A Darwinian view of Behçet’s disease

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Abstract

Behçet’s disease (BD) is a multisystem inflammatory disorder of unknown etiology, characterized by oral and genital ulceration, with other complications including eye, skin, joint, and central nervous system (CNS) lesions. Diagnosis is based on clinical findings, which may differ between patients. There is a strong genetic basis for BD; however, only a few genes have been associated with the disease across the geographical spread of BD. In this article, we discuss the history and combination of genes involved in this complex disease in relation to the geographical range and present our view that the disease has developed from a Darwinian perspective, with different gene polymorphisms that affect the same biological pathway. Moreover, these mutations individually are protective mechanisms against the disease relevant to each region, which affected both archaic and modern humans.

Keywords

genes • Behçet’s disease • introgression • neanderthal

Introduction

The sine qua non of Behçet’s disease (BD) is recurrent oral aphthous ulceration, which must be accompanied by two or more of the following major criteria: genital ulceration, uveitis, and skin lesions such as erythema nodosum and a positive pathergy test. A multitude of other clinical features, although not essential for diagnosis, is now recognized to be associated with the disease, such as seronegative arthropathy, increased risk of venous thrombosis, and gastrointestinal inflammation. The cause of BD is unknown, but an autoimmune inflammatory etiology, following infection by an as yet unidentified pathogen, has been suggested. In this scenario, a bacterial or viral infection of mucosa would induce an immune response, leading to cross-reactivity with self-proteins and producing the organ-specific damage, characteristic of BD. Although the precise etiology of BD remains mysterious, there is a strong genetic component to the disease, with a range of well-known associations with particular alleles.

HLA-B and chromosome 6

A heritable risk factor for BD was first identified in 1982 when an association with human leukocyte antigen-B5 (HLA-B5) in the human major histocompatibility complex (MHC) was first reported.[3] The HLA-B*5 locus includes the family of HLA-B51 alleles and HLA-B52. In the majority of populations, the greatest heritable risk factor for disease, and indeed for ocular disease severity, is HLA B51. In contrast, HLA-B*52, which differs from HLA-B*51 by only two amino acids in the peptide-binding groove, is not associated with BD in any population.[4] The geographical distribution of BD, concentrated mainly in countries from the Mediterranean basin to the Far East, led to the synonym of “Silk Road” disease. These regions lie between latitudes 30° and 45° north, and it is of interest that the reported prevalence of BD is associated with latitude and rarely encountered in more northern climes, the Americas, and Australasia. Furthermore, the geographical distribution of HLA-B*51 among healthy subjects roughly corresponds with global disease distribution.[6] This association
between geographical disease distribution and HLA-B*51 led to the hypothesis that genetic risk factors were propagated by migrant traders operating along the Silk Road some 2000 years ago. However, the new discipline of paleogenetics, involving recent advances in techniques for recovering and analyzing ancient DNA (aDNA), has produced evidence for much earlier acquisition of some HLA alleles, during Paleolithic migrations of modern humans (Homo sapiens) and admixture with archaic human species.

Following the draft sequence of the Neanderthal genome, it was estimated that the Neanderthal contribution represented between 1% and 4% of modern human genomes. Recent estimates of confidently inferred Neanderthal ancestry yield means of 1.38% and 1.15% in East Asian and European populations, respectively, but <0.1% of the genome in sub-Saharan African populations. Long stretches of modern Eurasian genomes lacking Neanderthal ancestry suggest that the genetic contribution was initially much larger, estimated at >3%, but that negative selection has operated to reduce the Neanderthal component now present in modern human genomes.

Evidence of another archaic population, genetically distinct from Neanderthals, comes from deoxyribonucleic acid (DNA) extracted from a finger bone found in the Denisova Cave in southern Siberia. It is not clear how this population relates to other known hominin species, so the genetically identified population is currently known simply as the “Denisovans”. While present-day Melanesian genomes are estimated to contain a Denisovan contribution of 4–6%, the Denisovan contribution to European genomes appears to be negligible. The Denisovan contribution to the genomes of living East Asians and Native Americans is higher, at around 0.2%, but this may be due to admixture with the ancestors of the modern-day inhabitants of Oceania, long after interbreeding with the Denisovans themselves. There is now genetic evidence for three to five instances of interbreeding between hominin populations, including modern humans and Neanderthals, although this is accepted to provide a low-resolution reconstruction of demographic history.

