Review

Behçet's disease: Familial clustering and immunogenetics

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ABSTRACT

Behçet's disease (BD) is a relapsing, multisystemic inflammatory disorder, characterized by major symptoms consisting of recurrent orogenital ulcerations, eye and skin lesions. Other clinical features may include musculoskeletal, vascular, gastrointestinal, renal, cardiopulmonary or neurological involvement. Vasculitis affecting all types and sizes of blood vessels is the main histopathologic process, in a third of cases complicated by thrombosis.

The etiopathogenesis is presently unknown, but BD likely represents the result of a peculiar immune response to hitherto unidentified environmental factors in genetically predisposed subjects. The prevalent distribution in a specific geographical area spanning the Mediterranean basin and Asia, the close association with human leukocyte antigen B*51 in different ethnic groups, and the familial clustering of BD are hallmarks accounting for the strong contribution of a genetic background.

The BD familial aggregation is characterized by both genetic anticipation and higher prevalence in childhood patients, likely defining a subset with stronger immunogenetic influences. Polymorphisms in genes encoding for host effector molecules may have a supplementary role in disease susceptibility and/or severity. The contribution of prothrombotic mutations and polymorphisms in the pathogenesis of BD thrombosis is controversial.

In this paper, the available reports on BD familial clustering and the evidence for the role of immunogenetic predisposing factors are reviewed.

Introduction

Behçet's disease (BD) is a relapsing, multisystemic inflammatory disorder, with a polymorphic clinical picture characterized by major symptoms consisting of recurrent orogenital ulcerations, eye and skin lesions (1). Musculoskeletal, vascular, gastrointestinal, renal, cardiopulmonary or neurological involvement may variably occur (1, 2). Vasculitis affecting all types and sizes of blood vessels is the main histopathologic process (1, 3), in a third of cases complicated by arterial and, more commonly, venous thrombosis (12% versus 88%) (3). Neurological involvement, major vessel disease and thrombosis account for most of the mortality (3, 4). Lacking pathognomonic symptoms or laboratory findings, the diagnosis is exclusively clinical, according to the International Study Group for BD criteria (5). Although the disease had been described as early as in the 5th century B.C. by Hippocrates (6), its etiopathogenesis is still unknown. The contribution of an antigen-driven immune response in genetically predisposed subjects has been suggested (7). The innate and adaptive immune systems are activated in BD, with evidence of neutrophil hyperfunction (8, 9) and of T helper (Th)1-type predominant response (7, 10), likely triggered by either extrinsic [Herpes simplex virus, *Streptococcus oralis/sanguis*, *Saccharomyces cerevisiae*, *Mycoplasma fermentas*, superantigens, microbial heat shock protein (HSP), environmental pollution agents] or intrinsic factors (human HSP, $\alpha\beta$-crystallin, retinal-S antigen, endothelial antigens or other organ-specific proteins) (1, 6, 7, 11). Recently, a direct role of Th1 lymphocytes in the mucocutaneous BD lesion development has been suggested (12).

The genetic predisposition to BD is supported by several findings, such as the disease peculiar ethnic distribution, association with human leukocyte antigens (HLA), evidence of the contribution of other immune response controlling genes, and familial clustering (6). As a matter of fact, even though BD may present a worldwide distribution, it endemically affects populations resident in a specific geographic area span-

ning from the Mediterranean basin to Japan, along the so-called Silk Road (6). The highest BD prevalence (80 to 370 cases/100,000 inhabitants) is observed in the Turkish population (1). The disease frequency ranges from 13.5 to 20 cases/100,000 citizens in Japan, Korea, northern China, Iran and Saudi Arabia (1), and from 2.62 to 0.12/100,000 in southern China (13), Switzerland (14), United Kingdom (UK) (1) and United States of America (USA) (1). BD is sporadic in the indigenous west African or Afro-Caribbean populations (15), and is virtually absent in indigenous Amerindians or among ethnic groups south of the equator (6). BD was likely spread in Asian and Eurasian populations through the migrations of nomadic or Turkish tribes, or even more earlier demographic movements (6, 7, 16). Mitochondrial DNA studies, that indicate trading of genes in populations along the Silk Road, suggest that the peculiar ethnic distribution of BD has a genetic basis (17).

Notably, Turks living in Germany present a disease frequency 18-fold lower than those resident in Anatolia, and Japanese immigrants in Hawaii and California rarely suffer from BD (1, 18). These regional variations in prevalence (19), as well as the observed geographical variability in phenotype, severity and outcome of BD (19-26), may account for the influence of environmental triggering factors, whose intermittent exposure might also explain the recurrent clinical expression of the disease.

BD generally occurs in the third or fourth decade of life, affecting both sexes (1, 2, 19) with a prevalent androtropism in the eastern Mediterranean area and a gynaecotropism in north European countries (18), that may suggest the modulating interaction between endocrine status and immunogenetic predisposing factors. Moreover, in adult patients, male sex and early onset of symptoms ($\leq$ 25 years) are usually associated with more severe disease (4, 19, 27-31).

Notably, the BD demographic distribution globally overlaps with the prevalence among the healthy populations of the class I HLA-B5, and particularly of

its B*51 split (6, 7). B*51 has been hitherto recognized as the strongest genetic marker of BD (1, 16, 32, 33), so that HLA typing has been proposed as useful for the diagnosis (1, 34). In B*51-negative patients, the influence of other(s) presently undefined genetic factor(s) has been suggested. In addition, polymorphisms in genes controlling the immunomodulatory milieu or the clotting cascade may have a supplementary role in the disease development or severity (6).

The genetic predisposition to BD is further underlined by the familial clustering (35) and concordance in monozygotic twins (36, 37). The familial recurrence of BD has not been clearly associated with a simple Mendelian model (38, 39). However, a recent formal segregation analysis after stratification of affected families into a paediatric and a non-paediatric group provided the evidence of a genetic heterogeneity in BD, focusing the existence of a Mendelian entity (autosomal recessive mode) only in the paediatric subgroup (40).

In this paper, the available reports on BD familial clustering and the evidence for the role of immunogenetic predisposing factors are reviewed, after a detailed search via the PubMed (National Library of Medicine) and Biosis indices. Some familial case descriptions, however, appeared before the International Study Group criteria publication (5).

Familial clustering of Behçet's disease

Familial clustering is one of the major epidemiological features of BD (35) and is characterized by genetic anticipation, accounting for the earlier disease onset in successive generations (41). The expansion of unstable trinucleotide repeats has been proposed as the genetic basis of the defect in familial cases (41), being the role of the genetic instability further underlined by sister chromatid exchange analyses (42).

The familial aggregation of BD has been observed in different ethnic groups (2, 35-41, 43-84) with a variable frequency. It is higher in Turks (18.2%),

Koreans (15.4%) and Jews (13.2%) than in Chinese (2.6%), Japanese (2.2%) and Europeans (1%) (19). The familial clustering is significantly more common in juvenile than adult patients (74-76), reaching 30.7% in a paediatric Moroccan study (81), so that the inclusion of family history has been proposed in the definition of paediatric BD (74). Recurrence mostly occurs between siblings, but also mother/son (35, 50, 54, 64, 67, 71, 76), mother/daughter (35, 54, 71, 76), father/son (35, 55, 71, 76), father/daughter (56, 71), cousin/cousin (35) and uncle/nephew (48) cases, and a four-generation BD family (38) have been reported. In Turks, the sibling recurrence risk ratio (λ_s) was estimated to be 11.4-52.5 (76), and these high λ_s values strongly support a hereditary basis for the disease (6, 76).

In familial BD, the HLA-B5 (B*51) positivity is much more frequent than in sporadic cases, being 68-83.3% in Turkish studies (61, 79) and ranging from 60.9% to 92% in Japanese reports (35, 69). The B*51 homozygosity is common in familial cases (79). Two pairs of Turkish monozygotic twins, all HLA-B*51 carriers and concordant for BD, have been reported (36, 37). The description of Turkish monozygotic twins, both HLA-B*51-negative and discordant for BD, further supports the role of B*51 or a very close non HLA-gene in the disease susceptibility (85). Among related pairs with a short interval in the disease onset, a great concordance of clinical symptoms was shown, suggesting the contribution of a common environmental trigger (35), whereas the report of 2 brothers brought up in separate homes and concordant for BD, seems to exclude it (53).

A high prevalence of isolated manifestations of the disease, such as recurrent orogenital ulcers or a positive skin pathergy test, was also observed among the patient first degree relatives, probably low-penetrant carriers of predisposing genes (76).

Immunogenetics of Behçet's disease
HLA associations
BD is known to be strongly associated with HLA-B5, and particularly with its split B*51, in various ethnic groups

mainly from the Far East and Mediterranean area (1,6,16). The prevalence of B*51 is up to 81% in Asian patients, whereas is 13% among white patients in western countries (1). This antigen was found to confer an odds ratio of 1.5 to 16 in different populations (11). In an ethnically homogeneous north west European population, such as the Irish people, despite a low B*51 prevalence (25%) in patients, a highly significant association of this antigen with BD was found, and the relative risk was 6.3 (86).

