ORIGINAL ARTICLE

IL-4 Gene Polymorphisms and Their Association With Atopic Asthma and Allergic Rhinitis in Pakistani Patients

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Abstract

Background and Objective: Interleukin (IL) 4 is a cytokine that mediates allergic responses. Different single nucleotide polymorphisms (SNPs) can influence the immune response mediated by cytokines. The aim of the present study was to investigate the possible association between *IL-4* polymorphisms and allergic rhinitis and atopic asthma.
Methods: A total of 214 atopic patients (108 with asthma and 106 with allergic rhinitis) and 120 healthy controls from Pakistan were genotyped for *IL-4* SNPs C-589T (rs2243250), T+2979G (rs2227284), and C-33T (rs2070874) using restriction fragment length polymorphism-polymerase chain reaction. Statistical analysis was performed using the statistical software package StatCalc, EpiInfo v.6.
Results: The SNP rs2243250 was significantly associated with both asthma (P=.004, χ^2=11.0) and allergic rhinitis (P<.001, χ^2=20.2), as was T-2979G (P<.001, χ^2=22.51 for asthma and P<.001, χ^2=57.6 for allergic rhinitis). The most frequent genotypes in the asthma and allergic rhinitis groups were TT for SNP rs2243250, and GG for SNP rs2227284. rs2070874 was not found to be associated with either of the 2 atopic respiratory diseases analyzed in the Pakistani cohort.
Conclusions: rs2243250 and rs2227284 are significantly associated with asthma and allergic rhinitis. The results of this study indicate that in addition to environmental factors, genetic risk factors also play an important role in the development of atopic respiratory diseases.

Key words: IL-4. Polymorphism. Disease-associated. Atopic asthma. Allergic rhinitis.

Resumen

Introducción y objetivo: La IL-4 es una citocina que media las reacciones alérgicas. Diferentes polimorfismos en nucleótidos simples (SNPs) pueden influir sobre la respuesta mediada por citocinas. El motivo de este trabajo fue investigar la posible asociación de polimorfismos de la IL-4 con rinitis alérgica (RA) o asma atópica.
Método: Se incluyó un total de 214 pacientes atópicos (asma n=108, RA n=106) y 120 controles sanos de Paquistán que fueron genotipados para SNPs IL-4 C-589T (rs2243250), T+2979G (rs2227284) y C-33T (rs2070874) mediante PCR. El análisis estadístico se llevó a cabo mediante el paquete StatCalc, EpiInfo v.6.
Resultados: Observamos que el SNPs rs2243250 se asocia de forma significativa a asma (p 0.004; χ^2=11.0) y a RA (p<0.001; χ^2=20.2). Los genotipos más frecuentes en asma y RA fueron TT para SNP rs2243250, y GG para SNP rs2227284. Por otra parte, el SNP rs2070874 no se asocia con ninguna enfermedad respiratoria en una cohorte pakistaní.
Conclusiones: rs2243250 y rs2227284 se asocian significativamente a asma y a RA. Los resultados de este estudio demuestran que además de los factores ambientales, los factores genéticos de riesgo juegan un papel importante en el desarrollo de las enfermedades atópicas respiratorias.

Palabras clave: IL-4. Polimorfismo. Enfermedad-asociada. Asma atópica. Rinitis alérgica.

Introduction

Cross-talk between genetic and epigenetic variations is crucial for disease manifestation and progression. Genetic variations, such as single nucleotide polymorphisms (SNPs), in different genes involved in complex inflammatory disorders, such as asthma, allergic rhinitis, and eczema have gained much attention. The cytokine-gene cluster located on chromosome 5 harbors the interleukin (IL) genes *IL-13*, *IL-4*, *IL-5*, *IL-3*, and the granulocyte-macrophage colony-stimulating factor gene *GM-CSF*, which all have an important role in atopic disease susceptibility [1-3].

Both *IL-4* and *IL-13* are key components of the immune system and are involved in functions such as immunoglobulin class switching in activated B lymphocytes, inhibition of the production of proinflammatory cytokines by monocytes, and increased endothelial cell surface expression of vascular cell adhesion molecule 1 [4]. *IL-4* mediates these responses by binding to the T-cell surface through a heterodimeric receptor called the *IL-4* receptor alpha chain (*IL-4Rα*). The gene that codes for *IL-4Rα* is located in the chromosome 16p region, which has been linked to atopy and increased serum immunoglobulin (Ig) E levels [5,6].

