Polymorphism of Interleukin 6 -174 G/C in Behcet Disease: Case Series and Review of Literature

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Abstract- To assess the association between polymorphisms of the IL-6 -174 G/C and Behçet's disease (BD) in Tunisian patients. DNA was extracted from blood samples taken from 43 Tunisian patients and 43 healthy controls. The polymorphisms were analyzed by PCR with the PCR-RFLP. No significant association was found between patients and controls concerning polymorphism of IL6 -174 G/C between the (allelic frequency: C (17.44 vs 8, 13%; *P*=0.17) et G (82,55 vs 91,86%; *P*= 0.21). Neither age of onset of BD nor sex appears to be influenced by allelic variation of SNP-174 G / C of IL6. Disease duration of BD was longer in patients having the form 174 G-allele. SNP -174G/C was more frequent in patients without significant association (32.5 vs 16,26% ; *P*=0.07). SNP -174 G/C was not associated with the HLA B51. Neither age of onset of BD nor sex appears to be influenced by SNP-174 G / C of IL6. Disease duration of BD was longer in the absence of the SNP-174 G/C IL6, with no significant difference (79.2 + / -45.095 *vs.*70.28 + / - 47.034 months, *P*=0.59). The polymorphism of IL6 -174 G/C does not modulate clinical expression of BD. The single nucleotide polymorphisms of the IL-6 do not appear to be associated with BD reconstruction.

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Introduction

Behçet's disease (BD) is a multisystem inflammatory disease characterized by recurrent urogenital ulceration, ocular inflammation, and skin lesions. The etiology of the disease is currently unknown, but evidence suggests that there is a strong genetic component mediating the chronicity of the disorder (1). It generally agrees that the inflammatory reaction in BD is mainly driven by type 1 helper T cell (Th1) cytokines. Moreover, the immune phenotyping and cytokine profiling of peripheral blood lymphocytes, and cytokine expressions within mucocutaneous lesions support the roles of Th1 cytokines in the pathogenesis of BD (2-5). Interleukin (IL)-6 is an important mediator of inflammatory and immune responses, and IL6 gene polymorphisms are known to play a part in chronic inflammatory and autoimmune disorders (6-10). Increased IL6 plasma concentrations and enhanced IL6 mRNA expression have been found in patients with active BD (11,12). Polymorphism of IL6 gene was rarely studied in BD (13,14). We aimed to investigate a possible association between polymorphism IL6-174 G/C and BD.

Material and Methods

Participants

This is a prospective, case control, descriptive, analytical and comparative study, including 43 patients with BD (23 men and 20 women; mean age of 40.63 years), fulfilling the International Study group criteria for the classification BD (15). They were recruited from the Department of Internal Medicine, Hospital Fattouma Bourguiba- Monastir- Tunisia. The control group consisted of 43 native Tunisian healthy volunteers' age and sex matched. Clinical features were recorded on a standard form; they are summarized in Table 1. An activity disease score was calculated for each patient according to the Bahkta score (16). The study was approved by our local ethics committee. Informed consent was obtained from all patients and controls.

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Table 1. Clinical features of the patients

	No of patients (%) N=177
Mean age (years)	43
Male/ Female	23/20
Mean duration of the disease (months)	75.62
Oral ulceration	43 (100)
Genital ulceration	34 (79.1)
Folliculitis	37 (86)
Skin pathergy response	13 (40.6)
Ocular inflammation	12 (27,9)
Vascular involvement	7 (16.3)
Neurological involvement	10 (23.2)
Articular involvement	25 (58.1)

Genetic study

The study of genetic polymorphism was performed in the laboratory of Genetics, University of pharmacology of Monastir.

The polymorphism IL 6 -174 G/C is a point mutation in the promoter of the gene encoding IL-6, consisting of a substitution of a guanine with a cytosine at position-174 from the transcription start site. The detection of this polymorphism was performed by PCR-RFLP with amplification same sequence of 527 pb using restriction enzyme NlaII.I.