In addition, B*51 positivity has been reported to associate with thrombophlebitis (87, 88) and more severe disease course and outcome (1, 18, 19, 89-92), but the evidence is conflicting (79).

However, notwithstanding the relevant role of B*51 itself has been strongly underlined by microsatellite comparative studies performed on Greek, Italian and Japanese patients (32), analyses on Turkish multicase families by the transmission disequilibrium test in affected sibling pairs estimated that its contribution to the overall genetic BD susceptibility was no more than 19% (80).

The exact pathogenic mechanism of the B*51 molecule is still unknown, but it might be primarily involved in the disease development through specific antigen presentation or molecular mimicry with microbial antigens, as well as participate in linkage disequilibrium with a presently unknown susceptibility gene (6, 16). Notably, HLA-B*5101 heavy chain transgenic mice do not develop BD-related manifestations, but show an increased neutrophil activity in response to stimulation, compared to non-transgenic mice (8). Similar neutrophil hyperactivity has been reported in B*51-positive healthy subjects (8).

The HLA-B exonic sequence that encodes the B*51 allele has been proposed to be the real pathogenic factor in BD (93). Among the twenty-four till now described B*51 molecular subtypes (B*5101-B*5124), B*5101 and B*5108 were significantly associated with BD in Spanish (94), Italian (95), Greek (96), German (97), Turkish (97), Iranian (98) and Saudi Arabian (99) pa-

tients, whereas Japanese patients were found to express the B*5101, but not B*5108 suballele (100). In a Greek report, the B*5101 subtype was expressed in 80% of patients and in 26% of controls, resulting in positive correlation with a younger disease onset and the development of erythema nodosum (101). In Israeli patients, HLA-B*51 and B*52 were both primarily associated with BD (102), being B*5101 and B*5201 the prevalent subtypes, while B*5108 and B*5104 were less expressed (103). In Chinese Han patients in Shanghai area, B*5101 predominated, and a novel association between BD and HLA-B*46 was found (104). Interestingly, in Turkish, Jordanian, Iranian and Japanese BD patients the entire nucleotide sequences of HLA-B*510101 genes, including the promoter and intron regions, are completely identical, suggesting that B*510101 may have the same phylogenetic origin (105).

Recently, a selective increase of B*2702 in Turkish BD patients was reported (106). Of note, the two main disease-related suballeles B*5101 and B*5108 share amino-acid residues at positions 63,67 and 77-83 (defining the Bw4 epitope), crucial for the antigen binding and natural killer (NK) cell interactions (7, 97). Moreover, B*2702 is the only HLA-B*27 subtype sharing with B*5101 and B*5108 the same killer immunoglobulin-like receptor (KIR) binding sequence (106). These findings suggest the role of an interaction between HLA molecules and KIRs on NK or T cells in the disease pathogenesis (106). Moreover, in Moroccan BD patients a previously unknown association with HLA-B*15 (107), as well as in UK white Caucasoid patients with HLA-B*5701 (108), has been reported. Therefore, the Bw4 motif expressed by HLA-B alleles variably associated with BD, such as B*51, B*27, B*15 and B*57, might be itself causally related to the disease (6).

Recently, allelic variants of the HLA-G gene have been associated with BD (109). Indeed, in Korean patients the frequency of the HLA-G haplotype containing 3741*+14 base pair (bp) and 1597*delC was increased more than 2-fold (109).

In Japanese patients with refractory ocular attacks, HLA-B*51 and DQw3 alleles, as well as the human complement factor 4 (C4)AQ0 allotype were significantly over-expressed (110); similarly, northern Han Chinese patients showed an enhanced frequency of B*51 and C4AQ0 (111). In Italian patients, a significant association between BD and the B51-DR5-DQw3 haplotype was found (112). Moreover, in Turkish patients the B5-DR5 alleles were in a strong positive linkage disequilibrium (113). In Chinese patients, the DR5-DQw1 and DRw8-DQw1 haplotypes were over-expressed (114). In Spanish patients from Andalucia, the DR11 and DQB1*0301 frequency was increased, and the DQB1*0303 allele was associated with severe uveitis, while the DQ5 expression was reduced particularly in HLA-B*51-positive subjects (89). Iraqi patients, both in sporadic and familial cases, showed a significantly high frequency of B51, DR2 and DQ3 alleles, and the A9,B51(5), Cw1,DR2,DQ3 haplotype was positively associated with BD, whereas the A2,A28,B35,DR12,DQ4 haplotype was negatively related (82). Mexican patients exhibited a markedly enhanced expression of HLA-B*44, B*52, B*56 alleles, as well as of DRB1*01 and DRB1*13 (115).

Moreover, in Turkish patients with erythema nodosum the HLA-Cw2 frequency was reduced, and patients with genital ulcerations under-expressed the Cw7 allele (88). In Japanese patients, the HLA-Cw*14 and Cw*15 carriage rate was significantly increased, and correlated with B*51 as a result of linkage disequilibrium (116). In patients from southern Spain, a significant association of HLA-Cw*1602 with BD was found, and the B*51-Cw*1602 haplotype was suggested as a new disease susceptibility marker in this population (117).

Tumour necrosis factor gene polymorphisms

The tumour necrosis factor (TNF)-α pathway is likely involved in BD pathophysiology (6,11), and studies have been performed to evaluate the role of polymorphisms in TNF-α, TNF-β, or

TNF receptor superfamily 1A (TNF-RSF1A/TNFRI) and 1B (TNFRSF1B/TNFRII) genes (108, 118-121).

In UK white Caucasoid patients, the TNF-α gene (*TNF*) promoter polymorphism -1031C was reported to be strongly associated with BD (108). Moreover, the *TNF*-1031C allele was strictly related to the disease even in B*51 or B*5701-negative patients. The contribution of *TNF*-1031C in BD was estimated at 35%. Two extended HLA haplotypes, both containing the *TNF*-1031C allele along with B*51 or B*5701, positively correlated with BD. A protective effect of the most common haplotype, *TNFH1*, which does not contain the *TNF*-1031C allele, was also identified. Furthermore, the *TNF*-238A variant was associated with the disease (108). These observations have been suggested to provide a crucial link between genetic risk and clinical observations (108).

In Turkish patients compared to controls, no significant difference was detected in the distribution of *TNF* promoter −308 and −376 polymorphisms, but a decreased frequency of the *TNF*-308A -378G haplotype was observed in patients with severe eye involvement (118). In patients of Middle Eastern descent, no association between the *TNF*-308 polymorphism and BD was observed, whereas the TNFB*2 allele of the lymphotoxin α gene was associated with HLA-B*51 among patients, and positively correlated with a poor visual outcome (119). In Korean BD patients, no differences were found in the distribution of the *TNF*-308 G/A, TNF-β gene +252 G/A or TNFRSF1B/TNFRII gene 196 R/M polymorphisms (120). In European BD patients, a high frequency of R92Q mutation in TNF-RSF1A/TNFRI gene was observed, and associated with an increased risk of extracranial venous thrombosis (121).

Major histocompatibility complex class I chain related gene A polymorphisms
A strict association between BD and the major histocompatibility complex class I chain-related (MIC) A gene (*MICA*)*A6 allele was demonstrated in Japanese (122), Korean (123, 124), Ir-

anian (125), Greek (126), Middle Eastern (127), Israeli Arab (128), but not in Spanish (129), Tunisian (130) and non-Ashkenazi Jewish (128) patients. In an Italian paediatric patient series, *A6 homozygous carriers were found to develop a more severe mucosal gut involvement (131). Moreover, in Korean (124), Middle Eastern (127) and Japanese patients (132), the MICA*009 allele was closely related to the disease. However, further stratification and linkage analyses have evidenced that, when exists, the association between BD and MICA alleles is due to a strong linkage disequilibrium with B*51 (127, 132, 133). Similar results were obtained in a comparative analysis of Japanese, Greek and Italian patients (32).

Interestingly, in HLA-B*51-positive Japanese patients with active BD, a specific cytotoxic T lymphocyte (CTL) response was detected to an amino-acid 294-302 sequence containing the HLA-B*51 binding motif in the MICA*009 protein (134) and including part of a triplet-repeat microsatellite polymorphism consisting of a 6 alanine residues, which was reported to be associated with BD (122). This finding suggests the potential involvement of B*51-restricted, MICA-autoreactive CTLs in the pathogenesis of BD (134).

Transporter associated with antigen processing gene polymorphisms
In Spanish BD patients compared to healthy controls, the complete absence of the transporter associated with antigen processing (TAP)1C alleles, as well as a linkage disequilibrium between TAP2B and HLA-DQB1*0501 were found (135).

In a Japanese report, the TAP2B/C genotype was absent in the patient group, while the allele frequency of TAP2C was increased in patients with erythema nodosum, and the TAP2C/H genotype was over-expressed in patients with skin involvement, suggesting that in Japanese BD patients TAP polymorphisms may play a role in the skin lesion development (136).

