

Several interleukin-4 and interleukin-13 gene single nucleotide polymorphisms among Chinese asthmatic patients

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ABSTRACT

Asthma is a common respiratory disease associated with airway hyperactivity and high total serum immunoglobulin E (IgE) levels. The asthmatic phenotypes are widely considered to be caused by environmental effects on genetically predisposed individuals. Interleukin (IL)-4 and IL-13 act on B cells and regulate the IgE production, and the single nucleotide polymorphisms (SNPs) in the IL-4 and IL-13 genes are implicated in the pathogenesis of asthma. To study the association of IL-4 and IL-13 gene polymorphisms with asthma, we sequenced the promoter regions and exons of IL-4 and IL-13 genes in two groups: one (spouses group) consisted of 13 pairs of asthmatic patients (cases) and their unaffected spouses (controls); the other (parents group) consisted of 11 pairs of asthmatic children (cases) and their unaffected father/mother (controls). In total, seven polymorphisms were identified, one novel and six previously reported. However, according to the results of the statistical analysis we performed, no statistically significant association of these SNPs and asthma was observed ($p > 0.05$). Asthma is largely determined by the accumulation of several certain genetic variations other than one or two SNPs. Future function studies may be able to help us reveal the significance of genetic variants we identified in this study.

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Key words: Asthma, children, gene, interleukin-4, interleukin-13, parent, phenotype, polymorphisms, SNPs, spouse

Asthma is a major public health burden that has shown a dramatic increase in prevalence over the past 20 years.¹ The development of asthma appeared to be caused by a variety of environmental exposures in genetically predisposed individuals.² Over the past 10 years, great efforts have been made to map the chromosomal locations of genes that contribute to asthma susceptibility.^{3–5} Chromosome 5q31–q33 contains a cluster of proinflammatory cytokines important in immune regulation. Two members in this region, interleukin (IL)-4 and IL-13, are implicated as asthma susceptibility genes.^{3,6} IL-4 is known to be essential in the pathogenesis of asthma and other allergic diseases^{7,8} by triggering isotype switching from immunoglobulin M (IgM) to IgE and IgG4.^{5,9} Although IL-4 and IL-13 exert similar biological activity,^{10,11} IL-13 shows unique activities in IgE-dependent inflammatory reactions.⁸ Recent studies have shown that asthma phenotype, including Airway hyper-responsiveness, inflammation, and airway remodeling,

could be suppressed by anti-human IL-13 monoclonal antibody.^{12,13} Therefore, both IL-4 and IL-13 are important to the study of asthma.

An exon polymorphism in the IL-13 gene was reported to be associated with elevated total serum IgE levels in different populations.^{14–17} This polymorphism leads to a substitution of Arg130Gln in the protein sequence and was predicted to have altered the IL-13 binding affinity to its receptor,^{15,18} although other studies doubted the statistical significance of this association.¹⁹ Furthermore, an association of C-589T in IL-4 gene with high total serum IgE levels^{20,21} and asthma²² was reported in some studies but could not be confirmed by others.^{23–25} Further study shows that the haplotype included IL-4 C-589T allele and other single nucleotide polymorphisms (SNPs) in the IL-4 gene were strongly associated with atopy.²⁴ To make clear the elusive genetic background of asthma, especially its uncertain association with IL-4 and IL-13 gene polymorphisms, we performed two groups of case-control experiments to study this association in a Chinese population.

METHODS

Subjects

We had a population of 24 pairs of asthmatic patients and controls. The diagnosis of bronchial asthma was made according to the National Asthma Education and Prevention program (Expert Panel Report-3) guidelines.²⁶ Clinical history, physical examination, and pul-

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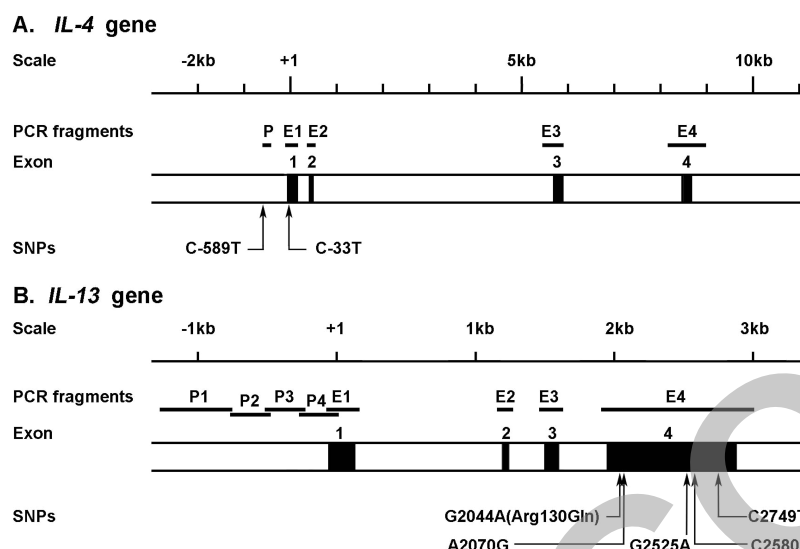


