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Interleukin-4 Gene Polymorphisms Confer Behçet's Disease in Turkish Population

H. B. Oral*, K. Dilek†, A. A. Özçimen‡, Ö. Taşkapılıoğlu§, Ü. Bingöl¶, A. Sarandöl**, H. Sarıcaoğlu††, M. Yurtkuran¶ & M. A. Yurtkuran†

*Department of Medical Microbiology, Immunology Unit, Uludag University Faculty of Medicine, Bursa, Turkey; †Department of Nephrology and Rheumatology, Uludag University Faculty of Medicine, Bursa, Turkey; ‡Department of Biology, Mersin University Faculty of Science and Art, Mersin, Turkey; §Department of Neurology, Uludag University Faculty of Medicine, Bursa, Turkey; ¶Department of Physical Medicine and Rehabilitation, Uludag University Faculty of Medicine, Bursa, Turkey; **Department of Psychiatry, Uludag University Faculty of Medicine, Bursa, Turkey; and ††Department of Dermatology, Uludag University Faculty of Medicine, Bursa, Turkey

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Correspondence to: H. B. Oral, Department of Medical Microbiology, Immunology Unit, Uludag University Faculty of Medicine, Gorukle Campus 16059, Nilufer, Bursa, Turkey.
E-mail: oralb@uludag.edu.tr

Abstract

Several cytokine genes may play crucial roles in host susceptibility to Behçet's Disease (BD), because the cytokine production capacity varies among individuals and depends on the cytokine gene polymorphisms. The association of the IL-4 and IL-4Rα gene polymorphisms with the susceptibility to BD was investigated in this study. DNA samples were obtained from a Turkish population of 97 patients with BD and 76 healthy control subjects. All genotyping (IL-4 and IL-4Rα) experiments were performed using PCR sequence-specific primers. When compared with the healthy controls, the frequency of IL-4 −1098 TG and −590 CT genotypes was higher in the patients with BD. Analysis of allele frequencies showed that IL-4 −1098 G and IL-4 −590 T alleles were more common in the patients with BD when compared with healthy controls. Also, IL-4 TTC and haplotypes were found to confer BD. Interestingly, we demonstrated that IL-4Rα gene polymorphism seems to be associated with the Pathergy test positivity in patients with BD. Our data suggest that IL-4 gene promoter polymorphisms may affect susceptibility to BD and increase risk of developing the disease. However, in order to confirm and assess the association of IL-4 and IL-4Rα gene polymorphisms with the BD, large cohort studies are needed.

Introduction

Behçet's disease (BD) is a systemic vasculitis characterized by inflammatory lesions of urogenital mucosa, eyes, skin, central nervous system and joints [1]. BD has a worldwide distribution, but it is most common in Japan, the Middle East and the Mediterranean countries. The prevalence of BD in Turkey is particularly high at 80–420/100,000 [2, 3]. Characteristic manifestations of BD are recurrent, which may last a few days to several weeks, some of them leaving permanent tissue damage and causing chronic manifestations or even death [4]. It usually starts in the third and forth decades. The male-to-female ratio is near to equal in big series of patients, although BD runs a more severe course in men and in those with a younger age of onset [4]. The pathogenesis of BD is unknown. An enhanced and dysregulated immune response has been suggested as the underlying pathology, and this can be triggered by environmental agents, mainly microbes, in genetically susceptible individuals [5–7]. There is a strong association with HLA-B51 [6, 8, 9] and an increased incidence in close family members. Gul *et al.* [10] have reported a sibling risk ratio λs value of 11.4–52.5 in Turkish patients with BD. There are also several reports suggesting that various host genetic factors, apart from HLA, play significant roles in susceptibility to BD [11–13]. Therefore, the identification of host genes responsible for susceptibility and resistance to BD should provide a significant contribution for understanding of the pathogenesis and may lead to the development of new prophylaxis and treatment strategies.