Intercellular adhesion molecule-1 gene polymorphisms
In Italian Caucasian patients, the distri-

bution of the E/K (glutamic acid for lysine) 469 genotype of the intercellular adhesion molecule-1 (ICAM-1) gene was similar in patients and controls, whereas the frequency of R/G (arginine for glycine) 241 polymorphism was different, being the R241 allele significantly over-expressed in the patient group (137). In addition, a trend toward a higher frequency of R241 in patients with joint involvement was found (137). Conversely, in Palestinian and Jordanian patients, a positive correlation between BD and E469 allele, but no significant differences in the R241 frequency were observed (138). These seemingly conflicting results may depend on the heterogeneous genetic background of the studied populations (137, 138). In Korean BD patients, the frequency of both ICAM-1 469 K/E and E/E genotypes was significantly higher in patients than in controls, and the E469 allele was over-expressed in patients with vasculitis, arthritis, genital ulcers, skin and ocular lesions (139).

Interleukin gene polymorphisms
In Turkish patients, a study on interleukin (IL)-1A, IL-1B, and IL-1 receptor antagonist gene (*IL1RN*) polymorphisms demonstrated an increase of the IL-1A -889C allele and CC genotype, as well as of the IL-1B +5887T allele and TT genotype (140). Moreover, the IL-1A -889C/IL-1B +5887T haplotype was associated with increased susceptibility, and the IL-1A -889 and IL-1B +5887 CC/TT combined genotype was over-expressed in patients, conferring a 2-fold increased risk of BD. No association was observed for other investigated polymorphisms in IL-1B gene and *IL1RN* (140). In another Turkish study no difference was found in the genotype or allele frequencies of IL-1A -889, IL-1B -511, and *IL1RN* between patients and controls, but the susceptibility to BD was increased in patients carrying the IL-1B +3953 T allele and TT genotype (141).

In Turkish patients, BD was associated with the TT genotype of *IL1RN* at mspal 11100, whereas the TC genotype was protective (142). In the same cohort, the GG genotype at −330 and

+166 of the IL-2 gene was negatively related to the disease, while the TG genotype significantly confirmed a susceptibility (142).

In Korean patients, the genotype and allele frequencies of polymorphisms in the 3' flanking region of the IL-6 gene (IL6*vntr*) significantly differed between patients and controls (143). These differences were particularly evident in both HLA-B51 negative and female patients. Susceptibility to BD was increased in subjects carrying the IL6*vntr**C allele, and the IL6*vntr**C/ IL6 gene promoter*G haplotype (143). In Italian patients, the study of single-nucleotide polymorphism at position –607 in the IL-18 gene promoter evidenced that the AA genotype associated with BD, and AA homozygous patients had a significantly higher frequency of central nervous system involvement than patients carrying a C allele (144).

Chemokine and chemokine receptor gene polymorphisms
In UK patients, the CC chemokine ligand (CCL)5 -403 AA genotype was only found in males (145). Similarly, the CCL2/monocyte chemoattractant protein (MCP)-1 genotypes 1/2 were predominant in males, whereas the genotype 4 was significantly associated with BD in females, suggesting that gender-specific polymorphisms affecting chemokine gene functions might influence the disease expression (145). In different ethnic groups, the CC chemokine receptor 5 (CCR5) Delta32 variant, a deletion mutation in the CCR5 gene, did not correlate with BD (146). In Korean BD patients, the CCL2/ MCP-1 promoter –2518 polymorphism was investigated (147). Serum CCL2/ MCP-1 levels were higher and symptoms of severe disease more frequent in G-allele carriers than in AA homozygotes. In addition, mononuclear cells of patients carrying the G-allele, when stimulated, showed a steeper increase in CCL2/MCP-1 production than those from AA homozygotes. However, the G-allele distribution in the patient group did not differ from controls, likely due to its dominance in general Korean population (147).

Mannose binding lectin gene polymorphisms
A study of mannose binding lectin (MBL) gene polymorphisms evidenced that Korean patients carried the –550*G/G homozygosity in the MBL2 gene promoter more frequently than controls (148). Moreover, the MBL2 HYPA haplotype, which is responsible for high MBL serum levels, positively correlated with both increased risk for the disease and worse prognosis, whereas the LYPA haplotype, which is associated with low MBL values, was negatively related to BD (148).
Japanese patients were found to express a significantly higher frequency of the A/B (wild/mutant) genotype in the codon 54 of MBL exon 1, irrespectively of HLA-B*51 status (149).

N-acetyltransferase gene polymorphism
Nonacetylated xenobiotics may induce autoimmunity (150), and genetic polymorphisms in xenobiotic-metabolizing enzymes, such as the arylamine N-acetyltranferase 2 (NAT2), have been investigated in BD (150, 151). In a Turkish report, NAT2*5A and NAT2*6A genotypes carried an increased risk of disease, suggesting that the NAT2 slow acetylator status may be determinant in the BD susceptibility (150). In another Turkish research, even if the frequency of the NAT2*5B allele (responsible for slow acetylation) was slightly higher in patients than controls, no association between NAT2 acetylator status and BD was found (151).

Endothelial nitric oxide synthase gene polymorphisms
In Italian BD patients, the Glu-Asp298 polymorphism in exon 7, as well as the 4 a/b polymorphism in intron 4 of the endothelial nitric oxide synthase (eNOS) gene were studied (152). The Asp298 allele frequency positively correlated with the disease, independently of the HLA-B*51 status, whereas the distribution of the 4 a/b genotype in intron 4 did not differ between patients and controls (152). Moreover, in Korean patients suffering from BD or other rheumatic affections with vasculitis, the Asp298 allele was over-represented in

comparison with controls (153). In addition, in Turkish patients the Glu-Asp-298 polymorphism was found to be a risk factor for BD, owing to the frequencies of Asp/Asp homozygosity and Glu/Asp heterozygosity were both significantly higher than in healthy controls (154).

Other genetic polymorphisms
Glutathione S-transferases (GSTs) are a multi-gene family of enzymes involved in detoxification of xenobiotics and in protection against oxidative stress (155). In Turkish BD patients compared with controls, a higher prevalence of the GSTM1 null genotype was observed, whereas GSTT1 (null) and GSTP1 (val/val) gene polymorphisms were not related to an enhanced risk of developing BD (155).
In Taiwanese BD patients, the manganese superoxide dismutase gene C1183T polymorphism did not differ between patients and controls (156). In the same study, the gene polymorphisms of cytochrome p450 1A1 (CYP1A1), a member of a superfamily of enzymes that catalyze oxidation of various xenobiotics, was evaluated, showing that the frequencies of CYP-1A1 4889G and 4887A alleles and CYP1A1 4889A/G and 4887C/A genotypes were significantly higher in patients. In addition, the simultaneous expression of CYP1A1 4889G and 4887A alleles was associated with a marked risk of disease development (156).
HSPs are a group of scavenger proteins expressed under denaturating stress conditions or microbial challenge, and likely implicated in autoimmune diseases, including BD (6, 11). In a Turkish research, the GAT/GAA heterozygote single nucleotide polymorphism in the region 136-150-II of the HSP 60 gene was identified in patients with vascular involvement (157).
Other polymorphisms in genes encoding for host effector molecules and vascular modulating factors have been evaluated in BD. In a Turkish patient cohort, the Fcγ fragment receptor (FcγR) gene polymorphisms appeared to correlate with the type of organ/system involvement, owing to the signifi-

cant relationships between FcγRIIIa allele and arthritis, as well as between FcγRIIIb and vascular disease (158).

In a Turkish study, the C/T single nucleotide polymorphism at position 450 in the C5a receptor gene was found to be equally frequent in patients and controls, but was negatively associated with uveitis in the BD group (159).

In Turkish patients compared to controls, the evaluation of the 3DL1/3DS1 polymorphism of the KIR gene demonstrated that the 3DS1 frequency was selectively decreased, especially in B*51-negative patients, suggesting its protective effect (160). In Chinese Han patients of Shanghai area, a negative correlation between BD and both KIR 3DL1 and 2DS genes was observed (161).

In Turkish reports, the distribution of the CTL antigen 4 (CTLA-4) A49G single nucleotide polymorphism did not significantly differ between patients and controls (162, 163). However, the CTLA-4 49A allele and the AA genotype frequencies were selectively increased in patients with ocular involvement and erythema nodosum-like lesions (162), whereas the A/G genotype positively correlated with arthritis (163).

In Italian BD patients, the +936C/T polymorphism in the 3' untranslated region (UTR) and the –634C/G polymorphism in the 5'-UTR of the vascular endothelial growth factor (VEGF) gene, as well as the 18 bp insertion/deletion (I/D) polymorphism at –2549 of the VEGF promoter region were investigated (164). The carriage rates of the –634C and I alleles were significantly higher in patients than in controls. While the distribution of the +936T allele was similar in the two groups, its frequency was significantly enhanced among patients with posterior uveitis/retinal vasculitis. In addition, VEGF levels released by lipopolysaccharide-stimulated peripheral blood mononuclear cells were significantly higher in II homozygous than in DD homozygous individuals (164).

