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No association of cytokine gene polymorphisms in Chinese patients with atopic dermatitis

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Summary

Background. Atopic dermatitis (AD) is a common chronically relapsing skin disease associated with the activation of T-helper 2 cells. Recent studies have shown that polymorphisms in the genes for interleukin (IL)-4, the IL-4 receptor, IL-13, and signal transducer and activator 6 (STAT6) may contribute to susceptibility of AD. To date, no cytokine gene polymorphism study has been conducted on Chinese patients with AD.
Aims. To determine whether genetic polymorphisms of the cytokine genes might influence the development of AD.
Methods. DNA samples were obtained from 94 patients and 186 control subjects. Using direct sequencing and microsatellite genotyping, we examined 22 polymorphisms in eight cytokine genes including the genes for IL-4, -10, -12B and -13, the IL-4 receptor, tumour necrosis factor (TNF)-α, STAT6, and interferon (IFN)-γ.
Results. No significantly different allelic and genotypic distributions of the cytokine gene polymorphisms could be found between patients and controls. Moreover, no association was observed with disease onset, gender, the presence of elevated serum total IgE level or blood eosinophilia.
Conclusion. Our study suggests that the analysed genetic polymorphisms of cytokine genes do not appear to be associated with AD susceptibility in our Chinese population.

Introduction

Atopic dermatitis (AD) is a common chronically relapsing skin disease that occurs most commonly during early infancy and childhood.[1] AD is frequently associated with elevated serum IgE levels and a personal or family history of AD, allergic rhinitis or asthma.[1] Although the pathogenesis of AD remains obscure, it probably results from a polygenic inheritance pattern that involves cytokine gene activation.[2]

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Evidence from several studies have shown an increased frequency of circulating allergen-specific interleukin (IL)-4 and IL-5-secreting T-helper (Th) 2 cells in AD, and the activation of Th2-like cells appears to be central to the immune dysregulation in AD.[3] In addition, high levels of IL-4, -5, -10, -12 and -13, and interferon (IFN)-γ have been detected in skin lesions and sera of patients with AD.[4–6]

Cytokine gene polymorphisms may affect constitutive and inducible cytokine production and contribute to the disease-associated cytokine imbalance. Recent studies have shown that polymorphisms in the genes encoding for IL-4, the IL-4 receptor (IL-4R), IL-13, and signal transducer and activator 6 (STAT6) may contribute to susceptibility for AD.[7–11] Although cytokine gene polymorphisms have been studied extensively in patients with AD in other populations, no report about cytokine

gene polymorphisms in Chinese AD patients has, to our knowledge, been published in the literature.

In order to investigate whether cytokine gene polymorphisms play a role in the pathogenesis of Chinese patients with AD, we performed a case–control association study by genotyping the polymorphisms of cytokine genes [genes encoding for IL-4, -10, -12B and -13, IL-4R, tumour necrosis factor (TNF)-α, STAT6, and IFN-γ] in a Chinese population.

Materials and methods

Patients and controls

In total, 94 patients with AD were recruited from the dermatological clinic at the Taipei Veterans General Hospital and Taipei Medical University Hospital. Their clinical records were reviewed by dermatologists specialized in AD. All patients were Chinese, residing in Taiwan, and genetically unrelated to each other. The diagnosis of AD was made according to diagnostic criteria for AD by Hanifin and Rajka, and patients having three or more major diagnostic criteria plus three or more minor features were enrolled in the study.[12] The control group comprised 186 healthy individuals (volunteer blood donors and hospital staff). The control subjects were selected to maximize matching for gender and geographical origin. Informed consent was obtained from each participant, under protocols approved by the research ethics boards of the hospitals.

Genotyping of cytokine genes

Genomic DNA was extracted from 5 mL of whole blood using a DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). The genotyping of single nucleotide polymorphisms (SNPs) of the cytokine genes (*IL-4, IL-10, IL-12B, IL-13, IL-4R* and *TNF-α*) of the study subjects were determined by direct sequencing. Their primer sequence and methods of genotyping are listed in Table 1. Firstly, the DNA sequence of cytokine genes was amplified by PCR. The amplified PCR products were then subjected to sequencing reaction using fluorescent-dye-terminator cycle chemistry (ABI Prism, PE Biosystems, CA, USA) in a 10 μL reaction. Sequences were obtained from both ends using the same primers used in the PCR reaction. The products of sequencing reactions were run on a DNA analyser (3730XL; ABI/Hitachi, Tokyo, Japan).