Regardless of the imprecision surrounding the events that led to the admixture and incorporation of archaic DNA into modern human genomes, it is now clear that modern humans have received input from archaic genomes and these contributions have variously been both beneficial and deleterious within modern human genomes. Focusing on HLA genes, it seems likely that modern humans acquired the HLA-B*73 allele through admixture with Denisovans. HLA-B*51 was identified in Neanderthal samples and is found in high frequencies in modern human populations in Eurasia but not in Africa. Simulations of HLA gene introgression strongly supported a Neanderthal origin for HLA-B*51 in modern genomes, among others. The evidence suggests that Neanderthal admixture contributed HLA-B*07, B*51, C*0702, and C*1602 to modern human genomes. Of these alleles, HLA-B*51 and HLA-C*1602 are associated with BD. Importantly, the frequency of these alleles in modern humans matches the prevalence of BD very closely [Figure 1]. The existence of these HLA alleles in archaic genomes and their preservation in modern genomes, when other Neanderthal genes were subject to negative selection, strongly suggest that these alleles were beneficial in the context of local pathogens encountered by both Neanderthal populations and modern human populations living in similar environments.

Indeed, Neanderthals and their ancestors had been living in Europe and Western Asia for hundreds of thousands of years before modern humans, expanding out of Africa or the Middle East, arrived in that territory. It seems likely that the immune system of Neanderthals had adapted to the local pathogen pool and that this genetic advantage was passed on to modern human populations through interbreeding. The links between admixture, retention of archaic DNA, and disease in human populations, archaic and modern, are likely to be related to genes that were and are protective against infectious diseases. Piga and Mathieu had proposed such a link with Neanderthal admixture and negative selection of HLA-B*5101 and Plasmodium falciparum infection and a positive selection in response to Yersinia pestis to describe the geoepidemiology distribution of BD.

The identification of single nucleotide polymorphisms (SNPs), of Neanderthal origin, that are the risk factors for a range of diseases including systemic lupus erythematosus, biliary cirrhosis, Crohn’s disease, smoking behavior, and type 2 diabetes (T2D) further supports a model of the introgression of Neanderthal genes, exerting a positive influence on the survival of early modern humans but leading to increased susceptibility to disease in modern individuals. The current Sars-CoV-2 pandemic has supported a role for Neanderthal-derived gene mutation and disease severity. Increased levels of a Neanderthal haplotype of 25′ oligoadenylate synthase (OAS1) in Europeans have a protective effect in Coronavirus-19 (COVID) infection. By comparison, a Neanderthal core haplotype of a gene cluster on Chr 3 is positively selected in South Asians, particularly Bangladeshis, is associated with a more severe response to COVID.

The link between archaic alleles and disease in modern humans is paradoxical; if such alleles were retained due to their protective nature, why are they linked with the disease in current populations? Several possibilities exist: first, until very recently, life expectancy was very short, and in particular, infant mortality very high. Genetic polymorphisms that helped to control pathogens in childhood and adolescence, allowing adulthood to be reached, would be retained—regardless of whether those polymorphisms caused “damage” later in
identified in multiple genetic studies as the strongest association, recent deep sequencing of the HLA loci in BD patients with imputation studies based on the 1000 Genomes Project was performed in two different independent BD cohorts. The robust association with HLA-B*51 was explained by a variant between B*51 and MHC class 1-related A (MICA; rs116799036), which was independent of B*51. Additional independent associations in psoriasis susceptibility locus (PSORS1), HLA-f-AS1, and HLA-Cw*1602 were also identified and replicated.\[17\] However, the independence of rs116799036 was not supported in the analysis of a larger cohort of patients that strongly backed HLA-B as the primary gene association on chromosome 6. Importantly, the amino acids relevant to the HLA-B*51 association were identified and included HLA-B*67, which is one of the two amino acid differences between B*51 and B*52. The latter is protective in Middle Eastern patients with BD.\[18\]

In support of the concept that HLA-B has been the major risk for BD, imputation studies identified SNP in endoplasmic reticulum-expressed aminopeptidase (ERAP1) (rs17482078). There was evidence of epistasis between ERAP1 and HLA-B*51. ERAP1 is a protein involved in trimming peptides to fit MHC class I molecules; mutations conferred risk for BD in HLA-B*51-positive individuals, with homozygosity of ERAP1 rs17482070 giving an OR of 3.78.\[19\] A haplotype, based on ERAP-1 sequencing, designated Hap10 with five non-ancestral amino acids was reported to be associated with BD.\[20\] Individual variants encoded by this haplotype rs2287987, rs10050860, and rs17482078 were previously reported by studies in various ethnic groups to be

