

Genetics and Epigenetics Pathogenesis of Behçet's Syndrome

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INTRODUCTION

Vasculitides are a group of diseases characterized by inflammation and destruction of blood vessel wall during the disease course. The disease itself exhibit non-specific signs and symptoms as well as differences in patho physiology, vessel involvement, and demographics [1]. Complexity of the disease related to the structural and function of the vessel, the tissue, and the associated microenvironment involved in the disease [2]. Behçet's syndrome (**BS**) is a vasculitides, which is also characterized numerous systemic manifestations, including oral aphthous ulcers, skin lesions, ocular diseases, neurologic diseases, rheumatologic diseases, gastrointestinal disease and genital ulcers. These clinical manifestations of BS are present mainly due to the inflammation of the blood vessels and it is known for its ability to affect both, arteries and the veins. However, the etiology of BS is only partially understood, and previous studies have demonstrated a role of genetic and epigenetic factors contributes to disease pathophysiology.

PATHOPHYSIOLOGY OF BEHÇET'S SYNDROME

Several studies indicate that the pathophysiology of BS is associated with the activities of T cells and monocytes, sometimes stimulated by heat shock proteins and streptococcal antigen [3]. Cellular activation and alterations in the numbers of T cell subpopulations are evident in BS [4]. In the pathogenesis of BS, auto-reactive T cells play a critical role in depletion of lymphocyte by an anti-CD52 monoclonal antibody (CAMPATH-1H) [5]. Furthermore, during disease exacerbations adenosine deaminase, which is an enzyme involved in lymphocyte proliferation, maturation, and differentiation, is activated in Behçet's syndrome [6,7].

Th1 lymphocytes were found to be increased in patients with BS [8-11]. Few studies have also shown that the serum levels of IL-8 and IL-12, and TNF- α receptors correlate with disease activity and progression [12,13]; in contrast, one study reported an increased production of these cytokines regardless of disease activity [14]. Elevated levels of IL-6, IL-8, IgA, C3, C4, and TNF- α were reported in patients with active ocular Behçet's. Elevated levels of IL-16 in cerebrospinal fluid have been reported in BS patients with active neurological disease and might be used as a marker of disease activity [15]. Furthermore, increased IL-8, IL12, monocyte chemo-attractant protein 1 and interferon-gamma were observed in patients with active Behçet's compared with healthy controls. However, IL-4 and IL-13 levels were not increased [16]. Increased expression of IL-23 mRNA has also been described in erythema nodosum-like lesions seen in BS [17].

Although T helper (**Th**) 1 predominantly responds in Behçet's, but some studies have also shown evidence for a Th2 response. There might be a combination of both Th1 and Th2 activity in this disease [8,18]. Mononuclear cells in peripheral blood obtained from patients with BS showed an increased amount of IL-4, IL-10, IL-13 [9,10,19]. Further, analysis of T-cell subpopulations in BS showed relative increase in the CD8:CD4 ratio, with a relative reduction in percentage of suppressor T cell subpopulation, particularly in patients with active disease. Levels of CD30 were found elevated in Behçet's patients with active disease. CD30, released from CD4+ Th2 cells, are a known marker of Th2 activity. Overall, Th2 might have an impact on disease course and severity, Th1 might be involved in the disease's active phases [20]. Furthermore, Th17 cells were found to be increased in active phase of BS and might be the reason of the apparently conflicting findings regarding Th1 and Th2 activity in this disorder [21,22].

Flow cytometric analysis of the aqueous humor obtained from the uveitis patients showed elevated levels of CD4+ and CD8+ T cells [23,24]. Th1 cytokines were also elevated in the aqueous humor and serum of the BS patients [25]. Ocular fluid from Behçet's patients with active uveitis demonstrated inflammatory cytokines, such as interferon, TNF, IL-2, IL-6, and IL-17. Activated CD4+ T cells in ocular fluid produced a large amount of TNF and IL-17. And this production was by the treatment with infliximab [26]. Synovial fluid in BS demonstrated lower levels of inflammatory cytokines compared to patients with rheumatoid arthritis [27]. Also, bronchoalveolar lavage from patients with Behçet's pulmonary disease shows higher levels of IL-18 and interferon-gamma before and after LPS stimulation than in controls.

Autoimmune activity against retinal self-antigens appears to be important in the pathogenesis of Behçet's uveitis [28]. A Retinal-S antigen is localized to the photoreceptor of the retina, and has been shown to be a potent uveitic autoantigen [29]. Peripheral blood S-antigen responsive lymphocytes are elevated during exacerbations of ocular inflammation in Behçet's patients [30]. There are a number of molecules known to mediate endothelial dysfunction in BS. Various studies have documented the role of Nitric oxide (**NO**), a highly reactive molecule associated with inflammatory activity, in endothelial activation in BS [31-33]. Polymorphisms of endothelial NO synthase genes have been associated with increase susceptibility to BS [34]. Elevated levels of symmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase, have been observed in Behçet's [35]. Vascular endothelial growth factor levels are higher in patients with Behçet's and have been associated with increased disease activity and NO concentrations [36-38]. Cerebrospinal fluid VEGF levels were higher in a group of Behçet's patients with neurological involvement compared with patients with non-inflammatory neurological diseases [39]. Polymorphisms of the VEGF gene are associated with Behçet's, but not in all populations [40,41].

