TNF polymorphisms in Lebanese patients with Behçet's disease

Sirs,

Behçet's disease (BD) is a inflammatory disorder of unclear etiology characterized by recurrent oral and genital ulcers, skin lesions and uveitis amongst other manifestations (1). HLA-B51 is strongly associated with the disease in various ethnic groups but its presence alone is not sufficient to explain all the disease manifestations. TNF-α is possibly implicated and several polymorphisms in the TNF gene have been studied in different ethnic groups with BD. The TNF-α-1031C allele has been the one most consistently associated with the disease compared to the other promoter area polymorphisms studied (2). The aim of our study was to investigate the association of TNF-α promoter region polymorphisms at locations -308, -238, -863, -857 and -1031 with susceptibility to BD in Lebanese patients.

A total 48 unrelated patients with a diagnosis of Behçet's disease, who met the International Study Group criteria were recruited from the American University of Beirut Medical Center Behçet's Disease Database and compared to 90 anonymized blood samples collected from randomly selected healthy Lebanese subjects at the Chronic Care Center, Lebanon. Five ml of blood was collected from each subject and DNA was extracted using standard methods. Genotyping for HLA-B51 and TNF-α promoter region polymorphisms was performed using primers that have been previously described (3, 4).

The chi-square and/or Fisher's exact test was used as appropriate to compare the genotype distributions and allele frequencies between the patients and controls.

We found no statistically significant differences (Table I) between the TNF-α -863, -857, -308, and -238 genotypes and allele frequencies between BD patients and healthy controls. We also observed no difference in the distribution of the -1031C allele in patients compared to controls. Three patients with BD were however homozygous for the -1031C allele whereas none in the control group were (p=0.03). All 3 of these patients were male.

We found no association between the clinical manifestations, disease severity and any of the TNF polymorphisms. HLA-B51 was significantly associated with the presence of ocular disease (p=0.004), but not with blindness or any of the other clinical manifestations. Disease severity was significantly associated with the presence of HLA-B51 (p=0.028) but not with any of the TNF-α polymorphisms studied.

In this study we evaluated the association of Behçet's disease and its clinical manifestations with the HLA-B51 and 5 TNF-α promoter SNPs in Lebanese patients. HLA-B51 was significantly more common in the patients compared to the controls (OR=3.4) and was associated with ocular disease and increased disease severity. This is in agreement with the data of Verity *et al*. (5), where HLA-B51 was associated with ocular disease in other Arab patients with Jordanian and Palestinian descendants.

With regard to the TNF-α polymorphisms studied, no differences were found in the distribution of the allelic or genotypic frequencies of TNF-α -308G/A, -238 G/A, -857C/T and -863C/A between BD patients and healthy controls. In addition, we found no difference in the -1031C allele, which has been identified in some populations (2, 6-8) to be a genetic marker in BD patients and is associated with an increase in TNF-α production (9). Three patients (6.5%), however, were homozygous for this allele compared to none in the control group (p=0.03), suggesting a possible role in our population. Previous studies of TNF-α polymorphisms in Lebanese patients failed to demonstrate an association between the -1031C allele and type I diabetes mellitus, but did show a gender difference in the CC genotype favouring males (10).

In summary, our study confirms the association of HLA-B51 with BD and suggests an association between the -1031CC genotype and the disease in Lebanese patients.

Table I. TNF-α -863, -857, -308, -238, and -1031 genotype and allele frequencies and HLA-B51 frequency in the study groups.

	Group		p-value
	Patients n (%)	Controls n (%)	
863 C>A			
CC genotype	37 (82.2)	66 (73.3)	0.44
CA genotype	4 (8.9)	15 (16.7)	
AA genotype	4 (8.9)	9 (10)	
A allele	8 (17.8)	24 (26.7)	0.25
857 C>T			
CC genotype	35 (79.5)	68 (75.6)	0.71
CT genotype	8 (18.2)	21 (23.3)	
TT genotype	1 (2.3)	1 (1.1)	
T allele	9 (20.5)	22 (24.4)	0.61
308 G>A			
GG genotype	37 (84.1)	75 (83.3)	0.78
GA genotype	7 (15.9)	14 (15.6)	
AA genotype	0 (0.0)	1 (1.1)	
A allele	7 (15.9)	15 (16.7)	0.91
238 G>A			
GG genotype	41 (93.2)	83 (92.2)	1.00
GA genotype	3 (6.8)	7 (7.8)	
AA genotype	0 (0.0)	0 (0.0)	
A allele	3 (6.8)	7 (7.8)	0.84
1031 T>C			
TT genotype	33 (71.7)	61 (67.8)	0.03
TC genotype	10 (21.7)	29 (32.2)	
CC genotype	3 (6.5)	0 (0.0)	
C allele	13 (28.3)	29 (32.2)	0.64
HLA-B51			
Absent	28 (63.6)	77 (85.6)	0.007
Present	16 (36.4)	13 (14.4)	

