ATG, but this polymorphism was silent.

IL-18-105A allele was associated with asthma

Next, the frequency of this polymorphism was studied in 497 asthmatic patients and 85 controls by PCR as shown in Fig. 2. Table 1 demonstrates that the frequency of the A allele for

patients with asthma was 0.891, which was significantly higher than that for controls (0.818) ($P < 0.01$). The OR of carrying one of the two A alleles vs. none was calculated as 1.83. This OR increased substantially to 1.92 for the data from asthmatic children (Table 2). This result demonstrates that the IL-18-105A/C polymorphism is associated with asthma. In order to exclude that this significance is due to the chance of population admixture, allele frequencies of $\beta 2$ -ADR-16-Arg/Gly were measured. The frequency of Arg allele for patients with asthma was similar to that from controls. We also examined the association of IL-18-105A/C polymorphism with the current severity of asthma and with atopic status. However, we failed to observe an association between the genotype and severity or atopic status (Table 2).

Linkage disequilibrium between 105A/C and -137G/C promoter polymorphism

Polymorphisms in the promoter region of the IL-18 gene have recently been identified [22]. The changes from C to A at position -607 (-607C/A) and from G to C at position -137 (-137G/C) were found to affect the transcription of the IL-18 gene. We next tried to determine whether there was a linkage disequilibrium between the coding polymorphism 105A/C and -607C/A or -137G/C. Table 3 shows the pairwise analysis of the polymorphisms, indicating a significant disequilibrium between 105A/C and -137G/C, and between 105A/C and -607C/A. A linkage disequilibrium between 105A/C and -137G/C ($D = 0.575$, $P < 0.0001$) was more significantly observed than that between 105A/C and -607C/A ($D = 0.283$, $P = 0.0034$). Finally, we verified the association of the genotype of 105A/C with serum IgE levels and IL-18 levels for asthmatic patients, but as shown in Table 4, no significant relation could be detected.

Discussion

In this study, we identified one polymorphism, 105A/C, in the coding regions of the IL-18 gene and demonstrated the association between this polymorphism and asthma. The OR was 1.83 for the asthmatic phenotype when the allele was A. Case-control studies are sometimes susceptible to the population admixture, but the frequency of Arg in patients with asthma was similar to that from controls. These results suggest that IL-18 may play a role in the pathogenesis of asthma, although it remains to be determined as to how the polymorphism influences the pathogenesis. The linkage disequilibrium between 105A and -137G suggests that the 105A allele may be associated with the up-regulation of IL-18

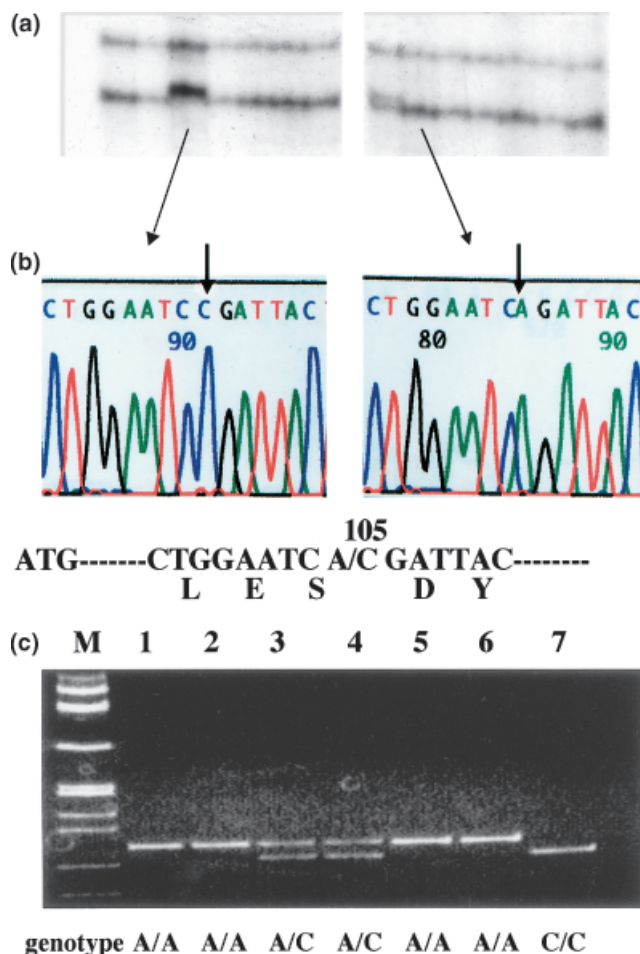


Fig. 2. IL-18-105A/C polymorphism RT-PCR-SSCP analysis of coding regions of the IL-18 gene. The coding region spanning exons 3–7 was amplified by RT-PCR and then digested with *MspI*. The products were then electrophoresed. Direct sequencing of the PCR products determined the existence of polymorphism located at the 105th site, A/C. Genotyping of IL-18-105A/C by RFLP-PCR.