Procoagulant polymorphisms and mutations

The contributing role of procoagulant polymorphisms and mutations has been evaluated in BD. In Turkish patients, the methylenetetrahydrofolate reductase (MTHFR) gene C677T mutation (producing a thermolabile and less active variant of the enzyme) did not increase the risk of deep venous thrombosis (DVT) (30, 165). However, plasma homocysteine levels were found significantly higher in patients with DVT history than in those without (165). In an Israeli series of patients with and without thrombosis, no differences either in the prevalence of MTHFR C677T homozygosity or in plasma concentrations of homocysteine were observed (166).

In a Turkish case control study, 37.5% of BD patients with DVT carried a single point mutation (replacement of arginine[506] with glutamine) at nucleotide 1691 in the coagulation factor V (FV) gene, known as the FV Leiden mutation (167). The contributing role of the FV Leiden mutation in BD venous thrombosis was confirmed in some studies (168-170), but not in others (30, 166, 171-174). Moreover, the Leiden mutation seemed to be a risk factor for ocular disease and, in particular, for retinal vascular occlusion among Middle Eastern (175) and Turkish patients (176), but not in UK white patients (177). In Turkish BD patients, the FV 4070G gene mutation did not appear to have a role in the pathogenesis of thrombosis (173).

In Turkish patients with DVT history compared to controls, the 31% of the thrombotic and 3% of the nonthrombotic subjects carried the substitution of guanine by adenine at position 20210 (G→A20210) in the 3'-UTR of the prothrombin (PT) gene (phenotypically expressing with an increase of PT plasma levels and hypercoagulability) (178). Moreover, two Italian BD patients with thrombotic events were reported to be heterozygous for the PT G→A20210 mutation (179). A young Spanish BD patient carrying the PT G→A20210 mutation presented right intracardiac thrombosis (180). However, other studies in different populations did not confirm the role of this PT mutation as a risk factor of thrombosis in BD (30, 166, 170, 172, 173). In an

Italian patient cohort, no association between PT G→A20210 and DVT was found, but the frequency of such a mutation was significantly higher in patients with posterior uveitis/retinal vasculitis (174).

In a Turkish patient cohort, the risk of thrombosis was significantly higher in subjects carrying the platelet glycoprotein Ia 807CT and 807TT genotypes than in those expressing the 807CC genotype (181).

Notably, in European BD patients the TNFRSF1A R92Q mutation was associated with an increased risk of venous thrombosis, especially in extracranial veins, suggesting the complexity of the thrombosis pathogenesis in BD (121).

Conclusions

A complex interaction of multiple factors, such as ethnic background, immunogenetic mechanisms and environmental influences, is likely involved in the BD etiopathogenesis. The strong role of a genetic predisposition is highlighted by the disease peculiar demographic distribution, the close association with HLA-B*51 (especially the B*5101 and B*5108 subtypes) in different ethnic groups, and the familial clustering. Presently, HLA-B*51 is the main BD genetic marker in many populations, likely contributing in the disease development through specific antigen presentation or molecular mimicry with microbial antigens, or participate in linkage disequilibrium with a presently unknown susceptibility gene. In B*51-negative BD patients, the influence of other(s) till now undefined genetic factor(s) is probably involved. HLA-B alleles other than B*51, such as B*27, B*15 and B*57, has been variably associated with BD, and their shared Bw4 motif might be the causally related factor to the disease.

The familial aggregation of BD is characterized by both genetic anticipation and higher prevalence in childhood patients, very likely defining a subset with stronger immunogenetic influences. Notably, in familial cases the HLA-B*51 positivity rate is much higher than in sporadic cases. The reported BD concordance in two pairs of monozygotic twins, all bearing B*51, further

supports the relevant role of such a genetic association. On the other hand, analyses of a small group of multicase families using the transmission disequilibrium test, even confirming the genetic link between BD and HLA-B locus, estimated that the B*51 contribution to overall genetic predisposition is no greater than 20%.

Polymorphisms in genes encoding for host effector molecules, such as TNF, TAP proteins, ICAM-1, ILs, CCL2/MCP-1, eNOS, VEGF and MBL, may contribute to the disease susceptibility and/or severity. Moreover, gender-specific polymorphisms affecting chemokine gene functions might influence the disease phenotypic expression.

The role of prothrombotic mutations and polymorphisms in the pathogenesis of BD thrombosis is presently controversial.

Ultimately, BD is likely a polygenic disease and further investigations are needed to clearly define the contributing role of immunogenetic predisposing factors and environmental triggers in its pathogenesis. At this regard, familial cases may constitute an amazing model of study.