The disease is characterized by infiltration of lymphocytes and neutrophils into the affected organs. It is now well known that cytokines play critical roles in the pathogenesis of BD, because they mediate many of the effector and regulatory functions of immune and inflammatory responses [6]. The Th1 subset produces interleukin (IL)-2, IFN-γ, TNF and lymphotoxin and

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facilitates cell-mediated immune responses, whereas the Th2 subset produces mainly IL-4, IL-5, IL-6 and IL-10 and assists in antibody production. More recently, IL-17-producing Th cells, referred as Th17 cells, were identified as a new subset of T helper cells unrelated to Th1 or Th2 cells, and several cytokines, e.g. IL-21 and IL-23, are involved in regulating their activation and differentiation [14, 15]. They not only play an important role in host defence against pathogens, but are also associated with the development of autoimmunity and inflammatory response [15–18]. Specifically, IL-4, whose gene is located in the long arm of chromosome 5, is produced mainly by activated Th2 cells and is shown to stimulate B cell proliferation, to regulate immunoglobulin (Ig) class switching to IgG1 and IgE, to promote T cell development and to inhibit the production of proinflammatory cytokines and proteases [19, 20]. Interleukin-4 can bind at least two types of receptors (IL-4Rα-γc and IL-4Rα-IL-13Rα1), both comprising the common α chain [21]. Interleukin-4 plays a major role as a Th2-type immune response mediator and induces naive Th cells to differentiate into Th2 cells while suppressing the development of Th1 cells [22].

The Th1/Th2 cell paradigm may be important in BD, because Th1 and Th2 cells imbalance may modulate some immune functions, sustain inflammatory reactions and aggravate the disease. The ratio of Th1 to Th2 cytokine-producing cells can reflect cytokine homoeostasis and indicate Th1 or Th2 predominance during disease activity. Although both Th1 (IFN-γ, IL-12) and Th2 (IL-4, IL-6 and IL-10) cytokines were demonstrated to be elevated in the sera of patients with BD, increase in the IFN-γ/IL-4 ratio was observed in active BD having pulmonary or neurological manifestations [23]. Therefore, it is possible that Th2 cytokines may also affect the development or severity of BD.

The *in vitro* maximal capacity of immune cells to produce different cytokines in response to mitogen stimulation has been shown to vary among individuals. Such differences can be attributed to several molecular mechanisms, including variations in transcription, translation and secretion pathways [24, 25]. An additional potential mechanism was described involving conservative mutations within cytokine-coding regions, and nucleotide variations within more pronounced regulatory regions [25–27]. Genetic polymorphisms in several cytokine genes have been described and demonstrated to influence gene transcription, leading to interindividual variations in cytokine production [26–28]. Therefore, it is reasonable to speculate that genetic polymorphisms that regulate the production of certain cytokines may be important determinants of susceptibility to BD and some of its clinical and laboratory features. In our previous study, we did not find any association between Th1 cytokine IFN-polymorphism and BD [12]. Therefore, in the present study, we aimed to determine the influence of single-nucleotide polymorphisms (SNPs) in Th2-type IL-4 and IL-4R genes on susceptibility to BD.

Materials and methods

Patients and controls. Blood samples, collected in ethylenediamine tetraacetate sterile tubes, were obtained from 97 Turkish patients affected with BD. All patients were diagnosed according to the diagnostic criteria prepared by the international study group for BD. Demographic characteristics and clinical features of the patients are summarized in Table 1.

A control group was composed of 76 healthy organ donors unrelated to each other or to the patients, matched for age (42.14 ± 8.02), sex (37 men and 39 women) and ethnicity and was from the same geographical area as the patients. The study was approved by the Ethical Committee of Uludağ University, Bursa, Turkey, and all subjects gave written informed consent.

DNA isolation and cytokine genotyping. Genomic DNA was extracted from blood samples by using Puregene Genomic DNA isolation kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Single-nucleotide polymorphisms were analysed in IL-4 and IL-4Rα for genotype assignment. Three different polymorphisms were examined for the IL-4 promoter region: positions −1098 (T versus G), −590 (C versus T) and −33 (C versus T). A single-nucleotide polymorphism at position +1902 (A versus G) was surveyed for IL-4Rα.

Table 1 Demographic characteristics and clinical features of 97 patients with Behcet's disease.