Figure 1. Interleukin (IL)-4 and IL-13 gene structure, polymerase chain reaction (PCR) segments used for single nucleotide polymorphism (SNP) detection, and the SNPs. (A) IL-4 gene has four exons, coding for a 153-amino acid peptide. Five PCR fragments were used, covering a piece of IL-4 promoter and all four exons. Two SNPs were found: C-589T in IL-4 P and C-33T in IL-4 E1 (5' untranslated region [5'-UTR]). (B) The IL-13 gene also has four exons, and it codes for a peptide of 132 amino-acids. Five overlapping fragments were used to cover the IL-13 promoter and exon 1. Three other segments were used, respectively, for the exons 2, 3, and 4. Five SNPs were identified, with only one (G2044A) leading to an amino acid polymorphism in IL-13 Protein (Arg130Gln). The rest of the SNPs are in the 3'-UTR of IL-13. Six of the SNPs were previously identified, and the IL-13 A2070G (exon 4, 3'-UTR) are novel. The position +1 refers to the beginning of the first open reading frame (ORF).

monary function test (forced expiratory volume in 1 second [FEV₁]) were performed for all subjects and they were examined for history of breathlessness and wheezing. The diagnosis of bronchial asthma was confirmed by a clear clinical history with clinical symptoms (episodic wheezing, chest tightness, and shortness of breath) and a 12% or great reversibility of FEV₁ spontaneously after administration of a bronchodilator.

The subjects were divided into two groups, the spouses group and the parents group. The spouses group consisted of 13 pairs of patients (cases) and their unaffected spouses (controls). It was assumed that the patient and his/her spouse were living in the same place and facing the same environmental changes, so that the effects of environment exposures could be eliminated. Considering the multigenic nature of asthma, another group of samples were used. In the parents group, 11 asthmatic children (cases), each paired with one of their unaffected parents (controls), were studied. An additional advantage of this group is that the paired case and control share not only the environment but also half of their genetic information. Therefore, the interference by other asthma susceptibility genes was weakened.

SNP Detection and Genotyping

The SNP identification is performed by sequencing of polymerase chain reaction (PCR) amplified frag-

ments covering the promoters and exons of IL-4 and IL-13 gene. In total, 13 PCR segments were used (Fig. 1 and Table 1). Genomic DNA was extracted from peripheral blood leukocytes, using the TaKaRa Blood Genome DNA Extraction Kit. PCRs were performed for 34 cycles in a total volume of 50 μ L containing 50 ng of genomic DNA, 100 μ mol/L each of dNTP, 200 pmol/L each of primer, and 4 U of ExTaq DNA polymerase (TaKaRa) in standard PCR buffer with magnesium chloride (TaKaRa). All of the PCR products were then purified using the TaKaRa Gel Extraction Kit. The purified fragments were sequenced in the DNA Sequencing Department at the TaKaRa Biotechnology (Dalian, China) Co., Ltd. The sequencing work was done with the ABI PRISM 377XL DNA Sequencer and ABI PRISM BIGDYE Terminator Cycle Sequencing Ready Reaction Kit.

For the C-589T polymorphism (or the IL-4 P fragment), restriction fragment length polymorphism technique was used for genotyping.²⁷ A substitution was introduced to the forward primer of IL-4 P (5'-TA-ACTTGGGCGAACATGGT-3') to generate a restriction site. The purified IL-4 P fragment was digested with 1 U of *Ava*II (Promega) overnight at 37°C, and the alleles resolved by electrophoresis on a 3.0% agarose gel. The fragment sizes were 195 bp for the T allele and 177 and 18 bp for the C allele. The other 12 PCR fragments were genotyped directly by sequencing.

