# INVESTIGATION OF HLA-B*51 SUB-ALLELES IN HLA-B*51 POSITIVE BEHCET PATIENTS
# HLA-B*51 POZİTİF BEHÇET HASTALARINDA HLA-B*51 ALLELLERİNİN ARAŞTIRILMASI

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**MAKALE BİLGİLERİ**
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**ABSTRACT**

**Aim:** The initial identification of Behçet's disease (BD) occurred in 1937, credited to the Turkish dermatologist Prof. Dr. Hulusi Behçet. Symptoms included repeated oral aphthae, genital ulcers, and uveitis. Then, it was revealed that the illness involves the joints, blood vessels, intestines, lungs, and brain system. Age, gender, psychological factors, infectious agents, immune responses, and genetic predispositions affect illness progression. The most common genetic risk factor is HLA-B*51. This study aimed to examine the relationship between HLA-B*51 sub-alleles and illness etiology.

**Materials and Method:** We compared the prevalence of two HLA-B*51 sub-alleles (HLA-B*51:01 and HLA-B*51:08) in patients with BD versus healthy individuals. The samples of 24 HLA-B*51 positive individuals were typed by Polymerase Chain Reaction with Sequence-Specific Primers (PCR-SSP). In the control group, 73 healthy bone marrow donors were HLA-B*51 subtyped by DNA sequencing.

**Results:** The prevalence of HLA-B*51:01 and 51:08 sub-alleles in patients was 80% and 20%, respectively. The frequencies of HLA-B*51:01, 02, 05, 07, and 08 in the control group were 90.4%, 4.1%, 2.7%, 1.4%, and 1.4%, respectively. There was no significant difference in HLA-B*51:01 allele frequency between patient and control groups ($p>0.05$; $p=0.457$ $RR<1$). Statistically significant differences were seen for the HLA-B*51:08 allele ($p<0.05$; $p=0.003$ $RR=18.8$). No statistically significant correlation was found between HLA-B*51:01 and HLA-B*51:08 sub-alleles and clinical symptoms (p-value > 0.05).

**Conclusion:** HLA-B*51:08 sub-allele may be an important risk factor for BD development. Future investigations can further highlight the significance of its role in the pathophysiology of the disease.

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## ÖZET

**Amaç:** 1937 yılında Türk Dermatolog Prof. Dr. Hulusi Behçet tarafından keşfedilen Behçet Hastalığının (BH) semptomları tekrar eden oral aft, genital ülserler ve üveyitti. Daha sonra bu hastalığın eklemleri, kan damarlarını, bağırsakları, akciğerleri ve beyin sistemini de kapsadığı bildirilmiştir. Hastalığın seyrini yaş, cinsiyet, fizyolojik faktörler, enfeksiyöz ajanlar, immün yanıt ve genetik geçişler etkiler. En yaygın görülen genetik risk faktörü İnsan Lökosit Antijeni (HLA)-B*51'dir. Bu çalışmada HLA-B*51 alt alleleri ile hastalık etyolojisi arasındaki ilişki irdelenmesi amaçlanmıştır.

**Gereç ve Yöntem:** BH hastaları ile kontrol grubu arasında HLA-B*51 alt allellerinin prevalanslarını karşılaştırdık. Sekans spesifik primer polimeraz zincir reaksiyonu (SSP-PZR) yöntemiyle HLA-B*51 pozitif olan 24 kişinin doku tiplemesi yapılmıştır. Kontrol grubunda ise kemik iliği donörü olan 73 sağlıklı bireyin HLA-B*51 alt allel tiplemesi DNA dizi analizi yöntemiyle belirlenmiştir.

**Bulgular:** Hasta grubunda HLA-B*51:01 ve 51:08 alt allellerinin prevalansı sırasıyla %80 ve %20 bulunmuştur. HLA-B*51:01, 02, 05, 07 ve 08 alt allellerinin kontrol grubundaki sıklıkları sırasıyla %90,4, %4,1, %2,7 %1,4 ve %1,4 olarak bulunmuştur. Hasta ve control grubu arasında HLA-B*51:01 alt allelinin sıklığı bakımından istatistiksel olarak anlamlı bir farklılık bulunmamıştır ($p>0.05$; $p=0.457$ $RR<1$). İstatistiksel olarak anlamlı sonuçlar HLA-B*51:08 alt alleli için bulunmuştur ($p<0.05$; $p=0.003$ $RR=18.8$). HLA-B*51:01 ve HLA-B*51:08 alt allelleri ile klinik semptomlar arasında anlamlı bir ilişki bulunmamıştır.

**Sonuç:** HLA-B*51:08 alt allelinin BH gelişiminde önemli bir faktör olabilir. Yapılacak çalışmalarla hastalığın patofizyolojisi açısından önemi gösterilebilir.