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References

1. ARAYSSI T, HAMDAN A: New insights into the pathogenesis and therapy of Behçet's disease. *Curr Opin Pharmacol* 2004; 4: 183-8.
2. AKMAN A, SALLAKCI N, COSKUN M *et al.*: TNF-alpha gene 1031 T/C polymorphism in Turkish patients with Behçet's disease. *Br J Dermatol* 2006; 155: 350-6.
3. WELSH K, BUNCE M: Molecular typing for the MHC with PCR-SSP. *Rev Immunogenet* 1999; 1: 157-76.
4. SKOOG T, VAN'T HOOFT FM, KALLIN B *et al.*: A common functional polymorphism (C --> A sub-

stitution at position -863) in the promoter region of the tumor necrosis factor-alpha (TNF-alpha) gene associated with reduced circulating levels of TNF-alpha. *Hum Mol Genet* 1999; 8: 1443-9.
5. VERITY DH, WALLACE GR, VAUGHAN RW *et al*.: HLA and tumour necrosis factor (TNF) polymorphisms in ocular Behçet's disease. *Tissue Antigens* 1999; 54: 264-72.
6. AHMAD T, WALLACE GR, JAMES T *et al*.: Mapping the HLA association in Behçet's disease: a role for tumor necrosis factor polymorphisms? *Arthritis Rheum* 2003; 48: 807-13.
7. KAMOUN M, CHELBI H, HOUMAN MH, LACHEB J, HAMZAOUI K: Tumor necrosis factor gene polymorphisms in Tunisian patients with Behçet's disease. *Hum Immunol* 2007; 68: 201-5.
8. PARK K, KIM N, NAM J, BANG D, LEE ES: Association of TNFA promoter region haplotype in Behçet's disease. *J Korean Med Sci* 2006; 21: 596-601.
9. HIGUCHI T, SEKI N, KAMIZONO S *et al*.: Polymorphism of the 5'-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. *Tissue Antigens* 1998; 51: 605-12.
10. SHBAKLO H, AZAR ST, TERWEDOW H, HALABY G, NAJA RP, ZALLOUA PA: No association between the -1031 polymorphism in the TNF-alpha promoter region and type 1 diabetes. *Hum Immunol* 2003; 64: 633-8.

Association of familial Mediterranean fever and celiac disease in a 14-year-old girl with recurrent arthritis

Sirs,

Celiac disease (CD) is an immune-mediated enteropathy associated with some autoimmune and inflammatory diseases (1). Familial Mediterranean fever (FMF) is a genetic inflammatory disease presenting with recurrent self-limiting attacks of joint, chest and abdominal pain associated with fever (2). FMF is also associated with several inflammatory diseases and vasculitides (2, 3). Herein we report a case of CD in association with FMF.

A 10-year-old girl was admited with a 4-year history of recurrent monoarthritis, failure to thrive, refractory iron deficiency anemia (IDA), and intermittant elevation of acute phase reactants. Her family history was unremarkable. Physical examination revealed paleness and failure to thrive. She had IDA. Fecal occult blood was negative. Investigations for recurrent arthritis (immunglobulins G, A and M, complements C3 and C4, prothrombin and partial thromboplastine times, anti-streptolysin O titer, rheumotoid factor, antinuclear antibody, anti-double strain DNA, antineutrophilic cytoplasmic antibody, serological tests for viral or bacterial infections, ocular examination, ankle and chest x-rays, electrocardiogram and echocardiogram) were normal. IgA anti-endomysial (EMA) antibody was positive. Duodenal biopsy revealed type III CD. She had HLA DQ2. With the diagnosis of CD, a gluten-free diet (GFD) was started, her EMA disappeared, and anemia improved within 9 months.