Table 1 Polymerase chain reaction (PCR) primers and assay conditions

Fragment Name		Primers (5'-3')	Fragment Size (bp)	PCR Condition* (°C)
IL-4	P§	11883#-TAAACTTGGGCGAACATGGT-11902 12077-TGGGGAAAGATAGAGTAATA-12058	195	52
	E1	12368-TCATTTTCCCTCGGTTTCAG-12387 12660-ACATCTGGAGAGATGGTGCC-12641	293	56
	E2	12839-CTTCTCTGTCCGGTTGGAGGTT-12860 13040-GCCCCGCTCGTTTTCCATTAGAC-13019	202	56
	E3	17930-GGCAAATCCTCCAAGAGAAAGG-17952 18403-CCACGCGGCCGGCTTGCCA-18385	474	56
	E4	20640-AGCATCTTCAAGTTCCACCCTC-20661	842	66
IL-13	P1	21481-ACTCAGTATAACAATCCTTGTGCC-21458 933-GTTGAGCCCCATCGAGGAC-951 1468-GGGTCTCACTATGTTGCCTGC-1448	536	54
	P2	1443-CCTAGGCAGGCAACATAGTG-462 1741-GCTATGGGAATTTGGGGAGT-1722	299	56
	P3	1692-TTTAAGAGACTGGTTCATCG-1711 1991-ACTTATTGAGAAGGGTCCAG-1972	298	52
	P4	1942-TAAACCCACCCAGATCTTGG-1961 2232-TGGTCAACAAAAGCGCCATG-2213	291	56
	E1	2134-GCTGCCACAAGACGCCAAGGCC-2155 2381-CCCTCATAGCTAGGACCCTGGC-2360	248	66
	E2	3367-GCCAGCACTCTGCTCACTGTCAC-3389 3488-GCCCCATCCTCCCTGCACCC-3469	122	56
	E3	3669-CCCAAGCAGGGCCTGACCCCTCGG-3692 3848-GCAGGGTGGGTGTGAGAGGG-3829	180	66
	E4	4117-GCCTCTGGCGTTCTACTCAC-4136 5223-CTACCCTCGCTGTAATCCATCC-5202	1107	66

*PCR annealing temperature.

#Nucleotide number with respect to the GenBank accession number of AF395008 for IL-4 and U31120 for IL-13.

§The forward primer of IL-4 P fragment was modified to generate a *Ava*II restriction site. After *Ava*II digestion, a 195-bp fragment represented the T allele, and 177 and 18 bp fragments represented the C allele.

IL = interleukin.

Statistical Analysis

Allele distributions were compared between the cases and controls in both groups by χ^2 -test, which was preformed with SPSS software package (Version 12.0; SPSS, Inc., Chicago, IL). No correction was made in this study for two reasons: individual allele is not independent of each other and previous studies revealed IL-4 and IL-13 genes to be asthma susceptibility genes.

RESULTS

In this study, a total of seven SNPs were identified (Fig. 1 and Table 2). Two SNPs were observed in IL-4 gene (C-589T and C-33T). Five SNPs were detected in IL-13 gene, and all of them were in exon 4. Only one of them (G2044A) could lead to an amino acid alteration in the IL-13 protein (glutamine for arginine substitution; Arg130Gln). The other four SNPs (A2070G,

G2525A, C2580A, and C2749T) were located in the 3'-UTR of the IL-13 gene.

Here, we report a newly identified SNP in the IL-13 gene: A2070G. According to the data we obtained, the frequency of the G allele is really low. Only two heterozygous individuals were found in a pool of 48 subjects, and they are a pair of daughter (asthmatic child) and mother (unaffected control). The rest of the SNPs have previously been reported in other population groups.^{19,25}

Because of the limited of number of subjects, the χ^2 -test results may be invalid when performed with the number of each genotype in cases and controls. Thus, we applied the χ^2 -tests to both the spouse and the parent groups using the number of each allele. However, this study failed to show statistically significant associations between asthma and any of the detected SNPs in either of the two groups ($p > 0.05$; Tables 2 and 3).

Table 2 Association between IL-4 and IL-13 gene polymorphisms and asthma in two groups

	SNPs	Alleles	Cases	Controls	χ^2	<i>p</i> Value
Spouses group						
IL-4	C-589T	C	8	11	0.746	0.388
		T	18	15		
IL-13	C-33T	C	6	7	0.103	0.749
		T	20	19		
	G2044A	G	23	23	0.000	1.000
		A	3	3		
	A2070G	A	26	26	NA	NA
		G	0	0		
	G2525A	G	22	23	0.165	0.685
		A	4	3		
C2580A	C	24	24	0.000	1.000	
	A	2	2			
C2749T	C	24	24	0.000	1.000	
	T	2	2			
Parents group						
IL-4	C-589T	C	6	7	0.109	0.741
		T	16	15		
IL-13	C-33T	C	1	3	1.100	0.294
		T	21	19		
	G2044A	G	19	18	0.170	0.680
		A	3	4		
	A2070G	A	21	21	0.000	1.000
		G	1	1		
	G2525A	G	20	20	0.000	1.000
		A	2	2		
C2580A	C	20	19	0.226	0.635	
	A	2	3			
C2749T	C	19	20	0.226	0.635	
	T	3	2			

The χ^2 tests were performed using the number of each allele in cases and controls.

