Association Between Single Nucleotide Polymorphisms of the Interleukin-4 Gene and Atopic Dermatitis

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ABSTRACT Atopic dermatitis (AD) is an inflammatory skin disease in which both genetic and environmental factors seem to be involved. Several studies investigated the association of certain genetic factors with AD in different ethnic groups, but conflicting data were obtained. This study was performed to check the possible association between single nucleotide polymorphisms (SNPs) of interleukin 4 (IL-4) and the IL-4 receptor α chain (IL-4Rα) and AD in a group of Iranian patients. The allele and genotype frequencies of genes encoding for IL-4 and IL-4Rα were investigated in 89 patients with AD in comparison with 139 healthy controls, using methods based on polymerase chain reaction sequence-specific primers. The most frequent alleles of IL-4 in patients were T at -1098 (P<0.001, odds ratio (OR)=2.35), C at -590 (P<0.001, OR=4.84) and C at -33 (P=0.002, OR=2.08). The most frequent genotypes of IL-4 in patients were TT, CC, and CC at positions -1098 (P<0.001, OR=3.59), -590 (P<0.001, OR=31.25) and -33 (P<0.001, OR=3.46), respectively. We found a significant lower frequency of GT at -1098 GT, TC at -590, and TC at -33 in patients. There were no statistically significant differences in the frequency of alleles and genotypes of IL-4Rα gene at position +1902. A strong positive association was seen between TCC haplotype and AD (68% in patients vs. 23.4% in controls, P<0.001, OR=8.91). We detected a significantly lower frequency of TTC, GCC, and TTT haplotypes (P<0.001, OR=0.02, P<0.001, OR=0.40, P<0.001, OR=0.39, respectively) in patients compared to controls. A significant association between the polymorphisms of the IL-4 gene promoter at positions -1098, -590, and -33 and AD was detected in the Iranian population.

KEY WORDS: atopic dermatitis; polymorphism, single nucleotide; interleukin-4 gene

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INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disorder, affecting 15 to 30% of children and 2 to 10% of adults. It can also occur with other atopic diseases such as asthma and allergic rhinitis (1).

In terms of pathogenesis, genetic disturbances and alterations in the immune system with presence of environmental triggers are involved in AD. Immunologically, strong association was established between AD and high serum immunoglobulin E (IgE) levels. Dysregulation of type 2 helper T (Th2) and type 1 helper T cells (Th1) contributes in the pathogenesis of AD. Th2 derived cytokines including interleukin (IL) 4, 5, and 13 up-regulate IgE synthesis leading to allergic reactions, whereas cytokines of Th1 down-regulate the production of IgE (2). On this account, variations in the genes encoding cytokines may have a role in the development of AD. In this regard, several genes have been proposed, using candidate gene and genome-wide association studies. Recent evidence suggests that single nucleotide polymorphisms (SNPs) of cytokine genes affect their serum level. Association of such SNPs with AD has been examined in different ethnic groups (3). Such associations have also been shown between AD and SNPs of IL-6 (4), TNF-α (5), and IL-1 (6). Association between IL-4/IL-4 receptor α chain (IL-4Rα) gene polymorphisms and several immunological diseases (7-12), including AD (13-16) has already been investigated with similar and contradictory results. In the present study, we aimed to conduct a case-control study to explore association between SNPs of IL-4 and IL-4Rα genes and AD among the Iranian population.

PATIENTS AND METHODS

Subjects

Eighty nine children with AD who were referred to the Immunology Clinic of the Children's Medical Center Hospital, affiliated to the Tehran University of Medical Sciences, Tehran, Iran, were included in the study. The diagnosis of AD was made based on the standard criteria of Hanifin and Rajka (17). One hundred and thirty nine unrelated healthy subjects with no history of atopy were also selected as a control group.

This project was approved by the ethical committee of the Tehran University of Medical Sciences. Written informed consent was obtained from patients' parents or their guardians.

Genotyping

DNA samples were extracted from blood samples. Polymerase chain reaction with sequence-specific primers (PCR-SSP assay Kit, Heidelberg University, Germany) was used for cytokine genotyping. Gene amplification was performed using Tedane Flexigene thermal cycler (Roche): initial denaturation at 94°C for 2 minutes, denaturation at 94°C for 10 seconds, annealing plus 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). The PCR products were read on 2% agarose gel electrophoresis. The frequencies of alleles, genotypes, and haplotypes of the IL-4 gene at positions -1098, -590, and -33 and of the IL-4Rα gene at position +1902 were counted.

Statistical analysis

SPSS statistical software (IBM, New York, NY, USA) was used for data analysis. Allele frequencies were determined by direct gene counting. Frequencies of alleles, genotypes, and haplotypes were compared between patients and controls, using the chi-square test. The odds ratios were calculated with 95% confidence intervals. Statistical significance was set at *P* value of <0.05.