Increased serum IgE levels are indicative of allergic response and correspond to a high level of *IL-4* messenger RNA (mRNA) synthesis. It has been suggested that enhanced IL-4 transcription is derived from genetic variations in the promoter region. In this regard, a promoter polymorphism (rs2243250; C-589T) has been reported to be associated with asthma and atopic dermatitis [7,8]. Along with promoter polymorphisms, various other SNPs have also been found to be associated with atopic disease in various populations. An association for T2979G and C-33T, also referred to as 3017 and +33, for example, has been reported in white asthmatic patients in Baltimore and German populations, respectively [9,10]. Replication of these association studies in other ethnic groups, however, has yielded controversial results. The aim of the present study was to determine the role of *IL-4* SNPs in patients with asthma and allergic rhinitis in a Pakistani cohort.

Study Participants and Methods

Study Participants

The current case-control study included 120 controls and 214 atopic patients (108 with atopic asthma and 106 with allergic rhinitis). All the patients were recruited from the Allergy Centre of the National Institute of Health in Islamabad, Pakistan. Inclusion criteria for controls were the absence of allergic reactions, negative skin prick tests, and total serum immunoglobulin (Ig) E levels of less than 100 IU/mL. Patients were diagnosed with atopic asthma according to the following criteria: a positive skin prick test reaction to at least 1 aeroallergen (pollen) and a history of shortness of breath

Table 1. List of Primers Used for Genotyping

Primers	Primer Sequence	°C[a]	PCR Product
rs2243250_F rs2243250_R	TAAACTTGGGAGAACATGGT TGGGGAAAGATAGAGTAATA	50	195 bp
rs2227284_ F rs2227284_R	CTACTCTTGGCAGTTGCTGGAA GGAACTCTCTGTAGAATTATGAACTTTAGGTC	58	220 bp
rs2070874_ F rs2070874_R	CAAGTTACTGACAATCTGGTGT CGGCACATGCTAGCAGGAA	58	223 bp

Abbreviations: bp, base pairs; PCR, polymerase chain reaction.
[a]°C, annealing temperature.

Table 2. Genomic Sequence Polymorphism Analysis of *IL-4* Gene by RFLP

rs Number	cDNA Coordinates of SNPs	Position	Enzyme Units	RE	RFLP Fragments
rs2243250	c.-589 C>T	Promoter	2.5	AvaII	C=177,18, T= 195
rs2227284	c.183+2527T>G	Intron 2	2.5	AluI	T=122,53,45, G=122,98
rs2070874	c.-33C>T	5′ UTR	5	BsmAI	C= 178,45, T= 140,45,38

Abbreviations: cDNA, complementary DNA; IL-4, interleukin 4; RE, restriction enzyme; RFLP, restriction fragment length polymorphism.

and wheezing due to chest tightness. Allergic rhinitis was diagnosed in patients with the following symptoms: sneezing, runny nose, nasal obstruction, itchy nose, and rhinorrhea.

Genotyping of SNPs

Genomic DNA was extracted using the standard phenol chloroform method. Genotyping was performed for the detection of 3 *IL-4* SNPs using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR).

PCR was performed using the Thermo Electron Corporation PCR system (PXE 0.2 Thermal cycler) and the Applied Biosystem Gene Amp PCR System 2700. The primer pairs used for the 3 SNPs are shown in Table 1. The PCR reaction contained 1X Taq Buffer (10 mM Tris-HCl, pH 9.0, 50m M KCl, 0.1% Triton X-100, 0.01% gelatin) (Fermentas), 10 pmol of each primer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1U of *Taq* DNA polymerase (Fermentas), and 100 ng of genomic DNA. The thermocycler profile consisted of 35 cycles. Before the first cycle, a 5-minute initial denaturation cycle was carried out at 95°C. Each cycle consisted of denaturation at 95ºC for 45 seconds followed by primer annealing for 45 seconds at 50°C for the -589(C/T) SNP and at 58°C for other 2 SNPs, with primer extension at 72°C for 45 seconds. Finally the temperature was held at 72°C for 7 minutes to allow the synthesis of unextended strands.