Endothelial cell activation and a generalized hypercoagulable state were observed in many Behçet's patients. Thrombin formation is increased, and fibrinolysis is decreased [42]. Lower activated protein C (**APC**) and soluble thrombomodulin concentrations in the plasma of patients with Behçet's syndrome have been observed. Thrombomodulin, present on the endothelial cell surface, binds thrombin, and the substrate specificity of thrombin thus bound is changed so that it preferentially activates protein C rather than cleaving fibrinogen. The APC generated at the endothelial surface is an important inhibitor of coagulation.

The findings of significantly decreased mean concentrations of plasma APC among 39 Spanish patients with Behçet's syndrome versus healthy controls, and of lower levels of APC among patients with Behçet's syndrome who had a prior thromboembolic episode versus those who had not, suggest that an acquired deficiency of APC in Behçet's syndrome may contribute to the risk of thrombosis [43]. Platelet activity is increased in Behçet's patients [44]. Studies have shown decreased erythrocyte deformability in Behçet's [45]. Tissue-type plasminogen activator levels are lower in patients with deep venous thrombosis who have Behçet's than in those who do not have Behçet's; this suggests a defect in fibrinolysis in Behçet's [46].

Thrombophilic factors such as factor V Leiden and prothrombin G20210A gene have not been linked to Behçet's, although Behçet's patients with coexisting thrombophilic factors may be at higher risk for thrombosis [47]. Levels of the fibrinolytic inhibitors thrombin-activatable fibrinolysis inhibitor (**TAFI**) and plasminogen activator inhibitor-1 (**PAI-1**) were higher in Behçet's patients than in controls, particularly in those patients with thrombosis [48]. However, the majority of available evidence suggests that the pathogenesis of thrombosis in Behçet's is probably not due to a hypercoagulable state but rather to vascular damage induced by inflammation or intrinsic endothelial dysfunction that, by itself, may serve as a source of thrombogenic stimuli [49].

In Behçet's syndrome, PMN motility is increased. Cell surface markers indicative of activation, such as CD64, are increased in patients with active disease to levels similar to those of patients with sepsis [50]. PMNs exhibit increased motility and enhanced adhesion to endothelial cells in vitro, a property due, in part, to increased expression of cell surface receptors including CD11a, CD18, and ICAM-1 [51]. Neutrophil binding to the endothelium is also facilitated by up regulated surface expression of adhesion molecules such as E-selectin. Increased E-selectin on the luminal surface favors neutrophil adhesion and facilitates neutrophil migration into the wall of the affected vessel and beyond [52].

Measurements of serum-soluble levels of this activity have been observed. Serum-soluble ICAM-1 is increased in patients with Behçet's syndrome, and the concentration may correlate with disease activity [53]. The serum of patients with active Behçet's syndrome may promote increased E-selectin presentation [23]. Some of this may be due to anti-endothelial antibodies [54]. Serum-soluble levels of selectin have been shown to be elevated in patients with active untreated disease but not if it is treated or inactive [55]. A study of patients with Behçet's retinal vasculitis showed elevated levels of soluble E-selectin, s-ICAM-1, and Interferon-beta. In vitro studies showed that these were produced by activated retinal vascular endothelial cells and that this could be inhibited by pretreatment with anti-Toll-like receptor 3 [56].

The protein actin is required for PMN mobility. The presence of high levels of a truncated actin with an N-terminus of Met-44 has been observed in neutrophils from Behçet's patients; PMN-elastase responsible for this cleavage has been isolated. Resulting altered PMN activity has been described [57]. Increased amount of reactive oxygen species suggest neutrophil mediated immunity [58]. Endogenous free radical scavenging enzymes appear to be reduced in Behçet's, creating an imbalance in the oxidant antioxidant equilibrium [7].

Serum granulocyte colony-stimulating factor (**G-CSF**) levels and neutrophil apoptosis are increased in active Behçet's patients compared with controls [59]. Markers of neutrophil activity have been proposed as possible indicators of disease activity and severity. These include polymorph nuclear elastase, plasma myeloperoxidase, and advanced oxidation protein products [60]. Matrix metalloproteinases play a role in leucocyte invasion of the central nervous system. Elevated levels of matrix metalloprotein-9 have been described in Behçet's patients compared with controls, and levels correlated with CSF neutrophil and mononuclear cells in a study of Behçet's patients with neurologic disease [61].

GENETIC PREDISPOSITION ASSOCIATED WITH BEHÇET'S SYNDROME

Increased risk of developing Behçet's syndrome is associated with the presence HLA-B51, a human leukocyte antigen. A meta-analysis of 4800 cases and 16,289 controls has shown a significant increase in the risk of HLA-B51/B5 carriers to develop Behçet's syndrome compared with non-carriers (pooled odds ratio of 5.8) [62]. This relationship was consistent across multiple

geographic locations, including Eastern Asia, Middle East/North Africa, Southern Europe, and Northern/Eastern Europe, but insufficient data were available for analysis of North American studies.