The short tandem repeats of the *STAT6* and *IFN-γ* genes were determined by the fluorescence-labelling technique. The forward primer was fluorescently labelled (with FAM) and the amplified PCR products were mixed with formamide containing a stop buffer, denatured for 5 min at 95 °C, and run on a performance optimized polymer 6

Table 1 Methods and primers used in genotyping of cytokine genes.

Gene polymorphism	Genotyping method	Primers
IL-4 –590T–C, +33T–C	Sequencing	F: ACTAGGCCT CACCTGATACG R:: CACTTGTGTCCGTGGACAAAG
IL-10 –1082 A–G, –819T–C, –592A–C	Sequencing	F: ATCCAAGACAACACTACTAA R:: TAAATATCCTCAAAGTTCC
IL-12B +4237G–A, +4496A–G, +4510G–A	Sequencing	F: ACCATCTGGAGAGCTTAAGAACC R:: TGCCTTACATTTGACTGAGGATT
Il-13 –1111C–T	Sequencing	F: ATGCCTTGTGAGGAGGGTCAC R:: CCAGTCTCTGCAGGATCAACC
+ 4464G–A	Sequencing	F: TGGCGTTCTACTCACGTGCT R:: CAGCACAGGCTGAGGTCTAA
IL-4R*E375A, L389L, C406R, S503P, Q576R	Sequencing	F: CAGCATGGTGCCCAGTGGAG R:: CTGCTGGCAAGCAGGCTTGA
TNF-α –1031T–C, –863C–A, –857C–T	Sequencing	F: TGGACTCACCAGGTGAGGCC R:: TCACTCCCTGGGGCCCTCTA
–308G–A, –238G–A	Sequencing	F: CAAACACAGGCCTCAGGACTC R:: AGGGAGCGTCTGCTGGCTG
STAT6: STR at exon 1	Microsatellite genotyping	F: GAGGGACCTGGGTAGAAGGA R:: CACCCCCATGCACTCATAG
IFN-γ: STR at first intron	Microsatellite genotyping	F: AGACATTCACAATTGATTTTATTCTTAC R:: CCTTCCTGTAGGGTATTATTATACG

STR, short tandem repeats; F, forward; R, reverse.

(POP6) gel in a DNA analyser (3700; ABI/Hitachi). Fragment sizes were determined using Genescan (version 3.1) and Genotyper (version 2.5) software.

Statistical analysis

The differences of the allele and genotype frequencies between the case and control subjects were assessed using the χ^2 test or Fisher's exact test. Odds ratios, confidence intervals, and significance values were calculated using the Epi Info program (version 3.3; CDC, Atlanta, GA, USA). The CLUMP program (D. Curtis, London Statistical Genetics Group, UK) was used to assess significance of case–control association studies with multiallelic markers. Hardy–Weinberg equilibrium was also tested using the χ^2 test.

Results

Characteristics of patients with AD

The 94 AD patients included 52 male and 42 female patients, with a mean age of 26.9 years (range 3 months to 81 years). Of these, 77 patients (81.9%) had the onset of AD before the age of 18 years and 17 patients (18.1%) had onset at 19 years or older (adult-onset AD).[13] Forty-five patients (47.9%) had concomitant manifestation of allergic rhinitis or asthma. Elevated serum total IgE level was detected in 76 patients (80.9%) and blood eosinophilia occurred in 31 (33%). Fifty-six patients (60%) reported a family history of AD, allergic rhinitis or asthma.

