

RESEARCH ARTICLE

TNF-alpha single nucleotide polymorphisms in atopic dermatitis

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ABSTRACT. Tumor necrosis factor-alpha (TNF- α) could be considered as potential biomarkers in atopic dermatitis (AD), while its level could be influenced by cytokine single gene polymorphisms (SNP). This study was performed in 89 pediatric patients with AD and 137 controls to assess polymorphisms of the TNF- α gene at positions -308 and -238, using the polymerase chain reaction and the sequence-specific primers method. The highest positive allelic association that made the patients susceptible to AD was seen for TNF- α -238/G ($p < 0.001$) and TNF- α -308/G ($p = 0.003$). The GG genotypes at TNF- α -238 and TNF- α -308, were both significantly higher in the patients with AD, compared to the controls ($p < 0.01$). The GG haplotype at TNF- α (-308,-238) was seen in 92.7% of the patients, which was significantly higher than the controls ($p < 0.001$), while a negative haplotypic association with AD was seen for TNF- α (-308, -238) AG and GA ($p < 0.01$). This study showed that the AG genotype of TNF- α -308, associated with a high production of cytokines, was significantly decreased in patients with AD, while the low-producing GG genotype, which could lead to low production of TNF- α , was over-expressed in the atopic patients.

Keywords: atopic dermatitis, cytokine, single gene polymorphisms, tumor necrosis factor-alpha

Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disease with a typical distribution of pruritic skin lesions and, as with any other inflammatory skin disease, it greatly affects the quality of life of patients with this condition [1-3]. AD combined with allergic rhinitis and bronchial asthma form an "atopic march" [4].

There is no distinct cause for AD; it is known to have a multifactorial pathogenesis, with environmental and genetic factors both having been implicated [1, 5-7].

Innate and adaptive immunity defects in AD seem to be both responsible for the biphasic nature of the disease [1, 8, 9]. Despite all of the information available regarding the cytokine dominance in different stages of AD, there are some theories suggesting that several single nucleotide polymorphisms (SNPs) in the regulatory regions of the cytokine genes can influence the cytokine secretion pattern [10], which can happen differently in every individual [11].

Several studies have been performed on cytokine gene polymorphisms, particularly proinflammatory cytokines, in different immunological disorders [12-17], but achieving consensus seems to be difficult as some studies show associations between specific cytokine polymorphisms and

AD, while others suggest completely different results [5]. There is some evidence concerning the possible influence of particular genotypes/haplotypes of the related genes on serum levels of the proinflammatory cytokines, including tumor necrosis factor-alpha (TNF- α) [18, 19], but to the best of our knowledge, no cytokine gene polymorphism study has ever been performed involving Iranian patients with AD.

This study was performed in a group of pediatric patients with AD to assess the association of SNPs in TNF- α at positions -308 and -238 with the disease.

PATIENTS AND METHODS

Subjects

Eighty nine Iranian patients with AD, who had been referred to the Immunology Clinic of the Children's Medical Center Hospital, the Pediatrics Center of Excellence in Iran, were enrolled in this study. Tehran, the capital of Iran, has more than 12 million inhabitants with a mixed population including all of the different, Iranian ethnic groups. Diagnosis of AD in the patients was based on

the standard criteria of Hanifin and Rajka [20]. Patients younger than six months old were excluded from the study. Only those patients with moderate to severe AD were enrolled in this study, while patients with mild AD were excluded. One hundred and thirty seven, unrelated, healthy subjects from Tehran with no evidence of atopy were also selected as the control group [21]. This study was approved by the Ethics Committee of Tehran University of Medical Sciences. Written informed consent was obtained from the parents of enrolled patients before sampling.

Genotyping

After DNA extraction from the blood samples, the polymerase chain reaction, with sequence-specific primers (PCR-SSP assay kit; Heidelberg University, Germany) was used for cytokine gene typing [21]. The frequencies of alleles, genotypes, and haplotypes of TNF- α SNPs at positions -308 and -238 were counted.

Statistics

Allele frequencies were estimated by direct gene counting. In order to test the Hardy-Weinberg equilibrium, the frequencies of various genotypes were compared using the *chi square* test. The odds ratios and 95% confidence intervals (CI) were calculated for each allele, genotype and haplotype. A *p*-value of less than 0.05 was considered significant.

RESULTS

Cytokine allele polymorphisms

The allele frequency (number and percentage), *p*-value, odds ratio and its 95% CI for both the atopic and healthy groups are presented in *table 1*. The highest, positive, allelic association that makes the patient susceptible to AD was seen for TNF- α -238/G [*p*<0.001, OR = 11.94, 95%CI: 4.06-34.44). A positive allelic association was also seen for TNF- α -308/G (*p* = 0.003).