**Figure 1:** BD, HLA-B*51, and archaic DNA. The frequency of HLA-B*51 is highest in the Middle East (red—high and green—low) and decreases across the Silk Road (dotted line) to China. The prevalence of BD follows a similar pattern (number/100,000 in boxes). Circles show the sites of bones used in typing archaic genomes. BD, Behçet’s disease; HLA-B51, human leukocyte antigen-B51.
associated with BD.\textsuperscript{[21, 22]} Such variants influence the binding of low-affinity peptidomes to HLA-B*5101, which may be relevant in BD. Specifically, 2 subpeptidomes were identified, whereby only peptides in the subpeptidome with Ala at position 2 are extensively destroyed, except when their position 1 residues are ERAP-1 resistant.\textsuperscript{[23, 24]} Recently, KIR3DL1 allele-level analysis revealed the KIR3DL1\textsuperscript{C}/*KIR3DS1 functional genotype to be implicated in disease pathogenesis and KIR3DL1\textsuperscript{HG}/*KIR3DL1\textsuperscript{HL} to be protective.\textsuperscript{[25]} These data suggest a complex mechanism by which HLA-B*5101 is involved in BD and more functional studies are required to elucidate the process.

Other loci on chromosome 6

Tumor necrosis factor (TNF) is a pro-inflammatory cytokine whose serum levels are raised in patients with BD and inhibition of TNF by various biologic drugs is effective in controlling the disease. The gene for TNF is on human chromosome 6. Many SNPs in TNF have been analyzed since the −1031 promoter allele was associated with BD in UK patients.\textsuperscript{[26]} Subsequently, −1031C has been linked with the disease in Turkish and Tunisian with BD.\textsuperscript{[27, 28]} Similarly, −308A/G in TNF has been associated with disease in Iranian Azeri Turks but not in Palestinian patients.\textsuperscript{[4, 29]} In Korean patients, none of the tested TNF SNP were associated with the disease.\textsuperscript{[30]} Therefore, SNPs in this important proinflammatory cytokine are associated with BD in many populations, but which polymorphism would appear to differ in different geographical cohorts.

Non-chromosome 6 associations

(a) Lymphocyte Signaling

Genome-wide analysis studies (GWAS) in a cohort of 379 Korean patients with Behçet’s and 800 controls have found a significant association with the GTPase of immunity-associated protein family (GIMAP) gene cluster on chromosome 7q36.1. Genome-wide significance was found using a dominant model of inheritance for 5 SNPs within the GIMAP cluster (rs1608157, rs1522596, rs10266069, rs10256482, and rs2286900) with subsequent fine-mapping efforts finding an association with 10 additional markers although these were distributed across 3 blocks of linkage disequilibrium (LD) within this ~200 kbp region. Replication of the signal was achieved for SNPs mapping to the GIMAP2 and GIMAP4 regions, but not GIMAP1, in a Japanese cohort. Additionally, functional data of 31 BD patients showed significantly reduced expression of GIMAP1, GIMAP4 (both in CD4 T cells), and GIMAP2 (in CD8 T cells) when compared to controls ($P < 0.05$). Further to this, using a luciferase reporter assay they found that a construct having the risk allele (C) within rs1608157 locus (the most significant GWAS SNP) had reduced luciferase activity compared to the G allele implying that the polymorphism negatively affected GIMAP4 promoter activity. However, GIMAP SNPs were not validated in the UK or European patients with BD.\textsuperscript{[31, 32]}