	Patients (%)
Demographic and laboratory features	
Sex (male/female)	48/49
Age (years, mean ± SD)	37.84 ± 10.37
Age of the disease onset (years, mean ± SD)	23.63 ± 8.82
Duration (years, mean ± SD)	14.21 ± 6.45
Pathergy positivity[a]	59/92 (64.13)
HLA-B51 positivity[b]	58/78 (74.35)
Clinical manifestations	
Oral ulcer	97/97 (100.00)
Genital ulcer	73/97 (75.26)
Ocular involvement	53/97 (54.64)
Skin lesions	52/97 (53.61)
Erythema nodosum	50/97 (51.55)
Vascular involvement	10/97 (10.31)
Neurological involvement	9/97 (9.28)
Arthralgia/arthritis	6/97 (6.19)

[a]Pathergy test was performed in 92 patients.
[b]HLA-B51 was evaluated in 78 patients.

All genotypes were determined with the use of PCR sequence-specific primers method by a commercially available kit (Protrans; Medizinische Diagnostische Produkte GMBH, Heidelberg, Germany) in accordance with the manufacturer's instructions. Briefly, amplification was carried out under the following conditions: initial denaturation at 94 °C for 2 min; denaturation at 94 °C for 10 s; annealing and extension at 65 °C for 1 min (10 cycles); denaturation at 94 °C, for 10 s; annealing at 61 °C for 50 s; extension at 72 °C for 30 s (20 cycles). The absence or presence of PCR products was visualized by 2% agarose gel electrophoresis. After electrophoresis, the gel was placed on a UV transilluminator and a Polaroid picture was taken for interpretation and documentation. The DNA extractions and PCR amplifications were performed by a technician blinded to the study groups.

Statistical analysis. Statistical analysis was performed by Epi Info Software version 3.2.2 (CDC, Atlanta, GA, USA). The distribution of cytokine gene polymorphisms was compared between patients with BD and healthy controls by the chi-squared or Fisher's exact test. *P* values smaller than 0.05 were considered significant. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated in cases of chi-squared or Fisher's exact test being significant. The data were analysed for appropriateness between the observed and expected genotype values and for their fit to Hardy–Weinberg equilibrium (Arlequin Software v. 2000; University of Geneva, Geneva, Switzerland). Also, significant probability values obtained were corrected for multiple testing (Bonferroni correction; *Pc*).

Results

Allele frequencies and genotype distributions of IL-4 and IL-4Rα are shown in Table 1. All allele and genotype frequencies were in Hardy–Weinberg equilibrium.

No statistically significant differences in the distribution of IL-4 −33 C/T and IL-4Rα A/G gene polymorphisms were observed between patients and controls (Table 2). The frequency of IL-4 −1098 TG genotype was higher in the BD group in comparison with the control group (52.08% versus 20.63%, $P = 0.000077$, $Pc = 0.00023$, OR = 4.18), whereas IL-4 −1098 TT genotype was more common in healthy controls when compared with the patients with BD (79.37% versus 45.84%, $P = 0.000028$, $Pc = 0.0008$, OR = 0.22). Analysis of allele frequencies showed that the frequency of IL-4 −1098 G allele was higher in the patients when compared with controls (28.12% versus 10.32%, *P*: 0.0001, respectively; OR = 3.40).

The frequency of IL-4 −590 CT genotype was found to be apparently higher in patients with BD compared with healthy control subjects (70.83% versus 34.92%, $P = 0.000008$, $Pc = 0.000025$, OR = 4.53) (Table 2). IL-4 −590 T allele was found more common in BD patients in comparison with healthy controls (35.42% versus 17.46%, $P = 0.00052$, $Pc = 0.001$, OR = 2.59).

The frequency of haplotypes of IL-4 (−1098, −590, −33) in patients with BD and healthy controls is shown in Table 3. The TCC was the most frequent haplotype in both patients and controls. However, the frequency of TCC was significantly lower in patients with BD than in the controls (38.5% versus 72.2%, $P = 0.0000$,

Table 2 Genotype and allele frequencies of the IL-4 and IL-4Rα gene polymorphisms in the patients with BD and controls.