The highest negative allelic association for AD was seen in TNF- α -238/A [*p*<0.001, OR = 0.08, 95%CI: 0.03-0.25]. A negative allelic association was also seen for TNF- α -308/A (*p* = 0.003).

Cytokine genotype polymorphisms

The GG genotype at TNF- α -238 in patients with AD was significantly more highly represented than in the con-

trols [*p*<0.001, OR = 15.60, 95%CI: 5.12-53.12]. Such a positive genotypic association was also seen for TNF- α -308/GG [*p* = 0.004, OR = 3.14, 95%CI: 1.40-7.20] (*table 1*).

Meanwhile the GA genotype frequency at TNF- α -238/GA in the patient group was significantly lower than in the control group [*p*<0.001, OR = 0.07, 95%CI (0.02-0.20)].

Cytokine haplotype polymorphisms

The GG haplotype at TNF- α (-308,-238) was seen in 92.7% of the patients, which was a significantly higher frequency than that seen in the controls (64.2%) [*p*<0.001, OR = 7.07, 95%CI (3.69-13.77)].

A negative haplotypic association for AD was seen for TNF- α (-308, -238)/ AG [5.1% of the patients *versus* 14.2% of the controls, *p* = 0.003, OR = 0.32, 95%CI (0.14-0.71)] and GA [2.2% of the patients *versus* 21.5% of the controls, *p*<0.001, OR = 0.08, 95%CI (0.02-0.24)].

DISCUSSION

The immune system plays a considerable role in the pathogenesis of AD. The susceptibility/protectivity of SNPs in AD might not be as highly penetrative as some other genetic SNPs, however, the high frequency of these polymorphisms makes AD a "high burden", public health issue. Identification of "at risk" individuals may help future surveillance and protection from the disease [22].

Several studies have examined the role of TNF- α in the pathogenesis of AD. TNF- α is coded by a segment of chromosome 6p21.3. Previous studies have shown no association between TNF- α -308G/A, -1031 T/C, -863C/A, -857C/T, -308G/A, -238G/A and AD [23-26]. Our data for TNF- α showed a decreased presence of the A allele at position -308, which highlighted the protective role for this allele in atopic patients, while the G allele in the same position showed an over-expression with an opposite effect. A significant decrease in TNF- α -308 AG was also seen in the atopic patients compared to the control group, but the GG genotype showed an over-expression in the AD group. Considering the fact that the AG genotype is associated with high production of TNF- α and the GG genotype is associated with low production of this cytokine, lower production of TNF- α is expected in those patients with AD. Similar, selective impairment of TLR2-mediated TNF- α production by monocytes was previously shown in patients with AD [27]. A significant increase in the G allele and

Table 1
TNF- α allele and genotype polymorphisms in Iranian patients with AD and a control group.

Position	Alleles/Genotypes	Control (n = 137) N (%)	AD (n = 89) N (%)	Pearson's <i>p</i> -value	Odds Ratio (95% Confidence Interval)
-308	A	39 (14.2)	9 (5.1)	0.003	0.32 (0.14-0.71)
	G	235 (85.8)	169 (94.4)	0.003	3.12 (1.41-7.11)
	AA	0 (0)	0	—	—
	AG	39 (28.5)	10 (11.2)	0.004	0.32 (0.14-0.71)
	GG	98 (71.5)	79 (88.8)	0.004	3.14 (1.40-7.20)
-238	A	59 (21.5)	4 (2.2)	<0.001	0.08 (0.03-0.25)
	G	215 (78.5)	174 (97.8)	<0.001	11.94 (4.06-39.44)
	AA	1 (0.7)	0	1.000	0.00 (0.00-26.89)
	GA	57 (41.6)	4 (4.5)	<0.001	0.07 (0.02-0.20)
	GG	79 (57.7)	85 (95.5)	<0.001	15.60 (5.12-53.12)

the GG genotype of TNF- α -238 was seen in AD. The GG haplotype also showed significant over-expression in AD, while the AG and GA haplotypes were significantly decreased in atopic patients. However, in contrast with our study, there was no association between either of the TNF- α positions with AD in German, Macedonian, and Chinese patients [24-26]. To the best of our knowledge, this is the first study showing such an association between TNF- α and AD. This might be due to the high variability between cytokine polymorphisms in the different ethnic groups [11]. However, it should be noted that the patients enrolled in this study and the controls were from the same region.

The results of this study of Iranian patients revealed significant differences in six allelic positions [TNF- α -308 (A and G) and TNF- α -238 (A and G)], six genotypic positions [TNF- α -308 (AG and GG), and TNF- α -238 (GA and GG)], and five haplotypic positions [TNF- α (-308, -238) in GG, AG, GA]. However, further, multi-centers studies with large numbers of atopic patients are needed to confirm the results of this study. Indeed, the study of TNF- α and stimulated cultured peripheral blood mononuclear cells in patients with AD could prove to be clinically significant.

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