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BRIEF REPORT — POLYMORPHISM REPORT

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Novel polymorphism in the 5′-untranslated region of the interleukin-4 gene

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Abstract We found a novel polymorphism C/T change at position -34 counting from the first ATG codon, of the interleukin-4 (*IL-4*) gene, located between the TATA box and the first ATG on exon 1. The allelic frequency of this C/T polymorphism in 64 independent Japanese samples was 0.37:0.63. In addition, PCR-restriction fragment length polymorphism analysis revealed that the -34T/T polymorphism was always associated with -590T/T, a known polymorphism that increases *IL-4* transcriptional activity.

Key words Interleukin-4 gene · Polymorphism · Atopic asthma · Translation

Introduction

Interleukin-4 (*IL-4*) is one of the chromosome 5q-cytokines that are involved in the development of atopic asthma. So far, *IL-4* promoter polymorphism C/T at position -590, counting from the first ATG codon (-590C/T), is the only reported polymorphism of the *IL-4* gene (Rosenwasser et al. 1995; Noguchi et al. 1998). This polymorphism is located upstream of all the known control elements of *IL-4*, such as the negative regulatory element, the positive regulatory elements, the NF-Y recognition sequence, the OAP40 recognition sequence, the NF-P recognition sequence, and the TATA box (Walley and Cookson 1996), and affects *IL-4* transcriptional activity (Rosenwasser et al. 1995; Song et al. 1996). In addition to the -590C/T polymorphism, we found a novel polymorphism of the *IL-4* gene at position -34 bp from the first ATG codon (-34C/T) and 33 bp from the transcriptional initiation site. We also studied the association between the -34C/T and -590C/T polymorphisms.

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Source and description

For the detection of polymorphisms of the *IL-4* gene near the control elements, we amplified the region including the upstream promoter of the *IL-4* gene by polymerase chain reaction (PCR). The PCR reaction was carried out on Ready-To-Go PCR Beads (Amersham Pharmacia Biotech, Uppsala, Sweden) in a total volume of 25 μl containing 20 ng of genomic DNA, and the primer pair 5′-CAGCAGCCCCAAGCTGATAAG-3′ and 5′-TTGCCGG CACATGCTAGCAG-3′ to amplify from the -358 to +58 region of the *IL-4* gene. The PCR conditions were: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. The PCR fragments of 408 bp were analyzed by direct sequencing, using an ABI PRISM 310 genetic analyzer (Perkin- Elmer, Foster City, CA, USA). We screened nine independent samples and found a single nucleotide C/T polymorphism at -34 bp from the first ATG.

Polymorphism and allele frequency

As this genetic alteration from C to T generated an *Mnl*I restriction site, we performed PCR-restriction fragment length polymorphism (RFLP) analysis. The PCR conditions were: initial denaturation at 94°C for 5 min, followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 15 s, with the primer pair 5′-CTCATTTTCCGTCGGTTTCAGC-3′ and 5′-GAAGCAGTTGGGACGTGAGA-3′ to amplify from the -126 to +23 region of the *IL-4* gene. The PCR products were digested with *Mnl*I (New England Biolab, Hertfordshire, UK), separated onto a 3% agarose gel, and visualized by ethidium bromide staining. By determining the genotypes of 64 Japanese samples, we estimated the allelic frequency of C:T to be 0.37:0.63 (Table 1).

Table 1. Genotype and allele frequencies of *IL-4* gene -34C/T polymorphism

	Genotypes			Alleles	
No	C/C	C/T	T/T	C	T
64	6	35	23	47	81
(%)	(9.4)	(54.7)	(35.9)	(36.7)	(63.3)

Further study of the association between the -34C/T and -590C/T polymorphisms of the *IL-4* gene showed that -34C/C and T/T were always associated with -590C/C and T/T, respectively, by PCR-RFLP analysis. Thus, it is most likely that -34C and -590C are on the one allele, while -34T and -590T are on the other. Since polymorphism of the translation initiation site causes a change in translation efficiency (Kanaji et al. 1998), further study will be needed to determine whether the -34C/T polymorphism near the translation initiation site has an effect on the translation of *IL-4* transcripts.

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