Frequencies of cytokine gene polymorphisms

The allele frequencies of the investigated cytokine gene polymorphisms are shown in Table 2. The genotype frequencies of all polymorphisms in the patients with AD and in the control subjects were in Hardy–Weinberg equilibrium ($P > 0.05$). There was complete linkage disequilibrium between the −590C and +33C alleles of the *IL-4* gene, between the −819C and −592C alleles of the *IL-10* gene, and between the A375, L389, R406 and P503 alleles of the *IL-4R* gene. No significantly different allelic and genotypic distributions of the analysed cytokine gene polymorphisms were found between the patients with AD and controls. No association was observed with disease onset (infant/childhood or adult-onset) (Table 3), gender, or the presence of elevated serum total IgE level or blood eosinophilia (data not shown).

Table 2 Cytokine gene polymorphisms in patients with atopic dermatitis and control subjects.

Polymorphism	Patients ($n = 188$)	Controls ($n = 372$)	*P*-value
IL-4			
−590C	40 (21.3%)	81 (21.8%)	0.98
+33C	40 (21.3%)	81 (21.8%)	0.98
IL-10			
−1082G	14 (7.4%)	28 (7.5%)	0.89
−819C	56 (29.8%)	122 (32.8%)	0.53
−592C	56 (29.8%)	122 (32.8%)	0.53
IL-12B			
+4237 A	33 (17.6%)	51 (13.7%)	0.28
+4496G	91 (48.4%)	181 (48.7%)	0.97
+4510 A	82 (43.6%)	172 (46.2%)	0.62
Il-13			
−1111T	38 (20.2%)	63 (16.9%)	0.4
+4464 A	69 (36.7%)	125 (33.6%)	0.53
IL-4R*			
A375	15 (8%)	21 (5.6%)	0.38
L389	15 (8%)	21 (5.6%)	0.38
R406	15 (8%)	21 (5.6%)	0.38
P503	15 (8%)	21 (5.6%)	0.38
R576	28 (14.9%)	46 (12.4%)	0.48
TNF-α			
−1031C	39 (20.7%)	76 (20.4%)	0.98
−863 A	38 (20.2%)	64 (17.2%)	0.45
−857T	22 (11.7%)	35 (9.4%)	0.48
−308 A	22 (11.7%)	36 (9.7%)	0.55
−238 A	1 (0.5%)	7 (1.9%)	0.28
STAT6			
13R (327 bp)	60 (31.9%)	95 (25.5%)	0.13
14R (329 bp)	4 (2.1%)	1 (0.3%)	
15R (331 bp)	114 (60.6%)	244 (65.6%)	
16R (333 bp)	10 (5.3%)	31 (8.3%)	
17R (335 bp)	0 (0%)	1 (0.3%)	
IFN-γ			
11R (122 bp)	26 (13.8%)	63 (16.9%)	0.88
12R (124 bp)	75 (39.9%)	133 (35.8%)	
13R (126 bp)	4 (2.1%)	6 (1.6%)	
14R (128 bp)	71 (37.8%)	147 (39.5%)	
15R (130 bp)	4 (2.1%)	7 (1.9%)	
16R (132 bp)	1 (0.5%)	2 (0.5%)	
17R (134 bp)	7 (3.7%)	14 (3.8%)	

Discussion

AD is one of the most common chronic disorders in childhood and its occurrence has increased steadily in industrialized countries over the past few decades.[14] The prevalence of AD in children ranges between 10 and 37% in white populations to 21% in the Chinese population.[14,15] Twin studies in white populations have shown that the concordance rate of AD in monozygotic twins is 72–86% compared with 21–23% in dizygotic twins, and the heritability is 0.96–1.4.[16] In our study, 60% of Chinese patients with AD reported a family

Table 3 Cytokine gene polymorphisms in infant/childhood-onset patients with atopic dermatitis and control subjects.

