ORIGINAL

Association Between the Interleukin 6 Genotype at Position –174 and Atopic Dermatitis

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Abstract

Background: Atopic dermatitis (AD) is a chronic skin disorder of unknown origin that usually manifests for the first time in early infancy. Different types of genetic predisposition and environmental factors seem to be associated with the disease.
Methods: This study was performed to evaluate the frequency of alleles, genotypes, and haplotypes of interleukin (IL) 6 single-nucleotide polymorphisms (SNPs) at positions –174 and nt565 in 89 Iranian children with AD and 139 healthy controls.
Results: The G allele was significantly more frequent at position –174 in *IL6* in atopic patients than in the healthy controls ($P<.001$; OR, 2.82). Genotype GG was found at the same position in 71% of the patients; this frequency was significantly higher than the frequency of 30% recorded in the controls ($P<.001$; OR, 5.60). The GG haplotype of *IL6* (–174, nt565) was significantly more frequent in the atopic patients than in the healthy controls ($P<.001$; OR, 2.99).
Conclusions: A significant increase in the frequency of the G allele and GG genotype at position –174 of *IL6* was found in patients with AD, thus suggesting that production of this cytokine is greater in atopic patients.

Key words: Atopic dermatitis. Cytokine. Single gene polymorphisms. Interleukin 6.

Resumen

Antecedentes: La dermatitis atópica (DA) es una alteración crónica de la piel de origen desconocido, que habitualmente comienza en la infancia. Diferentes predisposiciones y factores ambientales se asocian a esta enfermedad.
Métodos: Este estudio se realizó en 89 niños iraníes con DA para evaluar la frecuencia de alelos, genotipos y haplotipos de polimorfismos genéticos simples (SNPs) de la *IL6* en las posiciones 174 y nt565 en comparación con 139 controles sanos.
Resultados: Observamos un incremento significativo del alelo G de la IL6 en la posición –174 en los pacientes con DA comparado con el grupo control ($p<0.001$, OR=2.82). El genotipo GG de la misma posición se encontró en el 71% de los pacientes frente al 30% en los controles ($p<0.001$, OR=5.60). También se observa un incremento significativo en el haplotipo GG de la *IL6* (-174, nt565) en los pacientes con DA comparados con los controles sanos ($p<0.001$, OR=2.99).
Conclusiones: En conclusión observamos un aumento significativo del alelo G allele y del genotipo GG en la posición -174 de la *IL6* en pacientes con DA, lo que podría sugerir un aumento de la producción de esta citocina en los pacientes con DA.

Palabras clave: Dermatitis atóopica. Citocina. Polimorfismo genético simple. Interleucina 6.

Introduction

Atopic dermatitis (AD) is a chronic and recurring inflammation of the skin that generally affects children. In severe cases, the main clinical manifestations can appear as early as the neonatal period [1-2]. The etiology of AD seems to be multifactorial, and different types of genetic predisposition and environmental factors could be involved in the pathophysiology of the disease [3-6].

The immunologic aspects of AD are still being investigated. Cytokine imbalance could initiate inflammation in atopic patients, thus highlighting the important role of cytokines in AD. Early overproduction of some cytokines, including interleukin (IL) 4, IL-5, IL-6, IL-9, IL-10, and IL-13, could activate B lymphocytes to produce immunoglobulin (Ig) E and cause atopic manifestations [7].

As single-nucleotide polymorphisms (SNPs) of the cytokine genes can affect cytokine production, association studies have been conducted in different diseases [8-13]. Indeed, genomic studies have found several SNPs to be associated with AD in different populations [5,6].

This study was performed to determine the associations between SNPs of *IL6* and AD in a group of Iranian patients.

Patients and Methods

Participants

We enrolled 89 children with AD who were referred to the Immunology Clinic of the Children's Medical Center Hospital, the Pediatrics Center of Excellence in Tehran, Iran. The patients had AD (diagnosed according to standard criteria [14]), were older than 6 months of age, and had moderate-to-severe disease. The control group comprised 139 healthy unrelated individuals with no personal or family history of atopy [15].

We obtained written informed consent from the patients' parents or guardians before blood sampling. This study was approved by the Ethics Committee of Tehran University of Medical Sciences.

