IL-18 promoter polymorphisms confer susceptibility to Behçet's disease, particularly to the mucocutaneous form, in a Turkish population

Sirs,

Behçet's disease (BD) is an idiopathic systemic inflammatory disease characterized by a T helper 1 (Th 1) polarization. IL-18 was initially recognized as an interferon (INF)-gamma-inducing factor in T cells that acts in synergy with IL-12, leading to the development of T helper (Th)1 type immune response (1). However, further studies showed that IL-18 in the absence of IL-12 can promote the production of Th2 cytokines and take part in allergic inflammation, suggesting a possible role for IL-18 both in Th1 and Th2-type immune responses (2). Elevated expression of IL-18 is found in serum and pulmonary lavage fluid, and increased IL-18 production was observed in bronchoalveolar cells upon *in vitro* stimulation (3-5). We previously showed that serum IL-18 levels in all clinical presentations of BD patients were very high and correlated with disease activity (6). As reviewed recently, together with the polymorphisms located in the promoter region, more than 90 polymorphisms reported on the IL-18 gene. Among the reported polymorphisms, -607 C/A and -137 G/C were shown to be characterized by hyperfunctioning state (7). IL-18 single-nucleotide polymorphisms (SNP) in promoter region have been studied in BD patients by two different study groups from South Korea with contradictory results (8, 9). We hypothesized that SNP in the promoter region of IL-18 gene may be associated with BD in Turkish population.

We investigated the distribution of IL-18 promoter -607 C/A and -137 G/C polymorphisms in 123 BD patients (median age 23 years; range 14 years; 94 male, 29 female) and 101 healthy controls (median age 23 years; range 22 years; 78 male, 23 female) using Real-Time Polymerase Chain Reaction (Real-time PCR). Comparisons of the allele, genotype, genotype combination and haplotype frequencies of patients and healthy controls were performed by using Chi-Square test with Yates' correction or Fisher's exact test, which was appropriated. The SPSS 11.5 (SPSS Inc., Chicago, IL, USA) was used for these purposes. The consistency of the genotype frequencies with the Hardy-Weinberg equilibrium was also determined. The calculation of odds Ratios (OR) together with their confidence intervals (CI) was performed by using a calculator, which is available at the website http://www.hutchon.net/ConfidORselect.htm. A p-value of <0.05 was considered as indicating a significant difference.

As compared with healthy controls, BD patients had a significantly higher frequency of the -607 C allele (42% *vs*. 30.7%, OR=1.636, 95% CI=1.102-2.427, p=0.014) and, of the -607 CC genotype (20.1% *vs*. 9.9%, OR=2.298, 95% CI=1.041-5.074, p=0.036) (Table I). We could not find any difference between BD patients and healthy control with respect to -137 alleles and genotypes.

Haplotype analysis showed that BD patients had significantly higher frequency of -607C/-137C haplotype (3.1% *vs*. 0%, OR: ∞, 95% CI=NaN-∞, p=0.043), and of -607CC/-137GC genotype combination (6.1% *vs*. 0%, OR: ∞, 95% CI=NaN-∞, p=0.041) when compared to controls.

Although there was no association between BD patients with systemic involvement (ocular, articular, vascular or neurologic) and healthy controls according to allelic frequencies and genotypic distributions, BD patients with mucocutaneous involvement had a significantly higher frequency of the -607 CC genotype when compared with healthy controls (33.3% *vs*. 9.9% OR: 4.55, 95% CI: 1.671-12.384, p=0.004) (Table II).

Haplotype analysis showed that BD patients with mucocutaneous involvement had significantly higher frequency of -607C/-137C haplotype than BD patients with systemic involvements and healthy controls (OR: 11.101, 95% CI=1.268-97.130, p=0.015 and OR: ∞, 95% CI=NaN-∞, p=0.003, respectively). They also had higher frequency of -607CC/-137GC genotype combination compared with BD patients with systemic involvement and healthy controls (OR: 12.037, 95% CI=1.342-107.926, p=0.013 and OR:

Table I. Frequency of aleles and distribution of genotypes of IL-18 promoter polymorphisms in patients with Behçet's disease and healthy controls.

Polymorphisms	Patients n (%)*	Controls n (%)*	χ^2	p	OR	95% CI
Position -607 Genotypes						
CA	52 (43.6)	42 (41.6)	0.100	0.752	1.090	0.637-1.864
CC	24 (20.1)	10 (9.9)	4.408	0.036	2.298	1.041-5.074
AA	43 (36.1)	49 (48.5)	3.442	0.064	0.600	0.349-1.030
Alleles						
C	100 (42.0)	62 (30.7)	6.023	0.014	1.636	1.102-2.427
A	138 (57.9)	140 (69.3)				
Position -137 Genotypes						
GC	72 (58.5)	42 (54.5)	0.308	0.579	1.176	0.662-2.089
CC	–	–	–	–		
GG	51 (41.4)	35 (45.5)				
Alleles						
G	174 (70.7)	112 (72.7)	0.185	0.667	0.906	0.578-1.419
C	72 (29.2)	42 (27.3)				

*no. values of patients and controls are 119 *vs*. 101 and 123 *vs*. 77, respectively, for -607 and 137 polymorphisms.