References

1. SAKANE T, TAKENO M, SUZUKI N, INABA G: Behçet's disease. *N Engl J Med* 1999; 341: 1284-91.
2. GURLER A, BOYVAT A, TURSEN U: Clinical manifestations of Behçet's disease: an analysis of 2147 patients. *Yonsei Med J* 1997; 38: 423-7.
3. LEIBA M, SIDI Y, GUR H, LEIBA A, EHRENFELD M: Behçet's disease and thrombophilia. *Ann Rheum Dis* 2001; 60: 1081-5.
4. KURAL-SEYAHI EK, FRESKO I, SEYAHI N *et al.*: The long-term mortality and morbidity of Behçet's syndrome: a 2-decade outcome survey of 387 patients followed at a dedicated centre. *Medicine (Baltimore)* 2003; 82: 60-76.
5. INTERNATIONAL STUDY GROUP FOR BEHÇET'S DISEASE: Criteria for diagnosis of Behçet's disease. *Lancet* 1990; 335: 1078-80.
6. VERITY DH, WALLACE GR, VAUGHAN RW, STANFORD MR: Behçet's disease: from Hippocrates to the third millennium. *Br J Ophthalmol* 2003; 87: 1175-83.
7. GUL A: Behçet's disease: an update on the pathogenesis. *Clin Exp Rheumatol* 2001; 19 (Suppl. 24): S6-12.
8. TAKENO M, KARIYONE A, YAMASHITA N *et al.*: Excessive function of peripheral blood neutrophils from patients with Behçet's disease and from HLA-B51 transgenic mice. *Arthritis Rheum* 1995; 38: 426-33.
9. EKSIOGLU-DEMIRALP E, DIRESKENELI H, KIBAROGLU A, YAVUZ S, ERGUN T, AKOGLU T: Neutrophil activation in Behçet's disease. *Clin Exp Rheumatol* 2001; 19 (Suppl. 24): S19-24.
10. FRASSANITO MA, DAMMACCO R, CAFFORIO P, DAMMACCO F: Th1 polarization of the immune response in Behçet's disease. A putative pathogenetic role of interleukin-12. *Arthritis Rheum* 1999; 42: 1967-74.
11. DIRESKENELI H: Behçet's disease: infectious aetiology, new autoantigens, and HLA-B51. *Ann Rheum Dis* 2001; 60: 996-1002.
12. AHMED MB, HOUMAN H, MILED M, DELLAGI K, LOUZIR H: Involvement of chemokines and Th1 cytokines in the pathogenesis of mucocutaneous lesions of Behçet's disease. *Arthritis Rheum* 2004; 50: 2291-5.
13. MOK CC, CHEUNG TC, HO CTK, LEE KW, LAU CS, WONG RWS: Behçet's disease in southern Chinese patients. *J Rheumatol* 2002; 29: 1689-93.
14. AMBRESIN A, TRAN T, SPERTINI F, HERBORT C: Behçet's disease in Western Switzerland: epidemiology and analysis of ocular involvement. *Ocul Immunol Inflamm* 2002; 10: 53-63.
15. POON W, VERITY DH, LARKIN GL, GRAHAM EM, STANFORD MR: Behçet's disease in patients of west African and Afro-Caribbean origin. *Br J Ophthalmol* 2003; 87: 876-8.
16. MIZUKI N, INOKO H, OHNO S: Molecular genetics (HLA) of Behçet's disease. *Yonsei Med J* 1997; 38: 333-49.
17. COMAS D, CALAFELL F, MATEU E *et al.*: Trading genes along the silk road: mtDNA sequences and the origin of central Asian populations. *Am J Hum Genet* 1998; 63: 1824-38.
18. ZOUBOULIS CC, KOTTER I, DJAWARI D *et al.*: Epidemiological features of Adamantiades-Behçet's disease in Germany and in Europe. *Yonsei Med J* 1997; 38: 411-22.
19. ZOUBOULIS CC: Epidemiology of Adamantiades-Behçet's disease. *Ann Med Interne (Paris)* 1999; 150: 488-98.
20. O'NEILL T, RIGBY AS, MCHUGH S, SILMAN AJ, BARNES C: Regional differences in clinical manifestations of Behçet's disease. *In* WECHSLER B and GODEAU P (Eds.): *Behçet's Disease: Proceedings of the 6th International Conference on Behçet's Disease.* Amsterdam, Excerpta Medica 1993: 159-63.
21. DEMIROGLU H, BARISTA I, DUNDAR S: Risk factor assessment and prognosis of eye involvement in Behçet's disease in Turkey. *Ophthalmology* 1997; 104: 701-5.
22. KONE-PAUT I, YURDAKUL S, BAHABRI SA *et al.*: Clinical features of Behçet's disease in children: an international collaborative study of 86 cases. *J Pediatr* 1998; 132: 721-5.
23. GONZALEZ-GAY MA, GARCIA-PORRUA C, BRANAS F, LOPEZ-LAZARO L, OLIVIERI I: Epidemiologic and clinical aspects of Behçet's disease in a defined area of Northwestern Spain, 1988-1997. *J Rheumatol* 2000; 27: 703-7.
24. MUHAYA M, LIGTHMAN S, IKEDA E *et al.*: Behcet's disease in Japan and Great Britain: a comparative study. *Ocul Immunol Inflamm* 2000; 8: 141-8.
25. KRAUSE I, MADER R, SULKES J *et al.*: Behçet's disease in Israel: the influence of ethnic origin on disease expression and severity. *J Rheumatol* 2001; 28: 1033-6.
26. KOTTER I, VONTHEIN R, MULLER CA, GUNAYDIN I, ZIERHUT M, STUBIGER N: Behçet's disease in patients of German and Turkish origin living in Germany: a comparative analysis. *J Rheumatol* 2004; 31: 133-9.
27. YAZICI H, TUZUN Y, PAZARLI H *et al.*: Influence of age of onset and patient's sex on the prevalence and severity of manifestations of Behçet's syndrome. *Ann Rheum Dis* 1984; 43: 783-9.
28. TURSEN U, GURLER A, BOYVAT A: Evaluation of clinical findings according to sex in 2313 Turkish patients with Behçet's disease. *Int J Dermatol* 2003; 42: 346-51.
29. TUGAL-TUTKUN I, ONAL S, ALTAN-YAYCIOGLU R, HUSEYIN ALTUNBAS H, URGANCIOGLU M: Uveitis in Behçet's disease: an analysis of 880 patients. *Am J Ophthalmol* 2004; 138: 373-80.
30. TOYDEMIR PB, ELHAN AH, TUKUN A *et al.*: Effects of factor V gene G1691A, methylenetetrahydrofolate reductase gene C677T, and prothrombin gene G20210A mutations on deep venous thrombogenesis in Behçet's disease. *J Rheumatol* 2000; 27: 2849-54.
31. AMES PRJ, STEUER A, PAP A, DENMAN AM: Thrombosis in Behçet's disease: a retrospective survey from a single UK centre. *Rheumatology (Oxford)* 2001; 40: 652-5.
32. MIZUKI N, OTA M, YABUKI K *et al.*: Localization of the pathogenic gene of Behçet's disease by microsatellite analysis of three different populations. *Invest Ophthalmol Vis Sci* 2000; 41: 3702-8.
33. MIZUKI N, YABUKI K, OTA M *et al.*: Microsatellite mapping of a susceptible locus within the HLA region for Behçet's disease using Jordanian patients. *Hum Immunol* 2001; 62: 186-90.
34. YAZICI H, TUZUN Y, PAZARLI H, YALCIN B, YURDAKUL S, MUFTUOGLU A: The combined use of HLA-B5 and the pathergy test as diagnostic marker of Behçet's disease in Turkey. *J Rheumatol* 1980; 7: 206-10.
35. NISHIYAMA M, NAKAE K, KURIYAMA T, HASHIMOTO M, HSU ZN: A study among related pairs of Japanese patients with familial Behçet's disease: group comparison by interval of disease onsets. *J Rheumatol* 2002; 29: 743-7.
36. HAMURYUDAN V, YURDAKUL S, OZBAKIR F, YAZICI H, HEKIM H: Monozygotic twins concordant for Behçet's syndrome. *Arthritis Rheum* 1991; 34: 1071-2.
37. YAZGANOGLU KD, POLAT EA, AKDAG KOSE A, DILER S, AZIZLERLI G: HLA-B51 positive monozygotic twins concordant for Behçet's disease. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S90.
38. BERMAN L, TRAPPLER B, JENKINS T: Behçet's syndrome: a family study and the elucidation of a genetic role. *Ann Rheum Dis* 1979; 38: 118-21.
39. BIRD STEWART JA: Genetic analysis of families of patients with Behçet's syndrome: data incompatible with autosomal recessive inheritance. *Ann Rheum Dis* 1986; 45: 265-8.

40. MOLINARI N, KONE-PAUT I, MANNA R, DEMAILLE J, DAURES JP, TOUITOU I: Identification of an autosomal recessive mode of inheritance in paediatric Behçet's families by segregation analysis. *Am J Med Genet* 2003; 122A: 115-8.

41. FRESKO I, SOY M, HAMURYUDAN *et al.*: Genetic anticipation in Behçet's syndrome. *Ann Rheum Dis* 1998; 57: 45-8.

42. SONMEZ S, KAYA M, AKTAS A, IKBAL M, SENEL K: High frequency of sister chromatid exchanges in lymphocytes of the patients with Behçet's disease. *Mutat Res* 1998; 397: 235-8.

43. SEZER NF: Further investigation of the virus of Behçet's disease. *Am J Ophthalmol* 1956; 41: 41-55.

44. FORBES IJ, ROBSON HN: Familial recurrent orogenital ulceration. *Br Med J* 1960; 5173: 599-601.

45. FOWLER TJ, HUMPSTON DJ, NUSSEY AM, SMALL M: Behçet's syndrome with neurological manifestations in two sisters. *Br Med J* 1968; 2: 473-4.

46. MASON RM, BARNES CG: Behçet's syndrome with arthritis. *Ann Rheum Dis* 1969; 28: 95-103.

47. KORCZYN AD, BORNSTEIN B: Familial Behçet disease. *Harefuah* 1973; 84: 130-2.

48. CHAJEK T, FAINARU M: Behçet's disease. Report of 41 cases and a review of the literature. *Medicine (Baltimore)* 1975; 54: 179-96.

49. GOOLAMALI SK, COMAISH JS, HASSAN-YEK F, STEPHENS A: Familial Behçet's syndrome. *Br J Dermatol* 1976; 95: 637-42.

50. ABDEL-AZIZ AH, FAIRBURN EA: Familial Behçet's syndrome. *Cutis* 1978; 21: 649-52.

51. NAHIR M, SCHARF Y, GIDONI O, BRAZILAI A, FRIEDMAN-BIRNBAUM R, HAIM S: HL-A antigens in Behçet's disease. A family study. *Dermatologica* 1978; 156: 205-8.

52. AOKI K, OHNO S, OHGUSHI M, SUGIURA S: Familial Behçet's disease. *Jpn J Ophthalmol* 1978; 22: 72-5.

53. ARONSSON A, TEGNER E: Behçet's syndrome in two brothers. *Acta Derm Venereol* 1983; 63: 73-4.

54. GONZALEZ T, GANTES M, BUSTABAD S: HLA haplotypes in familial Behçet's disease. *J Rheumatol* 1984; 11: 405-6.

55. DUNDAR SV, GENCALP U, SIMSEK H: Familial cases of Behçet's disease. *Br J Dermatol* 1985; 113: 319-21.

56. YIM CW, WHITE RH: Behçet's syndrome in a family with inflammatory bowel disease. *Arch Intern Med* 1985; 145: 1047-50.

57. FALLINGBORG J, AMBROSIUS CHRISTENSEN L, GRUNNET N: HLA antigens in a family with Behçet's syndrome. *Acta Med Scand* 1986; 220: 375-8.

58. WILKINS MR, GOVE RI, ROBERTS SD, KENDALL MJ: Behçet's disease presenting as benign intracranial hypertension. *Postgrad Med J* 1986; 62: 39-41.

59. GRUFFERMAN S, BARTON JW 3RD, EBY NL: Increased sex concordance of sibling pairs with Behçet's disease, Hodgkin's disease, multiple sclerosis, and sarcoidosis. *Am J Epidemiol* 1987; 126: 365-9.

60. WOODROW JC, GRAHAM DR, EVANS CC: Behçet's syndrome in HLA-identical siblings. *Br J Rheumatol* 1990; 29: 225-7.

61. AKPOLAT T, KOC Y, YENIAY I *et al.*: Familial Behçet's disease. *Eur J Med* 1992; 1: 391-5.

62. KONE I, BERBIS P, PALIX C, BERNARD JL: Familial Behçet's disease in children. A report of 3 cases. *Clin Exp Rheumatol* 1992; 10: 627-8.

63. VILLANUEVA JL, GONZALEZ-DOMINGUEZ J, GONZALEZ-FERNANDEZ R, PRADA JL, PENA J, SOLANA R: HLA antigen familial study in complete Behçet's syndrome affecting three sisters. *Ann Rheum Dis* 1993; 52: 155-7.