Cytokine Gene	Allele frequency (%)		Genotype (%)		
IL-4 (−1098)	T[a]	G[a]	TT[a]	TG[a]	GG
Patients (*n* = 96)	138 (71.88)	54 (28.12)	44 (45.84)	50 (52.08)	2 (2.08)
Controls (*n* = 63)	113 (89.68)	13 (10.32)	50 (79.37)	13 (20.63)	0 (0.00)
P values	0.0001	0.0001	0.000028	0.000077	n.s.
ORs (95%CI)	0.29 (0.14–0.58)	3.40 (1.72–7.12)	0.22 (0.10–0.48)	4.18 (1.92–9.43)	
IL-4 (−590)	C[a]	T[a]	CC[a]	CT[a]	TT
Patients (*n* = 96)	124 (64.58)	68 (35.42)	28 (29.17)	68 (70.83)	0 (0.00)
Controls (*n* = 63)	104 (82.54)	22 (17.46)	41 (65.08)	22 (34.92)	0 (0.00)
P values	0.00052	0.00052	0.000008	0.000008	n.s.
ORs (95%CI)	0.39 (0.21–0.68)	2.59 (1.46–4.71)	0.22 (0.11–0.46)	4.53 (2.18–9.46)	
IL-4 (−33)	C	T	CC	CT	TT
Patients (*n* = 96)	150 (78.12)	42 (21.88)	54 (56.25)	42 (43.75)	0 (0.00)
Controls (*n* = 63)	108 (85.61)	18 (14.29)	45 (71.43)	18 (28.57)	0 (0.00)
P values	n.s.	n.s.	n.s.	n.s.	n.s.
IL-4Rα (+1902)	A	G	AA	AG	GG
Patients (*n* = 97)	163 (84.02)	31 (15.98)	68 (70.10)	27 (27.84)	2 (2.06)
Controls (*n* = 76)	127 (83.55)	25 (16.45)	55 (72.37)	17 (22.37)	4 (5.26)
P values	n.s.	n.s.	n.s.	n.s.	n.s.

[a]Statistically significant difference between patients with BD and control subjects ($P < 0.05$).
n.s., not statistically significant ($P > 0.05$); OR, odds ratios.

Table 3 Haplotype frequencies of the IL-4 polymorphisms in the patients with BD and controls.

Haplotypes	Patients (%)	Controls (%)	*P* values	Odds ratios (95% CI)
IL-4 (−1098, −590, −33)				
TCC[a]	74 (38.5)	91 (72.2)	0.0000	0.24 (0.14–0.40)
TTC[a]	33 (17.2)	5 (4.0)	0.0003	5.02 (1.86–16.90)
TTT	31 (16.1)	17 (13.5)	n.s.	
TCT	0 (0.0)	0 (0.0)	–	
GCC[a]	41 (21.5)	12 (9.6)	0.0057	2.58 (1.26–5.63)
GCT	9 (4.7)	1 (0.7)	n.s.	
GTC	2 (1.0)	0 (0.0)	n.s.	
GTT	2 (1.0)	0 (0.0)	n.s.	

[a]Statistically significant difference between patients with BD and control subjects ($P < 0.05$).
n.s., not statistically significant ($P > 0.05$).

OR = 0.24). Additionally, TTC haplotype was significantly more common in the patient group when compared with controls (17.2% versus 4.0%, $P = 0.0003$, $Pc = 0.0025$, OR = 5.02). The frequency of GCC haplotype was also higher in patients with BD, but the statistical significance for this haplotype was very close to the border when the *P* value was adjusted by Bonferroni correction (21.5% versus 9.6%, $P = 0.0057$, $Pc = 0.045$).

In order to investigate the association between clinical findings (Table 1) and cytokine gene polymorphisms in patients with BD, the patients were classified according to the clinical features of the disease, including oral ulcer, genital ulcer, ocular involvement, papulopustular lesions, erythema nodosum, large vascular involvement, neurological involvement and arthralgia/arthritis. It was found that the frequency of IL-4 −590 CT genotype was higher, but not statistically significant, in patients with ocular involvement when compared with those of the patients without pathergy positivity because the statistical significance for this polymorphism was lost when the *P* value was adjusted by Bonferroni correction (79.55% versus 60.47%, $P = 0.045$, $Pc = 0.14$) (Table 3).

