Behçet's Syndrome According to Classical and Population Genetics

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4.1 Introduction

The etiology of Behçet's disease (BD) remains unclear. However, as in many other inflammatory and/or immune-centered diseases, environmental factors are thought to trigger the symptomatology in individuals that harbor a particular genetic background. The strong association between BD and the human leukocyte antigen (HLA) class I allele, *HLA-B*51*, has been well established. This association indicates that the *HLA-B*51* allele is one of the genetic factors underlying BD. Still, the presence of *HLA-B*51*-negative BD patients suggests that other genetic

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factor(s) and/or various environmental or infectious agent(s) might also be risk factors for the development of BD. With the exception of the *HLA-B*51* allele, the molecular nature of the "genetic background" of BD has, until recently, remained mostly unknown. Novel candidate genes for BD are now being identified, with marked improvement in genetic analysis technology. This chapter describes classically known and recent genetic findings, as well as their possible involvement in the pathogenesis of BD.

4.2 HLA-B*51

HLA, located on chromosome 6p21.3, is the major histocompatibility complex in humans. HLA encodes genes involved in antigen processing and the presentation of antigenic peptides to T cells, and is instrumental in many innate and adaptive immune responses. Peptide binding to HLA molecules is the single most selective step in the recognition of pathogens by the adaptive immune system, and depends on specific amino acids in the peptide-binding groove of each HLA allele. The HLA genes are the most polymorphic ones in the human genome, and the alleles of the HLA genes vary widely among individuals. In addition, the distribution of HLA alleles varies widely according to ethnicity and geographic area. Therefore, variability in HLA allele types can lead to differences among individuals/ethnic groups regarding susceptibility to or protection from many diseases.

The HLA class I molecule *HLA-B*51* allele is strongly associated with BD in many different ethnic groups [1–4]. The allele frequency of *HLA-B*51* is markedly increased in BD patients compared with controls, while *HLA-B*52*, which is identical to *HLA-B*51* except for two amino acid residues, is not associated with BD. Therefore, it is assumed that the immune response against the specific peptides that bind to two *HLA-B*51*-specific amino acids contributes directly to the development of BD [5]. The HLA class I molecules bind peptides derived from endogenous proteins of host or pathogen origin and present them to $CD8^+$ cells. The characteristics of the peptide-binding motifs of *HLA-B*51* have already been reported (http://www.syfpeithi.de/); however, HLA-B51-restricted causative antigens for BD remain unclear, and thus further studies are needed to clarify the HLA-B51-driven immune response in the pathogenesis of BD.

BD exists worldwide but is clearly more prevalent in countries along the ancient Silk Route, which spans from East Asia to the Middle East and the Mediterranean basin [2, 6]. *HLA-B*51* is the most strongly associated risk factor for BD in these areas [2, 3]. In the Silk Route areas, BD has a prevalence ranging from 10 to 420 cases per 100,000 individuals; in contrast, it is rare in Western and Northern Europe and the United States (U.S.), accounting for less than 1 case per 100,000 individuals [6–8]. The frequency of the *HLA-B*51* allele is lower in general European and U.S. populations than in populations in the Silk Route areas, suggesting that the regional difference of *HLA-B*51* frequency reflects regional differences in the prevalence of BD. Although the frequency of *HLA-B*51* in

Italian, Portuguese, Alaskan Eskimo, and Canadian Inuit populations is similar to the frequency in the Silk Route areas, the prevalence of BD is only about 2 cases per 100,000 individuals in Italian and Portuguese populations, and no cases have ever been reported among Eskimo or Inuit populations [6]. In addition, BD has never been reported in U.S.-residing Japanese-Americans even though Japanese-Americans share the same genetic background as native Japanese, in whom the prevalence of BD is 13.5 cases per 100,000 individuals [6, 9, 10]. This fact implies that environmental factor(s) that interact with *HLA-B*51* and specifically distribute in the Silk Route areas are responsible for the development of BD.

More recently, Hughes et al. performed dense genotyping in the HLA region to locate the genetic association between *HLA-B*51* and BD [11]. They have shown that the genetic association between *HLA-B*51* and BD disappears completely in Turkish and Italian populations after controlling for the effect of rs116799036 (which is located in the approximately 24 kb upstream promoter region of *HLA-B*). Therefore, they have suggested that the risk previously ascribed to *HLA-B*51* is likely not causal with respect to BD. Further analysis is required to confirm their findings, with due consideration given to the extensive genetic diversity and the strong linkage disequilibrium in the HLA region.