Statistical analysis

Data were recorded and analyzed using SPSS 15.0. The results were expressed as mean and standard deviation for variables quantitative and percentages for categorical variables. The comparison of percentages was performed using the Chi2 test Pearson and Fisher's exact test. The comparison of means was performed using the Student t test. A *P*.value<0.05 was considered significant.

Results

Neither age of onset of BD nor sex appears to be influenced by allelic variation of SNP-174 G / C of IL6. Disease duration of BD was longer in patients having the form 174 G-allele (77.4±44.798 vs 67.2±46.87 months), with no significant statistically difference.

The frequencies of clinical manifestations of BD according to the presence of G or C allele of IL6 SNP-174G/C are shown in Table 2. The average of CRP (4.4±1.949 *vs.* 6.42 ± 4.14 mg/l) and ESR (25.4 ± 21.533 *vs.* 21.15 ± 16, 76 mmH1) were comparable across the 2 allelic forms of SNP IL6-174G/C. The activity index of Bhakta was not influenced by the allelic form of the SNP ILL6 -174 G/C (4.733±3.55 *vs.* 5.59±3.503). Since there was only one patient with the genotype- 174C / C of IL6, we studied the clinical expression and evolution of BD according to the existence of the SNP-174 G / C of IL6.

Neither age of onset of BD nor sex appears to be influenced by the presence of SNP-174 G / C of IL6 as shown in Table 3. Disease duration of BD was longer in the absence of the SNP- 174 G/C IL6, with no significant difference (79.2±45.095 *vs.*70.28±47.034 months, *P*=0.59). The frequencies of clinical manifestations of BD according to the presence of SNP-174 G/C of IL6 are shown in Table 4. It appears that the polymorphism of the IL6 -174 G/C does not modulate clinical expression of BD.

Table 2. Clinical involvement according to the allelic form of -174 G/C IL6

	-174 C (n=15) N (%)	-174 G (n=71) N (%)	*P*
Oral ulceration	15 (100)	71(100)	
Genital ulceration	13 (86.66)	55 (77.46)	
Folliculitis	13 (86.66)	48 (75.86)	
Positive Pathery test	5/11(45.45)	21/39 (53,84)	
Ocular involvement	2 (13.33)	20 (27.77)	
Anterior uveitis	0	12 (16.90)	
Posterior uveitis	2 (13.33)	12 (16.90)	*NS
Retinal vasculitis	2 (13.33)	6 (8.45)	
Neurological involvement	3 (20)	14 (19.71)	
Articular involvement	6 (40)	38 (53.52)	
Vascular involvement	6 (40)	10 (14.08)	
Venous thrombosis	3 (20)	8 (11.26)	
Arterial thrombosis	3 (20)	2 (2.81)	

*NS: Non-significant

Table 3. Gender and onset BD according to the presence of SNP-174 G/C IL6

		SNP -174(+) (n=14) N (%)	SNP -174(-) (n=29) N (%)	*P*
Age of onset (year)		28.58	29.36	
Gender	**Male**	9(64.28)	14(48.27)	NS
	Female	5(35.71)	15(51.72)	

Table 4. Clinical involvement according to the presence of SNP-174 G/C IL6

	SNP IL6-174(+) (n=14), N (%)	SNP IL6 -174(-) (n=29), N (%)	*P*
Oral ulceration	14 (100)	29 (100)	
Genital ulceration	12 (85.71)	22 (75.86)	
Folliculitis	13 (92.85)	24 (75.86)	
Positive Pathery test	4/10 (40)	9/22 (40.90)	
Ocular involvement	2 (14.28)	10 (34.48)	
Anterior uveitis	0	6 (20.68)	
Posterior uveitis	2 (14.28)	6 (20.68)	NS
Retinal vasculitis	2 (14.28)	3 (10.34)	
Neurological involvement	3 (21.42)	7 (24.13)	
Articular involvement	6 (42.85)	19 (65.55)	
Vascular involvement	3 (21.42)	5 (17.24)	
Venous thrombosis	3 (21.42)	4 (13.38)	
Arterial thrombosis	0	1 (3.44)	

The mean average of ESR (26.5±21.9 *vs.* 19.38±15.077 mmH1) and CRP (4.4±1.949 *vs.* 5.47±4.809) was comparable according to the presence or absence of SNP -174 G/C of IL6. The activity index of Bhakta was not influenced by the presence or absence of SNP-174 G / C IL6 (4.93±3.605 *vs.* 5.69±3.526).