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## Introduction

Prof. Dr. Hulusi Behçet, a famous Turkish dermatologist, discovered Behçet's Disease (BD) in 1937. Recurrent oral, vaginal, and uveitis are the most common BD symptoms. After that, the illness was linked to joint, blood vessel, gut, lung, and nervous system symptoms (1). BD is common worldwide, especially in Silk Road countries (2). The disease was most common in Turkey (3). Males are more likely to be diagnosed between 20 and 40. In teenage males, the disease's development is connected to poor clinical outcomes (4,5). For years, diagnostic criteria and classifications have been researched, but BD diagnosis has no definitive clinical finding. According to the 1990 I International Study Group for Behçet's Disease (ISGBD) diagnostic criteria, oral aphthous ulceration patients must have two other clinical symptoms: papulopustular lesions, erythema nodosum-like lesions, genital ulcers, superficial thrombophlebitis, eye involvement, and/or positive pathergy test.

Several studies have found that the HLA-B*51 allele is the most crucial genetic predisposition factor. Ohno et al. first linked BD to HLA-Bw51 in 1982 (6). Those with a genetic predisposition are known to get the disease through infections and environmental causes. Patients with vaginal and gastrointestinal ulcers have Herpes Simplex Virus Type I genomes in their saliva and peripheral lymphocytes. Due to their functional importance, infectious pathogens resemble human heat shock protein 65 (Hsp65). It is believed that Mycobacterium-derived Hsp56 activates T cells like human Hsp60. Identifying a BD pathogen or autoantigen is still difficult (7,8). The second hypothesis is that HLA-B*51 suppresses BD by binding to the Killer cell immunoglobulin-like receptor 3DL1 (KIR3DL1) receptor on natural killer (NK) cells. If HLA-B*51 expression on the cell surface stays normal, the observed cellular response protects cells from cytotoxicity (9). NK cells are suppressed by HLA-B*51 and KIR3DL1, although NKG2D (one of the NK cell activating receptors) and its ligand MHC class I polypeptide–related sequence A (MICA) counteract this. It has also been recommended that HLA-B*51 and KIR3DL1 lower allele combinations may contribute to this syndrome (10).

Cross-reactivity between HLA-B*51 and organ-specific antigens may play a major role in disease development (11). B alleles like B*51 and B*27 share amino acids with retinal soluble antigen peptides (12). Another possibility for the correlation between BD and genetics is this antigen's linkage disequilibrium (13). Inheritance of MICA*009, Tumor necrosis factor (TNF)-α, and HLA-B*51 genes may impact disease development (14). The sub-alleles HLA-B*51:01 and 51:08 are more common in BD (15,16). Asparagine at 63 and phenylalanine at 67 identify these compounds. However, amino acids at positions 67, 97, 116, and 152 may provide a significant risk of BD. Different amino acids may play different functions in antigen presentation (15).

The objective of this study was to conduct a comparative analysis of HLA-B*51 sub-alleles in both patients and healthy volunteers, to identify any potential link between certain sub-alleles and the progression of the disease. This will facilitate the diagnosis of BD. Furthermore, an investigation was conducted to examine the correlation between sub-alleles and the manifestation of organ involvement in individuals who tested positive for HLA-B*51.

## Material and Methods

### Study populations

This study included a total of 24 patients who tested positive for HLA-B*51. These patients were under the care of the Dermatology Department in two separate hospitals over the period from February to September 2017. The study comprised healthy patients who applied to the same laboratory as a donor for hematopoietic stem cell transplantation and underwent high-resolution HLA-B typing, serving as the control group. A statistical comparison was conducted

between a group of seventy-three healthy individuals who tested positive for the HLA-B*51 gene and the patients.

Signed Informed Consents were obtained from all patients. In compliance with the Declaration of Helsinki, our Institutional Non-Interventional Clinical Research Ethics Committee approved the study (Decision No: 17, 09.02.2017).

**DNA isolation**

The process of DNA isolation from whole blood was performed with the QiaAmp DNA Blood Mini Kit (Qiagen, USA) in accordance with the instructions provided by the manufacturer. Specifically, a 200µl blood sample was introduced into a 1.5 ml micro-centrifuge tube, and the pre-prepared kit was placed into the automated DNA Isolation device (QIAGEN Geno-M6, Alameda, CA). Ultimately, a DNA sample of 50 µl was obtained through the process of elution. The Nanodrop Spectrophotometer device (Thermo Scientific Nanodrop 2000, Wilmington, Delaware USA) was utilized to determine the purity and concentration of the samples. The samples that exhibited a concentration greater than 15 ng/µl and a purity range of 1.80-1.90 were deemed suitable for inclusion in the study.