A higher than baseline prevalence of HLA-B51 has been found in Behçet's patients from Italy (odds ratio of 5.9), Germany, Middle Eastern and Far Eastern countries (63 versus 9 percent in controls), of HLA-B52 (21 versus 9 percent) in Israel, and of HLA-B57 in the United Kingdom [1,63]. HLA-B5101 and, to a lesser extent, HLA-5108 alleles have been most closely linked in patients along the Silk Road [9]. Other HLA alleles may increase (HLA-B15, HLA-B27, HLA-B57, HLA-26) or decrease (HLA-B49, HLA-A03) the risk for Behçet's in various populations and in men and women [64]. However, the HLA contribution was estimated to be less than 20 percent in one study [63].

There may also be a genetic contribution to disease severity. Presence of a HLA-B51 allele has been associated with worse disease in several studies [1,65]. Several possible mechanisms for the genetic linkage to HLA-B51 includes, alterations in the B pocket of the antigen-binding groove by HLA-B51 [66], cross-reactivity between HLA-B51 and organ-specific antigens [67], and linkage disequilibrium with other disease-associated genes [68].

Though most cases of Behçet's are sporadic, families with multiple affected members, known as familial clustering, have been reported, and having a first-degree relative with Behçet's does increase risk for the disease [69]. HLA-B51 rates are higher in familial than in sporadic cases. Affected children of patients with Behçet's syndrome show genetic anticipation [70]. A triplet repeat microsatellite polymorphism of a tri-nucleotide repeat (**GCT**) was found within a major histocompatibility complex (**MHC**) Class I related gene in a study on Japanese patients [71].

Non-HLA genes also play a role in determining susceptibility to disease. Genome-wide screening of affected families with more than one affected member has identified additional, non-HLA regions of potential interest [72]. Associations between Behçet's syndrome and polymorphisms of the following genes have been reported.

1. The intercellular adhesion molecule (**ICAM**)-1 gene,
2. The endothelial nitric oxide synthase gene,
3. TNF genes,
4. The vascular endothelial growth factor (**VEGF**) gene,
5. Manganese superoxide dismutase gene,
6. Cytochrome P450 gene,
7. Endoplasmic reticulum amino peptidase 1 (**ERAP1**) gene,
8. The interleukin (**IL**)-10 genes,
9. The IL-23 receptor gene.

Furthermore, disease activity correlates with a pro-inflammatory shift in peripheral T cell populations featuring the expansion of both Th17 and Th22 subsets and a reduction in Tregs [73]. Dysregulation in miRNAs mediating immune response pathways and effector cell development may be involved in these population shifts. In a recent study by Zhou et al., active uveitis in BD patients of Han Chinese descent was associated with downregulation of miR-155 expression in both PBMCs and monocyte-derived dendritic cells (**mc-DCs**) [74]. In addition, the overexpression of miR-155 in transfected DCs was shown to down regulate pro-inflammatory IL-6 and IL-1 β while increasing anti-inflammatory IL-10. Further, miR-155 expression in DCs was inversely related to IL-17 production in allogeneic CD4⁺ T cells. Previous studies have shown that miR-155 also modulates inflammatory responses in DCs through its role in a negative-feedback loop that controls TLR/IL-1 signaling [75]. In contrast, miR-155 was shown to promote differentiation of pro-inflammatory Th17 and Th1 T cell subsets in other forms of autoimmunity, such as experimental autoimmune encephalitis models [76]. This suggests that miR-155 might have pleiotropic and heterogeneous effects on disease pathogenesis influenced by the nature of the predominant immune response.

The function of miRNAs can be influenced by genetic variants located in or around pre-miRNA or within target mRNA binding sites [77]. This form of miRNA dysregulation was demonstrated in Han Chinese BD patients in miR-146a/rs2910164, miR-196a2/rs11614913, and miR-182/rs76481776. In the first study, BD was associated with variant rs2910164 within miR-146a, a key regulator of the type I interferon pathway [78]. Healthy individuals with the homozygous risk allele for miR-146a/rs2910164 had increased miR-146a expression and increased serum levels of pro-inflammatory cytokines IL-1 β , IL-17, and TNF- α . In another study, a variant in the pre-miRNA region of miR-196a2, rs11614913, was associated with BD, as well as arthritis in BD patients [79]. The homozygous risk allele was associated with decreased expression of miR-196a, increased PBMC production of IL-1 β and MCP-1, and increased expression of the miR-196a target gene BACH1, a transcriptional repressor of the anti-inflammatory heme oxygenase 1 (**HO-1**) enzyme which protects against oxidative stress [80]. In the third study, BD was associated with variant rs76481776 in a pre-miRNA region of miR-182, an IL-2-induced miRNA that is a critical switch in T-reg differentiation [81]. In healthy individuals with either the homozygous or heterozygous risk allele, miR-182 expression was increased in CD3/CD28-stimulated CD4⁺ T cells. Combined, these BD-associated genetic variations in miRNA genes were shown to promote a disease phenotype involving increased production of pro-inflammatory cytokines and a decreased expansion of anti-inflammatory T-reg cells.