4.3 Possible Non-HLA Candidate Genes

The *HLA-B*51* allele is the major susceptibility gene responsible for BD. In many ethnic groups, approximately 50–80 % of BD patients possess the *HLA-B*51* allele [3]. Hence, about 20–50 % of BD patients lack the *HLA-B*51* allele, suggesting that other genetic and/or environmental factor(s) might also be risk factors for the development of BD. To identify novel susceptibility genes for BD outside HLA, many candidate gene investigations based on plausible biological functions related to the disease pathogenesis have been performed. Possible susceptibility genes for BD in the non-HLA region that have been identified by candidate gene approaches include *ICAM1*, *coagulation factor V*, *VEGF*, and *eNOS*.

4.3.1 ICAM1

Intercellular adhesion molecule-1 (ICAM-1), a cell-adhesion molecule that regulates cell–cell interactions in the immune system, is mainly expressed on endothelial cells. ICAM-1 is involved in the T-cell activation and the transendothelial migration of neutrophils [12]. ICAM-1 expression is increased during inflammation, and elevated serum levels of soluble ICAM-1 are observed in patients with active BD [13, 14]. These facts suggest that ICAM-1 plays an important role in the induction and development of inflammation in BD. Previous studies have reported the association of *ICAM1* polymorphisms with BD. The lysine to glutamic acid variation of codon 469 (K469E) is associated with an increased risk of BD in Palestinian, Jordanian, Korean, and Lebanese populations [15–17]; in contrast, the

allele frequency of the glycine to arginine allele of codon 241 (G241R), but not E469, was significantly higher in the Italian population [18]. The E469 allele has also been associated with clinical manifestations of BD in Korean and Tunisian populations [16, 19].

4.3.2 Coagulation Factor V

BD is a systemic inflammatory disease that carries a high risk of venous thrombosis (the formation of a thrombus within a vein). BD confers a 14-fold relative risk of developing venous thrombosis compared with BD-free controls [20]. A G-to-A substitution at nucleotide +1691 of the *coagulation factor V* gene (+1691 G/A), called factor V Leiden mutation, is one of the most prevalent genetic risk factors for venous thrombosis [21, 22]. A recent meta-analysis [23] that includes 27 previous studies assessing the association of BD with possible genetic risk factors for venous thrombosis in BD patients demonstrated that factor V Leiden is significantly associated with BD and the presence of thrombosis. Importantly, these results were only relevant in Turkish patients with BD, suggesting that this mutation might contribute to venous thrombosis in at least Turkish BD population, but not in all populations. Meanwhile, the meta-analysis revealed no significant association between other factors (+20210 G/A in the prothrombin gene and 677 C/T in the methyltetrahydrofolate reductase gene) and thrombosis in the context of BD.

4.3.3 VEGF

Vascular endothelial growth factor (VEGF) is a potent endothelial-cell-specific mitogen and permeability factor with key roles in angiogenesis, vascular maintenance, and inflammation [24–27]. VEGF augments interferon-γ and inhibits interleukin (IL)-10 secretion by T cells responding to mitogens or antigens; thus, VEGF can enhance a Th1 phenotype [28], which is evident in patients with active BD [29, 30]. Increased serum VEGF concentrations have been observed in patients with BD, and patients with active disease have significantly higher VEGF levels than patients with inactive disease [31]. In addition, ocular BD patients exhibit higher VEGF levels than non-ocular patients [31]. Therefore, VEGF may participate in the course of BD, especially during the active stage, and increased serum VEGF concentrations may be a risk factor for the development of ocular disease. The *VEGF* promoter polymorphisms −634C (the C allele of −634 C/G) and −2549I (an 18-bp insertion at −2549) are reportedly associated with BD susceptibility in the Italian population [32]. Moreover, −634C and −2549I have been involved in the development of ocular inflammation of BD in Korean and Tunisian populations, respectively, but demonstrated no association with disease susceptibility [33, 34].