***High-resolution sequence-specific primer (PCR-SSP) assay***

HLA-B*51 positive Behçet patients' sub-alleles were determined by this assay. Following the manufacturer's recommendations, SSP HLA-B*51 Kit (HLA-B51 Excl Taq Lifecodes, Stamford, USA) was used: A mix was created by adding 264 µl master mix, 176 µl DNA sample, 433 µl dH2O, and 7 µl Taq DNA polymerase (Lifecodes, USA). For each well, a 10 µl sample was added from the mix. PCR conditions included 1 cycle of denaturation at 94°C for 2 minutes, 10 cycles of denaturation, annealing, and extension at 94°C and 65°C for 10 and 60 seconds, respectively, 20 cycles at 94°C for 10 seconds, 61°C for 50 seconds, and 72°C for 30 seconds. Amplicons were kept at 4°C for analysis. Amplification was followed by 2% Agarose gel electrophoresis (0.5X tris-boric acid-EDTA). Electrophoresis was 20 minutes at 140V and 400A. Under the UV transilluminator, gel bands were seen. The findings were evaluated using Olerup SSP Start Score Version 5.00.41T/07 (Stockholm, Sweden).

***Statistical analysis***

The Statistical Package for Social Sciences for Windows Version 22.0 (SPSS 22.0 Inc, Chicago, USA) for Windows 7 Software Program was utilized to conduct the statistical analyses. The numerical parameters were compared using the Mann-Whitney U Test. The statistical tests of Pearson Chi-square and Fisher's Exact test were utilized to examine the association between qualitative variables. A significance level of $p<0.05$ was deemed as statistically significant. The assessment of the likelihood of developing BD with HLA-B*51 sub-alleles was conducted based on the Relative Risk (RR) associated with the disease (17).

**Results**

The frequency of the HLA-B*51 allele among BD patients admitted to our laboratory between 2014 and 2017 was 71.4%. The study found that 62.5% (n=15) of the patients were male, whereas 37.5% (n=9) were female. Among the patient population, a majority of 79.1% (n=19) exhibited the HLA-B*51:01 sub-allele, while a smaller proportion of 16.6% (n=4) displayed the HLA-B*51:08 sub-allele. Additionally, a minority of 4.1% (n=1) presented with both the HLA-B*51:01 and HLA-B*51:08 sub-alleles. Among the cohort of healthy participants, it was observed that 90.4% (n=66) carried the HLA-B*51:01 allele, while 4.1%

(n=3) had the HLA-B*51:02 allele. Additionally, 2.7% (n=2) of the participants exhibited the HLA-B*51:05 allele, while 1.4% (n=1) had the HLA-B*51:07 allele. Lastly, another 1.4% (n=1) of the healthy individuals carried the HLA-B*51:08 sub-allele. The frequencies of HLA-B*51 sub-alleles in patients with BD and the control group were presented in Figure 1 and Figure 2, respectively.