EPIGENETIC PREDISPOSITION ASSOCIATED TO BEHÇET'S SYNDROME

Epigenetics is the study of stable and heritable changes in the function of genes which occur without altering the DNA sequence and include DNA methylation, histone modification, and

microRNAs [82]. These epigenetic modifications can be stable to maintain the specific lineage of cells or dynamic to respond to developmental and environmental signals. Alterations in the epigenetic state of the cell can lead to aberrancies in phenotype which are then passable to daughter cells. The most extensively studied epigenetic mechanism is DNA methylation, a stable modification that commonly refers to the addition of a methyl group to the 5 prime carbon of a cytosine residue of a CG dinucleotide. Non-CG methylation is also known to occur, particularly in embryonic cells, and more recently also described in double-negative T cells; however, the regulatory mechanisms and functions associated with non-CG methylation patterns are not well-known [83]. DNA methylation is a very common modification in the human genome occurring at 70-80 % of CG dinucleotides [84]. Clusters of unmethylated CG dinucleotides, called CpG Islands, are often found within the promoter regions of genes. Further, methylation of these CpG Islands is typically associated with transcriptional repression [85]. The mechanisms by which DNA methylation silences gene expression are not entirely understood; however, one common hypothesis is that DNA modification hinders the binding of transcription factors. In addition, DNA methylation may silence gene expression by interacting with methyl-CpG-binding proteins that initiate chromatin remodeling [86]. In either event, DNA methylation is an important epigenetic mechanism involved in regulating developmental processes, genomic imprinting, and X chromosome inactivation [87]. Aberrancies in DNA methylation have been associated with several forms of cancer, autoimmune diseases, and inflammatory conditions [87].

Another form of epigenetic variation is the modification of histones which are proteins wrapped as an octamer by DNA to form the nucleosome subunits of chromatin structures. Histones can be post-translationally modified by the acetylation, methylation, phosphorylation, sumoylation, ubiquitination, ADP ribosylation, deamination, and proline isomerization of amino acid residues within their protein tail [88]. Histone modifications most commonly studied are those of lysine residues of histone H3 (**H3K**) for which acetylation is often associated with less compact chromatin structure, whereas mono-, di-, and tri-methylation are often associated with a more condensed structure [89].

A central feature in BD pathogenesis is dysregulated cytoskeletal remodeling, a central process in both the adhesion and infiltration of leukocytes into inflamed tissues [90]. In a genome-wide DNA methylation study that compared DNA methylation in active untreated BD patients to age, sex, and ethnicity matched healthy controls, aberrant CG methylation of both monocytes and CD4+ T cells was found in and around genes significantly enriched for functions related to cytoskeletal remodeling [90]. These include genes such as RAC1, RGS14, and FSCN2 among many others, which are involved in cytoskeletal action polymerization and bundling that is crucial to filopodia and lamellipodia formation during cell migration and tissue invasion. In both cell types, several CG sites that were differentially methylated in patients compared with controls had their aberrant methylation levels reversed and restored to normal levels when the same patients were studied following disease remission. These findings suggested an important role for epigenetic

remodeling in cytoskeleton-related genes in the pathogenesis and the therapeutic response in BD. In addition, dynamic differentially methylated CG sites that are reversed following disease remission might warrant further investigation to develop epigenetic biomarkers for disease activity and remission in BD.

