Association of IL-4 gene VNTR variant with deep venous thrombosis in Behçet’s disease and its effect on ocular involvement

Ahmet Inanir,¹ Sengul Tural,² Serbulent Yigit,³ Goknur Kalkan,⁴ Gunseli Sefika Pancar,⁵ Helin Deniz Demir,⁶ Omer Ates⁷

¹Gaziosmanpaşa University, Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Tokat, Turkey; ²Onokav Mayis University, Faculty of Medicine, Department of Blood Center, Samsun, Turkey; ³Gaziosmanpaşa University, Faculty of Medicine, Department of Medical Biology, Tokat, Turkey; ⁴Gazi Osmanpaşa University, Faculty of Medicine, Department of Dermatology, Tokat, Turkey; ⁵Tokat Public Hospital, Department of Dermatology, Tokat, Turkey; ⁶Tokat Gazi Osmanpaşa University, Faculty of Medicine, Department of Ophthalmology, Tokat, Turkey

Purpose: Behçet’s disease (BD) is a systemic vasculitis characterized by inflammatory lesions of the urogenital mucosa, eyes, skin, central nervous system, and joints. Vein thrombosis constitutes the most frequent vascular manifestation of the disease, and may cause such ocular vascular thrombotic events as central retinal vein and central retinal artery thrombosis. Thrombosis is a serious problem, and often leads to irreversible vision loss. Previous studies have shown that genetic factors predispose individuals to BD. Several cytokine genes might play crucial roles in host susceptibility to BD and to thrombophilia. Various polymorphic regions of the interleukin-4 (IL-4) gene (~1098G and 590T) are associated with BD in the Turkish population. This study was conducted in Turkish patients with BD to determine the frequency of the IL-4 gene 70 bp variable number of tandem repeats (VNTR) variant, and its association with clinical findings.

Methods: Genomic DNA obtained from 488 individuals (238 patients with Behçet’s disease and 250 healthy controls) was used in the study. Genomic DNA was isolated and genotyped using PCR assay for the IL-4 gene 70 bp VNTR polymorphism determined by using PCR with the specific primers.

Results: There was statistical significance between the groups regarding IL-4 genotype distribution (p<0.001, odds ratio: 2.55 [1.629–4.052], 95% confidence interval) and allele frequencies (p<0.001, 2.381[1.586–3.617], 95% confidence interval). When we examined IL-4 genotype frequencies according to the clinical characteristics, we observed a statistically significant association between the P.P. genotype and deep venous thrombosis (p=0.01). Deep venous thrombosis was also associated with ocular involvement in our study group (p=0.014).

Conclusions: Our findings suggest that the IL-4 gene 70 bp VNTR polymorphism is associated with susceptibility to development of BD. Deep venous thrombosis is also associated with ocular involvement in BD. The IL-4 gene could be a genetic biomarker in Behçet’s disease in a Turkish study population.

Behçet’s disease (BD) is a chronic multisystem inflammatory disorder characterized by mucocutaneous, ocular, vascular, and central nervous system manifestations. The common manifestations are recurrent oral and genital ulcers and ocular involvement. Venous or arterial thromboses occur in 7% to 38% of patients [1]. Venous thrombosis is more common than arterial thrombosis, with relative frequencies of 90% and 10%, respectively [2,3]. Although vascular lesions are not included in the major diagnostic criteria of BD, one-quarter to one half of patients are likely to develop this complication [4-6]. Venous thrombosis is a major vascular involvement reported in 7% to 33% of patients with BD [6]. BD has a worldwide distribution but is most common in Japan, the Middle East, and Mediterranean countries. The prevalence of BD in Turkey is particularly high, at 80–420 per 100,000 individuals [7,8]. BD occurs more commonly in men than in women and primarily affects individuals between the second and fourth decades of their life, with a more aggressive course in young male adults. BD is characterized by infiltration of lymphocytes and neutrophils into the affected organs. Cytokines play critical roles in the pathogenesis of BD [9,10]. Several cytokine genes may play crucial roles in host susceptibility to BD, because cytokine production capacity varies among individuals and depends on the cytokine gene polymorphisms [11]. Cytokines are signaling molecules that contribute to inflammatory response and protect the body from pathogens and other environmental factors. Interleukin-4 (IL-4) is a key cytokine that induces the activation and differentiation of B cells and is involved in the development of the T helper-2 subset of lymphocytes. IL-4 has cytotoxic, antitumor effects, inhibits induction of nitric oxide synthase, inhibits release of superoxide by macrophages, and has numerous anti-inflammatory effects [12-14]. IL-4 also plays a role in the function of macrophages, B-cell
and T-cell chemotaxis, the formation of endothelial cell adhesion molecules, and hematopoiesis. Based on these findings, we hypothesized that the genotype of IL-4 in patients with BD may be a determining factor in BD pathogenesis.