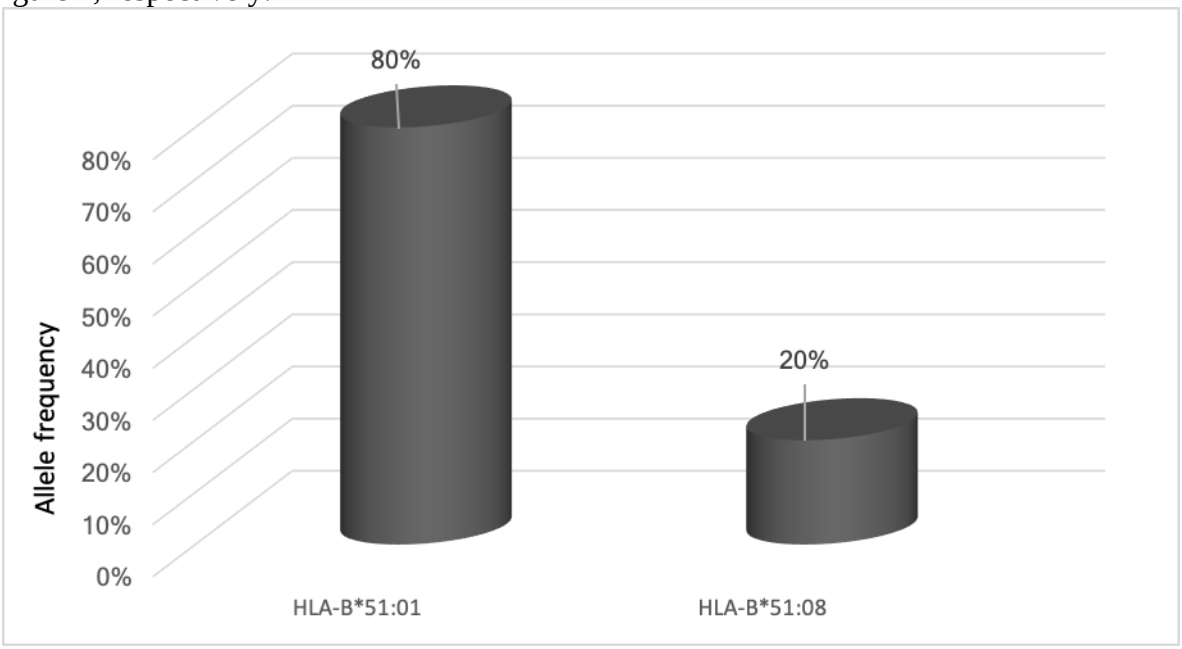


**Figure 1.** HLA-B*51 sub-allele frequencies of the patient group

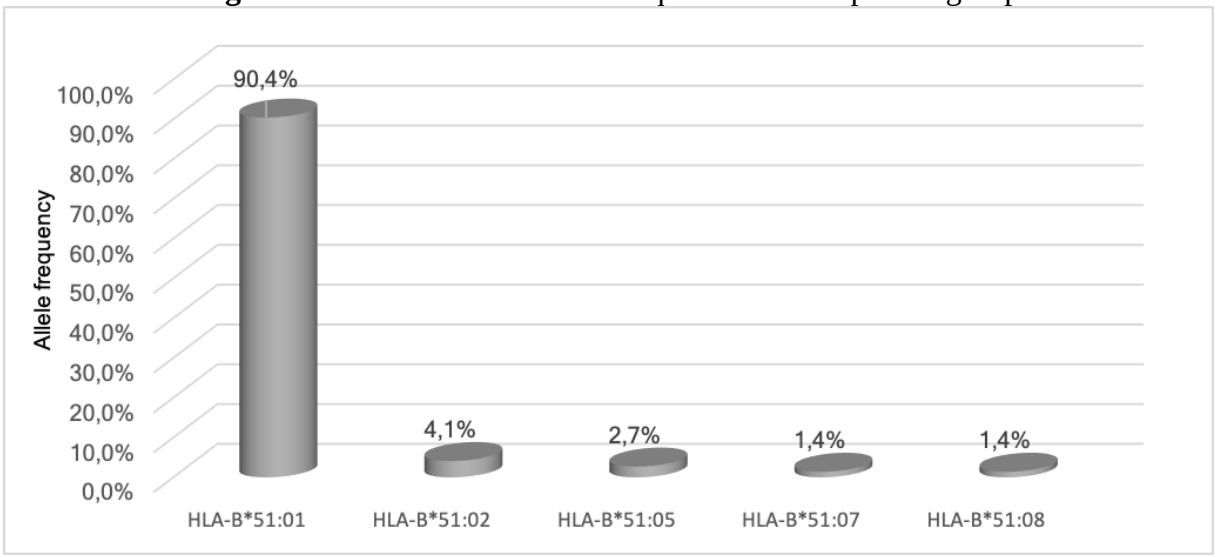


**Figure 2.** HLA-B*51 sub-allele frequencies of the control group

The clinical manifestations were categorized into two groups: major symptoms, which included oral aphthae, vaginal aphthae, dermatological complaints, and ophthalmic involvement, and minor findings, which encompassed neurological, joint, and vascular involvement. This classification was employed to investigate the potential correlation between HLA-B*51 and the clinical symptoms.

The most common HLA-B*51 sub-allele in both groups was 51:01. HLA-B*51:08 was also common in patients (Table 1).

**Table 1:** Comparison of HLA-B*51 suballeles frequency of Behçet's patients and control group

| **HLA-B*51 suballeles** | **Patients (*n*=24)** | **Control (*n*=73)** | ***p*** | **Relative risk (RR)** |
|---|---|---|---|---|
| **HLA-B*51:01** | 20 (80%) | 66 (90.4%) | 0.457 | 0.88 |
| **HLA-B*51:02** | 0 (0%) | 3 (4.1%) | - | |
| **HLA-B*51:05** | 0 (0%) | 2 (2.7%) | - | |
| **HLA-B*51:07** | 0 (%0) | 1 (%1.4) | - | |
| **HLA-B*51:08** | 5 (%20) | 1 (%1.4) | **0.003** | **18.8*** |

*95% Confidence Interval 18.8 (2.08-166)

There was no statistically significant difference observed in the prevalence of the HLA-B*51:01 sub-allele between patients diagnosed with BD and the control group (p=0.457, relative risk (RR)=0.88). Given that the RR is smaller than 1, there is a decreased likelihood of disease occurrence in individuals who carry the HLA-B*51:01 allele. There was a statistically significant difference in the frequency of HLA-B*51:08 between the sick group and the control group (p=0.003, RR=18.8). In this particular scenario, individuals who carry the HLA-B*51:08 allele exhibit a significantly elevated risk, approximately 18.8 times greater, of developing the condition in question.