References

1. Sakane T, Takeno M, Suzuki N, Inaba G. Behçet's disease. *N Engl J Med*. 1999; 341: 1284.
2. Mahr A, Belarbi L, Wechsler B, Jeanneret D, Dhote R, et al. Population-based prevalence study of Behçet's disease: differences by ethnic origin and low variation by age at immigration. *Arthritis Rheum*. 2008; 58: 3951-3959.
3. Gunesacar R, Erken E, Bozkurt B, Ozer HT, Dinkci S, et al. Analysis of CD28 and CTLA-4 gene polymorphisms in Turkish patients with Behçet's disease. *Int J Immunogenet*. 2007; 34: 45-49.
4. Skin hypersensitivity to streptococcal antigens and the induction of systemic symptoms by the antigens in Behçet's disease-a multicenter study. The Behçet's Disease Research Committee of Japan. *J Rheumatol*. 1989; 16: 506-511.
5. Direskeneli H. Behçet's disease: infectious aetiology, new autoantigens, and HLA-B51. *Ann Rheum Dis* 2001; 60: 996-1002.
6. Lockwood CM, Hale G, Waldman H, Jayne DR. Remission induction in Behçet's disease following lymphocyte depletion by the anti-CD52 antibody CAMPATH 1-H. *Rheumatology*. 2003; 42: 1539-1544.
7. Erkiilic K, Evereklioglu C, Cekmen M, Abdullah O, Fuat D, et al. Adenosine deaminase enzyme activity is increased and negatively correlates with catalase, superoxide dismutase and glutathione peroxidase in patients with Behçet's disease: original contributions/clinical and laboratory investigations. *Mediators Inflamm*. 2003; 12: 107-116.
8. Canpolat F, Unver M, Eskioğlu F, Kösebalaban S, Durmazlar SP. Serum and erythrocyte adenosine deaminase activities in patients with Behçet's disease. *Int J Dermatol*. 2006; 45: 1053-1056.
9. Evereklioglu C. Current concepts in the etiology and treatment of Behçet disease. *Surv Ophthalmol*. 2005; 50: 297-350.
10. Hamzaoui K, Hamzaoui A, Guemira F, Bessioud M, Hamza M, et al. Cytokine profile in Behçet's disease patients. Relationship with disease activity. *Scand J Rheumatol*. 2002; 31: 205-210.
11. Sugi-Ikai N, Nakazawa M, Nakamura S, Ohno S, Minami M. Increased frequencies of interleukin-2- and interferon-gamma-producing T cells in patients with active Behçet's disease. *Invest Ophthalmol Vis Sci* 1998; 39: 996-1004.
12. Imamura Y, Kurokawa MS, Yoshikawa H, Nara K, Takada E, et al. Involvement of Th1 cells and heat shock protein 60 in the pathogenesis of intestinal Behçet's disease. *Clin Exp Immunol*. 2005; 139: 371-378.
13. Turan B, Pfister K, Diener PA, Hell M, Möller B, et al. Soluble tumour necrosis factor receptors s TNFR1 and sTNFR2 are produced at sites of inflammation and are markers of arthritis activity in Behçet's disease. *Scand J Rheumatol*. 2008; 37: 135-141.
14. Durmazlar SP, Ulkar GB, Eskioğlu F, Tatlıcan S, Mert A, et al. Significance of serum interleukin-8 levels in patients with Behçet's disease: high levels may indicate vascular involvement. *Int J Dermatol*. 2009; 48: 259-264.
15. Mege JL, Dilsen N, Sanguedolce V, Gul A, Bongrand P, et al. Overproduction of monocyte derived tumor necrosis factor alpha, interleukin (IL) 6, IL-8 and increased neutrophil superoxide generation in Behçet's disease. A comparative study with familial Mediterranean fever and healthy subjects. *J Rheumatol*. 1993; 20: 1544-1549.
16. Akman-Demir G, Tüzün E, İçöz S, Yeşilot N, Yentür SP, et al. Interleukin-6 in neuro-Behçet's disease: association with disease subsets and long-term outcome. *Cytokine*. 2008; 44: 373-376.
17. Ben Ahmed M, Houman H, Miled M, Dellagi K, Louzir H, et al. Involvement of chemokines and Th1 cytokines in the pathogenesis of mucocutaneous lesions of Behçet's disease. *Arthritis Rheum*. 2004; 50: 2291-2295.
18. Lew W, Chang JY, Jung JY, Bang D. Increased expression of interleukin-23 p19 mRNA in erythema nodosum-like lesions of Behçet's disease. *Br J Dermatol*. 2008; 158: 505.
19. Dalghous AM, Freysdottir J, Fortune F. Expression of cytokines, chemokines, and chemokine receptors in oral ulcers of patients with Behçet's disease (BD) and recurrent aphthous stomatitis is Th1-associated, although Th2-association is also observed in patients with BD. *Scand J Rheumatol*. 2006; 35: 472.
20. Akpolat T, Koç Y, Yeniay I. Familial Behçet's disease. *Eur J Med*. 1992; 1: 391.
21. Raziuddin S, al-Dalaan A, Bahabri S, Siraj AK, al-Sedairy S. Divergent cytokine production profile in Behçet's disease. Altered Th1/Th2 cell cytokine pattern. *J Rheumatol*. 1998; 25: 329-333.