Polymorphism	Patients (n = 154)	Controls (n = 372)	P-value
IL-4			
−590C	30 (19.5%)	81 (21.8%)	0.64
+33C	30 (19.5%)	81 (21.8%)	0.64
IL-10			
−1082G	7 (4.5%)	28 (7.5%)	0.29
−819C	43 (28%)	122 (32.8%)	0.32
−592C	43 (28%)	122 (32.8%)	0.32
IL-12B			
+4237 A	28 (18.2%)	51 (13.7%)	0.24
+4496G	71 (46.1%)	181 (48.7%)	0.66
+4510 A	70 (45.5%)	172 (46.2%)	0.95
Il-13			
−1111T	31 (20.1%)	63 (16.9%)	0.46
+4464 A	55 (35.7%)	125 (33.6%)	0.72
IL-4R*			
A375	11 (7.1%)	21 (5.6%)	0.65
L389	11 (7.1%)	21 (5.6%)	0.65
R406	11 (7.1%)	21 (5.6%)	0.65
P503	11 (7.1%)	21 (5.6%)	0.65
R576	22 (14.3%)	46 (12.4%)	0.65
TNF-α			
−1031C	34 (22.1%)	76 (20.4%)	0.76
−863 A	33 (21.4%)	64 (17.2%)	0.31
−857T	21 (13.6%)	35 (9.4%)	0.2
−308 A	18 (11.7%)	36 (9.7%)	0.59
−238 A	1 (0.6%)	7 (1.9%)	0.45
STAT6			
13R (327 bp)	50 (32.5%)	95 (25.5%)	0.14
14R (329 bp)	3 (1.9%)	1 (0.3%)	
15R (331 bp)	91 (59.1%)	244 (65.6%)	
16R (333 bp)	10 (6.5%)	31 (8.3%)	
17R (335 bp)	0 (0%)	1 (0.3%)	
IFN-γ			
11R (122 bp)	20 (13%)	63 (16.9%)	0.46
12R (124 bp)	67 (43.5%)	133 (35.8%)	
13R (126 bp)	4 (2.6%)	6 (1.6%)	
14R (128 bp)	57 (37%)	147 (39.5%)	
15R (130 bp)	1 (0.6%)	7 (1.9%)	
16R (132 bp)	1 (0.6%)	2 (0.5%)	
17R (134 bp)	4 (2.6%)	14 (3.8%)	

history of AD, allergic rhinitis or asthma. This indicated the presence of strong genetic factors underlying the development of AD.

Acute skin lesions in AD patients showed a higher number of IL-4, -5 and -13 mRNA-expressing cells whereas chronic AD skin lesions contained a larger number of cells expressing IL-12 and IFN-γ mRNA.[17,18] Therefore, a biphasic T-cell response in the skin (Th2 cells in acute AD and Th1 cells in chronic AD) has been proposed.[1] Because the aberrant cytokine expression could be important in the pathogenesis of AD, functional relevant cytokine gene polymorphisms might affect cytokine production and therefore could determine disease susceptibility of AD. Previous studies have shown that the *IL-4–590T, IL-13 +4464A, IL-4R*R576, STAT6*13R, IL-12B +4237A,* and *TNF*-α −308A alleles are strongly associated with AD or atopic disease in white and Japanese populations.[7–11,19,20] However, other studies revealed contradictory results.[21,22] In the present study, no significantly different allelic or genotypic distributions of the analysed cytokine gene polymorphisms were found between our AD patients and controls. Moreover, no association with disease onset, gender, elevated serum total IgE level or blood eosinophilia was observed. It has been suggested that AD is a multifactorial disease that results from the interactions between susceptibility genes, environmental factors, defective skin barrier function and immunological responses.[1] Whether a particular gene or mutation causes AD depends on the overall genetic background of the host and may vary substantially in different ethnic groups. Although the case number in our series is limited, our results indicated that the analysed cytokine gene polymorphisms do not appear to be associated with AD susceptibility in a Chinese population. In fact, it is not yet clear whether the genetic contribution for AD could be explained by variation in a limited number of genes.[2]

Genome-wide linkage studies in white populations revealed highly significant evidence for linkage on chromosomes 1, 3, 5, 13, 15, 17, 18 and 20.[23–25] However, no such study has been conducted in Chinese populations. Although the present case–control candidate gene study did not support cytokine genes as important susceptibility genes of AD in the Chinese population, large-scale genome-wide screens for AD in Chinese families is still warranted to unravel the genetic base of AD.

In conclusion, our study did not find association between the examined genetic polymorphisms of cytokine genes and AD in our Chinese population.

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