ARTHRITIS & RHEUMATISM
Vol. 44, No. 1, January 2001, pp 239–241

CONCISE COMMUNICATIONS

Evidence for linkage of the HLA–B locus in Behçet's disease, obtained using the transmission disequilibrium test

Behçet's disease is a systemic vasculitic disorder characterized mainly by recurrent oral and genital ulceration, skin lesions, and uveitis. The etiology of Behçet's disease is unknown, but available data support the possible involvement of some microbial agents in genetically susceptible individuals (1).

The majority of Behçet's disease patients have sporadic disease with no family history; however, the familial recurrence risk is probably increased. A recent study revealed a sibling recurrence risk of 4.2% and a λ_S value of 11.4–52.5 for Behçet's disease in Turkey (2). Association of HLA–B51 with Behçet's disease is regarded as being the strongest evidence of genetic contribution described to date (3). However, whether HLA–B51 is a Behçet's disease susceptibility gene itself or if this strong association reflects linkage disequilibrium with another gene has yet to be elucidated. Recent studies with polymorphic microsatellite DNA markers suggested that the disease susceptibility locus is probably located within a 46-kb segment between the major histocompatibility complex class I chain–related gene family A (MICA) and HLA–B genes (4,5).

Although the strong association of HLA–B51 with Behçet's disease has been confirmed in different ethnic groups, genetic linkage of this region to Behçet's disease has not yet been documented. Association studies are subject to error based on selection of cases and healthy controls, with population stratification rather than disease possibly explaining any differences observed. We have therefore ascertained whether there is genetic linkage between HLA–B and Behçet's disease using the transmission disequilibrium test (TDT), since it is a test for linkage in the presence of association and is not sensitive to a stratified population (6).

The study group consisted of 81 individuals from 12 multicase families of Turkish origin. Both of the parents were available in 9 families, and 1 parent was missing in 3. Thirty-three of the study subjects (22 male, 11 female) met the criteria of the International Study Group (ISG) for the diagnosis of Behçet's disease (7). There were 8 sib-pairs, 1 sib-trio, and 3 sib-quadruples; in 2 families, 1 of the parents was also affected. Ten individuals with isolated recurrent oral ulcers and/or other Behçet's disease–related manifestations who did not fulfill the ISG criteria were classified as "unknown" cases. Statistical analyses were carried out using the data only from 33 individuals who met the ISG criteria.

Data on HLA–B and HLA–A antigens, determined by standard serologic methods, were available in all but 1 family (72 individuals). To cover a region between HLA–A and HLA–DQ, 5 different polymorphic microsatellite markers, i.e., MICA5, tumor necrosis factor a (TNFa), TNFb, TNFd, and DQCAR, were analyzed by polymerase chain reaction amplification and electrophoresis on denaturing polyacrylamide gel using a 373A automated DNA sequencer (PE Applied Biosystems, Foster City, CA).

TDT was performed using the Extended TDT (ETDT) package, which is a version of TDT for markers with multiple alleles, which uses a logistic regression method (8). For evaluation of the empiric significance, we performed a Monte Carlo simulation of ETDT (9) with 1,000 replicates.

ETDT analysis revealed evidence of linkage to Behçet's disease only for HLA–B, with both allele-wise statistics and the Monte Carlo simulation (Table 1). Chi-square analysis of the individual alleles revealed a positive result only for HLA–B51 (Table 2) ($P = 0.0003$, $P_{corr} = 0.0042$). No significant results were found for the other markers.

Table 1. Allele-wise ETDT results for HLA–A, HLA–B, and 5 microsatellite markers in multicase Behçet's disease families*

Marker	χ^2	df	Asymptotic P	Empiric P (SE)†
HLA–A	12.5	9	0.18	0.25 (0.01)
HLA–B	28.4	14	0.01	0.02 (0.005)
MICA5	4.0	4	0.41	0.47 (0.02)
TNFb	9.0	5	0.11	0.20 (0.01)
TNFa	6.9	8	0.55	0.73 (0.01)
TNFd	4.3	4	0.37	0.49 (0.02)
DQCAR	15.3	8	0.054	0.11 (0.01)

* ETDT = Extended transmission disequilibrium test; df = degrees of freedom; MICA5 = microsatellite marker in exon 5 of the major histocompatibility complex class I chain–related gene family A; TNFb = tumor necrosis factor microsatellite marker b; SE = standard error.
† Results of a Monte Carlo simulation with 1,000 replicates.