Several other genes linked to the severity of particular manifestations of BD, associated with T cell signaling, have been reported and many of these have been validated in multiple studies. Certain gene polymorphisms associated with other autoimmune diseases, including protein tyrosine phosphatase non-receptor type 22 (PTPN22) and cytotoxic T-lymphocyte antigen 4 (CTLA-4), were shown to have a negative association—results that support the concept that BD is an autoinflammatory rather than an autoimmune disease.\textsuperscript{[33]} PTPN22 R620W polymorphism is most prevalent in northern Europe and protective against Mycobacteria tuberculosis but induces susceptibility to Mycobacteria leprae, which was more common in the Middle East, where PTPN22 R620W is very rare. PTPN22 R620W was inversely associated with BD in patients particularly in a UK cohort.\textsuperscript{[34]} This association was seen neither in patients from the Middle East and Southern Europe, where the prevalence of the polymorphism is much lower, nor in Chinese patients with BD, where the polymorphism does not exist.\textsuperscript{[35–37]} PTPN22 encodes a protein Lyp that binds to Csk, an inhibitory kinase, and together disrupt T cell signaling through dephosphorylation of Lck. The R620W SNP disrupts this interaction.\textsuperscript{[38]} In myeloid cells, PTPN22 positively increases type 1 interferon production, and dependent viral infection and gut homeostasis.\textsuperscript{[39]} In mice, carriage of 620W led to increased autoreactive T cell clones in new bone-marrow emigrants.\textsuperscript{[40]} Inhibition of PTPN22 activity restored central B cell tolerance.\textsuperscript{[41]} Superresolution microscopy showed that while wild type PTPN22 forms large clusters in unstimulated T cells and dissociates on stimulation by LFA-1, on ICAM-1 monolayers, enabling association with Lck and ZAP70 at the leading edge. By comparison, PTPN22 620W was not retained at the leading edge resulting in increased LFA-1 clustering and integrin-mediated adhesion.\textsuperscript{[42]} PTPN22 is also highly expressed in neutrophils. R620W enhanced neutrophil migration and increased Ca$^{2+}$ release, in humans, and is a critical regulator of FcR activation and interaction with immune complexes by neutrophils in mice.\textsuperscript{[43–45]}

For CTLA4 polymorphisms, the situation is even more complex. Early studies on Turkish and Tunisian patients with BD found associations with certain manifestations of the disease.\textsuperscript{[46, 47]} However, studies on the UK, Middle Eastern, and Chinese patients showed no association.\textsuperscript{[48–50]} Meta-analysis of eight studies including those above showed no association with BD.\textsuperscript{[51]} However, whether certain features of BD are linked to CTLA4 polymorphisms or they are associated with other combinations of genes will need larger studies.

A signal transducer and activator of transcription 4 (STAT4) association are supported by a study on Han Chinese patients with BD, with SNP rs7574070 and additional SNPs rs7572482 and rs897200, all associated although in strong
LD. In addition, Hou et al. showed that BD patients' homozygous for the rs897200 risk allele had increased expression of the STAT4 mRNA and protein, as well as IL-17, which is regulated by the gene.[52] Furthermore, ENCODE data reveal that the rs897200 polymorphism determines the formation of a transcription factor binding site, and the functional studies by the authors indicate that this SNP is likely to be an expression of quantitative trait locus (eQTL) of STAT4. Association of STAT4 was further reported in patients with BD from Turkey and Iran.[24, 63] Although a STAT4 gene association (rs7574865) has been reported in a number of autoimmune diseases including RA and systemic lupus erythematosus, variants associated with BD are in low LD, with this particular marker implying that such associations are independent of each other and, therefore, may confer different mechanisms of pathogenesis.[24]

Therefore, several polymorphisms in genes involved with T cell signaling are reported in BD but not in all populations. However, that many genes signaling through different, though interconnected, pathways in different geographical populations influence the same outcome that supports a Darwinian concept of BD [Figure 2].

(b) Interleukin-10 (IL10)/IL12R/IL23R

GWAS identified polymorphisms in the IL10 and IL23R/IL12RB2 genes, which may help to explain the inflammatory response in BD.[54, 55] Both IL10 as an anti-inflammatory molecule and IL23R as a member of the pro-inflammatory IL-17 pathway have been identified as associated with BD in candidate gene studies and, therefore, their detection in GWAS is intriguing.[56, 57] The interleukin genes IL10’s and IL23R’s roles within BD pathogenesis have been elucidated from the above-described independent but concurrent Turkish and Japanese GWAS. Genotyping identified an association with the IL10 intronic rs1518111 variant in a combined cohort, which correlated with IL-10 production by blood monocytes, implicating low IL-10 as a risk factor of BD.[55] A second intronic SNP rs1554286 was initially associated with BD in the Japanese study; however, further investigation and LD analysis fine mapped the association to the IL10 promoter region with variants rs1800872 and rs1800871 having a stronger association. A subsequent meta-analysis of the data with additional Korean samples and Turkish GWAS data identified rs1800871 as the most significant risk marker. Replication of the IL-10 association has since been achieved in other geographical cohorts of patients with BD.[58]