64. CHONG VF, PATHMANATHAN R: Familial Behçet's syndrome with intestinal involvement-case reports and a review of the literature. *Ann Acad Med Singapore* 1993; 22: 807-10.

65. KONE-PAUT I, BERNARD JL: Behçet's disease in children in France. *Arch Fr Pediatr* 1993; 50: 561-5.

66. HAYASAKA S, KUROME H, NODA S: HLA antigens in a Japanese family with Behçet's disease. *Graefes Arch Clin Exp Ophthalmol* 1994; 232: 589-90.

67. MORI M, KURIYAMA T, MITSUDA T *et al.*: Familial Behçet's disease-a case report. *Ryumachi* 1994; 34: 988-92.

68. VAIOPOULOS G, SFIKAKIS PP, HATZINIKOLAOU P, STAMATELOS G, KAKLAMANIS P: Adamantiadis-Behçet's disease in sisters. *Clin Rheumatol* 1996; 15: 382-4.

69. NISHIURA K, KOTAKE S, ICHIISHI A, MATSUDA H: Familial occurrence of Behçet's disease. *Jpn J Ophthalmol* 1996; 40: 255-9.

70. ANDO H, MIZUKI N, OHNO S *et al.*: Identification of a novel HLA-B allele (B*4202) in a Saudi Arabian family with Behçet's disease. *Tissue Antigens* 1997; 49: 526-8.

71. BANG D, YOON KH, CHUNG HG, CHOI EH, LEE ES, LEE S: Epidemiological and clinical features of Behçet's disease in Korea. *Yonsei Med J* 1997; 38: 428-36.

72. MACGREGOR AJ, SEAMAN G, DENMAN E, DENMAN AM: Disease recurrence risk and the risk of lymphoproliferative disorders in the relatives of patients with Behçet's syndrome: results of a nationwide survey. *Br J Rheumatol* 1997; 36 (Suppl. 1): 34.

73. SANT SM, KILMARTIN D, ACHESON RA: HLA antigens in familial Behçet's disease in Ireland. *Br J Rheumatol* 1998; 37: 1250-57.

74. KONE-PAUT I, GEISLER I, WECHSLER B *et al.*: Familial aggregation in Behçet's disease: high frequency in siblings and parents of paediatric probands. *J Pediatr* 1999; 135: 89-93.

75. TREUDLER R, ORFANOS CE, ZOUBOULIS CC: Twenty-eight cases of juvenile-onset Adamantiades-Behçet's disease in Germany. *Dermatology* 1999; 199: 15-9.

76. GUL A, INANC M, OCAL L, ARAL O, KONICE M: Familial aggregation of Behçet's disease in Turkey. *Ann Rheum Dis* 2000; 59: 622-5.

77. ONAL S, TUGAL-TUTKUN I, URGANCIOGLU M, GUL A: Clinical course of ocular Behçet's disease in siblings. *Ocul Immunol Inflamm* 2001; 9: 111-24.

78. NISHIYAMA M, NAKAE K, UMEHARA T: A study of familial occurrence of Behçet's disease with and without ocular lesions. *Jpn J*

79. *Ophthalmol* 2001; 45: 313-6.
GUL A, UYAR FA, INANC M *et al.*: Lack of association of HLA-B*51 with a severe disease course in Behçet's disease. *Rheumatology (Oxford)* 2001; 40: 668-72.

80. GUL A, HAJEER AH, WORTHINGTON J, BARRETT JH, OLLIER WER, SILMAN AJ: Evidence for linkage of the HLA-B locus in Behçet's disease, obtained using the transmission disequilibrium test. *Arthritis Rheum* 2001; 44: 239-40.

81. LAGHMARI M, KARIM A, ALLALI F *et al.*: Childhood Behçet's disease: clinical and evolutive aspects. About 13 cases. *J Fr Ophthalmol* 2002; 25: 904-8.

82. AL-JOOFY IK, MUSLIH RK, MOUSAWY KM, AL-WAIZ MM: HLA typing class I and II antigens in Iraqi patients with Behçet's disease (sporadic and familial cases). *Adv Exp Med Biol* 2003; 528: 217-20.

83. ZOUBOULIS CC, VAIOPOULOS G, MARCOMICHELAKIS N *et al.*: Onset signs, clinical course, prognosis, treatment and outcome of adult patients with Adamantiades-Behçet's disease in Greece. *Clin Exp Rheumatol* 2003; 21 (Suppl. 30): S19-26.

84. ALLALI F, BENOMAR A, KARIM A *et al.*: Behçet's disease in Moroccan children: a report of 12 cases. *Scand J Rheumatol* 2004; 33: 362-3.

85. GUL A, INANC M, OCAL L, ARAL O, CARIN M, KONICE M: HLA-B51 negative monozygotic twins discordant for Behçet's disease. *Br J Rheumatol* 1997; 36: 922-7.

86. KILMARTIN DJ, FINCH A, ACHESON RW: Primary association of HLA-B51 with Behçet's disease in Ireland. *Br J Ophthalmol* 1997; 81: 649-53.

87. KRAUSE I, LEIBOVICI L, GUEDJ D, MOLAD Y, UZIEL Y, WEINBERGER A: Disease patterns of patients with Behçet's disease demonstrated by factor analysis. *Clin Exp Rheumatol* 1999; 17: 347-50.

88. KAYA TI, DUR H, TURSEN U, GURLER A: Association of class I HLA antigens with the clinical manifestations of Turkish patients with Behçet's disease. *Clin Exp Dermatol* 2002; 27: 498-501.

89. CASTILLO PALMA MJ, SANCHEZ ROMAN J, OCANA MEDINA C, GONZALEZ ESCRIBANO MF, NUNEZ ROLDAN A, LOPEZ-CHECA F: Serologic and molecular HLA typing in patients from Andalucia with Behçet's disease. Genetic and clinical correlations. *Med Clin (Barc)* 1996; 106: 121-5.

90. CHOUKRI F, CHAKIB A, HIMMICH H, MARIH L, CAILLAT-ZUCMAN S: HLA-B phenotype modifies the course of Behçet's disease in Moroccan patients. *Tissue Antigens* 2003; 61: 92-6.

91. CHANG HK, KIM JU, CHEON KS, CHUNG HR, LEE KW, LEE IH: HLA-B51 and its allelic types in association with Behçet's disease and recurrent aphthous stomatitis in Korea. *Clin Exp Rheumatol* 2001; (Suppl. 24): S31-5.

92. ERMAKOVA NA, ALEKBEROVA ZS, PROKAEVA TB, POLJANSKAJA IB: Frequency of HLA in patients with Behçet's disease and association with occlusive retinal vasculitis. *Adv Exp Med Biol* 2003; 528: 231-3.

93. SANO K, YABUKI K, IMAGAWA Y *et al.*:

The absence of disease-specific polymorphisms within the HLA-B51 that is the susceptible locus for Behçet's disease. *Tissue Antigens* 2001; 58: 77-82.

94. GONZALEZ-ESCRIBANO MF, RODRIGUEZ MR, WALTER K, SANCHEZ-ROMAN J, GARCIA-LOZANO JR, NUNEZ-ROLDAN A: Association of HLA-B51 subtypes and Behçet's disease in Spain. *Tissue Antigens* 1998; 52: 78-80.

95. KERA J, MIZUKI N, OTA M *et al.*: Significant associations of HLA-B*5101 and B*5108, and lack of associations of class II alleles with Behçet's disease in Italian patients. *Tissue Antigens* 1999; 54: 565-71.

96. MIZUKI N, OTA M, KATSUYAMA Y *et al.*: Sequencing-based typing of HLA-B*51 alleles and the significant association of HLA-B*5101 and B*5108 with Behçet's disease in Greek patients. *Tissue Antigens* 2002; 59: 118-21.

97. KOTTER I, GUNAYDIN I, STUBIGER N *et al.*: Comparative analysis of the association of HLA-B*51 suballeles with Behçet's disease in patients of German and Turkish origin. *Tissue Antigens* 2001; 58: 166-70.

98. MIZUKI N, OTA M, KATSUYAMA Y *et al.*: HLA class I genotyping including HLA-B*51 allele typing in the Iranian patients with Behçet's disease. *Tissue Antigens* 2001; 57: 457-62.

99. YABUKI K, OHNO S, MIZUKI N *et al.*: HLA class I and II typing of the patients with Behçet's disease in Saudi Arabia. *Tissue Antigens* 1999; 54: 273-7.

100. MIZUKI N, OTA M, KATSUYAMA Y *et al.*: HLA-B*51 allele analysis by the PCR-SBT method and a strong association of HLA-B*5101 with Japanese patients with Behçet's disease. *Tissue Antigens* 2001; 58: 181-4.

101. KOUMANTAKI Y, STAVROPOULOS C, SPYROPOULOU M *et al.*: HLA-B*5101 in Greek patients with Behçet's disease. *Hum Immunol* 1998; 59: 250-5.