4.3.4 eNOS

Nitric oxide (NO), which is mainly produced in endothelial cells, plays an important role in regulating vascular homeostasis and contributes to vascular dilation, inhibition of platelet agglutination and cell-adhesion molecule expression, and vascular smooth muscle relaxation [35]. NO concentration is reportedly decreased in BD patients during the active period of the disease [36], suggesting that the decreased NO level may be centrally involved in endothelial dysfunction and thrombosis in BD patients. NO is synthesized from L-arginine by at least three distinct isoforms of the enzyme NO synthase (NOS). Three isoforms of NOS identified so far are NOS1 (neuronal NOS), NOS2 (inducible NOS), and NOS3 (endothelial NOS, or eNOS) [37]. Of these, eNOS is mainly expressed in the endothelial cells and contributes to the inhibition of platelet and leukocyte adhesion, inhibition of the proliferation and migration of vascular smooth muscle cells, and vascular dilation [35]. Several studies have been performed to assess the association between *eNOS* gene polymorphisms (+894 G/T and a 27-bp tandem repeat in intron 4) and BD; some, but not all, have reported that these polymorphisms are associated with BD [38]. A recent meta-analysis that includes these previous studies [38] has suggested that these polymorphisms do not confer susceptibility to BD in Turkish or Asian populations. Moreover, the meta-analysis also demonstrated that +894 G/T revealed a significant association with BD in European and Tunisian populations, while the risk allele of +894 G/T differed between these populations: the T allele was associated with increased disease risk in Europeans and decreased risk in Tunisians. There is still no clear explanation for this controversial result.

Although these four genes might indeed be susceptibility genes for BD, the genetic contributions of these genes to BD remain unclear. Most of the previously reported candidate gene approaches for BD were characterized by low statistical power because of small sample sizes and might therefore lead to false positive or false negative results. Aside from *HLA-B*51*, genetic factors with small or moderate effects appear to be involved in the risk of developing BD. To overcome this issue, large sample sizes (which usually allow even small effects to be statistically significant) would be required.

4.4 Genome-Wide Association Study Findings

A genome-wide association study (GWAS) is an approach that involves rapidly genotyping a dense panel of genetic markers that covers the entire genome and has great power to detect genetic variants that contribute to the risk of developing common and complex diseases. BD susceptibility genes/loci that have been successfully identified by GWASs include *HLA-A*26*, *IL10*, *IL23R-IL12RB2*, *STAT4*, *ERAP1*, *CCR1-CCR3*.

4.4.1 HLA-A*26

A GWAS of BD that employed 23,465 microsatellite markers was published in 2009 [39]. This GWAS identified one marker in the HLA class I region that is not in linkage disequilibrium with *HLA-B*51*. A comprehensive analysis of the HLA class I region found that the *HLA-A*26* allele was significantly associated with BD independently of *HLA-B*51*, suggesting that *HLA-A*26* is the second major susceptibility allele for BD. Individuals with *HLA-B*51*, *HLA-A*26*, or both account for approximately 80 % of all BD patients in Japan. The association of BD with *HLA-A*26* has also been reported in Taiwan, Greece, and Korea [40–42]. In addition, it has been reported that the phenotype frequency of *HLA-A*26* was increased ~7-fold in Saudi Arabian patients compared with healthy controls; however, this difference was not statistically significant [43]. Moreover, it has been suggested that *HLA-A*2601*, one of the major *HLA-A*26* subtypes, might be associated with ocular BD in the Japanese population and might also be a marker for poor visual prognosis [44]. In a Korean population study, *HLA-A*2601* and two other alleles (*HLA-A*0207* and *HLA-A*3004*) were significantly associated with an increased risk of developing BD, and *HLA-A*2601* was associated with uveitis; *HLA-A*0207* was associated with skin lesions and arthritis; and *HLA-A*3004* was associated with vascular lesions, genital ulcers, and a positive pathergy test, suggesting that certain *HLA-A* alleles are responsible for the unique clinical features of BD [42]. *HLA-A*26* was not associated with BD in Palestine, Jordan, Iran, Ireland, Italy, or Turkey [45–49], although Remmers et al. [50] have reported a strong association between the *HLA-A* region and BD independent of *HLA-B*51* in a Turkish population.

There are at least four possible reasons why this association has not been observed in all populations. The first reason is that the frequency of the *HLA-A*26* allele differs among ethnicities. It is more common in Japan, Taiwan, and Korea than in other areas; therefore, the association between *HLA-A*26* and BD may have been easily observed in these countries. Actually, Hughes et al. [11] reported the low allele frequency of *HLA-A*26* in Turkish and Italian populations, in which the association with BD cannot be assessed, while a strong association was observed for *HLA-A*0201*. Second, there have been differences between studies regarding sample size and research strategy. Previous studies with negative results in *HLA-A*26* did not recruit enough samples to provide statistically significant results; they also did not stratify the study population according to *HLA-B*51* status. Third, the environmental factor(s) required for the development of BD associated with the *HLA-A*26* antigen are distributed unevenly throughout the planet. Finally, other genetic factors are highly important in the development of BD in populations in which no association has been observed between *HLA-A*26* and BD. Thus, the association between BD and *HLA-A*26* has not clearly been verified, and further studies are needed to assess the association of BD with *HLA-A*26*.