Within a ~50 kbp intergenic region between the IL23R and IL12RB2 genes on chromosome 1, SNPs rs12119179, rs1495695, rs17375018, and rs924080 have all been associated with BD from GWAS. SNP rs12119179 was initially associated with the Japanese cohort.[59] Fine mapping showed a stronger association with rs1495965 which in meta-analysis of a Korean cohort.[59] No association with this region was detected in the Turkish GWAS alone although SNP rs924080 was significantly associated in their combined cohort.[55] Recent functional analysis in PBMCs has further demonstrated that the rs924080 risk allele increases expression of IL6 and augments the expression capacity of both IL23R and TNF-α when stimulated with lipopolysaccharide, leading the authors to suggest that the risk allele underpins an inappropriately increased inflammatory response to bacterial pathogens that ultimately increases the risk of BD onset.[61] In addition to this, an earlier study investigating the IL23 signaling pathway genes had shown evidence of BD association with IL23R SNPs rs17375018, rs11209032, and rs924080 in Chinese cohorts, although there has been no GWAS association with this region within Chinese Han to date.[62, 63]

(c) Pathogen Recognition

Pathogens are recognized by a family of molecules, the best known of which are Toll-like receptors (TLR). Non-synonymous variants identified by deep-sequencing of 10 identified genes (GWAS), and 11 identified by association with innate immunity, Table 1 showed TLR4 was associated with BD, while rare nucleotide-binding oligomerization domain-2 (NOD2) variants were nominally significant. TLR4 variants that are risk factors in Crohn’s are protective in BD.[64] TLR expression in buccal lesions from BD patients showed an increase in positive cells, but this was no different from other inflammatory conditions tested. Polymorphisms in TLR2 and TLR4 showed no association with BD; however, a polymorphism in

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Figure 2: GIMAP and PTPN22, identified with BD in different geographic populations are linked to many similar molecules that are involved in lymphocyte activation such as STAT3 (signal transducer and activator of transcription 3). Image in Biorender.
Table 1: GWAS published to date on BD highlighting where the established genes have been identified

| Study                      | Population | Cohort type | Cases | Controls | HLA-BS1 (6p21) | IL10 (1q31-q32) | IL23R-IL12RB2 (1p31) | CCR1-CCR3 (3p21) | ERAP1 (5q15) | STAT4 (2q32) | KLRC4 (12p13-p12) | GIMAP1-4 (7q36) | UBAC2 (13q32) |
|----------------------------|------------|-------------|-------|----------|----------------|------------------|---------------------|------------------|--------------|--------------|------------------|-----------------|-------------|
| Fei et al.[74]             | Turkish    | Discovery   | 156   | 172      | MS             | MS               | P < 0.05            | P > 0.05         |              |              |                  |                  |             |
| Salwaha et al.[75]         | Turkish    | Replication | 376   | 369      |                | MS               | P < 0.05            | P > 0.05         |              |              | MS               |                  |             |
| Fine mapping & meta-analysis | Italian    | Replication | 144   | 560      |                | MS               | P < 0.05            | P > 0.05         |              |              | MS               |                  |             |
| Remmers et al.[56]         | Turkish    | Discovery   | 1215  | 1278     | GWAS           | GWAS             | MS                  |                  |              |              |                  |                  |             |
| Middle Eastern Arab        | Turkish    | Replication | 110   | 224      | P < 0.05       | P > 0.05         |                    |                  |              |              |                  |                  |             |
| Greek                      | Greek      | Replication | 107   | 84       | P < 0.05       | P > 0.05         |                    |                  |              |              |                  |                  |             |
| Korean                     | Korean     | Replication | 77    | 52       | P < 0.05       | P > 0.05         |                    |                  |              |              |                  |                  |             |
| UK Caucasian              | Replication | 120      | 119    | P < 0.05  | P > 0.05       |                    |                    |                  |              |              |                  |                  |             |
| All                        | Meta-analysis | 2374  | 2600   | GWAS       | GWAS            |                    |                    |                  |              |              |                  |                  |             |
| Japanese                   | Japanese   | Discovery   | 612   | 740      | GWAS           | GWAS             | GWAS                |                  |              |              | GWAS            |                  |             |
| Turkish                    | Turkish    | Replication | 1215  | 1279     | P < 0.05       | P < 0.05         |                    |                  |              |              | GWAS            |                  |             |
| Chinese                    | Chinese    | Discovery   | 149   | 951      | GWAS           |                  |                    |                  |              |              |                  |                  |             |
| Hox et al.[114]            | Chinese    | Replication | 554   | 1159     | P < 0.05       |                  |                    |                  |              |              |                  |                  |             |
| Combined                   | Meta-analysis | 703   | 2010   | GWAS       |                  |                    |                    |                  |              |              |                  |                  |             |
| Lee et al.[31]             | Korean     | Discovery   | 379   | 800      | GWAS           |                  |                    |                  |              |              | GWAS            | P < 0.05        |             |
| Japanese                   | Japanese   | Replication | 363   | 272      |                |                  |                    |                  |              |              |                  |                  |             |
| Turkish                    | Imputed    | 1209        | 1278  | GWAS       | GWAS           | GWAS             | GWAS                | GWAS             |              |              | MS               | MS             |             |
| Kirino et al.[63]          | Turkish    | Replication | 838   | 630      | P < 0.05       | P < 0.05         |                    |                  |              |              | P < 0.05        | MS             | MS          |
| Japanese                   | Japanese   | Replication | 612   | 740      | P > 0.05       | Mono             | P < 0.05            |                  |              |              | P < 0.05        | MS             | MS          |
| Combined                   | Meta-analysis | 2659  | 2648   | GWAS       | GWAS           | GWAS             | GWAS                | GWAS             |              |              | GWAS            | GWAS           |             |