Restriction enzyme digestion for the PCR products of *IL-4* C-589T, T2979G, and C-33T was carried out by adding 2 µL 10X Buffer R (10 mM Tris-HCl [pH 8.5 at 37°C], 10 mM $MgCl_2$, 100 mM KCl, and 0.1 mg/mL bovine serum albumin) and the respective restriction enzyme (Table 2) to a PCR tube containing 15 µL of PCR product and 5 µL of DNAase and RNAase free water. The mixture was spinned down and incubated at 37°C for 16 hours. The enzyme was deactivated at 65°C for 15 minutes.

Statistical Analysis

Statistical differences for genotype and allele frequencies between patients and controls were determined by computing the Pearson χ^2 and odds ratios (ORs), with 95% CIs, using the statistical software package StatCalc EpiInfo v.6.

Results

Three *IL-4* SNPs, C-589T (rs2243250), T 2979G (rs2227284), and C-33T (rs2070874), were genotyped in healthy controls and patients with atopic asthma and allergic rhinitis.

Genotyping Analysis

The C-589T SNP was found to be significantly associated with both asthma (P=.004, χ^2=11.0) and allergic rhinitis (P<.001, χ^2=20.2) compared with controls. Of the 3 allelic combinations, the TT genotype was significantly associated with asthma and allergic rhinitis, with P values of <.001 (χ^2=10.88) and <.001 (χ^2=19.72), respectively.

Similarly, a highly significant association was observed for

Table 3. Genotype Frequencies of *IL-4* Polymorphisms in Controls and in Patients With Asthma and Allergic Rhinitis (AR)[a]

Genotypes	Controls (n=120)	Asthma Patients (n=108)	*P* Value (χ^2)		OR (95% CI)	AR Patients (n=106)	*P* Value (χ^2)		*P* Value (χ^2)	OR (95% CI)
rs2243250										
CC	31 (25.8)	26 (24.1)			1.10 (0.58-2.09)	18 (17)	<.001 (20.2)		.10 (2.60)	1.70 (0.85-3.44)
CT	84 (70)	63 (58.3)	.004 (11.0)		1.67 (0.93-2.99)	62 (58.5)			.07 (3.26)	1.66 (0.92-2.98)
TT	5 (4.2)	19 (17.6)			0.20 (0.06-0.61)	26 (24.5)			<.001 (19.72)	0.13 (0.04-0.39)
rs2227284										
TT	64 (53.3)	27 (25)			3.43 (1.88-6.28)	17 (16)	<.001 (57.66)		<.001 (34.05)	5.98 (3.05-11.84)
TG	56 (46.7)	76 (70.4)	<.001 (22.51)		0.37 (0.21-0.66)	58 (54.7)			.22 (1.46)	0.72 (0.41-1.27)
GG	0	5 (4.6)			0.00 (0.00-1.03)	31 (29.3)			<.001 (40.67)	0.00 (0.00-0.10)
rs2070874										
CC	93 (77.5)	77 (71.3)			1.39 (0.73-2.63)	92 (86.8)	.07 (3.27)		.07 (3.27)	0.52 (0.24-1.12)
CT	27 (22.5)	31 (28.7)	.28 (1.15)		0.72 (0.30-1.37)	14 (13.2)			.07 (3.27)	1.91 (0.89-4.11)
TT	0	0			–	0			–	–

Abbreviations: IL-4, interleukin 4; OR, odds ratio.
[a]Data are presented as number (%) unless otherwise indicated.

Table 4. Allele Frequencies of *IL-4* Polymorphisms in Controls and in Patients With Asthma and Allergic Rhinitis (AR)[a]

Alleles	Controls	Asthma Patients	*P* Value (χ^2)	OR (95% CI)	AR Patients	*P* Value (χ^2)	OR (95% CI)
rs2243250							
C	146 (60.8)	115 (53.2)	·10 (2.68)	1.36 (0.92-2.01)	98 (46.2)	.001 (9.67)	1.81 (1.22-2.67)
T	94 (39.2)	101 (46.8)			114 (53.8)		
rs2227284							
T	184 (76.7)	130 (60.2)	<.001 (14.40)	2.17 (1.42-3.33)	92 (43.3)	<.001 (52.41)	4.29 (2.81-6.56)
G	56 (23.3)	86 (39.8)			120 (56.6)		
rs2070874							
C	213 (88.75)	185 (85.6)	.32 (0.99)	1.32 (0.74-2.38)	198 (93.4)	0.08 (2.95)	0.56 (0.27-1.14)
T	27 (11.25)	31 (14.4)			14 (6.60)		