RESULTS

Patient characteristics

This study was performed on 89 patients (52 men and 37 women) with mean age of 2.2±1.2 years. Scoring AD (SCORAD) index was measured for all the cases, indicating severe and moderate AD in 37 and 52 cases, respectively. A family history of atopy was found in 80.9% of patients. Mean eosinophil count was 269/mm^3, and median total serum IgE was 33 IU/mL. Skin prick test was performed in 60 patients, which was positive in 33 patients (to at least one allergen); the results are shown in Table 1.

Table 1. The results of skin prick test in 60 enrolled patients with atopic dermatitis

Allergen	Frequency (%)
Mite	5 (8.33)
Wheat	4 (6.66)
Fish	4 (6.66)
Tomato	2 (3.33)
Walnut	5 (8.33)
Hazelnut	3 (5.00)
Cow's milk	11 (18.33)
Egg	16 (26.66)
Soybean	11 (18.33)
Peanut	1 (1.66)

Alleles and genotypes frequencies

The results of allele and genotype frequencies are shown in Table 2. The most frequent alleles of IL-4 in patients were T at -1098 (P<0.001, odds ratio (OR)=2.35), C at -590 (P<0.001, OR=4.84) and C at -33 (P=0.002, OR=2.08). The most frequent genotypes of IL-4 in patients were TT, CC, and CC at positions -1098 (P<0.001, OR=3.59), -590 (P<0.001, OR=31.25), and -33 (P<0.001, OR=3.46), respectively. We found a significantly lower frequency of GT at -1098 GT, TC at -590, and TC at -33 in our patients. There were no statistically significant differences in the frequency of alleles and genotypes of the IL-4Rα gene at position +1902.

Haplotype frequencies

The results of haplotype analysis are shown in Table 3. A strong positive association was seen between the TCC haplotype and AD (68% in patients vs. 23.4% in controls, P<0.001, OR=8.91). We detected a significantly lower frequency of TTC, GCC, and TTT haplotypes (P<0.001, OR=0.02; P<0.001, OR=0.40; P<0.001, OR=0.39, respectively) in patients in comparison to controls.

DISCUSSION

AD results from interaction between genetic and environmental factors. To date, loss of the function mutation in the filaggrin gene (FLG) is believed to be the strongest genetic alteration that causes epidermal barrier dysfunction, increasing predisposition to AD. Apart from that, recent evidence suggests that immune imbalance has a prominent influence in the manifestation of AD. It is well known that IL-4 promotes IgE synthesis through both isotype switching in B cells and inhibition of interferon gamma (IFNγ) production. Furthermore, high levels of IL-4 have been observed in acute skin lesions of patients with AD. Increased production of Th2 cytokines IL-4 could also impair the function of the epidermal barrier. It has been shown that IL-4 causes the under-expression of filaggrin and calcium-binding A11 proteins by keratinocytes. On the other hand, IL-4 increases the presence and function of the serine protease kallikrein 7 of kerationcytes, leading to increased desquamation (18-20).

It has been documented that SNPs in the genes encoding such cytokines could affect their production. Polymorphisms in genes encoding IL-4 and IL-4Rα (located on chromosomes 5q31 and 16p12, respectively) may be associated with total serum level of IgE. The IL-4Rα chain is a component of the IL-4 receptor and required for signal transduction of IL-4. Several studies showed association between SNPs of the IL-4 and IL-4Rα genes and AD, with conflicting outcomes (21); however, demographic characteristics of these studies were different.

In the present study, we investigated AD for association with the IL-4 gene promoter at -1098, -590, and -33 and the IL4Rα gene at +1902 positions. We detected an over-expression of the T allele at position

Table 2. Interleukin-4 (IL-4) and IL4RA gene polymorphisms in patients with atopic dermatitis and controls