The occurrence of significant clinical observations, such as oral aphthae, vaginal aphthae, and ocular involvement, was noted in a comparable sequence, as indicated in Table 2. There was no statistically significant association seen between HLA-B*51:01 and HLA-B*51:08 and organ involvement, as indicated by a p-value greater than 0.05. The occurrence rates of significant clinical observations, including dermatological, neurological, joint, and vascular involvement, exhibited comparable frequencies (see Figure 3).

**Table 2**: Relationship between HLA-B * 51 sub-alleles and clinical symptoms in BD patients

| | **HLA-B*51 sub-alleles (*n*=24)** | | | | $\chi^2$ | |
|---|---|---|---|---|---|---|
| | HLA-B*51:01 (*n*=20) | | HLA-B*51:08 (*n*=5) | | HLA-B*51:01 (*n*=20) | HLA-B*51:08 (*n*=5) |
| | Positive | Negative | Positive | Negative | | |
| **Major clinical findings** | | | | | | |
| Oral aphthae | 90% (n=18) | 10% (n=2) | 100% (n=5) | 0% (n=0) | 0.461 | 0.461 |
| Genital Aphthae | 50% (n=10) | 50% (n=10) | 80% (n=4) | 20% (n=1) | 1 | 1 |
| Eye involvement | 40% (n=8) | 60% (n=12) | 60% (n=3) | 40% (n=2) | 0.341 | 0.341 |
| **Minor clinical findings (**dermatological, neurological, joint and vascular involvement**)** | %55 (n=11) | %45 (n=9) | %40 (n=2) | %60 (n=3) | 1 | 1 |

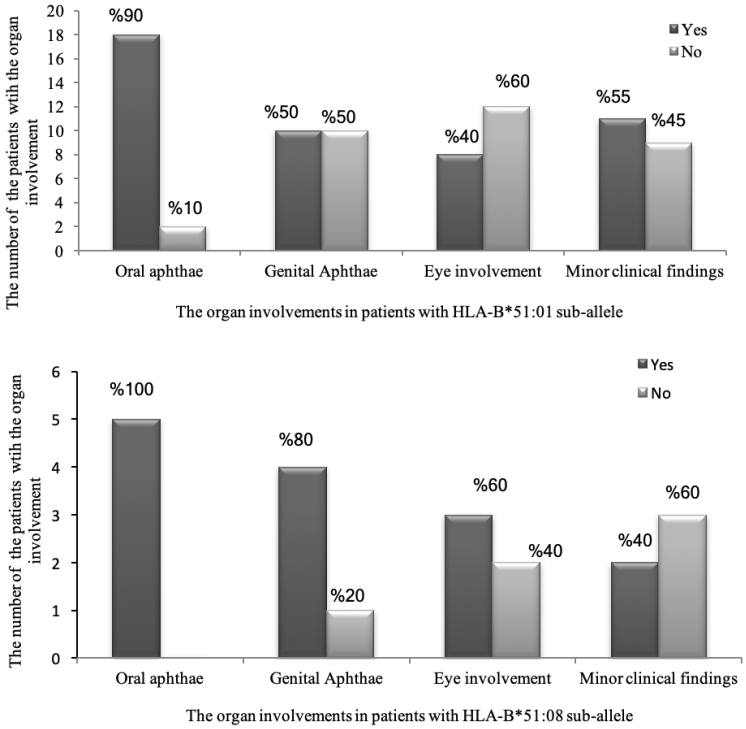


**Figure 3.** The comparison of organ involvement in Behçet's patients with HLA-B*51:01 and HLA-B*51:08 sub-alleles

## Discussion

The human genome's most variable region is the 4 Mb HLA region (18). Over two hundred genes reside in the region, and twenty-two are linked to the immune system (19) and other illnesses (17). HLA-B5 and its variation, HLA-B*51, were documented by Ohno (20) in Japan and by Yazıcı (21), Soylu (22), (23), and Azizlerli (24) in Turkey. Zierhut et al. (25) helped Germans grasp this subgroup. Several methods linked HLA-B*51 polymorphism to BD. With the development of molecular technologies, HLA gene DNA analysis became possible. After the discovery of HLA-B*51 in BD, sub-alleles have garnered attention. Based on 2017 data, 280 HLA-B*51 sub-alleles were discovered. The findings linked BD to the HLA-B*51:01 and 51:08 sub-alleles (26).