METHODS

Study population: The present study included 238 patients with BD and 250 controls, recruited from the Gazi Osmanpaşa University Department of Physical Medicine and Rehabilitation (Tokat, Turkey). The ethics committee of Gazi Osmanpaşa University Medical Faculty approved informed consent in accordance with the study protocol. Patients with BD fulfilled the International Criteria of Behçet’s Disease for classification [15]. All patients signed a written consent form after being informed about the details of the study. A complete clinical evaluation was performed for all patients. The controls were selected by excluding a diagnosis of BD. All the individuals in the control group were healthy. The data collection sheet included information such as age, disease duration, deep venous thrombosis, and several clinical characteristics. Individual features of patients with BD and controls are summarized in Table 1 and Table 2. Genotype determination DNA was extracted from 2 ml venous blood according to the kit procedure (Sigma-Aldrich, Taufkirchen, Germany) and stored at −20 °C. To detect 70 bp VNTR polymorphism in the third intron of the IL-4 gene, PCR assay was used as described by Mout et al. [16]. PCR was performed with a 25 µl reaction mixture containing 50 ng DNA, 20 pM of each primer, 200 mM of deoxynucleotide triphosphate (dNTP), 2.5 mM MgCl₂, 0.5 U Taq polymerase, 10 mM KCl buffer (Fermentas, Shenzhen, China). Amplification was carried out using primers F5’ AGG CTG AAA GGG GGA AAG C-3’, with initial denaturation at 95 ºC for 5 min, 30 cycles of denaturation at 94 ºC for 30 s, annealing at 58 ºC for 45 s, extension at 72 ºC for 1 min, and final extension at 72 ºC for 10 min. P₁P₁ genotype was homozygous wild type, P₁P₂ genotype heterozygous mutant, P₂P₂ genotype homozygous mutation type, wild type allele was P₁, and mutant type allele was P₂, respectively. The PCR products were visualized on three percent agarose gel stained with ethidium bromide. 

Statistical analysis: Analysis of the data was performed using SPSS 15.0 (SPSS, Chicago, IL) and the OpenEpi Info software package program [17]. Continuous data were given as mean±SD (standard deviation) and (minimum-maximum values). The frequencies of the alleles and genotypes in the patients and the controls were compared with ñ² analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. A p value smaller than 0.05 (two-tailed) was statistically significant.

RESULTS

The demographic variables and baseline characteristics of the patients are shown in Table 1 and Table 2. The mean±SD age was 36.36±9.62 in the patient group and 35.84±11.36 in the control group, respectively. There were 114 (47.90%) women and 123 (52.10%) men in the patient group; in the control group, these figures were 155 (62%) and 95 (38%), respectively. Table 3 presents the distribution of IL-4 70 bp VNTR polymorphic genotypes in the patient group and the control group. Our results showed that there was a statistically significant difference between the groups regarding IL-4 genotype distribution (p<0.001) and allele frequencies (p<0.001; Table 3). In the present study, the P₂ allele was a 2.3-fold risk factor for BD (OR, 95% CI; 2.381 [1.586–3.617]). When we examined the IL-4 genotype frequencies according to the clinical characteristics, we found a statistically significant

| Table 1. Demographic variables and baseline characteristics of the patients. |
|---------------------------------------------------------------|
| **Individual characteristics** | **Mean±SD** | **Min-Max** |
|--------------------------------|------------|------------|
| Average age of patients | 36.36±9.62 | 20–70      |
| Average age of controls   | 35.84±11.36| 18–65      |
| Disease duration, years   | 6.87±5.96  | 1–29       |

Min-Max: Minimum-maximum values.

| Table 2. Clinical findings of BD patients |
|-----------------------------------------|
| **Clinical characteristics**            | **Number of Patients (%)** |
|-----------------------------------------|-----------------------------|
| Skin lesions                            | 234(98.3)                   |
| Oral ulcers                             | 234(98.3)                   |
| Genital ulcers                          | 168(70.6)                   |
| Ocular inflammation                     | 180(75.6)                   |
| Deep venous thrombosis                  | 71(29.8)                    |
| Colchicine use                          | 234(98.3)                   |
| Response to colchicine                  | 238(100)                    |
| Papulopustule                           | 96(40.3)                    |
| Erythema nodosus                        | 63(26.5)                    |