Cytokine	Position	Alleles/ Genotypes	Control (n=139) No. (%)	Atopic Dermatitis (n=89) No. (%)	*P* value	Odds ratios (95% confidence interval)
IL-4	-1098	G	84 (30.2)	28 (15.7)	**<.001**	**0.43 (0.26-0.70)**
		T	194 (69.8)	150 (84.3)	**<.001**	**2.35 (1.42-3.90)**
		GG	1 (0.7)	2 (2.2)	0.563	3.14 (0.22-88.72)
		GT	82 (59.0)	24 (27.0)	**<.001**	**0.25 (0.14-0.47)**
		TT	56 (40.3)	63 (70.8)	**<.001**	**3.59 (2.03-6.34)**
	-590	C	149 (53.6)	151 (84.8)	**<.001**	**4.84 (3.01-7.76)**
		T	129 (46.4)	27 (15.2)	**<.001**	**0.20 (0.12-0.33)**
		CC	10 (7.2)	63 (70.8)	**<.001**	**31.25 (14.19-68.81)**
		TC	129 (92.8)	25 (28.1)	**<.001**	**0.03 (0.01-0.07)**
		TT	0	1 (1.1)	0.393	-
	-33	C	200 (71.9)	150 (84.30)	**.002**	**2.08 (1.29-3.37)**
		T	78 (28.1)	28 (15.70)	**.003**	**0.47 (0.28-0.78)**
		CC	61 (43.9)	65 (73.00)	**<.001**	**3.46 (1.96-6.15)**
		TC	78 (56.1)	20 (22.5)	**<.001**	**0.22 (0.12-0.42)**
		TT	0	4 (4.5)	**0.023**	**-**
IL-4Rα	+1902	A	242 (87.7)	147 (82.6)	0.130	0.66 (0.39-1.12)
		G	34 (12.3)	31 (17.4)	0.184	1.48 (0.85-2.59)
		AA	106 (76.8)	61 (68.5)	0.168	0.65 (0.36-1.19)
		GA	30 (21.7)	25 (28.1)	0.377	1.38 (0.72-2.67)
		GG	2 (1.5)	3 (3.4)	0.385	2.34 (0.31-20.50)

Table 3. Interleukin-4 (IL-4) haplotype polymorphisms in patients with atopic dermatitis and controls

Haplotype	Controls (n=139)	Atopic Dermatitis (n=89)	*P* value	Odds ratio (95% confidence interval)
	No. (%)	No. (%)		
TTC	51 (18.3)	1 (0.6)	**<.001**	**0.02 (0.00-0.17)**
GCC	83 (30.0)	26 (14.6)	**<.001**	**0.40 (0.24-0.66)**
TTT	76 (27.3)	23 (12.9)	**<.001**	**0.39 (0.23-0.67)**
TCC	65 (23.4)	121 (68.0)	**<.001**	**8.91 (5.88-13.50)**
TCT	2 (0.7)	5 (2.8)	0.117	3.94 (0.67-29.65)
GTT	1 (0.3)	0	1.00	0.00 (0.00-26.85)
GCT	0	0	-	-
GTC	0	2 (1.1)	0.153	-

-1098 in patients with AD in comparison to controls, while the presence of the G allele was decreased at the same position indicating the protective role of this allele against AD. A significant increase in the frequency of the -1098/TT genotype was also found. Similarly, Stavric *et al.* (13) found the protective role of the G allele at this position in Macedonian pediatric patients.

At position -590, we found an over-expression of the C allele and of the CC genotype in patients with AD, whereas the frequencies of the T allele and TC genotype were lower in patients with AD than controls. It has been shown that the presence of the CC genotype at this position is associated with low production of IL-4 (22). Therefore, decreased level of IL-4 could be expected in our patients. Contrary to our data, Kawashima *et al.* (16) reported that an over-expression of the -590/T allele increased the risk of AD in Japanese patients. Rosenwasser *et al.* (23) associated over expression of the -590/T allele with increased IgE levels in an American population. deGuia *et al.* (14) showed a positive association between the TT genotype of IL-4 -590 and high IgE levels. Interestingly, Elliott *et al.* found no association between -590 C/T polymorphisms and AD in a cohort of Australian patients (24). Notably, they suggest there may be linkage between -590C/-34C haplotypes and AD.

Our data also showed significant positive association between AD and the C allele and CC genotype of IL-4 at position -33. In contrast, Stavric *et al.* stated that the C allele of IL-4 -33 has a protective role against AD. Although some authors found association between SNPs of IL-4Rα (25), our study failed to show significant allelic and genotypic association between IL4Rα +1902 A/G and AD. A similar finding was found in the studies conducted by Stavric *et al.* and Kayserova *et al.* (26); however, Kayserova *et al.* showed significant differences between IL-4Rα +1902 A/G and positivity of tree pollen-specific IgE in the AD group that may be a predictive factor in the development of respiratory allergy. A gain-of-function mutation in IL-4Rα at position +1902 was associated with atopy (27). A significant increase was also seen in the frequency of TCC haplotype of IL-4 in patients with AD. Inconsistently, Stavric *et al.* found no association between the TCC haplotype and AD. They found a significant increase in the frequency of TTT haplotype in patients with AD, while our result was the opposite.

CONCLUSION

It is of note that such incompatibilities in different studies may be due to racial differences as well as sample sizes. In conclusion, we have identified genotypes that make the patients susceptible to AD, including TT at position-1098, CC at position -590, and CC at position -33. Moreover, the TCC haplotype had a role in development of AD. This is the first Iranian study showing association between AD and IL-4 polymorphisms. However, further observations with larger sample sizes of patients with AD are required to support the results of this study.

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