Rec: Recessive genotypic model; GWAS: Genome wide significance; MS: Moderate levels of significance; Turkish: Same Turkish cohort; Same Japanese cohort; Mono: polymorphism monomorphic in studied population; Any blank cells: No data / information disclosed. Chromosomal position of genes specified in brackets below gene name.
BD, Behçet’s disease; ERAP1, endoplasmic reticulum-expressed aminopeptidase; GWAS, genome-wide analysis studies; GIMAP, GTPase of immunity associated protein family; HLA-BS, human leukocyte antigen-BS; IL10, interleukin-10.
the downstream TLR signaling molecule, TIRAP, that leads to gain of function is associated with BD.\textsuperscript{[66]} SNPs in TLR2 rs2289318 and rs3804099 were associated with ocular disease in Han Chinese patients with BD. Both polymorphisms were linked to altered TLR2 mRNA expression in peripheral blood mononuclear cells stimulated with peptidoglycan.\textsuperscript{[66]} NOD2 is an intracellular recognition molecule that recognizes muramyl dipeptide present in some bacteria. SNPs in NOD2 associated with Crohn’s disease were not associated with BD in British, Turkish, or Arab patients in initial studies, with one SNP inversely associated with the disease.\textsuperscript{[67]} A potential protective association was reported in a second study with SNP to be protective in Caucasian patients with BD.\textsuperscript{[68]} In a recent study of a Spanish cohort, a single rare SNP was associated with the protection of patients with BD.\textsuperscript{[69]}

FUT2 encodes an \(\alpha\)-(1,2) fucosyltransferase that regulates the secretion of the H antigen (precursor of the human ABO blood group antigens) in body fluids and intestinal mucosa. About 80% of people have the secretor phenotype, which is determined by the presence of at least one functional FUT2 allele. FUT2 is expressed in the intestinal epithelial cells and fucosylated proteins are shed into the lumen. Fucose, which is metabolized by certain gut bacteria that induce short-chain fatty acids such as butyrate and downregulating virulence genes in other bacterial spp. FUT2 is increased in epithelial cells via TLR signaling.\textsuperscript{[70]} Five coding SNPs were identified in Iranian patients with BD.\textsuperscript{[71]} Meta-analysis of Iranian and Turkish GWAS data supported the initial report that rs601338 allele is associated with disease in Turks and Iranians patients with BD and rs1047781 allele is associated with disease in Japanese patients.\textsuperscript{[72]} These non-secretor genotypes have been associated with increased predisposition or resistance to different infectious agents such as norovirus but are not associated with the gut microbiome composition.\textsuperscript{[73]}