We also estimated the contribution of HLA–B (λ_{HLA-B}) to the overall genetic susceptibility to Behçet's disease, using identity-by-descent (IBD) sharing of HLA–B alleles in these families (10). The frequencies of IBD-shared HLA–B alleles in Behçet's disease patients were found to be 15.4%, 50%, and 34.6% for 0-allele, 1-allele, and 2-allele sharing, respectively, using the SPLINK program (11). Based on the proportion of affected pairs sharing 0 alleles, we calculated λ_{HLA-B} to be 1.6 (10). Thus, the highest contribution of HLA–B to the overall genetic susceptibility to Behçet's disease was estimated to be 19%, using the method of Risch (10) and assuming a multiplicative interaction between disease susceptibility loci (19% for $\lambda_S = 11.4$ and 12% for $\lambda_S = 52.5$).

A strong association between HLA–B51 and Behçet's disease has long been known, but this study is the first to demonstrate evidence for linkage of the HLA–B locus with Behçet's disease. Although the recent fine mapping study in Behçet's disease cases and controls, covering a 900-kb region surrounding the HLA–B locus, refined the critical region for Behçet's disease to a 46-kb segment between MICA and HLA–B, HLA–B51 showed the strongest association with Behçet's disease, and no other functioning gene could be identified by sequencing of this segment (4,5,12).

The contribution of the HLA–B locus to the overall genetic susceptibility to Behçet's disease is relatively small,

Table 2. Number of transmissions of individual HLA–B alleles from parents to the children with Behçet's disease

	HLA–B allele														
	7	8	13	18	27	35	38	44	50	51	52	55	57	60	62
Passed	0	1	1	2	0	3	0	2	1	23	1	2	0	3	2
Not passed	2	3	3	5	4	3	4	4	1	4	1	4	2	1	0

even with an assumption of multiplicative interaction between Behçet's disease susceptibility loci based on our observations in other extended families (2,10). Further studies are needed to map the susceptibility genes for Behçet's disease on the other chromosomal regions, with larger numbers of families as well as sporadic disease cases and matched controls.

Dr. Gül's work was supported by the European Science Exchange Programme (the Royal Society and Scientific and Technical Research Council of Turkey).

Ahmet Gül, MD
University of Istanbul
Istanbul, Turkey
Ali H. Hajeer, PhD
Jane Worthington, PhD
Jennifer H. Barrett, PhD
William E. R. Ollier, PhD
Alan J. Silman, MD
University of Manchester
Mancheter, UK

1. Sakane T, Takeno M, Suzuki N, Inaba G. Behçet's disease. N Engl J Med 1999;341:1284–91.
2. Gül A, Inanc M, Ocal L, Aral O, Konice M. Familial aggregation of Behçet's disease in Turkey. Ann Rheum Dis 2000;59:622–5.
3. Mizuki N, Inoko H, Ohno S. Pathogenic gene responsible for the predisposition of Behçet's disease. Int Rev Immunol 1997;14:33–48.
4. Ota M, Mizuki N, Katsuyama Y, Tamiya G, Shiina T, Oka A, et al. The critical region for Behçet's disease in the human major histocompatibility complex is reduced to a 46-kb segment centromeric of HLA-B, by association analysis using refined microsatellite mapping. Am J Hum Genet 1999;64:1406–10.
5. Mizuki N, Ota M, Katsuyama Y, Yabuki K, Ando H, Goto K, et al. Association analysis between the MIC-A and HLA–B alleles in Japanese patients with Behçet's disease. Arthritis Rheum 1999;42: 1961–6.
6. Schaid DJ. Transmission disequilibrium, family controls, and great expectations. Am J Hum Genet 1998;63:935–41.
7. International Study Group for Behçet's disease. Criteria for diagnosis of Behçet's disease. Lancet 1990;335:1078–80.
8. Sham PC, Curtis D. An extended transmission/disequilibrium test (TDT) for multi-allele marker loci. Ann Hum Genet 1995;59:323–36.
9. Zhao JH, Sham PC, Curtis D. A program for the Monte Carlo evaluation of significance of the extended transmission/disequilibrium test. Am J Hum Genet 1999;64:1484–5.
10. Risch N. Assessing the role of HLA-linked and unlinked determinants of disease. Am J Hum Genet 1987;40:1–14.
11. Holmans P, Clayton D. Efficiency of typing unaffected relatives in an affected-sib-pair linkage study with single-locus and multiple tightly linked markers. Am J Hum Genet 1995;57:1221–32.
12. Shiina T, Tamiya G, Oka A, Takishima N, Inoko H. Genome sequencing analysis of the 1.8 Mb entire human MHC class I region. Immunol Rev 1999;167:193–9.