One year later, the child was admitted with arthritis of the right ankle, chest pain, and dyspnea without fever. Acute phase reactants were elevated, while routine tests, chest x-rays, electrocardiogram, echocardiogram and repeated investigations for any possible causes of arthritis were normal. EMA IgA was negative. The arthritis improved within 5 days. The patient was suspected to have FMF because of her ethnic origin, and clinical and laboratory findings that revealed a homozygous M694V mutation. A diagnosis of FMF was made and colchicine was started. Her acute phase reactants normalized, physical development returned to normal within 2 years, and she has had no complaints for 2 years.

Although CD may occur together with other immune-mediated disorders, its coexistence with FMF has never been described before. Our patient met all the diagnostic criteria of both CD and FMF. Initially we thought that her arthropathy was an extra-intestinal finding of CD. Although rare, arthropathy may be the first and even the only symptom of CD, which takes the form of non-erosive oligoarthritis or polyarthritis affecting primarily the knees and ankles, resolves on GFD, and generally does not relapse (4).

Arthritis in FMF is recurrent and transient, as was seen in our patient, and irreversible joint destruction is rare (5). FMF is associated with several inflammatory diseases and vasculitides including inflammatory bowel disease, polyarteritis nodosa, Henoch-Schönlein purpura, and Behçet's disease (2, 3). The factors underlying these associations are not completely understood. The FMF gene (MEFV) has been suggested to act as a modifier affecting the expression of other inflammatory diseases (3). MEFV encodes pyrin, which probably normally assists in controlling inflammation by deactivating the immune response. Defective pyrin results in uncontrolled inflammation (3). In FMF patients increased transcription of the proinflammatory cytokines (TNF-α, IL-6 and IL-8) has been reported (6). Cytokines also play role in the pathogenesis of CD by stimulating intestinal mucosal immune cells, leading to inflammation that induces the intestinal lesion (7, 8). Inflammation induced by FMF may trigger potentially pathogenic intra-epithelial lymphocytes, and in genetically susceptible individuals this may turn into persistent pathogenic signalling. Although the underlying mechanism is unclear, we hypothesize that interactions between immunological and genetic factors may play a key role in the association of these two disorders.

In Turkey, the prevalence of CD and FMF is 0.09% and 2.8/10,000, respectively (9, 10). The theoretical prevalence of both diseases in a single individual would be 0.25/100,000. This report is the first description of CD and FMF found in a child patient, but studies with larger populations are needed to investigate whether this coexistence is coincidental or whether a relationship between the two diseases actually exists.

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References

1. RAVIKUMARA M, TUTHILL DP, JENKINS HR: The changing clinical presentation of coeliac disease. *Arch Dis Child* 2006; 91: 969-71.
2. TUNCA M, AKAR S, ONEN F *et al*.: Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. *Medicine* 2005; 84: 1-11.
3. FIDDER H, CHOWERS Y, ACKERMAN Z *et al*.: The familial Mediterranean fever (*MEVF*) gene as a modifier of Crohn's disease. *Am J Gastroenterol* 2005; 100: 338-43.
4. COLLIN P, KORPELA M, HALLSTROM O, VIANDER M, KEYRLAINEN O, MAKI M: Rheumatic complaints as a presenting symptom in patients with celiac disease. *Scand J Rheumatol* 1992; 21: 20-3.
5. INCE E, CAKAR N, TEKIN M *et al*.: Arthritis in children with familial Mediterranean fever. *Rheumatol Int* 2002; 21: 213-17.
6. NOTARNICOLA C, DIDELOT MN, SEGURET F *et al*.: Enhanced cytokine mRNA levels in attack-free patients with familial Mediterranean fever. *Genes Immun* 2002; 3: 43-5.
7. KAGNOFF MF: Celiac disease: pathogenesis of a model immunogenetic disease. *J Clin Invest* 2007; 117: 41-9.
8. DI SABATINO A, CICCOCIOPPO R, CUPELLI F *et al*.: Epithelium-derived interleukin 15 regulates intraepithelial lymphocyte Th1 cytokine production, cytotoxicity, and survival in coeliac disease. *Gut* 2006; 55: 469-77.
9. DEMIRÇEKEN F, KANSU A, KULOĞLU Z, GIRGIN N, GÜRIZ H, ENSARI A: Human tissue transglutaminase antibody screening by immunochromatographic line immunoassay for early diagnosis of celiac disease in Turkish children. *Turk J Gastroenterol* 2008; 19: 14-21.
10. OZEN S, KARAASLAN Y, OZDEMIR O *et al*.: Prevalence of juvenile chronic arthritis and familial Mediterranean fever in Turkey: a field study. *J Rheumatol* 1999; 26: 1638-9.