**Conclusion**

The strongest association with BD is HLA-B*51 on chromosome 6, a link that has been confirmed in several studies in different geographical groups. HLA-B*51 is derived from Neanderthal admixture and is likely to have been maintained in modern humans as part of a protective mechanism against a disease that affected both archaic and modern humans. Beyond chromosome 6, SNPs in genes involved in lymphocyte signaling and function, along with ubiquitination and pathogen recognition, which may be protective in evolutionary time but when brought together represent further evidence supporting a Darwinian view of BD. The identification of different polymorphisms in different geographical cohorts of patients with BD supports the accumulation of small mutations that, when combined with HLA-B*51, lead to the disease. However, it is now becoming clear that these different, positively selected SNPs are, in many cases, affecting the same biological pathways. An excellent, comprehensive genetic and epigenetic analysis identified a heritability of 60% in BD incorporating mutations in genes associated with the immune response, including interferon-gamma and IL-12 production, regulation of lymphocyte-mediated immunity cytokine–cytokine receptor interaction, and regulation of innate immune response. Genetic cumulative risk scores supported the known prevalence of BD globally.\textsuperscript{[74]} These new insights cast light on the biochemical pathway involved in BD, beyond links with individual genes. This emerging understanding of the evolutionary, genetic, and biochemical basis of BD may explain much of the variation in BD prevalence with geography and may even suggest new directions for therapeutic research. In conclusion, protective genes inherited from archaic humans in the distant past, interacting with other polymorphisms in modern humans, may explain the strong link that we observe between HLA-B and BD.

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**References**

[1] Saadoun D, Wechsler B. Behcet’s Disease. Orphanet J Rare Dis, 2012;7(1):1–6.
[2] Gul A. Genetics of Behcet’s Disease: Lessons Learned from Genomewide Association Studies. Curr Opin Rheumatol, 2014;26(1):56–63.
[3] Ohno S, Ohguchi M, Hirose S, et al. Close Association of HLA-Bw51 with Behcet’s Disease. Arch Ophthalmol, 1982;100(9):1455–1458.
[4] Verity DH, Marr JE, Ohno S, et al. Behcet’s Disease, the Silk Road and HLA-B51: Historical and Geographical Perspectives. Tissue Antigens, 1999;54(3):213–220.
[5] Green RE, Krause J, Briggs AW, et al. A Draft Sequence of the Neandertal Genome. Science, 2010;328(5979):710–722.
[6] Sankararaman S, Mallick S, Dannemann M, et al. The Genomic Landscape of Neandertal Ancestry in Present-day Humans. Nature, 2014;507(7492):354–357.
[7] Krause J, Fu Q, Good JM, et al. The Complete Mitochondrial DNA