Beneficial effect of etanercept on rheumatoid lymphedema

Since 1998, rheumatologists nationwide have been able to prescribe anti–tumor necrosis factor (anti-TNF) therapy for their patients with rheumatoid arthritis (RA), often with dramatic improvement in the arthropathy (1,2). There is little information, however, on the benefit of this therapy for the extraarticular manifestations of RA. Common extraarticular features include rheumatoid nodules, interstitial lung disease, vasculitis, neuropathy, and Sjögren's syndrome. A less common complication is rheumatoid lymphedema (3,4). Described herein is a patient with longstanding seropositive RA complicated by rheumatoid lymphedema, who was prescribed etanercept in March 2000 after failure to respond to other agents. She experienced a dramatic response of the arthritis as well as the lymphedema, for the first time in her disease course.

The patient presented in 1993 at 51 years of age, with rheumatoid factor–positive polyarthritis. Antinuclear antibody was negative, and her erythrocyte sedimentation rate (ESR) was 105 mm/hour. She was treated initially with nonsteroidal antiinflammatory drugs (NSAIDs) and low-dose prednisone; after 1 year, methotrexate (MTX) was instituted. She improved clinically, and the corticosteroids were discontinued in 1995. In May 1995, she developed diffuse right wrist and forearm swelling with no evidence of vascular compromise. She had no adenopathy, breast masses, or skin changes. The area was nontender, and there was no warmth or erythema. Her other joints appeared normal, although her ESR was 41 mm/hour. The MTX dosage was increased from 12.5 mg/week to 17.5 mg/week, without any change in the arm. Corticosteroids were reinstituted, again with no change noted. Her disease followed a fluctuating course over the next 4½ years, requiring 5–10 mg/day of prednisone in addition to the MTX and NSAIDs.

In March 2000, etanercept was instituted at 25 mg subcutaneously twice weekly. Within 8 weeks her synovitis improved, and by 12 weeks the lymphedema was dramatically reduced. Except for some residual tightness and swelling of the palm and dorsum of the hand, the swelling of the forearm up to the elbow had virtually resolved.

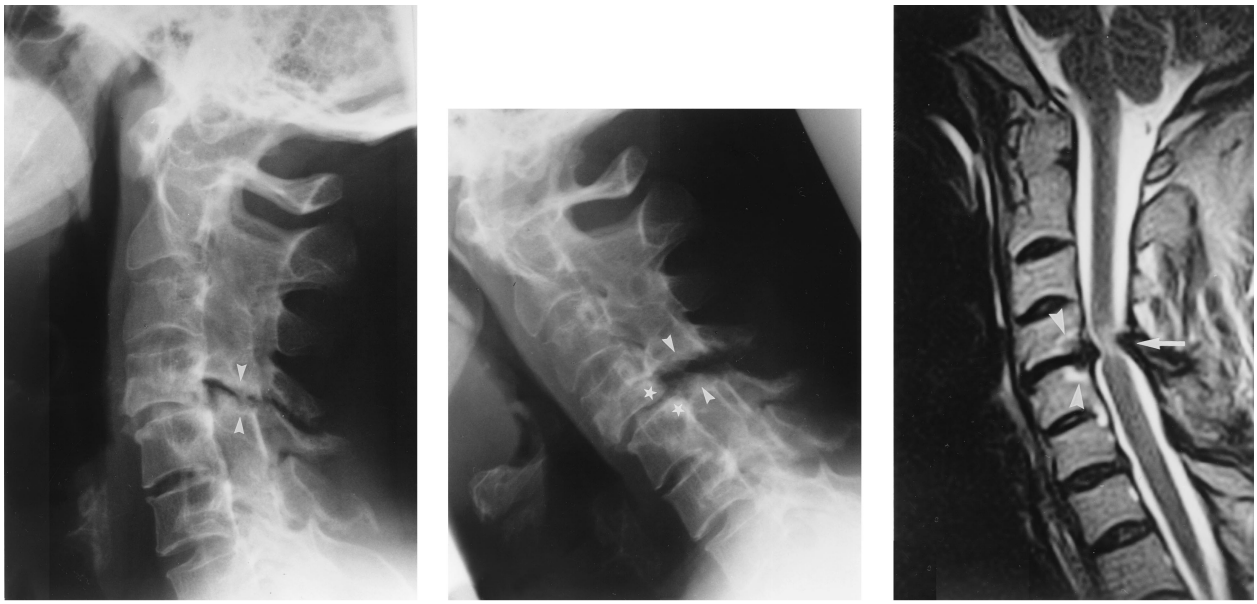
Rheumatoid lymphedema is an uncommon extraarticular feature of RA, which has been reported in <30 patients in the literature. It is thought to be due to complications of synovitis with the development of inflammation and possible fibrosis of the superficial lymph vessels, with subsequent obstruction of lymphatic flow (3–5). Treatment is usually symptomatic, with pressure stockings and, in rare instances, surgery. These approaches have been only partially successful. Previously, medical management of RA has not been of consistent benefit for the lymphedema, and in the majority of patients, it is a longstanding problem even with good control of the inflammatory joint disease. Anti-TNF therapy in this patient dramatically reduced the lymphedema after <4 months of therapy. The mechanism of action of etanercept in the treatment of lymphedema is speculative. Assuming synovitis causes adjacent lymphatic inflammation and ultimate fibrosis, maximal control of active rheumatoid synovitis could abrogate this reaction. Further reports of improvement and/or resolution of a variety of extraarticular features of RA, including rheumatoid lymphedema, with the use of anti-TNF agents will likely be forthcoming in the next few years.