Genotyping

DNA was extracted from peripheral blood. Cytokine genotyping was performed using polymerase chain reaction with sequence-specific primers (PCR-SSP assay kit, Heidelberg University) [15]. Briefly, the gene was amplified using a Tedane Flexigene thermal cycler (Roche) under the following conditions: initial denaturation at 94°C for 2 minutes; denaturation at 94°C for 10 seconds; annealing + extension at 65°C for 1 minute (10 cycles); denaturation at 94°C for 10 seconds; annealing at 61°C for 50 seconds; and extension at 72°C for 30 seconds (20 cycles). Thereafter, the availability of the PCR products was assessed using 2% agarose gel electrophoresis. The gel was placed on a UV transilluminator, and a digital image was taken for analysis and documentation. The frequencies of alleles, genotypes, and haplotypes of *IL6* at positions –174 and nt565 were recorded.

Statistical Analysis

We estimated allele frequencies by direct gene counting and compared them using the chi-square test. We calculated the odds ratio (OR) and Wald's 95% confidence interval (CI) for each allele, genotype, and haplotype. We considered a *P* value <.05 to be statistically significant.

Results

Patient Characteristics

The study sample comprised 89 atopic patients (52 male and 37 female) with moderate AD (n=52) or severe AD (n=37) according to the Scoring AD (SCORAD) index. A family history of atopy was recorded in 80.9% of patients. Median total IgE was 33 IU/mL, and the mean eosinophil count was 269/mm^3.

IL6 Allele Polymorphisms

Table 1 presents the allele frequency (number and percentage), *P* value, and OR with its 95%CI in both the

Table 1. *IL6* Allele and Genotype Polymorphism in Iranian Atopic Patients and Healthy Controls

Position	Alleles/Genotypes	Control (n=139) No. (%)	Atopic Dermatitis (n=89) No. (%)	*P* Value	OR (95% CI [Wald])
–174	C	101 (36.3)	30 (16.9)	<.001	0.36 (0.22-0.58)
	G	177 (63.7)	148 (83.1)	<.001	2.82 (1.73-4.59)
	CC	4 (2.9)	4 (4.5)	.715	1.59 (0.32-7.80)
	CG	93 (66.9)	22 (24.7)	<.001	0.16 (0.09-0.31)
	GG	42 (30.2)	63 (70.8)	<.001	5.60 (3.01-10.48)
nt565	A	50 (18)	30 (16.9)	.854	0.92 (0.55-1.56)
	G	228 (82)	148 (83.1)	.854	1.08 (0.64-1.83)
	AA	4 (2.9)	4 (4.5)	.714	1.59 (0.32-7.80)
	GA	42 (30.2)	22 (24.7)	.453	0.76 (0.40-1.44)
	GG	93 (66.9)	63 (70.8)	.639	1.20 (0.65-2.23)

atopic children and the healthy controls. We found a significant decrease in the frequency of the C allele at position –174 in *IL6* among the atopic patients (OR, 0.36; 95%CI, 0.22-0.58; *P*<.001); the frequency of the G allele at the same position was significantly greater than among the healthy controls OR, 2.82; 95%CI, 1.73-4.59; *P*<.001). No significant differences were found between the 2 groups for the nt565 position.

IL6 Genotype Polymorphisms

The significant differences in genotype frequency between the groups are shown in Table 1. A significant negative association was observed between the –174 CG genotype and AD in the patients (*P*<.001; OR, 0.16; 95%CI, 0.09-0.31). This association could represent a protective effect against AD. In addition, a strongly positive association was found between AD and the –174 GG genotype (OR, 5.60; 95%CI, 3.01-10.48; *P*<.001), thus revealing that patients were more susceptible to AD. No significant differences in genotype frequency were found between the groups for the nt565 position.

IL6 Haplotype Polymorphisms

The data revealed the frequency of the GG haplotype (–174, nt565) to be significantly higher for atopic patients than for the healthy controls (OR, 2.99; 95%CI, 1.84-4.88; P<.001). A significant negative association was found between AD and the CG haplotype (–174, nt565) (OR, 0.00; 95%CI, 0.00-0.11; P<.001), whereas no association was found for the CA and GA haplotypes at the same position (Table 2).