22. Mantaş Ç, Direskeneli H, Ekşioğlu-Demiralp E, Akoglu T. Serum levels of Th2 cytokines IL-4 and IL-10 in Behçet's disease. *J Rheumatol.* 1999; 26: 510.
23. Vaccarino L, Triolo G, Accardo-Palombo A, Scola L, Palmeri M, et al. Pathological implications of Th1/Th2 cytokine genetic variants in Behçet's disease: Data from a pilot study in a Sicilian population. *Biochem Genet.* 2013; 51: 967-975.
24. Sugita S, Kawazoe Y, Imai A, Yamada Y, Horie S, et al. Inhibition of Th17 differentiation by anti-TNF-alpha therapy in uveitis patients with Behçet's disease. *Arthritis Res Ther.* 2012; 14: R99.
25. Lee YJ, Horie Y, Wallace GR, Choi YS, Park JA, et al. Genome-wide association study identifies GIMAP as a novel susceptibility locus for Behçet's disease. *Ann Rheum Dis.* 2013; 72: 1510-1516.
26. Yu HG, Lee DS, Seo JM, Ahn JK, Yu YS et al. The number of CD8+ T cells and NKT cells increases in the aqueous humor of patients with Behçet's uveitis. *Clin Exp Immunol.* 2004; 137: 437-443.
27. Keino H, Sakai J, Nishioka K, Sumida T, Usui M. Clonally accumulating T cells in the anterior chamber of Behçet disease. *Am J Ophthalmol.* 2000; 130: 243-245.
28. Ahn JK, Chung H, Lee DS, Yu YS, Yu HG. CD8brightCD56+ T cells are cytotoxic effectors in patients with active Behçet's uveitis. *J Immunol.* 2005; 175: 6133-6142.
29. Ahn JK, Yu HG, Chung H, Park YG. Intraocular cytokine environment in active Behçet uveitis. *Am J Ophthalmol.* 2006; 142: 429.
30. Guenane H, Hartani D, Chachoua L. Production of Th1/Th2 cytokines and nitric oxide in Behçet's uveitis and idiopathic uveitis. *J Fr Ophthalmol.* 2006; 29: 146.
31. Pay S, Erdem H, Pekel A, Simsek I, Musabak U, et al. Synovial proinflammatory cytokines and their correlation with matrix metalloproteinase-3 expression in Behçet's disease. Does interleukin-1beta play a major role in Behçet's synovitis? *Rheumatol Int.* 2006; 26: 608-613.
32. Kurhan-Yavuz S, Direskeneli H, Bozkurt N, Y Özyazgan, T Bavbek, et al. Anti-MHC autoimmunity in Behçet's disease: T cell responses to an HLA-B-derived peptide cross-reactive with retinal-S antigen in patients with uveitis. *Clin Exp Immunol* 2000; 120: 162-166.
33. Yamamoto JH, Minami M, Inaba G, Masuda K, Mochizuki M. Cellular autoimmunity to retinal specific antigens in patients with Behçet's disease. *Br J Ophthalmol.* 1993; 77: 584-589.
34. de Smet MD, Dayan M. Prospective determination of T-cell responses to S-antigen in Behçet's disease patients and controls. *Invest Ophthalmol Vis Sci.* 2000; 41: 3480.
35. Zhao C, Yang P, He H, Lin X, Li B, et al. S-antigen specific T helper type 1 response is present in Behçet's disease. *Mol Vis.* 2008; 14: 1456-1464.
36. Direskeneli H, Keser G, D'Cruz D, Khamashta MA, Akoğlu T, et al. Anti-endothelial cell antibodies, endothelial proliferation and von Willebrand factor antigen in Behçet's disease. *Clin Rheumatol.* 1995; 14: 55-61.
37. Evereklioglu C, Turkoz Y, Er H, Serhat Inaloz H, Emin O, et al. Increased nitric oxide production in patients with Behçet's disease: is it a new activity marker? *J Am Acad Dermatol.* 2002; 46: 50-54.
38. Duygulu F, Evereklioglu C, Calis M, Borlu M, Cekmen M, et al. Synovial nitric oxide concentrations are increased and correlated with serum levels in patients with active Behçet's disease: a pilot study. *Clin Rheumatol.* 2005; 24: 324-330.
39. Nakao K, Isashiki Y, Sonoda S, Uchino E, Shimonagano Y, et al. Nitric oxide synthase and superoxide dismutase gene polymorphisms in Behçet disease. *Arch Ophthalmol.* 2007; 125: 246-251.
40. Sahin M, Arslan C, Naziroglu M, Tunc SE, Demirci M, et al. Asymmetric dimethylarginine and nitric oxide levels as signs of endothelial dysfunction in Behçet's disease. *Ann Clin Lab Sci.* 2006; 36: 449-454.
41. Kamoun M, Houman MH, Hamzaoui A, Hamzaoui K. Vascular endothelial growth factor gene polymorphisms and serum levels in Behçet's disease. *Tissue Antigens* 2008; 72: 581.
42. Yalçın B, Arda N, Tezel GG, Erman M, Alli N. Expressions of vascular endothelial growth factor and CD34 in oral aphthous lesions of Behçet's disease. *Anal Quant Cytol Histol.* 2006; 28: 303-306.
43. Hamzaoui K, Ayed K, Hamza M, Hamzaoui A. VEGF and mRNA VEGF expression in CSF from Behçet's disease with neurological involvement. *J Neuroimmunol.* 2009; 213: 148.
44. Nam EJ, Han SW, Kim SU, Choa JH, Saa KH, et al. Association of vascular endothelial growth factor gene polymorphisms with behçet disease in a Korean population. *Hum Immunol.* 2005; 66: 1068-1073.
45. Espinosa G, Font J, Tàssies D, Vidaller A, Deulofeu R, et al. Vascular involvement in Behçet's disease: relation with thrombophilic factors, coagulation activation, and thrombomodulin. *Am J Med.* 2002; 112: 37-43.