Barbara E. Ostrov, MD
Milton S. Hershey Medical Center
Hershey, PA

1. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischman RM, Fox RI, et al. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med 1999;340:253–9.
2. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, et al. Therapeutic efficacy of multiple intravenous infusions of anti–tumor necrosis factor α monoclonal antibody

combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum 1998;41:1552–63.
3. Dacre JE, Scott DL, Huskisson EC. Lymphedema of the limbs as an extra-articular feature of RA. Ann Rheum Dis 1990;49:722–4.
4. Athreya BH, Ostrov BE, Eichenfeld AH, Goldsmith DP. Lymphedema associated with juvenile rheumatoid arthritis. J Rheumatol 1989;16:1338–40.
5. Mulherin DM, FitzGerald O, Bresnihan B. Lymphedema of the upper limb in patients with psoriatic arthritis. Semin Arthritis Rheum 1993;22:350–6.

Clinical Images: Cervical spinal cord compression



A **B** **C**

The patient, a 38-year-old woman, had had juvenile rheumatoid arthritis since 1973. On admission to the hospital in 1998, she reported having experienced numbness, tingling, and pain in her arms for several months, with no deterioration in arm function. She had felt minor symptoms in her neck. On clinical examination, the neck was stiff and the biceps tendon and patella reflexes hyperactive. Laboratory tests revealed an erythrocyte sedimentation rate of 42 mm/hour, a C-reactive protein level of 20 mg/liter, and a serum alkaline phosphatase level of 130 units/liter. Cervical spine radiographs taken at extension **(A)** and flexion **(B)** revealed apophyseal joint ankylosis at C2–4 and C5–6, and instability at C4–5. The joint surfaces of the C4–5 apophyseal joint were uneven and the joint space widened due to erosions **(A; arrowheads)**. The width of the apophyseal joint space was increased in flexion **(B; arrowheads)**, and there was mild anterior displacement of the C4 vertebral body **(B; asterisks)**. T2-weighted sagittal magnetic resonance images of the cervical spine (fast spin echo, 4,000 ms/160 ms, 4.0 mm, 270 mm, 256 × 256) showed spinal canal stenosis, cord compression, and medullopathy (bright signal) at the C4–5 level **(C; arrow)**. Further erosions were seen at the posterior portions of vertebral end plates **(C; arrowheads)**. Spinal canal decompression and spondylodesis at C4–5 were performed. Postoperatively, numbness remained in the hands.

Kari Laiho, MD
Markku Kauppi, MD, PhD
Irma Soini, MD, PhD
Rheumatism Foundation Hospital
Heinola, Finland