4.4.2 IL10 and IL23R-IL12RB2

In 2010, two GWASs by Remmers et al. [50] and Mizuki et al. [51] that employed a dense panel of single-nucleotide polymorphism (SNP) markers reported the genes that encoded IL-10 (*IL10*), IL-23 receptor (*IL23R*), and IL-12 receptor beta (*IL12B2*) as novel BD susceptibility loci, and demonstrated that polymorphisms in these loci are associated with the risk of BD in Turkish, Japanese, and Korean populations. The association of BD with *IL10* polymorphisms has also been reported in Iranian, UK, Jordanian, and Palestinian populations [52, 53], while the association of BD with *IL23R* polymorphisms has also been observed in Chinese populations [54]. In addition, in an Iranian population, *IL23R* and *IL12RB2* polymorphisms were significantly associated with BD and the importance of *IL23R* regulatory regions has been highlighted with respect to susceptibility to BD [53]. Therefore, it is highly possible that an immune response involving *IL10* and *IL23R* (or *IL12RB2*) contributes to BD development.

As *IL10* polymorphisms are associated with decreased *IL10* mRNA expression, it has been suggested that decreased expression of IL-10, which down-regulates Th1-type immune responses [55, 56], is related to the development of BD. *IL12RB2*, which encodes an IL-12 receptor chain, is expressed on Th1 and natural killer cells [57, 58]. *IL12RB2* polymorphisms might enhance responsiveness against IL-12, leading to excessive Th1 immune responses. Thus, the activation of a Th1 immune response derived from *IL10* and *IL12RB2* polymorphisms might be involved in BD development.

IL23R encodes a subunit of the IL-23 receptor. *IL23R* is expressed on Th17 cells and macrophages. Recent studies have suggested that Th17 cells are closely correlated with clearance of extracellular bacterial infection, neutrophil chemotaxis, and autoimmune disease development [59–61]. In BD patients, immune response and protection against certain streptococcal infections (e.g., *Streptococcus sanguinis* are enhanced) suggest that these bacterial infections may serve as triggers for the disease development [62]. As a result, excessive migration of neutrophils into the disease lesions might be induced by enhanced neutrophil functions, contributing to the pathogenesis of BD. *IL23R* polymorphisms might enhance responsiveness to IL-23 in Th17 cells and other IL23R positive cells, and accordingly, the Th17 cell-mediated adaptive immune response as well as an innate response might be activated and promote BD development.

4.4.3 STAT4

Two SNP GWASs (by Hou et al. in 2012 [63] and Kirino et al. in 2013 [64]) identified the *STAT4* (signal transducer and activator of transcription 4) gene as a susceptibility gene for BD and demonstrated that *STAT4* polymorphisms are associated with the risk of BD in Turkish, Japanese, and Chinese populations. A study in a Korean population has also reported the association of intestinal BD

with a *STAT4* polymorphism [65]. In addition, the study also reported that polymorphisms in *IL17A* and *IL23R* were associated with intestinal BD and that gene–gene interactions were observed between *IL17A*, *IL23R*, and *STAT4* polymorphisms, suggesting that the joint effect of SNPs in *IL17A*, *IL23R*, and *STAT4* genes may modulate susceptibility to intestinal BD.

STAT4 encodes a transcription factor that transmits signals induced by several key cytokines, including IL-12 and IL-23 [66]. STAT4 is an essential element in the early events of Th1 differentiation [67]. STAT4 has also been implicated in the production of IL-17 by the IL-23-differentiated cells, suggesting that it may be involved in the survival or maintenance of Th17 cells [68]. *STAT4* mRNA expression is reportedly higher in individuals with the BD-associated alleles (A alleles of rs7574070 and rs7572482) than in individuals lacking these alleles in a European population [64]. Since both IL-12 and IL-23 act through STAT4, the BD-associated *STAT4* alleles may induce upregulated IL-12 and IL-23 activity, which can lead to the development of BD. In a Chinese population, a BD-associated *STAT4* allele (the A allele of rs897200) was also associated with the upregulation of *STAT4* and the transcription and protein expression of IL-17 (a Th17 cytokine), but not interferon-γ (a Th1 cytokine) [63]. These findings suggest that the A allele of rs897200 in *STAT4* might contribute to the pathogenesis of BD through the Th17 pathway, but not the Th1 pathway.