Discussion

An increase in plasma IL6 and the expression of mRNA IL6 in the MB was already described (2,17,18). Few studies have investigated the polymorphism of the IL6 in BD. In the literature, three studies were published: one affecting the polymorphism of the IL6 receptor (14) and the other two on IL6 polymorphism (5,13). These studies have involved populations of Korean, Turkish and German. Their data are summarized in Table 5.Authors believe that the scarcity of studies of polymorphism of IL6 in BD is related to the fact that IL6 is a pro-inflammatory cytokine of Th2, whereas BD is a Th1 disease.

To our knowledge, only two studies have focused on the SNP-174 G / C IL6 in BD; the present study is the third in the world and the first in a Tunisian population of patients with BD.

Table 5. Polymorphism of IL6 in BD

Study	Patients/ Controls	Origin	SNPs
Chang (2006) (13)	89/123	Korea	IL6prom IL6vntr
Storz (2008) (14)	121/71	Germany/ Turkey	IL6R* 24 013 A / G 48892 A / C
Dilek (2009)(5)	97/127	Turkey	-174G/C

In current study, comparison of allele frequencies of the polymorphism gene coding for IL6 at position -174 between patients and the group control indicated no statistically significant difference in the C allele frequency and G. The genotype frequencies of SNP-174g / C IL6 were comparable between the two groups. Furthermore, the genotype G / G was more frequently found in controls, but the difference was not statistically significant. The SNP-174 G/C IL-6 was more frequently found in patients but the association was not significant, which allowed us to conclude that the presence of SNP-174 G / C IL6 was not associated with BD in patients of

present. Current results are in keeping with the other two studies (5,13).

Chang et al. through the study of 89 Korean patients with BDlooking for a possible association of two functional IL6 gene polymorphisms, the SNP -174G/C and IL6vntr and BD, concluded that there was no association between polymorphism IL6-174g / C and susceptibility to BD (13). Moreover, the genotype IL6vntr B / C was more frequently found and what it statistically significant patients with BD compared to controls and allele C of IL6vntr. These differences were more significant in HLA-B51 negative patients (genotypes: P= 0.01, allele: + 0.04).

The authors showed that the haplotype IL6promG * / * IL6vntrC was more frequently found in patients with DB, the OR of the BD in these patients were 7.3. For Dilek *et al*., there was no association of SNP-174g / C and susceptibility to BD (5).

The study of polymorphism of the IL6 receptor: 24,013 A / G and 48892 A / C in 121 patients with BD (93 Germans and 28 Turks) and 71 healthy controls (51 Turks and 20 Germans), found no statistical difference between patients and controls and between German and Turkish patients (14). To confirm these results, further investigations are needed in other ethnic populations.

In current study, neither age of onset of BD, nor sex and disease duration of BD seem to be influenced by changes in allele or genotyping of SNP-174g / C. It was the same for different clinical manifestations and activity BD.

A small number of patients and therefore the low statistical power is one major limitation of the present study. Nevertheless, current results confirm those of Chang et al. who did not show any association between the two IL6 polymorphisms' and characteristics including average age of onset, duration of disease progression, and manifestations and their clinical severity (13). It appeared that the genotype B/ C of IL6vntr was more frequent among female patients (male: 8.3 *vs.* women: 91.7%, P=0.048).

In Dilek's study, the genotype -174 G / G was less frequent in patients with BD and positive pathergy test (5).

It is interesting to report the results of the study Barrafshani *et al*., which found that the -573 polymorphism of IL6 was associated with a higher risk of recurrent ulceration (OR:8.5) (19). Given the scarcity of studies, we cannot draw any conclusion on the role of the IL6 polymorphism-174g / C in the clinical expression of BD. Further studies on other populations, to include a wider workforce are necessary.

IL6 gene polymorphisms may influence the susceptibility and the disease expression of BD.

To confirm these findings, further investigations are required in other ethnic populations.

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