Association between the diagnostic tests and cytokine gene polymorphism showed that the frequency of IL-4Ra +1902 AA genotype was lower in patients with pathergy positivity, when compared with that of patients without this finding (62.71% versus 84.85%, $P = 0.023$, $Pc = 0.047$, OR = 0.30 [CI 95% 0.00–0.96]) (Table 4). Analysis of allele frequency demonstrated that the presence of G allele at position +1902 of IL-4Rα is a risk factor for positive pathergy reaction (20.3% versus 7.68%, $P = 0.023$, $Pc = 0.047$, OR = 3.11 [1.00–10.96]) (data not shown).

Moreover, no association was demonstrated between genital ulcer, papulopustuler skin lesions, large vascular involvement, ocular involvement, neurological involvement, arthralgia/arthritis, HLA-B51 positivity and age of the disease onset and cytokine gene polymorphisms (Table 3). Also, none of the haplotypes were associated with clinical and diagnostic findings (data not shown).

Discussion

Although the aetiology of BD is not yet known, it is thought that genetic predisposition and immune dysregulation seem to be critical factors in the pathogenesis. The role of cytokines is complicated in BD. On stimulation with mitogen, $CD3^+$ T cells from patients with active BD demonstrated a strong polarization to Th1 [23]. In addition, PBMC from patients with BD and active uveitis showed increased expression of mitogen-activated mRNA of T-bet, which is the transcription factor expressed in only Th1 cells [29]. Several previous studies have examined cytokine production in patients with BD. In one study, PBMC from 24 patients was stimulated and compared with healthy controls. There was enhanced entry into the Th1 pathway, with IFN-γ^+ cells increasing, compared with PBMC taken from normal subjects. Dividing $CD4^+$ T cells showed the phenotype of activated effector/memory cells [30]. In a second study, paired blood and serum were taken from 25 patients during clinically active BD and 20 patients during remission. Serum IL-4, IL-6, IL-10, IL-12, IL-17, IL-18 and IFN-γ were significantly higher in patients compared with controls. Similar levels of IL-4, IL-10 and IL-12 were seen in active and remission patients, while IL-6, IL-17, IL-18 and IFN-γ were significantly higher in patients with active disease versus those in remission. On stimulation with phorbol myristate acetate/ionomycin, the PBMC of active patients produced more IFN-γ than those in remission or controls although there was no difference in the production of IL-4 using intracellular cytokine staining [23].

Although BD has been defined primarily as a Th1-driven disease, IL-6, which increases in active BD, promotes Th2 cell differentiation, while inhibiting IFN-γ production and Th1 cell differentiation, a process that conflicts with the Th1 polarization described in BD [31, 32]. In a more recent study, levels of 10 cytokines were analysed in serum samples from 52 patients with BD, 20 patients with recurrent aphthous stomatitis and 15 healthy volunteers. Cytokines associated with an adaptive immune response such as IFN-γ and IL-2 were found in few samples, while IL-4 and IL-10 were not detected in any sample [33]. Recent studies have suggested that IL-23/IL-17 axis may be crucial to BD development. One study showed that the expression of IL-23p19 mRNA, IL-23, IL-17 and IFN-γ was markedly elevated in BD patients with active uveitis. Meanwhile, the frequencies of IL-17-producing and IFN-γ-producing T cells from PBMCs were significantly upregulated in BD patients with active uveitis [18]. Similarly, another study reported that the ratio of Th17/Th1 cells, but not the ratios of Th1/Th2

Table 4 Association of the most common clinical findings, HLA-B51 positivity, age of the disease onset and IL-4 and IL4Rα gene polymorphisms in the patients with BD. Because the size of samples are small, only Bonferroni correction of the *P* values (*Pc*) < 0.05 is considered significant (in bold).