4.4.4 ERAP1

The endoplasmic reticulum aminopeptidase 1 (ERAP1) is centrally involved in peptide trimming before HLA class I presentation. Previous GWASs have shown that *ERAP1* polymorphisms are associated with psoriasis and ankylosing spondylitis [69–71]. There is evidence for gene–gene interactions between *ERAP1* polymorphisms and the disease-associated HLA alleles in both diseases [70, 72]: *ERAP1* polymorphisms affected psoriasis and ankylosing spondylitis susceptibility only in individuals carrying *HLA-C*06* and *HLA-B*27*, respectively.

The SNP GWAS by Kirino et al. in 2013 [64] revealed that *ERAP1* polymorphisms are associated with BD in Turkish populations. The study also identified evidence of an interaction between *HLA-B*51* and *ERAP1*; *ERAP1* variants affected the risk for BD only in *HLA-B*51*-positive individuals, and homozygosity for the risk allele T at the *ERAP1* locus rs17482078 was associated with an odds ratio (OR) for BD of 3.78 among *HLA-B*51*-positive individuals and an OR of 1.48 among *HLA-B*51*-negative individuals. These findings indicate that the BD-associated *ERAP1* variant contributes to disease susceptibility through an interaction with the *HLA-B*51* protein and that modulation of *ERAP1* may be effective in treating BD, especially in *HLA-B*51*-positive patients.

4.4.5 CCR1-CCR3

The SNP GWAS by Kirino et al. in 2013 [64] also identified BD-associated polymorphisms in the *CCR1* (chemokine (C–C motif) receptor 1)-*CCR3* region in Turkish and Japanese populations. Strong association signals were located in the 3′ untranslated region (UTR) of *CCR1*. The strongest, rs7616215, is located within DNase I hypersensitivity and histone 3 lysine 4 methylation sites, suggesting that the polymorphisms have an effect on transcription. The study actually demonstrated a significant correlation between the disease risk T allele of rs7616215 and enhanced expression of *CCR1* mRNA, but not *CCR3* mRNA. In addition, the study also found that the migration of monocytes in response to a gradient of the CCR1 ligand MIP1-α was less pronounced in T allele-positive individuals than in T allele-negative individuals, and that *CCR1* mRNA expression correlated significantly with the chemotactic activity of monocytes against a gradient of MIP1-α. Therefore, the study results suggest that impaired clearance of pathogens may contribute to the etiology of BD. Hou et al. reported that the *CCR1-CCR3* polymorphisms were also associated with BD in a Chinese population: rs13084057 in the 3′ UTR of *CCR1*, and rs13075270 and rs13092160 in the intergenic region between *CCR1* and *CCR3* (the 5′ UTR of *CCR1* or the 5′ UTR of *CCR3*) [73]. The study demonstrated that *CCR1* and *CCR3* mRNA expression were reduced in individuals with the TT genotype of rs13092160 (homozygosity for the risk allele), compared with those carrying the CT genotype (heterozygosity for the risk allele), suggesting that both *CCR1* and *CCR3* genes may contribute to the development of BD.

CCR1 and *CCR3* encode the beta chemokine receptor family, which belongs to the G protein-coupled receptor super family. These receptors play an important role in the recruitment and activation of inflammatory cells and contribute to autoimmune and allergic diseases [74, 75]. They are closely located in chromosome 3p21.3 and are considered to have originated from a common ancestral gene, suggesting that the expression of these genes may be regulated through similar pathways [76, 77]. Further investigation is needed to clarify the significance of *CCR1* and *CCR3* in the etiology of BD.

4.5 Conclusion

This chapter summarizes the genetic susceptibility factors for BD identified to date. In addition to the genes discussed above, the *KLRK1* (killer cell lectin-like receptor subfamily K, member 1)-*KLRC4* (killer cell lectin-like receptor subfamily C, member 4) locus on chromosome 12p13.2-p12.3 [64] and the *GIMAP* (GTPases of immunity-associated protein) locus on chromosome 7q36.1 [78] have been identified by GWASs and might also be important for the development of BD. Recent GWAS findings provide new insights into the genetic tendency underlying BD by connecting classically known findings and allow for clearer interpretation

of the etiology and pathophysiology of BD at the molecular level. Thus, findings from genetic studies can provide useful clinical information and open the door to the development of more accurate and reliable diagnostic and treatment approaches for BD.

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