102. ARBER N, KLEIN T, MEINER Z, PRAS E, WEINBERGER A: Close association of HLA-B51 and B52 in Israeli patients with Behçet's syndrome. *Ann Rheum Dis* 1991; 50: 351-3.

103. PAUL M, KLEIN T, KRAUSE I, MOLAD Y, NARINSKY R, WEINBERGER A: Allelic distribution of HLA-B*5 in HLA-B5-positive Israeli patients with Behçet's disease. *Tissue Antigens* 2001; 58: 185-6.

104. XU L, YU L, FAN L: Study on association of HLA-B*51 alleles with Behçet's disease in Chinese Han population in Shanghai area. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S89.

105. TAKEMOTO Y, NARUSE T, ITO N *et al.*: Comparison of HLA-B51 haplotypes and microsatellite polymorphisms in Behçet's disease among multiple populations. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S89.

106. GUL A, UYAR FA, INANC M *et al.*: A weak association of HLA-B*2702 with Behçet's disease. *Genes Immun* 2002; 3: 368-72.

107. CHOUKRI F, CHAKIB A, HIMMICH H, HUE S, CAILLAT-ZUCMAN S: HLA-B*51 and B*15 alleles confer predisposition to Behçet's disease in Moroccan patients. *Hum Immunol* 2001; 62: 180-5.

108. AHMAD T, WALLACE GR, JAMES T *et al.*: Mapping the HLA association in Behçet's disease. A role for tumour necrosis factor polymorphisms ? *Arthritis Rheum* 2003; 48: 807-13.

109. CHOI JS, NAM JH, BANG D, LEE ES, LEE S, PARK KS: HLA-G 3741 14bp insertion in the 3'-UTR in Behçet's disease. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S91.

110. MATSUKI K, JUJI T, TOKUNAGA K *et al.*: HLA antigens in Behçet's disease with refractory ocular attacks. *Tissue Antigens* 1987; 29: 208-13.

111. MINESHITA S, TIAN D, WANG LM *et al.*: Histocompatibility antigens associated with Behçet's disease in northern Han Chinese. *Intern Med* 1992; 31: 1073-5.

112. BALBONI A, PIVETTI-PEZZI P, ORLANDO P *et al.*: Serological and molecular HLA typing in Italian Behçet's patients: significant association to B51-DR5-DQw3 haplotype. *Tissue Antigens* 1992; 39: 141-3.

113. ALPSOY E, YILMAZ E, COSKUN M, SAVAS A, YEGIN O: HLA antigens and linkage disequilibrium patterns in Turkish Behçet's patients. *J Dermatol* 1998; 25: 158-62.

114. SUN A, HSIEH RP, CHU CT, WANG JT, LIU BY, CHIANG CP: Some specific human leukocyte antigen (HLA)-DR/DQ haplotypes are more important than individual HLA-DR and -DQ phenotypes for the development of mucocutaneous type of Behçet's disease and for disease shift from recurrent aphthous stomatitis to mucocutaneous type of Behçet's disease. *J Oral Pathol Med* 2001; 30: 402-7.

115. SOTO-VEGA E, GARCIA-MUNOZ R, RICHAUD-PATIN Y *et al.*: Class I and class II MHC polymorphisms in Mexican patients with Behçet's disease. *Immunol Lett* 2004; 93: 211-5.

116. MIZUKI N, OHNO S, ANDO H *et al.*: HLA-C genotyping of patient with Behçet's disease in the Japanese population. *Hum Immunol* 1996; 15: 47-53.

117. SANZ L, GONZALEZ-ESCRIBANO F, DE PABLO R, NUNEZ-ROLDAN A, KREISLER M, VILCHES C: HLA-Cw*1602: a new susceptibility marker of Behçet's disease in southern Spain. *Tissue Antigens* 1998; 51: 111-4.

118. DUYMAZ-TOZKIR J, GUL A, UYAR FA, OZBEK U, SARUHAN-DIRESKENELI G: Tumour necrosis factor-alpha gene promoter region −308 and −376 G→A polymorphisms in Behçet's disease. *Clin Exp Rheumatol* 2003; 21 (Suppl. 30): S15-8.

119. 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.

120. LEE EB, KIM JY, LEE YJ, PARK MH, SONG YW: TNF and TNF receptor polymorphisms in Korean Behçet's disease patients. *Hum Immunol* 2003; 64: 614-20.

121. AMOURA Z, DODE C, HUE S *et al.*: Association of the R92Q TNFRSF1A mutation and extracranial deep vein thrombosis in patients with Behçet's disease. *Arthritis Rheum* 2005; 52: 608-11.

122. MIZUKI N, OTA M, KIMURA M *et al.*: Triplet repeat polymorphism in the transmembrane region of the MICA gene: a strong association of six GCT repetitions with Behçet's disease. *Proc Natl Acad Sci USA* 1997; 94: 1298-303.

123. PARK SH, PARK KS, SEO YI *et al.*: Association of MICA polymorphism with HLA-B51 and disease severity in Korean patients with Behçet's disease. *J Korean Med Sci* 2002; 17: 366-70.

124. MOK J, BANG D, LEE ES, LEE S, PARK KS: Strong association of MIC-A*009 of extracellular domains and MIC-A*A6 of transmembrane domain in Korean patients with Behçet's disease. *Adv Exp Med Biol* 2003; 528: 221-4.

125. MIZUKI N, YABUKI K, OTA M *et al.*: Analysis of microsatellite polymorphism around the HLA-B locus in Iranian patients with Behçet's disease. *Tissue Antigens* 2002; 60: 396-9.

126. YABUKI K, MIZUKI N, OTA M *et al.*: Association with MICA gene and HLA-B*5101 with Behçet's disease in Greece. *Invest Ophthalmol Vis Sci* 1999; 40: 1921-6.

127. WALLACE GR, VERITY DH, DELAMAINE LJ *et al.*: MIC-A allele profiles and HLA class I associations in Behçet's disease. *Immunogenetics* 1999; 49: 613-7.

128. COHEN R, METZGER S, NAHIR M, CHAJEK-SHAUL T: Association of the MIC-A gene and HLA-B51 with Behçet's disease in Arabs and non-Ashkenazi Jews in Israel. *Ann Rheum Dis* 2002; 61: 157-60.

129. GONZALEZ-ESCRIBANO MF, RODRIGUEZ MR, AGUILAR F, ALVAREZ A, SANCHEZ-ROMAN J, NUNEZ-ROLDAN A: Lack of association of MICA transmembrane region polymorphism and Behçet's disease in Spain. *Tissue Antigens* 1999; 54: 278-81.

130. BEN AHMED M, HOUMAN H, ABDELHAK S *et al.*: MICA transmembrane region polymorphism and HLA B51 in Tunisian Behçet's disease patients. *Adv Exp Med Biol* 2003; 528: 225-8.

131. PICCO P, PORFIRIO B, GATTORNO M *et al.*: MICA gene polymorphisms in an Italian paediatric series of juvenile Behçet disease. *Int J Mol Med* 2002; 10: 575-8.

132. MIZUKI N, OTA M, KATSUYAMA Y *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.

133. SALVARANI C, BOIARDI L, MANTOVANI V *et al.*: Association of MICA alleles and HLA-B51 in Italian patients with Behçet's disease. *J Rheumatol* 2001; 28: 1867-70.

134. YASUOKA H, OKAZAKI Y, KAWAKAMI Y *et al.*: Autoreactive CD8+ cytotoxic T lymphocytes to major histocompatibility complex class I chain-related gene A in patients with Behçet's disease. *Arthritis Rheum* 2004; 50: 3658-62.

135. GONZALEZ-ESCRIBANO MF, MORALES J, GARCIA-LOZANO JR *et al.*: TAP polymorphism in patients with Behçet's disease. *Ann Rheum Dis* 1995; 54: 386-8.

136. TAKIZAWA K, TAKEUCHI F, NABETA H *et al.*: Association of transporter associated with antigen processing genes with Behçet's disease in Japanese. *Autoimmunity* 2003; 36:

161-5.

137. BOIARDI L, SALVARANI C, CASALI B *et al.*: Intercellular adhesion molecule-1 gene polymorphisms in Behçet's disease. *J Rheumatol* 2001; 28: 1283-7.

138. VERITY DH, VAUGHAN RW, KONDEATIS E *et al.*: Intercellular adhesion molecule-1 gene polymorphisms in Behçet's disease. *Eur J Immunogenet* 2000; 27: 73-6.

139. KIM EH, MOK JW, BANG DS, LEE ES, LEE SN, PARK KS: Intercellular adhesion molecule-1 gene polymorphisms in Korean patients with Behçet's disease. *J Korean Med Sci* 2003; 18: 415-8.

140. KARASNEH J, HAJEER AH, BARRETT J, OLLIER WE, THORNHILL M, GUL A: Association of specific interleukin 1 gene cluster polymorphisms with increased susceptibility for Behçet's disease. *Rheumatology (Oxford)* 2003; 42: 860-4.