Six Turkish research examined sub-alleles and HLA-B*51-positive BD. Balkan et al. found HLA-B*51:01 (97.5%) and HLA-B*51:09 (2.5%) sub-alleles in 2017 HLA-B*51 positive patients (27). Müller et al.'s 2005 study found that HLA-B*51:01 (87.5%) and HLA-B*51:08 (14.2%) were the most common sub-alleles (28). Pirim et al. (2004) found HLA-B*51 sub-alleles: HLA-B*51:01 (45.5%), HLA-B*51:08 (25%), HLA-B*51:05 (9.1%), HLA-B*51:11 (6.81%), and HLA-B*51:04 (4.54%) (29). Demirseren et al. (2014) found HLA-B*51:01 (68.6%), 51:02 (33.3%), 51:09 (21.5%), and 51:22 (17.6%) sub-alleles (30). Kötter et al. found HLA-B*51:01 (81%), HLA-B*51:08 (11%), and HLA-B*51:05 (2%) in 2001 (31). Atalay et al. (1998) found 94% of patients had HLA-B*51:01 and 6% had 51:08/09 (32).

The present investigation examined the prevalence of HLA-B*51 sub-alleles in patients who tested positive for HLA-B*51. The results revealed that HLA-B*51:01 was observed in 80% of the patients, whereas HLA-B*51:08 was found in 20% of the patients. The findings of this study were in line with previous research conducted on Turkish patients diagnosed with Behçet's disease. A higher prevalence of the condition was noted among males in both the Turkish population and Mediterranean countries (3). In the present investigation, a total of 24

patients were included, with 62.5% (n=15) being male and 37.5% (n=9) being female. Among the male patients, 75% exhibited the presence of HLA-B*51:01, whereas the remaining 25% displayed HLA-B*51:08. All female patients in the study had the presence of HLA-B51*01. The observation of a male patient possessing both alleles is noteworthy.

This study revealed a significant association between the presence of the HLA-B*51:08 sub-allele and an increased illness risk, with patients carrying this sub-allele exhibiting an 18.8-fold higher susceptibility compared to those with alternative sub-alleles. The study conducted by Belem et al. (2020) revealed that HLA-B*51:08 was only detected in patients, while other sub-alleles were observed in both patients and healthy individuals within the Brazilian community (33). A prior meta-analysis has additionally demonstrated a robust correlation between the susceptibility to the disease and the existence of HLA*B51. Nevertheless, the sub-alleles were not taken into account in the study (34).

Behçet's disease's key criteria, such as recurrent mucosal and skin symptoms, ocular findings, and pathergy skin test, vary by ethnicity. Clinical criteria are used to diagnose Behçet's illness since there is no pathognomonic characteristic. In 2005, Davatchi et al. found oral aphthae in 100%, genital in 88%, eye involvement in 29%, joint involvement in 16%, and neurological complaints in 2.2% of BD patients regardless of tissue type, but no dermatological complaints (35). Demirseren et al. showed that 100% of patients had oral aphthae, 82.4% had genital, 35.3% had ocular, 47.1% had joint, and 7.8% had neurological problems (30). We found oral aphthae in 23 patients (95%), genital in 14 (58.3%), eye involvement in 11 (45.8%), and dermatologic/neurological/joint/vascular symptoms in 13 (65%). HLA-B*51:01 patients had the same order of key clinical findings as previously reported (19). We found that HLA-B*51:08 sub-alleles increased organ involvement (oral, vaginal, and ocular). The tiny size of our cohort may have hampered our investigation.

**Conclusion**

In conclusion, while there have been previous investigations on the sub-alleles of HLA-B*51 in Turkish individuals with BD, we posit that the identification of sub-alleles among patients residing in the Izmir region will make a valuable contribution to the existing body of literature. This study identified the HLA-B*51:08 sub-allele as a significant factor in the chance of developing BD. The findings of this study indicate that the presence of the HLA-B*51:08 sub-allele, as opposed to the HLA-B*51:01 allele, may offer significant insights into the pathophysiology of the disease.

## References

1. Kaklamani VG, Vaiopoulos G, Kaklamanis PG. Behçet’s Disease. Semin Arthritis Rheum. 1998;27(4):197–217. doi: 10.1016/s0049-0172(98)80001-2.
2. Skef W, Hamilton MJ, Arayssi T. Gastrointestinal Behçet’s disease: A review. World J Gastroentero 2015 Apr 4;21(13):3801. doi: 10.3748/wjg.v21.i13.3801
3. Azizlerli G, Köse AA, Sarica R, et al. Prevalence of Behçet’s disease in Istanbul, Turkey. Int J Dermatol. 2003 Jan 1;42(10):803–6. doi: 10.1046/j.1365-4362.2003.01893.x.
4. Gürler A, Boyvat A, Türsen Ü. Clinical manifestations of Behçet’s disease: an analysis of 2147 patients. Yonsei Med J. 1997;38(6):423–7. doi: 10.3349/ymj.1997.38.6.423.
5. Alpsoy E, Constantin Zouboulis C, Ehrlich GE. Mucocutaneous Lesions of Behçet’s disease. Yonsei Med J. 2007;48(4):573–85. Available from: https://synapse.koreamed.org/upload/synapsedata/pdfdata/0069ymj/ymj-48-573.pdf