46. Navarro S, Ricart JM, Medina P, Vayá A, Villa P, et al. Activated protein C levels in Behçet's disease and risk of venous thrombosis. *Br J Haematol.* 2004; 126: 550-556.
47. Akar S, Ozcan MA, Ateş H, Gürler O, Alacacioglu I, et al. Circulated activated platelets and increased platelet reactivity in patients with Behçet's disease. *Clin Appl Thromb Hemost.* 2006; 12: 451-457.
48. Tatlican S, Duran FS, Eren C, Eskioglu F, Dikmenoglu N, et al. Reduced erythrocyte deformability in active and untreated Behçet's disease patients. *Int J Dermatol.* 2010; 49: 167-171.
49. Yurdakul S, Hekim N, Soysal T, Fresko I, Bavunoglu I, et al. Fibrinolytic activity and d-dimer levels in Behçet's syndrome. *Clin Exp Rheumatol.* 2005; 23: S53-S58.
50. Caramaschi P, Poli G, Bonora A, Volpe A, Tinazzi I, et al. A study on thrombophilic factors in Italian Behçet's patients. *Joint Bone Spine.* 2010; 77: 330-334.
51. Ricart JM, Ramón LA, Vayá A, España F, Santaolaria ML, et al. Fibrinolytic inhibitor levels and polymorphisms in Behçet disease and their association with thrombosis. *Br J Haematol.* 2008; 141: 716-719.
52. Leiba M, Seligsohn U, Sidi Y, Harats D, Sela BA, et al. Thrombophilic factors are not the leading cause of thrombosis in Behçet's disease. *Ann Rheum Dis.* 2004; 63: 1445-1449.
53. Ureten K, Ertenli I, Oztürk MA, Kiraz S, Onat AM, et al. Neutrophil CD64 expression in Behçet's disease. *J Rheumatol.* 2005; 32: 849-852.
54. Sahin S, Akoğlu T, Direskeneli H, Sen LS, Lawrence R. Neutrophil adhesion to endothelial cells and factors affecting adhesion in patients with Behçet's disease. *Ann Rheum Dis.* 1996; 55: 128-133.
55. Buldanlioglu S, Turkmen S, Ayabakan HB, Yenice N, Vardar M, et al. Nitric oxide, lipid peroxidation and antioxidant defence system in patients with active or inactive Behçet's disease. *Br J Dermatol.* 2005; 153: 526-530.
56. Saglam K, Yilmaz MI, Saglam A, Ulgey M, Bulucu F, et al. Levels of circulating intercellular adhesion molecule-1 in patients with Behçet's disease. *Rheumatol Int.* 2002; 21: 146-148.
57. Ateş A, Tiryaki OA, Olmez U, Tutkak H. Serum-soluble selectin levels in patients with Behçet's disease. *Clin Rheumatol.* 2007; 26: 411.
58. Lee MT, Hooper LC, Kump L, Hayashi K, Nussenblatt R, et al. Interferon-beta and adhesion molecules (E-selectin and s-intracellular adhesion molecule-1) are detected in sera from patients with retinal vasculitis and are induced in retinal vascular endothelial cells by Toll-like receptor 3 signalling. *Clin Exp Immunol.* 2007; 147: 71-80.
59. Yamashita S, Suzuki A, Kamada M, Yanagita T, Hirohata S, et al. Possible physiological roles of proteolytic products of actin in neutrophils of patients with Behçet's disease. *Biol Pharm Bull.* 2001; 24: 733-737.
60. Yoshida T, Tanaka M, Sotomatsu A, Okamoto K, Hirai S. Serum of Behçet's disease enhances superoxide production of normal neutrophils. *Free Radic Res.* 1998; 28: 39-44.
61. Kawakami T, Ohashi S, Kawa Y, Takahama H, Ito M, et al. Elevated serum granulocyte colony-stimulating factor levels in patients with active phase of sweet syndrome and patients with active behcet disease: implication in neutrophil apoptosis dysfunction. *Arch Dermatol.* 2004; 140: 570-574.
62. Yazici C, Köse K, Caliş M, Demir M, Kirnap M, et al. Increased advanced oxidation protein products in Behçet's disease: a new activity marker? *Br J Dermatol.* 2004; 151: 105-111.
63. Hamzaoui K, Maître B, Hamzaoui A. Elevated levels of MMP-9 and TIMP-1 in the cerebrospinal fluid of neuro-Behçet's disease. *Clin Exp Rheumatol.* 2009; 27: S52.
64. de Menthon M, Lavalley MP, Maldini C, Guillevin L, Mahr A. HLA-B51/B5 and the risk of Behçet's disease: a systematic review and meta-analysis of case-control genetic association studies. *Arthritis Rheum* 2009; 61: 1287.
65. Gül A, Hajeer AH, Worthington J, Ollier WE, Silman AJ. Linkage mapping of a novel susceptibility locus for Behçet's disease to chromosome 6p22-23. *Arthritis Rheum.* 2001; 44: 2693-2696.
66. Takeuchi M, Kastner DL, Rimmens EF. The immunogenetics of Behçet's disease: A comprehensive review. *J Autoimmun.* 2015; 64: 137.
67. 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: 92-96.
68. Gebreselassie D, Spiegel H, Vukmanovic S. Sampling of major histocompatibility complex class I-associated peptidome suggests relatively looser global association of HLA-B*5101 with peptides. *Hum Immunol.* 2006; 67: 894.
69. Wildner G, Thureau SR. Cross-reactivity between an HLA-B27-derived peptide and a retinal autoantigen peptide: a clue to major histocompatibility complex association with autoimmune disease. *Eur J Immunol.* 1994; 24: 2579.