	Genital ulcer		Ocular involvement		Papulopustular lesions		Erythema nodosum		Pathergy positivity		HLA-B51 positivity		Age of the disease onset (years)	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	≤18	>18
IL-4 (−1098) (*n*)	(72)	(24)	(53)	(43)	(51)	(45)	(50)	(46)	(58)	(33)	(58)	(20)	(26)	(71)
TT	33 (45.83)	11 (45.83)	25 (47.17)	19 (44.19)	22 (43.14)	22 (48.89)	23 (46.00)	21 (45.65)	26 (44.83)	17 (51.52)	27 (46.55)	10 (50.00)	9 (34.62)	35 (49.30)
TG	38 (52.78)	12 (50.00)	27 (50.94)	23 (53.49)	27 (52.94)	23 (51.11)	26 (52.00)	24 (52.18)	30 (51.72)	16 (48.48)	29 (50.00)	10 (50.00)	16 (61.54)	35 (49.30)
GG	1 (1.39)	1(4.17)	1 (1.89)	1 (2.32)	2 (3.92)	0 (0.00)	1 (2.00)	1 (2.17)	2 (3.45)	0 (0.00)	2 (3.45)	0 (0.0)	1 (3.84)	1 (1.40)
IL-4 (−590) (*n*)	(72)	(24)	(53)	(43)	(51)	(45)	(50)	(46)	(58)	(33)	(58)	(20)	(26)	(71)
CC	23 (31.94)	5 (20.83)	11 (20.75)	17 (39.53)	13 (29.17)	15 (33.33)	11 (22.00)	17 (36.96)	18 (31.03)	10 (30.30)	16 (27.59)	8 (40.00)	7 (28.00)	21 (29.58)
CT	49 (68.06)	19 (79.17)	42 (79.55)	26 (60.47)	38 (70.83)	30 (66.67)	39 (78.00)	29 (63.04)	40 (68.97)	23 (69.70)	42 (72.41)	12 (60.00)	19 (72.00)	50 (70.42)
TT	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
IL-4 (−33) (*n*)	(72)	(24)	(53)	(43)	(51)	(45)	(50)	(46)	(58)	(33)	(58)	(20)	(26)	(71)
CC	39 (54.17)	15 (62.50)	26 (57.69)	28 (65.91)	31 (60.78)	23 (51.11)	27 (54.00)	27 (58.70)	36 (62.07)	15 (45.45)	33 (56.89)	11 (55.00)	16 (61.54)	40 (56.34)
CT	33 (45.83)	9 (37.50)	27 (36.54)	15 (29.55)	20 (39.22)	22 (48.89)	23 (46.00)	19 (41.30)	22 (37.93)	18 (54.55)	25 (43.11)	9 (45.00)	10 (38.46)	31 (43.66)
TT	0 (0.00)	0 (0.00)	0 (5.77)	0 (4.54)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
IL-4Rα (+1902) (*n*)	(73)	(24)	(53)	(44)	(52)	(45)	(50)	(47)	(59)	(33)	(58)	(20)	(26)	(71)
AA	53 (72.60)	15 (62.50)	37 (69.81)	31 (70.45)	35 (67.31)	33 (73.33)	36 (72.00)	32 (68.09)	**37 (62.71)**	**28 (84.85)**	38 (65.52)	14 (70.0)	18 (69.23)	50 (70.42)
AG	18 (24.66)	9 (37.50)	15 (28.30)	12 (27.27)	16 (30.77)	11 (24.44)	12 (24.00)	15 (31.91)	20 (33.90)	5 (15.15)	18 (31.03)	6 (30.00)	8 (30.77)	19 (26.76)
GG	2 (2.74)	0 (0.00)	1 (1.89)	1 (2.28)	1 (1.92)	1 (2.23)	2 (4.00)	0 (0.00)	2 (3.39)	0 (0.00)	2 (3.45)	0 (0.00)	0 (0.00)	2 (2.82)

or Th17/Th2 cells, was significantly increased in patients with BD, especially with uveitis or folliculitis, compared with healthy controls. Collectively, these data show that not only Th1 but also Th17 pathway may make a contribution for immunological aberrations of BD [34].