Abbreviations: IL-4, interleukin 4; OR, odds ratio.
[a]Data are presented as number (%) unless otherwise indicated.

the T2979G SNP in both asthma and allergic rhinitis patients, with *P* values of <.001 (χ^2=22.51) and <.001 (χ^2=57.66), respectively. The TT genotype might play a protective role as 53.3% of controls had this genotype, compared with 25% of patients with asthma (*P*<.001; χ^2=19.03; OR, 3.43 [95% CI, 1.88-6.28]) and 16% of those with allergic rhinitis (*P*<.001; χ^2=34.05; OR, 5.98 [95% CI, 3.05-11.84). The GG genotype, in contrast, was significantly associated with disease, as it was detected in 29.3% of allergic rhinitis patients but in none of the controls.

For the third polymorphism, C-33T, no significant difference was observed between patients and controls in terms of genotype or allele frequencies (Tables 3 and 4).

A significant difference in allele frequencies was observed for C-589T in patients with allergic rhinitis (*P*<.001; χ^2=9.67; OR, 1.81 [95% CI, 1.22-2.67]) but not in those with asthma (*P*<.10; χ^2=2.68; OR, 1.36 [95% CI, 0.92-2.01]) compared with controls. Similarly for T2979G, allele frequencies differed significantly between controls and patients with asthma (*P*<.001; χ^2=15.45; OR, 2.21 [95% CI, 1.46-3.37]) and allergic rhinitis (*P*<.001; χ^2=54.41; OR, 4.29 [95% CI, 2.81-6.56]) (Table 4).

Discussion

Asthma and other atopic diseases are considered to be multifactorial, with immunological, environmental, and genetic factors all contributing towards disease manifestation and progression. In recent genome-wide association studies, it has been proposed that genetic variations in the genes of the immunological pathways such as IL-4/IL-13 might be associated with disease phenotype. However, contradictory reports exist regarding the association of C-589T with allergy susceptibility. In asthma patients in the United Kingdom [11], Kuwait [12], China [13], Brazil [14], and India [15,16], for instance, no significant associations were observed for this SNP. In the current study, a highly significant association was observed between C-589T and both atopic asthma and allergic rhinitis. Our results are consistent with those reported by studies of asthmatic patients in Japan [17], Germany [18], and Taiwan [19].

In German asthmatic patients, a significant association was observed for the C-33T SNP; 2979G, in contrast, reached only borderline significance [10]. These findings are not consistent with ours, as we detected no association for C-33T but did find a strong association for T2979G. In the German study, T2979G was denoted G2979T. We used the dbSNP database notation T2979G. The authors of another study reported that T2979G (also referred to as 3017G/T) might be involved in transcriptional regulation of *IL-4* as it is located in the putative transcription factor binding site for the peroxisome proliferator-activated receptor alpha (PPARα). The T allele was found to be located within the core consensus sequence for the PPARα and its activators were proposed to have the ability to inhibit *IL-2, IL-6, IL-18*, and the tumor necrosis factor gene *TNF* [20] which is responsible for the shifting of T cells towards type 2 helper T cells. This theory is not consistent with the findings of a study in which IL-4 levels were measured in human $CD4^+$ T cells upon activation with a PPARα activator [21]; the drawback of that study, however, was that the authors were not sure about the allele they referred to as G2979T at the 3017 position.

The C-33T SNP has been found to be strongly associated with asthma in the Russian population, with a highly significant *P* value of <.001. The results of the current study for C-33T are similar to those of a recent study in the Chinese population [22]. In another study, Gervaziev et al [23] postulated that the locus containing C-33T (referred as T-33C) is part of the cAMP responsive element binding protein (CREB)-binding site; CREB is a regulatory protein involved in the activation of gene transcription in the cAMP-dependent pathway, and it is therefore possible that C-33T might be involved in the regulation of IL-4 expression through the modification of the CREB-binding site [23].

Comparing current and previous studies, we can conclude that it might be interesting to analyze a block of SNPs (i.e. a

haplotype) within a gene rather than studying single SNPs. We were not able to perform haplotype analysis, because we did not have sufficient DNA for certain individuals. We therefore recommend the performance of further studies in the same patients and controls to help understand the combined effect of different SNPs in a gene.

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