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ORIGINAL ARTICLE

IL-12B Promoter Polymorphism Associated with Asthma and *IL-12B* Transcriptional Activity

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ABSTRACT

Background: The interleukin-12B gene (*IL-12B*) encodes the p40 chain of interleukin-12 (IL-12), which promotes cell-mediated Th1 responses and the production of interferon-gamma (IFN-γ) that downregulates IgE production. Chromosome 5q31-q33 near the *IL-12B* locus is significantly linked to asthma, as determined by a genome-wide search in the Japanese population.

Methods: We sequenced exons 1-8 including parts of the introns and promoter region of *IL-12B* in asthmatic patients and healthy controls. We examined plasma IL-12 concentrations, IL-12 production by Derf1-stimulated peripheral blood mononuclear cells (PBMCs) and the *IL-12B* transcriptional activity.

Results: *IL-12B* promoter polymorphism existed as $^{-2703}$CTCTAA/GC and $^{-2403}$T/C alleles, which were linked to each other. Homozygosity for haplotype 1 ($^{-2703}$CTCTAA /$^{-2403}$T) was associated with asthma susceptibility in Japanese children ($P < 0.001$). Both plasma IL-12 concentrations and IL-12 production by Derf1-stimulated PBMCs in the subjects with homozygotes for haplotype 1 were lower than those with homozygotes for haplotype 2 ($^{-2703}$GC /$^{-2403}$C) ($P < 0.001$). The transcriptional activity of the construct with haplotype 1 was lower than that of the construct with haplotype 2, and the *IL-12B* transcriptional activity was influenced by the $^{-2403}$T/C allele rather than by the $^{-2703}$CTCTAA/GC allele.

Conclusions: Homozygosity for haplotype 1, which is associated with reduced *IL-12B* transcriptional activity and reduced IL-12 production, is a predisposing factor for asthma susceptibility in Japanese children.

KEY WORDS

asthma, IgE, interferon-gamma, interleukin-12B promoter polymorphism

INTRODUCTION

Interleukin-12 (IL-12) is a heterodimeric molecule that is composed of two disulfide-linked subunits, p35 and p40. It is produced by macrophages, B cells and other antigen-presenting cells (APCs),[1,2] and plays important roles in interferon-gamma (IFN-γ) production by T cells and natural killer (NK) cells.

Genome-wide linkage screens, in which the genetic factors of the diseases can be identified, have been performed for asthma and recognized many regions linked to asthma.[3] Asthma is associated with Th2 cytokines, such as IL-4, IL-5, IL-9, IL-13, which are mapped to chromosome 5q31-q33. Polymorphisms of the IL-4 receptor α chain and IL-13 are associated with asthma.[4-6] Yokouchi *et al.* have reported significant evidence for linkage of asthma to 5 q31-33 near the *IL-12B* locus but not the IL-4 and IL-13 loci in the Japanese population.[7] Therefore, *IL-12B* is one of the candidate genes for asthma. Several polymorphisms have been identified in *IL-12B*,[8,9] including a single-nucleotide polymorphism in the 3' untranslated region, which has been associated with the susceptibility to type 1 diabetes and atopic dermatitis[10,11] but not to asthma and allergic rhinitis in the Japanese population.[12] Recently, it has been reported that the polymorphism exists in the *IL-12B* promoter region.[13,14]

In this study, we sequenced exons 1-8 including parts of the introns and region 3 kb upstream from

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Table 1 Sequence of oligonucleotides for PCR.