Wide variations have been documented in the frequencies of cytokine polymorphisms among different healthy populations, including the IL-4 −590 polymorphism, which has been most widely investigated in healthy populations. We found a −590 C allele in 82.5% of our healthy volunteers, a rate that is different from the prevalence rates reported in different regions of the world. The rates reported were 17.3% in Chinese [35], 32.3% in Japanese [36], 19.8% in Koreans [37], 88.7% in British caucasians [38], 63.3% in Germans [39], 78.8% in Greeks [40] and 77.1% in Macedonians [41]. All these data demonstrate that the IL-4 −590 C/T genotypes were significantly different between the East Asian and Caucasian population, as also recently mentioned by Zhu *et al.* [42]. The implications of this heterogeneity underline the necessity of including a local control group when clinical studies are carried out.

Some studies have shown that the maximal capacity of cytokine production varies among individuals and correlate with single-nucleotide polymorphism in various cytokine genes [43–47]. In 1995, Rosenwasser *et al.* [48] reported that a functional polymorphism at position −590 T>C in the IL-4 gene and the T allele is associated with an increased IL-4 gene expression *in vitro*. Additionally, individuals who carry the T allele in −590C/T polymorphism of the IL4 gene have been shown to have a higher proportion of IL-4-producing T helper cells [49]. Interleukin-4 acts through IL-4R consisting of an IL-4Rα chain and a common γ chain. In the polymorphic IL-4Rα-chain, guanine (G) is substituted for adenine (A) at position 1902 (according to the IL-4Rα-chain sequence reported by Idzerda *et al.* [50]), causing a change from glutamine (Q) to arginine (R) at position 576 of the precursor protein (Q576R). It was demonstrated that The R576 allele (G allele) enhances IL-4 production by Th2 cells and total and antigen-specific IgE responses [51]. In the light of these data, it may be suggested that IL-4 or IL-4R gene polymorphisms may contribute to Th2-skewed cytokine balance through increment of IL-4 gene expression.

Three cytokine SNPs situated at positions −1098 (G/T), −590 (C/T) and −33 (C/T) in the promoter region of IL-4 gene were investigated in this study. The IL-4 −590 C/T polymorphism, the most frequently studied IL-4 gene polymorphism, was associated with many types of diseases, such as asthma, schizophrenia, Alzheimer's disease, psoriasis, several autoimmune diseases and various cancers [35, 37, 39, 42, 48, 52–54]. In our study, the presence of IL-4 −590 T allele was associated with an increased risk of BD, which was also demonstrated by increased frequency of IL-4 −590 CT genotype in the patients with BD when compared with those of healthy subjects. To the best of our knowledge, so far no reports have been published regarding the role of the IL-4 −590 T/C polymorphism in BD. Similarly, we also showed that IL-4 −1098 T/G polymorphism was associated with BD, and the presence of G allele seemed to be a risk factor for the disease susceptibility. Additionally, we demonstrated that The TTC and GCC haplotypes seemed to cause susceptibility to BD. The latter data confirm that conversion of T allele to G allele at position −1098 or C allele to T allele at position −590 in the most common haplotype TCC lead to susceptibility to the disease. However, the most important limitation of our present study is small sample size, which may restrict the statistical power of this study. However, we suggest that, after validation by larger studies, our data together with HLA-B51 positivity may be helpful to identify at-risk population for BD.

Interestingly, we demonstrated that IL-4Rα gene polymorphism seems to confer the Pathergy test positivity in patients with BD, whereas none of the IL-4 gene polymorphisms were associated with clinical findings and specific diagnostic tests for BD. But, the association of IL-4Rα gene polymorphism with the Pathergy test needs to be interpreted with caution until they can be replicated in a large-scale study and/or, maybe, in different ethnic groups.

In conclusion, our study demonstrates that polymorphisms within the IL-4 gene seems to be involved in the susceptibility to BD, and IL-4 −590 and IL-4 −1098 polymorphisms may be valuable markers to predict the risk for the development of BD. Finally, we suggest that large cohort studies are necessary in order to assess the true association of IL-4 and IL-4Rα alleles, genotypes or haplotypes with the BD, because cytokine genetic polymorphisms and their functional expression are very different in various populations of same ethnic origin.

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