6. Ohno S, Ohguchi M, Hirose S, Matsuda H, Wakisaka A, Aizawa M. Close association of HLA-Bw51 with Behçet's disease. Arch Ophthalmol. 1982;100(9):1455–8. Doi: 10.1001/archopht.1982.01030040433013
7. Kotake S, Namba K, Higashi K, et al. The change of clinical manifestations of patients with Behçet's disease in Japan. Adv Exp Med Biol. 2003;528:83–4. doi: 10.1007/0-306-48382-3_16.
8. Turna I, Pabuççuoğlu U, Akin C, Lebe B, Gunes AT. Histopathologic and direct immunofluorescence findings of the papulopustular lesions in Behçet's disease. Eur J Dermatol. 2006;16(2):146–50. Available from: https://pubmed.ncbi.nlm.nih.gov/16581565/
9. Kulkarni S, Martin MP, Carrington M. The Yin and Yang of HLA and KIR in human disease. Semin Immunol. 2008 Dec;20(6):343–52. doi: 10.1016/j.smim.2008.06.003.
10. Shafi S, Vantourout P, Wallace G, et al. An NKG2D-mediated human lymphoid stress surveillance response with high interindividual variation. Sci Transl Med. 2011 Dec 14;3(113). doi: 10.1126/scitranslmed.3002922.
11. Song YW, Kang EH. Behçet's disease and genes within the major histocompatibility complex region. Mod Rheumatol. 2012 Apr;22(2):178–85. doi: 10.1007/s10165-011-0542-4
12. Takeuchi M, Kastner DL, Remmers EF. The immunogenetics of Behçet's disease: A comprehensive review. J Autoimmun. 2015 Nov 1;64:137–48. doi: 10.1016/j.jaut.2015.08.013.
13. Arayssi T, Hamdan A. New insights into the pathogenesis and therapy of Behçet's disease. Curr Opin Pharmacol. 2004 Apr;4(2):183–8. doi: 10.1016/j.coph.2003.10.009.
14. Marshall SE. Behçet's disease. Best Pract Res Clin Rheumatol. 2004 Jun;18(3):291–311. doi: 10.1016/j.berh.2004.02.008.
15. Mizuki N, Inoko H, Ando H, et al. Behçet's disease associated with one of the HLA-B51 subantigens, HLA-B* 5101. Am J Ophthalmol. 1993;116(4):406–9. doi: 10.1016/s0002-9394(14)71396-0.
16. Guasp P, Alvarez-Navarro C, Gomez-Molina P, et al. The Peptidome of Behçet's Disease-Associated HLA-B*51:01 Includes Two Subpeptidomes Differentially Shaped by Endoplasmic Reticulum Aminopeptidase 1. Arthritis Rheumatol. 2016 Feb 1;68(2):505–15. doi: 10.1002/art.39430.
17. Gül A, Hajeer AH, Worthington J, Barrett JH, Ollier WER, Silman AJ. Evidence for linkage of the HLA–B locus in Behçet's disease, obtained using the transmission disequilibrium test. Arthritis Rheum. 2001;44(1):239–41. doi: 10.1002/1529-0131(200101)44:1<239::AID-ANR31>3.0.CO;2-X.
18. Choo SY. The HLA system: genetics, immunology, clinical testing, and clinical implications. Yonsei Med J. 2007 Feb;48(1):11–23. doi: 10.3349/ymj.2007.48.1.11.
19. BOYVAT A. Behçet Hastalığında Tanı ve Ayırıcı Tanı. Türkiye Klinikleri J Dermatol - Özel Konular. 2017;10(4):315–20. Available from: https://www.turkiyeklinikleri.com/article/tr-Behçet-hastaliginda-tani-ve-ayirici-tani-80555.html
20. Ohno S, Asanuma T, Sugiura S, Wakisaka A, Aizawa M, Itakura K. HLA-Bw51 and Behçet's Disease. JAMA. 1978 Aug 11;240(6):529–529. doi:10.1001/jama.1978.03290060031006