IL = interleukin; NA = not available; SNPs = single nucleotide polymorphisms.

DISCUSSION

Asthma is an inflammatory airway disease that is characterized by episodic airway obstruction for which an allergic underpinning is common. The development of this disease is largely considered to be determined by the interaction between genetic susceptibility of the host and various environmental exposures. Over the past 10 years, numerous genetic studies have provided the abundance of asthma susceptibility genes: IL genes (IL-4,^{5,20-22,25,27,28} IL-13,^{5,15,19,29} IL-9,^{30,31} and IL-5^{6,32}), CD14,^{6,33} ADRB2 (B2 adrenergic receptor),³⁴ IL-4R α ,³⁵ STAT6 (signal transducer and activator of transcription 6),³⁶ ADAM33,³⁷ DPP10,³⁸ PHF11,³⁹ etc. To date, more than 60 genes have been identified as the asthma susceptibility genes.³⁵ It is quite possible that asthma susceptibility is determined by the accumulation of several certain genetic variations other than one or two SNPs. On the other hand, we are not able to predict the

actual risk factors for getting asthma for an individual who carries the susceptibility genotypes because of the genetic complexity of this disease. The multigenic nature of asthma creates additional obstacles for us to truly understand the genetic background of this common disease.

In this study we focused on the IL-4 and IL-13 gene. We have identified two SNPs in IL-4 and five SNPs in IL-13, with one novel and six previously reported. This study is the first time that a group of paired parents and children were used for case-control studies. The asthmatic children were living together with their parents. Thus, the children and parents experienced similar environmental exposures. Moreover, the parents group had additional advantage for the analysis of complicated genetic disorders. The affected children got one-half of the genetic information from their unaffected fathers/mothers. Hence, the interference

Table 3 Association between IL-4 C-33T and IL-4 C-589T genotype and asthma in two groups (the other single nucleotide polymorphisms data were not shown because of low mutation frequency)

	SNPs	Genotype	Cases	Controls	χ^2	<i>p</i> Value
Spouses group IL-4	C-589T	TT	5	3	1.559	0.459
		CT	8	9		
		CC	0	1		
	C-33T	TT	7	5	1.091	0.580
		CT	6	8		
		CC	0	1		
Parents group IL-4	C-589T	TT	6	5	0.202	0.904
		CT	4	5		
		CC	1	1		
	C-33T	TT	10	9	1.053	0.591
		CT	1	1		
		CC	0	1		

IL = interleukin.

caused by other known or unknown susceptibility genes could be reduced. Our hypothesis was that by comparing the difference between the spouses group and parents group, we would have a deeper understanding of the importance of certain variants in the IL-4 and IL-13 genes to asthma. However, in this study, none of the SNPs could be associated with asthma in the spouses or the parents groups. As previously reported, the IL-4 C-589T was related to asthma²² and elevated IgE levels in the American,²¹ European,²⁰ and Japanese⁴⁰ populations, but subsequent studies indicated conflicting results.^{23–25} In both the Japanese and the British populations,¹⁵ significant associations were observed between IL-13 G2044A (Arg130Gln) and asthma, although this result could not be repeated in the Dutch population.¹⁹ The possible reason is that asthma is a complex multigenic disorder other than the only contribution from one or two SNPs.^{24,41–43} The most important reason for these differences is the in differing number of the subjects. The greater subject amount could make the more convincing results of the genetic association study. Another potential cause of differences in the results of these studies is that they were done in different ethnic groups. The allele frequencies of IL-4 C-589T in this study were similar to that in the Japanese population but differed from that of a white population. Thus, it is quite reasonable that the ethnic background affects the results from a variety of populations.

Heinzmann *et al.* used a computer model to study the biological activity of IL-13 Arg130Gln.¹⁵ In their study, the replacement of Arg with Gln would lead to either a closer or a reduced binding, and seems to alter the IL-13 signaling. *In vitro* studies have indicated that IL-4

–589T allele may cause greater transcription factor binding and hence this IL-4 promoter polymorphism is likely to correlate with enhanced IL-4 activity *in vivo*.²¹ Additional functional studies are needed for understanding asthma susceptible genes and raising the potential for new therapeutic strategies.

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