Table 2. *IL6* Haplotype (–174, nt565) Polymorphism in Iranian Atopic Patients and Healthy Controls

Haplotype	Control (n=139) No. (%)	Atopic Dermatitis (n=89) No. (%)	*P* Value	OR (95%CI [Wald])
GG	173 (62.2)	148 (83.15)	<.001	2.99 (1.84-4.88)
CG	55 (19.8)	0	<.001	0.00 (0.00-0.11)
CA	46 (16.6)	30 (16.85)	.966	1.02 (0.60-1.74)
GA	4 (1.4)	0	.160	0.00 (0.00-2.38)

Discussion

The results of genomic studies analyzing genetic susceptibility to AD suggest that several genes are responsible for the disease [5,6]. As with any other type of genetic predisposition, SNPs can affect the expression pattern of related genes and, consequently, an individual's susceptibility to AD. Several population-based studies involving different ethnic groups have reported associations between AD and cytokine gene polymorphisms that could mediate cytokine production, resulting in massive helper T cell (T_H) polarization imbalance [16-18]. We analyzed the SNPs at positions –174 and nt565 in *IL6*.

IL-6 is a key cytokine in the host defense mechanism and can function as both a proinflammatory cytokine and an anti-inflammatory cytokine [19,20]. In its role as a proinflammatory cytokine, it initiates production of IL-4 and IL-5 as the predominant T_H2 cytokines in acute AD; as an anti-inflammatory cytokine, it is responsible for the suppressor effect of IL-6 on IL-1 and tumor necrosis factor α and for activating IL-1Rα and IL-10 [21]. IL-6 also plays a central role in the acute phase response and in production of fever, thus making it one of the most relevant cytokines and immunological pathways [22]. Increased expression of cyclooxygenase 2, nuclear factor κB and C-reactive protein can result in the acute phase response, which is induced by IL-6. These inflammatory elements can exercise an effect on activated leukocytes by playing a role in the production of reactive oxygen species. Oxidative damage to DNA molecules due to reactive oxygen species could facilitate carcinogenesis through the natural course of chronic inflammatory diseases such as AD or asthma [23].

In this study, we investigated –174 and nt565 in chromosome 7p21, which codes for *IL6*. We observed a significant decrease in the frequency of the C allele at position –174 in patients with AD, whereas the frequency of the G allele was significantly increased in atopic patients. By contrast, we did not find any allelic association between position nt565 of the *IL6* gene and AD. Similarly, other authors were unable to find an association between –174 CG and AD in European American children [18] or in children from Germany [24] and Macedonia [25]. Moreover, Hoffjan et al [18] found no association between AD and SNPs at position –922 A/G in *IL6* and AD, and Starvic et al [25], who also examined *IL6* nt565, found no significant association, as was the case in our study.

At the genotype level, we observed underexpression of –174 for the CG genotype and significant overexpression for the GG genotype. However, we found no association between nt565 and AD. As the GG genotype at this position seems to be associated with higher production of IL-6 [26], increased levels of this cytokine could be expected in patients with AD. Our data also showed that polymorphisms in *IL6* could lead to a significant increase in the frequency of the GG haplotype, whereas the frequency of the CG haplotype was significantly lower in atopic patients than in healthy individuals. Our results are consistent with those of earlier studies on the SNPs of *IL6* that suggested that the presence of the G allele at position –174 could cause an increased inflammatory response, such as the one we expect in atopic patients. This response is more intense in the case of a homozygous genotype of the G allele [27]. Imboden et al [28] previously confirmed the clinical aspects of this issue by showing higher serum IgE production in a group with hay fever and the –174 GG genotype.

In conclusion, ours is the first study on polymorphisms in the *IL6* gene in Iranian patients with AD. Our findings indicate potentially significant associations between these polymorphisms and inflammatory disease. Significant results were observed for position –174 of the alleles (C and G),

2 genotypes (CG and GG), and 2 haplotypes (–174, nt565) of *IL6*. Further multicenter studies in different regions are needed to confirm the results of this study, which could help to guide future preventive efforts in the field of AD as a high-burden inflammatory disease. Indeed, measurement of IL-6 in the serum or stimulated cultured peripheral blood mononuclear cells of patients with AD could increase the clinical significance of the results.

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