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**Conflict of Interest**

Robert J. Moots is the Co-Editor-in-Chief of the journal. The article was subject to the journal’s standard procedures, with peer review handled independently of this editor and his research groups.
Genome of an Unknown Hominin from Southern Siberia. Nature, 2010;464(7290):894–897.
[8] Reich D, Green RE, Kircher M, et al. Genetic History of an Archaic Hominin Group from Denisova Cave in Siberia. Nature, 2010;468(7327):1053–1060.
[9] Prufer K, Racimo F, Patterson N, et al. The Complete Genome Sequence of a Neanderthal from the Altai Mountains. Nature, 2014;505(7481):43–49.
[10] Abi-Rached L, Jobin MJ, Kulkarni S, et al. The Shaping of Modern Human Immune Systems by Multiregional Admixture with Archaic Humans. Science, 2011;334(6052):89–94.
[11] Piga M, Mathieu A. The Origin of Behçet’s Disease Geodepidemiology: Possible Role of a Dual Microbial-driven Genetic Selection. Clin Exp Rheumatol, 2014;32(S84):S123–S129.
[12] Zeberg H, Pääbo S. A Genomic Region Associated with Protection Against Severe COVID-19 is Inherited from Neandertals. Proc Natl Acad Sci U S A, 2021;118(9):e2026309118. doi: 10.1073/pnas.2026309118.
[13] Zhou S, Butler-Laporte G, Nakanishi T, et al. A Neanderthal OAS1 Isoform Protects Individuals of European Ancestry Against COVID-19 Susceptibility and Severity. Nat Med, 2021;27(4):659–667. doi: 10.1038/s41591-021-01281-1.
[14] Williams GC. Pleiotropy, Natural Selection, and the Evolution of Senescence. Evolution (BioOne), 1957:11;398–411.
[15] Pfeffer-Gík T, Levine A. Dietary Clues to the Pathogenesis of Crohn’s Disease. Dig Dis, 2013;32(4):389–394.
[16] Horowitz A, Strauss-Albee DM, Leipold M, et al. Genetic and Environmental Determinants of Human NK Cell Diversity Revealed by Mass Cytometry. Sci Transl Med, 2013;5(208):208ra145.
[17] Hughes T, Coit P, Adler A, et al. Identification of Multiple Independent Susceptibility Loci in the HLA Region in Behçet’s Disease. Nat Genet, 2013;45(3):319–324.
[18] Ombrello MJ, Kirino Y, de Bakker PI, et al. Behçet Disease-associated MHC Class I Residues Implicate Antigen Binding and Regulation of Cell-mediated Cytotoxicity. Proc Natl Acad Sci U S A, 2014;111(24):8867–8872.
[19] Kirino Y, Bertsias G, Ishigatsubo Y, 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(2):202–207.
[20] Takeuchi M, Ombrello MJ, Kirino Y, et al. A Single Endoplasmic Reticulum Aminopeptidase-1 Protein Allele is a Strong Risk Factor for Behçet’s Disease in HLA-B*51 Carriers. Ann Rheum Dis, 2016;75(12):2208–2211.
[21] Zhang L, Yu H, Zheng M, et al. Association of ERAP1 Gene Polymorphisms with Behçet’s Disease in Han Chinese. Invest Ophthalmol Vis Sci, 2015;56(10):6029–6035.
[22] Padula MC, Lecesse P, Pellizzieri E, et al. Distribution of rs17482078 and rs27044 ERAP1 Polymorphisms in a Group of Italian Behçet’s Syndrome Patients: A Preliminary Case-control Study. Intern Emerg Med, 2019;14(5):713–718.
[23] Guasp P, Barnea E, González-Escribano MF, et al. The Behçet’s Disease-associated Variant of the Aminopeptidase ERAP1 Shapes a Low-affinity HLA-B*51 Peptidome by Differential Subpeptidome Processing. J Biol Chem, 2017;292(23):9680–9689.
Analysis of CD28 Genome-wide Association PTPN22 Is a Critical CTLA-4+49A/G Low Prevalence of Dense Genotyping of No Association of CTLA-4 TIRAP Ser180Leu

[51] Lee YH, Song GG. CTLA-4 Polymorphisms and Susceptibility to Behcet’s Disease in a Chinese Han Population. Ann Rheum Dis, 2007;68(2):122–127.

[52] Hou S, Yang Z, Du L, et al. No Association of CTLA-4 Polymorphisms with Susceptibility to Behcet’s Disease. Br J Ophthalmol, 2009;93(10):1378–1381.

[53] Sousa I, Shahram F, Francisco D, et al. Brief Report: Association of CCR1, KLRC4, IL12A-AS1, STAT4, and ERAP1 with Behçet’s Disease in Iranians. Arthritis Rheumatol, 2015;67(10):2742–2748.

[54] Mizuki N, Meguro A, Ota M, et al. Genome-wide Association Studies Identify IL23R-IL12RB2 and IL10 as Behçet’s Disease Susceptibility Loci. Nat Genet, 2010;42(8):703–706.

[55] Remmers EF, Cosan F, Kirino Y, et al. Genome-wide Association Study Identifies Variants in the MHC Class I, IL10, and IL23R-IL12RB2 Regions Associated with Behçet’s Disease. Nat Genet, 2010;42(8):698–702.

[56] Wallace GR, Kondeatis E, Vaughan RW, et al. IL-10 Genotype Analysis in Patients with Behcet’s Disease. Hum Immunol, 2007;68(2):122–127.

[57] Jiang Z, Yang P, Hou S, et al. IL-23R Gene Confers Susceptibility to Behçet’s Disease in a Chinese Han Population. Ann Rheum Dis, 2010;69(7):1325–1328.

[58] Jung JH, Song GG, Lee YH. Meta-Analysis of Associations Between Interleukin-10 Polymorphisms and Susceptibility to Vasculitis. Immunol Invest, 2015;44(6):553–565.