70. Kirino Y, Bertsias G, Ishigatsubo Y, Mizuki N, Tugal-Tutkun I, et al. Genome-wide association analysis identifies new susceptibility loci for Behçet's disease and epistasis between HLA-B*51 and ERAP1. *Nat Genet.* 2013; 45: 202-207.
71. Koné-Paut I, Geisler I, Wechsler B, Ozen S, Ozdogan H, et al. Familial aggregation in Behçet's disease: high frequency in siblings and parents of pediatric probands. *J Pediatr.* 1999; 135: 89-93.
72. Fresko I, Soy M, Hamuryudan V, Yurdakul S, Yavuz S, et al. Genetic anticipation in Behçet's syndrome. *Ann Rheum Dis.* 1998; 57: 45-48.
73. Mizuki N, Ota M, Kimura M, Ohno S, Ando H, et al. Triplet repeat polymorphism in the transmembrane region of the MICA gene: a strong association of six GCT repetitions with Behçet disease. *Proc Natl Acad Sci. USA.* 1997; 94: 1298-1303.
74. Karasneh J, Gül A, Ollier WE, Silman AJ, Worthington J. Whole-genome screening for susceptibility genes in multicase families with Behçet's disease. *Arthritis Rheum.* 2005; 52: 1836-1842.
75. Gül A. Pathogenesis of Behçet's disease: autoinflammatory features and beyond. *Semin Immunopathol.* 2015; 37: 413.
76. Consolandi C, Turrone S, Emmi G, Severgnini M, Fiori J, et al. Behçet's syndrome patients exhibit specific microbiome signature. *Autoimmun Rev.* 2015; 14: 269-276.
77. Ombrello MJ, Kastner DL, Remmers EF. Endoplasmic reticulum-associated amino-peptidase 1 and rheumatic disease: genetics. *Curr Opin Rheumatol.* 2015; 27: 349.
78. Yokota K, Hayashi S, Fujii N, Yoshikawa K, Kotake S, et al. Antibody response to oral streptococci in Behçet's disease. *Microbiol Immunol.* 1992; 36: 815-822.
79. Calgüneri M, Ertenli I, Kiraz S, Erman M, Celik I. Effect of prophylactic benzathine penicillin on mucocutaneous symptoms of Behçet's disease. *Dermatology.* 1996; 192: 125-128.
80. Yalçın B, Atakan N, Dogan S. Association of interleukin-23 receptor gene polymorphism with Behçet disease. *Clin Exp Dermatol.* 2014; 39: 881.
81. Kogan A, Shinar Y, Lidar M, Revivo A, Langevitz P, et al. Common MEFV mutations among Jewish ethnic groups in Israel: high frequency of carrier and phenotype III states and absence of a perceptible biological advantage for the carrier state. *Am J Med Genet.* 2001; 102: 272-276.
82. Renauer PA, Coit P, Sawalha AH. The DNA methylation signature of human TCRalpha-beta+CD4-CD8- double negative T cells reveals CG demethylation and a unique epigenetic architecture permissive to a broad stimulatory immune response. *Clin Immunol.* 2015; 156: 19-27.
83. Balada E, Ordi-Ros J, Vilardell-Tarres M. DNA methylation and systemic lupus erythematosus. *Ann N Y Acad Sci.* 2007; 1108: 127-136.
84. Deaton AM, Bird A. CpG islands and the regulation of transcription. *Genes Dev* 2011; 25: 1010-1022.
85. Bird AP, Wolffe AP. Methylation-induced repression-belts, braces, and chromatin. *Cell* 1999; 99: 451-454.
86. Surani MA. Imprinting and the initiation of gene silencing in the germ line. *Cell* 1998; 93: 309-312.
87. Gupta B, Hawkins RD. Epigenomics of autoimmune diseases. *Immunol Cell Biol.* 2015; 93: 271-276.
88. Bhaumik SR, Smith E, Shilatifard A. Covalent modifications of histones during development and disease pathogenesis. *Nat Struct Mol Biol.* 2007; 14: 1008-1016.
89. Kouzarides T. Chromatin modifications and their function. *Cell* 2007; 128: 693-705.
90. Hughes T et al. Epigenome-wide scan identifies a treatment responsive pattern of altered DNA methylation among cytoskeletal remodeling genes in monocytes and CD4+ T cells from patients with Behçet's disease. *Arthritis Rheumatol.* 2014; 66: 1648-1658.