21. Yazici H, Tuzun Y, Pazarli H, Yalcin B, Yurdakul S, Muftuoglu A. The combined use of HLA-B5 and the pathergy test as diagnostic markers of Behçet's disease in Turkey - PubMed. J Rheumatol. 1980 Mar-Apr; 7(2):206-10. Available from: https://pubmed.ncbi.nlm.nih.gov/7373623/
22. Soylu M, Ersöz TR, Erken E. The association between HLA B5 and ocular involvement in Behçet's disease in southern Turkey. Acta Ophthalmol. 1992 Dec 1;70(6):786–9. Doi: 10.1111/j.1755-3768.1992.tb04888.x
23. Erkılıç K, Patıroğlu T, Doğan H, Mirza E, Çağıl N. HLA Antigenes and Their Associations with Organ Lesions in Behçet's Disease. Journal of Retina-Vitreous. 1996;4(3). Available from: https://retinavitreus.com/abstract.php?lang=tr&id=322
24. Azizlerli G, Aksungur VL, Sarica R, Akyol E, Övül C. The association of HLA-B5 antigen with specific manifestations of Behçet's disease. Dermatology. 1994;188(4):293–5. doi: 10.1159/000247169.
25. Zierhut M, Saal J, Pleyer U, Kötter I, Dürk H, Fierlbeck G. Behçet's disease: epidemiology and eye manifestations in German and Mediterranian patients - PubMed. Ger J Ophtalmol. 1995;446–51. Available from: https://pubmed.ncbi.nlm.nih.gov/7492938/
26. Mizuki N, Inoko H, Ohno S. Molecular Genetics (HLA) of Behçet's disease. Yonsei Med J. 1997;38(6):333–9. Available from: https://www.eymj.org/Synapse/Data/PDFData/0069YMJ/ymj-38-333.pdf
27. Balkan E, Eyerci N, Keleş S, et al. Evaluation of HLA-B*51 Subtypes in Behçet's Patients with Uveitis Behçet Üveitli Hastalarda HLA-B*51 Alt Tipinin Değerlendirilmesi. Kafkas J Med Sci. 2017;7(3):193–6. doi: 10.5505/kjms.2017.39306
28. Müller CA, Günaydın İ, Stübiger N, et al. 2003. Association of HLA-B51 Suballeles with Behçet's Disease in Patients of German and Turkish Origin, In: Ohno S, Zierhut M, editors. Immunology of Behçet's Disease. 1st Edition. Lisse: Swets and Zeitlinger Publishers; pp. 121. Available from: https://books.google.com.tr/books?id=3TCHv9RqFwcC&printsec=frontcover&hl=tr&source=gbs_ge_summary_r&cad=0#v=onepage&q=Association%20of%20HLA-B51%20Suballeles%20with%20Beh%C3%A7et%E2%80%99s%20Disease%20in%20Patients%20of&f=false
29. Pirim I, Atasoy M, Ikbal M, Erdem T, Aliagaoglu C. HLA class I and class II genotyping in patients with Behçet's disease: a regional study of eastern part of Turkey. Tissue Antigens. 2004 Sep;64(3):293–7. doi: 10.1111/j.1399-0039.2004.00280.x.
30. Demirseren DD, Ceylan GG, Akoglu G, et al. HLA-B51 subtypes in Turkish patients with Behçet's disease and their correlation with clinical manifestations. Genet Mol Res. 2014 Jul 2;13(3):4788–96. doi: 10.4238/2014.July.2.8.
31. Kötter I, Günaydin I, Stübiger N, et al. Comparative analysis of the association of HLA-B*51 suballeles with Behçet's disease in patients of German and Turkish origin. Tissue Antigens. 2001;58(3):166–70. doi: 10.1034/j.1399-0039.2001.580304.x.
32. Kaya-Atalay A. Behçet Hastalarında HLA-B51 Genotiplerinin İncelenmesi [PhD thesis]. [Istanbul, Turkey]:Marmara University;1998. Available from: https://www.proquest.com/docview/2564506524?pq-origsite=gscholar&fromopenview=true

33. Belem JMFM, Fraga AM, Andrade LEC, de Souza AWS. HLA-B*51 and its main subtypes in Brazilian patients with Behçet's disease. Clin Exp Rheumatol. 2020;38:53–9. PMID: 33124572.
34. 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 Oct 10;61(10):1287–96. doi: 10.1002/art.24642.
35. Davatchi F, Shahram F, Chams C, et al. 2003. Non-Ocular Manifestations. In: Ohno S, Zierhut M, editors. Immunology of Behçet's Disease. 1st Edition. Lisse: Swets and Zeitlinger Publishers; pp. 21. Available from: https://books.google.com.tr/books?hl=tr&lr=&id=3TCHv9RqFwcC&oi=fnd&pg=PP11&dq=Non-Ocular+Manifestations+zierhut&ots=MUm2Cm6wCN&sig=VQZzIPEt2CSQVC07-_C-cbdveTc&redir_esc=y#v=onepage&q=Non-Ocular%20Manifestations&f=false

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