Letters to the editor

CTLA-4 gene variants are not associated with Behçet's disease or its clinical manifestations

Sirs,

CTLA-4 has emerged as an important susceptibility locus for many autoimmune diseases. Several polymorphisms have been described in the CTLA-4 gene: -1722T/C and -319C/T, both within the promoter region; +49A/G in exon 1; a microsatellite $(AT)_n$ 3´UTR polymorphism; and a recently described CT60A/G dismorphism (single-nucleotide polymorphisms) [SNP] 3087243 that is associated with a variety of autoimmune disease, with the CT60 A allele being protective and the G allele increasing susceptibility (1). Since Behçet's disease (BD) is a chronic multisystem inflammatory disorder that possibly shares a common genetic background with other autoimmune diseases, we investigated the possible influence of the CT60A/G dimorphism of the CTLA-A gene in the susceptibility to Behçet's disease in Lebanese patients (2).

A total of 46 unrelated patients with a diagnosis of BD who met the International Study Group criteria and 53 unrelated healthy control individuals matched for age and sex were enrolled (3, 4). The disease severity score was calculated using the method described by Krause (5). Genomic DNA was extracted from peripheral blood lymphocytes, the CT60 polymorphisms were determined, and the 3´UTR polymorphism of the CTLA-4 was genotyped (6). The allele and genotype frequencies of the CTLA-4 and CTLA4-CT60 (GA and GG) polymorphisms were obtained by direct counting in the BD patients and controls. Chi-square and Fisher's exact tests were conducted and SPSS 13.0 was used to perform the analysis. $P<0.05$ was considered to be statistically significant.

The average age of the patients was 32.50±11.38 years, and 82% were male. All had oral ulcerations and 75% had genital ulcerations. Anterior uveitis, posterior uveitis and blindness were present in 47%, 51% and 8% of the patients, respectively. With regard to the CTLA-4 3´UTR microsatellite, a total of 25 different genotypes and 17 alleles were identified. No significant association between any genotype and occurrence of the disease was found. There was no significant difference in the distribution of all alleles between the two groups and no significant association could be found between any allele and BD. Moreover, there was no significant association between the genotype of the CT60 marker and having BD (p=0.12) (Table I). When the genotypes of CTLA4-CT60 were reclassified as having the AA genotype versus having other genotypes (GA or GG), there was still no significant association between the genotype and the clinical manifestations of the patients. Patients and controls were also compared with respect to the CTLA4-CT60 alleles (G *vs*. A) and there was no significant difference between the two groups. In addition, there was no significant association between the CTLA4-CT60 genotype and clinical findings. The average severity score was 7.7±3.3, 7.6±3.4, and 6.7±4.2 for the GG, GA and AA genotypes, respectively; these differences were not statistically significant (p=0.82).

This is the first study in which the association between CT-60A/G polymorphism in the CTLA-4 gene and susceptibility to BD has been evaluated. Two publications have focused on CTLA-4 49A/G polymorphism in the Turkish BD population (7, 8). The +49A allele of the CTLA-4 gene was found to be associated with ocular involvement and erythema nodosum, but in the other study no such association was noted and the CTLA4 +49GG genotype was found to be negatively associated with BD (7, 8). Here we evaluated CT-60A/G in Lebanese patients and found no association with the clinical manifestations or disease severity. With regard to the CTLA-4 3´UTR microsatellite, no significant association was found between any genotype or allele and having the disease. Our study is limited by its small sample size. Further larger studies should be conducted on the polymorphisms examined here and other polymorphic positions located within the 3´ region of the CTLA-4 gene.

Table I. Distribution of CTLA-4 polymorphisms and CT60 marker genotypes in controls and Behçet's disease patients.

Genotype	Group		*p*-value
	Patients	Controls	
CTLA-4 polymorphism	(n=46)	(n=53)	
88-104	1 (2.2%)	2 (3.8%)	1.00
88-106	9 (19.6%)	4 (7.5%)	0.13
88-108	0 (0.0%)	3 (5.7%)	0.25
88-110	3 (6.5%)	8 (15.1%)	0.21
88-112	1 (2.2%)	2 (3.8%)	1.00
88-114	1 (2.2%)	2 (3.8%)	1.00
88-116	1 (2.2%)	0 (0.0%)	0.46
88-118	0 (0.0%)	1 (1.9%)	1.00
88-122	2 (4.3%)	1 (1.9%)	0.48
88-124	2 (4.3%)	1 (1.9%)	0.60
88-126	2 (4.3%)	4 (7.5%)	0.68
88-128	3 (6.5%)	3 (5.7%)	1.00
88-130	3 (6.5%)	1 (1.9%)	0.34
88-88	8 (17.4%)	17 (32.1%)	0.09
88-96	1 (2.2%)	0 (0.0%)	0.46
96-114	0 (0.0%)	1 (1.9%)	1.00
100-118	0 (0.0%)	1 (1.9%)	1.00
104-122	1 (2.2%)	0 (0.0%)	0.46
106-126	1 (2.2%)	0 (0.0%)	0.46
110-114	1 (2.2%)	1 (1.9%)	1.00
110-124	2 (4.3%)	0 (0.0%)	0.21
114-114	1 (2.2%)	0 (0.0%)	0.46
114-120	1 (2.2%)	0 (0.0%)	0.46
122-122	1 (2.2%)	0 (0.0%)	0.46
126-130	1 (2.2%)	1 (1.9%)	1.00
CT60			
G/G	8 (19.05%)	20 (37.74%)	0.12
G/A	26 (61.9%)	23 (43.4%)	
A/A	8 (19.05%)	10 (18.87%)	

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