Table II. Frequency of aleles and distribution of genotypes of IL-18 promoter polymorphisms in the subgroups of patients with Behçet's disease and healthy controls.

Polymorphisms	Mucocutaneous Involvem. no. (%)*	Systemic Ocular Involvem. no. (%)*	Articular Involvem. no. (%)*	Vascular Involvem. no. (%)*	Neurologic Involvem. no. (%)*	Controls no. (%)*	χ^2	p
Position -607 Genotypes								
CA	11 (36.6)	22 (47.8)	4 (33.3)	7 (31.8)	8 (88.8)	42 (41.6)	10.333	0.066
CC	10 (33.3) [a]	7 (15.2)	2 (16.6)	5 (22.7)	–	10 (9.9) [b]	12.275	0.031
AA	9 (30.0)	17 (36.9)	6 (50.0)	10 (45.4)	1 (11.1)	49 (48.5)	7.968	0.158
Alleles								
C	31 (51.6)	36 (39.1)	8 (33.3)	17 (38.6)	8 (44.4)	62 (30.7)	9.794	0.081
A	29 (48.3)	56 (60.8)	16 (66.6)	27 (61.3)	10 (55.5)	140 (69.3)		
Position -137 Genotypes								
GC	23 (65.7)	25 (55.5)	7 (63,6)	11 (47.8)	6 (66.6)	42 (54.5)	2.643	0.755
CC	–	–	–	–	–			
GG	12 (34.2)	20 (44.4)	4 (36.3)	12 (52.1)	3 (33.3)	35 (45.5)		
Alleles								
G	47 (67.1)	65 (72.2)	15 (68.1)	35 (76.0)	12 (66.6)	112 (72.7)	1.589	0.903
C	23 (32.8)	25 (27.7)	7 (31.8)	11 (23.9)	6 (33.3)	42 (27.3)		

*no. values of patients with mucocutaneous, ocular, articular, vascular, neurologic involvements, and controls are 30, 46, 12, 22, 9 *vs*. 101 and 35, 45, 11, 33, 9 *vs*. 77, respectively, for -607 and 137 polymorphisms.
p=0.004; OR: 4.55; 95 % CI: 1.671-12.384 for **a** *vs*. **b**.

Table III. Frequency of haplotype and genotype combinations of two IL-18 promoter bi-allelic polymorphisms in the subgroups of patients with Behçet's disease and healthy controls.

	Mucocutaneous involvement no. (%)*	Systemic involvement no. (%)*	Controls no. (%)*	χ^2	*P*
Haplotypes					
-607A/-137C	16 (25.0)	35 (26.5)	36 (25.7)	0.056	0.973
-607A/-137G	16 (25.0)	45 (34.1)	51 (36.4)	2.638	0.267
-607C/-137C	5 (7.8) [a]	1 (0.8) [b]	– [c]	16.596	<0.001
-607C/-137G	27 (42.2)	51 (38.6)	53 (37.9)	0.358	0. 836
Genotype combinations					
-607AA/-137GC	10 (31.3)	20 (30.3)	21 (30.0)	0.016	0.992
-607AA/-137GG	–	5 (7.6)	4 (5.7)	2.470	0.291
-607CA/-137GC	6 (18.8)	15 (22.7)	15 (21.4)	0.202	0.904
-607CA/-137GG	6 (18.8)	15 (22.7)	22 (31.4)	2.323	0.313
-607CC/-137GC	5 (15.6) [d]	1 (1.5) [e]	– [f]	16.903	<0.001
-607CC/-137GG	5 (15.6)	10 (15.2)	8 (11.4)	0.524	0.770

*The calculations of frequency of haplotypes and genotype combinations were performed in 32 and 66 of patients with mucocutaneous and systemic involvement, respectively, and 70 controls.
p=0.015; OR: 11.101; 95 % CI: 1.268 to 97.130 for [a] *vs.* [b]; *p*=0.003; OR: •; 95 % CI: NaN-• for [a] *vs.* [c].
p=0.013; OR: 12.037; 95 % CI: 1.342-107.926 for [d] *vs.* [e]; *p*=0.002; OR: •; 95 % CI: NaN-• for [d] *vs.* [f].

∞, 95% CI=NaN-∞, *p*=0.002, respectively) (Table III). The findings of the present study are contrasting with the results of our previous work which showed elevated serum levels of IL-18 in BD patients with all types of involvement including, mucocutaneous, ocular, articular, and vascular (6). Considering the results of the present study, one might suggests that IL-18 polymorphisms may not be the sole mechanism responsible for the elevated serum IL-18 levels, particularly in those patients having systemic involvement. When we compare the findings of our study with other studies investigating IL-18 polymorphism, our findings are contrasting with those of Jang and co-workers who found no relationship between IL-18 polymorphism and BD in Korean patients (8). However, our results are in agreement with the results of Lee and co-workers from Korea (9). The quantitative differences between the study of Lee and ours such as the frequency of -607 CC genotype (42.7% *vs.* 20.1% for BD, 23.3% *vs.* 9.9% for healthy donors, Lee's and the present study, respectively) might result from the ethnic differences between the two study populations.

We concluded that the IL-18 promoter gene is a candidate susceptibility gene in Turkish BD patients, particularly those with mucocutaneous involvement.

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This study was supported by the Research Center of Gülhane Military Medical Academy,

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Competing interests: none declared.

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