Primer	Sequence	Primer	Sequence	position
1S	5'-GAGAAGCATTCAGAAGCTCT-3'	1A	5'-GTCCCACTTCACAATCCAGA-3'	promoter
2S	5'-GTTTGTCAGCAGACCTTCCT-3'	2A	5'-GGAACAGGGCTCTGAATTGT-3'	promoter
3S	5'-GACAAGTGATTTCACTGCGG-3'	3A	5'-GGGCTAGTCCTATATGAAAG-3'	promoter
4S	5'-GGTATCCAGCTCTCTAACTC-3'	4A	5'-GACTTTGCCTTTTAGCCTTC-3'	promoter
5S	5'-GCAATCTGCTTTGTCCACTT-3'	5A	5'-GCTAAGAGGTATGCAAAGGT-3'	promoter
6S	5'-GCAGGTACATGTTCCTGTTC-3'	6A	5'-GGTTCTTCCCAAGTCAGAGA-3'	promoter
7S	5'-GCCAAGATGGGTGGTAAATA-3'	7A	5'-GAGGAGGGAACATAGACATC-3'	promoter
8S	5'-GCATCTCCATCTCCTTCCTT-3'	8A	5'-GCACACTAACGGTTTCTACA-3'	exon1
9S	5'-GGCTTAAAGGGGCCAAGT-3'	9A	5'-AGGGAGCACTATCCCTCAGC-3'	intron1-1
10S	5'-ATGTTATCTCATTGCCTTTC-3'	10A	5'-AAGTGGTTCTGAAACCACTG-3'	intron1-2
11S	5'-GTATCAGATGGCTTGCCTTA-3'	11A	5'-GTGCATGGTTGCCCATTTCA-3'	exon2
12S	5'-GGGAAGACTAAGCTCTACTG-3'	12A	5'-CAACGAACCAAGACTGTCAT-3'	intron2
13S	5'-GTCTTGTGCTGTTTGCAGTT-3'	13A	5'-GCATCTCCAAACTCTTTGAC-3'	exon3-1
14S	5'-GTGACACCCCTGAAGAAGAT-3'	14A	5'-GAGGCTAAGCATTCAGACTG-3'	exon3-2
15S	5'-GATAGTGTATCACTCTGCAC-3'	15A	5'-GCTGAGAAACCAGAGCAGTT-3'	exon4
16S	5'-TACTTCTGCTGACACCACTA-3'	16A	5'-GAACTAGGATCAAATTGTATAC-3'	intron4-1
17S	5'-GGTTACATAATCATATGTA-3'	17A	5'-GTTAGGATTTCAGGTGTGAG-3'	intron4-2
18S	5'-TCCAGAGACATGTAAGTGC-3'	18A	5'-GAGATGATGCTTGTCAACCA-3'	exon5
19S	5'-GCATCTCTCAGATCCTGCAA-3'	19A	5'-GCACCTGAATCACTTCTTAC-3'	exon6
20S	5'-GCTAGAAAGATGAAAGCTGG-3'	20A	5'-GTTTCTGATTCTGGCAACTG-3'	exon7
21S	5'-TAGCTCATCTTGGAGCGAAT-3'	21A	5'-GCTTGCCAGAGGCTTTCTTG-3'	intron7
22S	5'-GCAAGCTTGCAGGACTCAGA-3'	22A	5'-GATGGATCAGGTCATAAGAG-3'	exon8-1
23S	5'-GCCAGGATGTATGGAATGTT-3'	23A	5'-GACAGGGTCTCATTCTGTCA-3'	exon8-2
24S	5'-GCCTAGGTGACAGAATGAGA-3'	24A	5'-GCAAGCAGAGTACTCAAATC-3'	exon8-3

the transcriptional start site of *IL-12B* in 30 patients. Furthermore, we investigated *IL-12B* promoter polymorphism in 111 asthmatic patients and 78 controls, and examined the relationship between *IL-12B* polymorphism and asthma prevalence, IL-12 production and *IL-12B* transcriptional activity. We showed that *IL-12B* promoter polymorphism is associated with asthma and influences IL-12 production and *IL-12B* transcriptional activity.

METHODS

PATIENTS AND CONTROL SUBJECTS

One hundred and eleven asthmatic patients and 78 controls were enrolled in this study. Asthma was diagnosed on the basis of the American Thoracic Society guidelines. All the asthmatic patients showed total IgE levels above 200 IU/mL or specific sensitization to major allergens such as house dust and mite. The mean age ± SD of the asthmatic patients was 5.6 ± 2.9 years, and their mean IgE ± SD level was 906.8 ± 1347.4 IU/mL. All the controls had a negative history of atopic diseases. Their plasma IgE levels were lower than 150 IU/mL and their specific IgE levels were negative. The mean age ± SD of the controls was 4.7 ± 3.4 years, and their mean IgE ± SD level was 52.3 ± 52.4 IU/mL. Informed consent was obtained from all the subjects or their parents.

DETECTION OF *IL-12B* POLYMORPHISM

Genome DNA was extracted from neutrophils with a Sepa-gene kit (Sanko Junyaku, Tokyo, Japan). Exons 1-8 including parts of the introns and region 3 kb upstream from the transcriptional start site of *IL-12B* were amplified and sequenced using an ABI 3100 DNA sequencer (Applied Biosystems, CA, USA). We also sequenced previously identified polymorphisms in the introns.[9] The conditions for the PCR were 94℃ for 1 minute, 60℃ for 1 minute, and 72℃ for 1 minute. The primers used are shown in Table 1.

CELL PREPARATION

Peripheral blood mononuclear cells (PBMCs) were separated from the heparinized blood of the subjects by gradient centrifugation in Ficoll-Paque (Pharmacia, Uppsala, Sweden). PBMCs were suspended at a density of 1×10^6/mL in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mmol/L L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin.

CELL CULTURE

PBMCs (1×10^6/mL) were cultured in the presence or absence of 5 IU/mL recombinant human IL-12 (R &D Systems, Inc, Wiesbaden, Germany) or 5 μg/mL Derf1 (Asahi, Tokyo, Japan) for 24 hours in a final volume of 1 mL in a round-bottom tube (Falcon 2059,