Table 1. Allele and genotype frequencies of IL-18-105A/C polymorphisms in patients with asthma and controls

Allele	Asthma (n = 497)	Control (n = 85)	Genotype	Asthma (n = 497)	Control (n = 85)
A	0.891	0.818	AA	0.792	0.682
C	0.109	0.182	AC	0.197	0.271
$P = 0.0062$			CC	0.010	0.047
			$P = 0.0089$		

Table 2. Allele frequencies of IL-18-105A/C and β 2-ADR-16-Arg/Gly in asthmatic adults and children (upper panel) and allele frequencies of IL-18-105A/C in asthmatic patients classified by severity and atopics (lower panel)

Allele	Control	Asthma	Adult	Children
<i>IL-18-105A/C</i>				
A	139	886	490	396
C	31	108	62	46
Odds ratio		1.83	1.76	1.92
(CI)		(1.37–2.26)	(1.10–2.82)	(1.18–3.10)
P-value		0.0062	0.0172	0.0089
<i>β2-ADR-16</i>				
Arg	71	484	269	215
Gly	81	506	283	223
Odds ratio		1.09	1.08	1.10
(CI)		(0.78–1.53)	(0.75–1.55)	(0.76–1.59)
P-value		0.6169	0.6588	0.6135

Table 2a

Allele	Control	Severity of asthma			Type of asthma	
		Intermittent	Mild to moderate	Severe	Atopics	Non-atopics
A	139	523	206	77	722	164
C	31	64	26	9	92	16
Odds ratio		1.82	1.76	1.91	1.75	2.29
(CI)		(1.43–2.31)	(1.01–3.08)	(0.87–4.18)	(1.11–2.75)	(1.20–4.37)
P-value		0.0110	0.0460	0.1058	0.0129	0.0104

Table 3. Combined genotype frequencies of IL-18-105A/C, IL-18-137G/C and IL-18-607C/A

	105A/C			
	A/A	A/C	C/C	
– 137G/C				
G/G	59	0	0	$P < 0.0001$
G/C	1	17	1	$n = 84$
C/C	0	0	6	$D = 0.575$
– 607C/A				
C/C	16	0	0	$P = 0.0034$
C/A	28	10	1	$n = 83$
A/A	15	7	6	$D = 0.283$

Linkage disequilibrium between 105A/C and – 137G/C was more significantly observed than that between 105A/C and – 607C/A.

Table 4. Serum levels of IgE and IL-18 from patients with asthma

	IgE (IU/mL)	IL-18 (pg/mL)
<i>Genotype</i>		
A/A	869 ± 1852 ($n = 360$)	220 ± 148 ($n = 57$)
A/C	1176 ± 2973 ($n = 87$)	224 ± 102 ($n = 16$)
C/C	1274 ± 1176 ($n = 4$)	ND

Serum levels of IgE and IL-18 were compared with the IL-18-105A/C genotype. Results are indicated as means ± SD. There was no significant difference. ND = Not determined.

expression through the –137G allele, although we did not find an increase in the serum IL-18 levels of asthmatic patients with the AA haplotype of the 105 polymorphism. A previous report regarding polymorphisms in the promoter region showed that the –137G allele enhanced the transcriptional activity of the IL-18 gene when cells were stimulated with PMA plus ionomycin, but that there were no significant differences in promoter activity between –137G and –137C alleles in the absence of stimulants [22]. Alternatively, it is possible that 105A/C, although quiescent, directly affects the modulation of transcription of the gene or RNA stabilization [24] or that 105A/C is linked to another polymorphism, which alters the functional property of IL-18 or its gene regulation. Further studies are required to clarify these points.

Recently, several lines of *in vitro* and *in vivo* studies have generated contradictory results about the function of IL-18 in the pathophysiology of allergic diseases [10]. The activities of IL-18, including induction of IFN- γ synthesis by T cells or NK cells and of Th1 induction [1–3] as well as inhibition of IgE production by B cells when expressed together with IL-12 [5], show that this cytokine may be a negative regulator in view of the pathogenesis of allergic diseases. However, IL-18 has also been reported to induce the release of both histamine and IL-4 from basophils [6], and cause Th2 differentiation [8]. The effect of IL-18 on the asthma mouse model thus seems to be paradoxical. For instance, administration of IL-18 was found to enhance Ag-induced eosinophils recruitment into the trachea and bronchoalveolar lavage fluid of sensitized mice *in vivo* [25], and to increase Th2 cytokines and airway eosinophilia in a mouse model of allergic asthma [26]. In

contrast, coadministration of IL-12 and IL-18 was shown to inhibit antigen-induced airway hyper-responsiveness, eosinophilia and serum IgE levels [27], and IL-18 deficiency to cause enhancement of allergen-induced eosinophilia accumulation [28]. Although the reasons for such contradictory results remain to be determined, it is likely that they are related to the responding cells or to whether or not IL-18 together with IL-12 acts on the cells. On the other hand, changes in IL-18 properties or expression through genetic polymorphisms could lead to the exacerbation or suppression of allergic responses.

Polymorphisms in candidate genes have been identified and associated with asthma [29, 30]. The IL-18 gene is located at chromosomal region 11q23 [31]. Previous studies of genome-wide research for asthma have demonstrated that the region of 11q21–24 was linked to asthma phenotype, specific IgE to a group of common aeroallergens and positive skin test to house dust mite [32, 33]. Candidate genes in this region include matrix metalloproteinase genes 1, 3 and 8, and the δ , ϵ and γ subunits of CD3. Moreover, the newly identified IL-18-105A/C polymorphism and/or the previously identified IL-18-137G/C polymorphism can be considered to be one of such polymorphisms associated with asthma. We expect that our observations are going to contribute to an understanding of the pathophysiology of asthma and result in the establishment of predictive markers for asthma.

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