141. COSKUN M, BACANLI A, SALLAKCI N, ALPSOY E, YAVUZER U, YEGIN O: Specific interleukin-1 gene polymorphisms in Turkish patients with Behçet's disease. *Exp Dermatol* 2005; 14: 124-9.

142. BICAKCIGIL M, YILMAZ V, DIRESKENELI H, SARUHAN-DIRESKENELI G: Cytokine polymorphisms associated with Behçet's disease in Turkey. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S91.

143. CHANG HK, JANG WC, PARK SB *et al.*: Association between interleukin 6 gene polymorphisms and Behçet's disease in Korean people. *Ann Rheum Dis* 2005; 64: 339-40.

144. BOIARDI L, SALVARANI C, CASALI B *et al.*: Single-nucleotide polymorphism located at position –607 of the interleukin-18 gene promoter region in Behçet's disease. *Arthritis Rheum* 2004; 50 (Suppl. 9): S232.

145. CHEN Y, VAUGHAN RW, KONDEATIS E *et al.*: Chemokine gene polymorphisms associate with gender in patients with uveitis. *Tissue Antigens* 2004; 63: 41-5.

146. YANG X, AHMAD T, GOGUS F *et al.*: Analysis of the CC chemokine receptor 5 (CCR5) Delta32 polymorphism in Behçet's disease. *Eur J Immunogenet* 2004; 31: 11-4.

147. CHO ML, KIM JY, KO HJ *et al.*: The MCP-1 promoter –2518 polymorphism in Behçet's disease: correlation between allele types, MCP-1 production and clinical symptoms among Korean patients. *Autoimmunity* 2004; 37: 77-80.

148. PARK KS, MIN K, NAM JH, BANG D, LEE ES, LEE S: HYPA haplotype in the mannose binding lectin gene (MBL2) is associated with Behçet's disease. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S90.

149. WANG H, NAKAMURA K, INOUE T *et al.*: Mannose-binding lectin polymorphisms in patients with Behçet's disease. *J Dermatol Sci* 2004; 36: 115-7.

150. TAMER L, TURSEN U, ESKANDARI G *et al.*: N-acetyltransferase 2 polymorphisms in patients with Behçet's disease. *Clin Exp Dermatol* 2005; 30: 56-60.

151. AYNACIOGLU AS, BOZKURT A, NACAK M *et al.*: N-acetyltransferase polymorphism in patients with Behçet's disease. *Eur J Clin Pharmacol* 2001; 57: 659-62.

152. SALVARANI C, BOIARDI L, CASALI B *et al.*: Endothelial nitric oxide synthase gene polymorphisms in Behçet's disease. *J Rheumatol* 2002; 29: 535-40.

153. KIM JU, CHANG HK, LEE SS *et al.*: Endothelial nitric oxide synthase gene polymorphisms in Behçet's disease and rheumatic diseases with vasculitis. *Ann Rheum Dis* 2003; 62: 1083-7.

154. OKSEL F, OZMEN M, AKSU K, KESER G, BERDELI A: Endothelial nitric oxide synthase gene polymorphisms in Turkish patients with Behçet's disease. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S92.

155. TURSEN U, TAMER L, ESKANDARI G *et al.*: Glutathione S-tranferase polymorphisms in patients with Behçet's disease. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S90.

156. YEN JH, TSAI WC, LIN CH, OU TT, HU CJ, LIU HW: Cytochrome p450 1A1 and manganese superoxide dismutase gene polymorphisms in Behçet's disease. *J Rheumatol* 2004; 31: 736-40.

157. OZBALKAN Z, ERTENLI I, KIRAZ S *et al.*: Heat shock protein (HSP) 60/65 gene polymorphism in Behçet's disease. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S92.

158. AKSU K, BERDELI A, KESER G *et al.*: Fcγ–RIIa, IIIa and IIIb receptor gene polymorphism in Behçet's disease. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S92.

159. ERKEN E, GUNESACAR R, OZER HTE: C5a receptor polymorphism in Behçet's disease. Negative association with uveitis. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S91.

160. DUYMAZ-TOZKIR J, DUYAR FA, SARUHAN-DIRESKENELI G, GUL A: Killer immunoglobulin-like receptor gene 3DL1/3DS1 polymorphism in Behçet's disease. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S91.

161. XU L, FAN L, YU L: Association of killer immunoglobulin-like receptor (KIR) gene polymorphism with Behçet's disease. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S91.

162. SALLAKCI N, BACANLI A, COSKUN M, YAVUZER U, YEGIN O, ALPSOY E: CTLA-4 gene polymorphism in Turkish patients with Behçet's disease. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S92.

163. GUNESACAR R, ERKEN E, OZER HTE: Analysis of CTLA-4 and C28 gene polymorphisms in patients with Behçet's disease. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S92.

164. SALVARANI C, BOIARDI L, CASALI B *et al.*: Vascular endothelial growth factor gene polymorphisms in Behçet's disease. *J Rheumatol* 2004; 31: 1785-9.

165. CANATAROGLU A, TANRIVERDI K, INAL T *et al.*: Methylenetetrahydrofolate reductase gene C677T mutation and plasma homocysteine levels in Behçet's disease. *Rheumatol Int* 2003; 23: 236-40.

166. LEIBA M, SELIGSOHN U, SIDI Y *et al.*: Thrombophilic factors are not the leading cause of thrombosis in Behçet's disease. *Ann Rheum Dis* 2004; 63: 1445-9.

167. GUL A, OZBEK U, OZTURK C, INANC M, KONICE M, OZCELIK T: Coagulation factor V gene mutation increases the risk of venous thrombosis in Behçet's disease. *Br J Rheumatol* 1996; 35: 1178-80.

168. MAMMO L, AL-DALAAN A, BAHABRI SS, SAOUR JN: Association of factor V Leiden with Behçet's disease. *J Rheumatol* 1997; 24: 2196-8.

169. ONER AF, GURGEY A, GURLER A, MESCI L: Factor V Leiden mutation in patients with Behçet's disease. *J Rheumatol* 1998; 25: 496-8.

170. GURGEY A, BALTA G, BOYVAT A: Factor V Leiden mutation and PAI-1 gene 4G/5G genotype in thrombotic patients with Behçet's disease. *Blood Coagul Fibrinolysis* 2003; 14: 121-4.

171. LESPRIT P, WECHSLER B, PIETTE JC *et al.*: Activated protein C resistance caused by factor V Arg 506→Gln mutation has no role in thrombotic manifestations of Behçet's disease. *Ann Rheum Dis* 1995; 54: 860.

172. ESPINOSA G, FONT J, TASSIES D *et al.*: Vascular involvement in Behçet's disease: relation with thrombophilic factors, coagulation activation, and thrombomodulin. *Am J Med* 2002; 112: 37-43.

173. ATES A, DUZGUN N, ULU A, TIRYAKI AO, AKAR N: Factor V gene (1691A and 4070G) and prothrombin gene 20210A mutations in patients with Behçet's disease. *Pathophysiol Haemost Thromb* 2003; 33: 157-63.

174. SILINGARDI M, SALVARANI C, BOIARDI L *et al.*: Factor V Leiden and prothrombin gene G20210A mutations in Italian patients with Behçet's disease and deep vein thrombosis. *Arthritis Care Res* 2004; 51: 177-83.

175. VERITY DH, VAUGHAN RW, MADANAT W *et al.*: Factor V Leiden mutation is associated with ocular involvement in Behçet disease. *Am J Ophthalmol* 1999; 128: 352-6.

176. BATIOGLU F, ATMACA LS, KARABULUT HG, BEYZA SAYIN D: Factor V Leiden and prothrombin gene G20210A mutations in ocular Behçet disease. *Acta Ophthalmol Scand* 2003; 81: 283-5.

177. CHEN Y, STANFORD MR, WALLACE GR, VAUGHAN RW, KONDEATIS E, FORTUNE F: Factor V Leiden mutation does not correlate with retinal vascular occlusion in white patients with Behçet's disease. *Br J Ophthalmol* 2003; 87: 1048-9.

178. GUL A, ASLANTAS AB, TEKINAY T, KONICE M, OZCELIK T: Procoagulant mutations and venous thrombosis in Behçet's disease. *Rheumatology (Oxford)* 1999; 38: 1298-99.

179. SALVARANI C, CALAMIA K, SILINGARDI M, GHIRARDUZZI A, OLIVIERI I. Thrombosis associated with the prothrombin gene G→A 20210 mutation in Behçet's disease. *J Rheumatol* 2000; 27: 515-6.

180. VAYA A, FORNER MJ, ESTELLES A *et al.*: Intracardiac thrombosis in a case of Behçet's disease associated with the prothrombin 20210G-A mutation. *Haematologica* 2000; 85: 425-8.

181. POLAT G, ESKANDARI G, KAYA TI *et al.*: Association of the platelet glycoprotein Ia C807T/G873A gene polymorphism and thrombosis in Behçet patients. *Haematologia (Budap)* 2002; 32: 121-8.