Min-Max: Minimum-maximum values.
association between the \(P_1P_2\) genotype and deep venous thrombosis according to the genotype frequencies (\(p=0.01\); Table 4). There was also a statistically significant association between the \(P_1\) allele and deep venous thrombosis according to the allele frequencies (\(p=0.008\); Table 5). We examined the ocular involvement distribution according to deep venous thrombosis and found a statistically significant association between ocular involvement and deep venous thrombosis (\(p=0.014\); Table 6). In addition, we examined the IL-4 gene 70 bp VNTR genotype and allele frequencies according to clinical status and found that cases with ocular involvement (\(+)\) and deep venous thrombosis (\(+)\) were associated with the \(P_2\) allele (\(p=0.01\); Table 7). The IL-4 gene \(P_1\) allele frequency was 16% in the patient group and 7% in the control group. \(P_2\) allele occurrence was 84% in the patient group and 93% in the control group (Table 3). The frequencies of the \(P_1P_1\), \(P_1P_2\), and \(P_2P_2\) genotypes of the intron 3 VNTR polymorphism in the patient group were 3.4%, 26.1%, and 70.5%, respectively, and 1.2%, 12.8%, and 86.0%, respectively, in the control group. The homozygote \(P_1P_1\) genotype frequency of the IL-4 gene was 3.4% in the patient group and 1.2% in the control group. The heterozygote \(P_1P_2\) genotype frequency was 26.10% in the patient group and 12.8% in the control group. The homozygote \(P_2P_2\) genotype frequency was 70.50% in the patient group and 86% in the control group.

**DISCUSSION**

Behçet’s disease is a multisystem vasculitis that can affect all sizes of blood vessels [18-22]. The disease was first defined by Hulusi Behçet, a Turkish professor of dermatology, in 1937 as a triad of recurrent aphthous stomatitis, genital aphthae, and relapsing uveitis [23]. BD has been reported worldwide but has a distinct geographic distribution with highest prevalences in countries along the ancient Silk Road route [23]. No specific pathological testing or technique is available for diagnosing the disease [23]. Ocular disease is usually bilateral and characteristically occurs within 2 to 3 years of disease onset. Ocular involvement is reported in 30% to 70% of patients with Behçet’s disease. Uveitis involving the anterior and posterior uveal tracts is a significant cause of morbidity. Uveitis, posterior uveitis, and retinal vasculitis may cause visual loss in up to 25% of patients [24]. Although the etiology of BD is not yet known, immune dysregulation is critical factor in the pathogenesis [11]. Genetic factors that predispose individuals to BD play especially important roles in the development of the disease. Familial aggregation studies in patients with BD indicate a strong genetic background and a complex inheritance model [25]. There is a strong association with HLA-B51 and an increased incidence among close family members [26-28]. Venous thrombosis, a clinical finding of BD, is also a common multifactorial disease associated with a major public health burden. Vascular lesions, in particular subcutaneous thrombophlebitis and deep vein thrombosis, may also occur, being detected in 10% to 30% of patients with active disease [4,5]. Vasculitis is the pathological lesion underlying most clinical manifestations of BD, including venous thrombosis. However, thrombophilia may also play an important role in the pathogenesis of the thrombotic manifestations observed in BD [6]. Retinal vein occlusion is associated with increased levels of vascular endothelial growth factor; antivascular endothelial growth factor therapy has been proposed as a promising strategy for retinal vein occlusion [29]. Genetic factors are known to contribute to the susceptibility to venous thrombosis, but how many genes are involved and their contribution to venous thrombosis risk remain obscure.

| Genotype | Patients (n=238; \%) | Controls (n=250; \%) | \(\chi^2\) | \(p\) value | OR (95%CI) |
|----------|----------------------|----------------------|------------|-------------|------------|
| \(P_1P_1\) | 8 (3.40) | 3 (1.2) | 17.33 | \(p<0.001\) |          |
| \(P_1P_2\) | 62 (26.10) | 32 (12.8) |          |             | 2.555 (1.629–4.052) |
| \(P_2P_2\) | 168 (70.50) | 215 (86) |          |             | 0.349 (0.074–1.295) |
| \(P_1P_1 + P_1P_2; P_2P_2\) | 70:168 | 35:215 | 17.15 | \(p<0.001\) |          |
| \(I P_1P_2 + P_2P_2; P_1P_1\) | 230:8 | 247:3 | 2.585 | \(p=0.107\) |          |

**Allele frequency**

- \(P_1\): 78 (16) 38 (7) 17.98 \(p<0.001\) 2.381 (1.586–3.617)
- \(P_2\): 398 (84) 462 (93)

The results that are statistically significant are typed in bold. (Homzygous wild type (\(P_1P_1\)) heterozygous mutant type (\(P_1P_2\)) homozygous mutation type (\(P_2P_2\)). Wild type allele (\(P_1\)) mutant type allele (\(P_2\)).
For BD, possible candidate antigens include vascular proteins, because the central histopathological finding in BD is a vasculitis, and environmental factors such as infectious agents, which may cause cross-reactivity to human antigens and result in immune activation [24].

### Table 4. IL-4 genotype frequencies according to the clinical characteristics in BD patients (n=238).

| Clinical Characteristics | P_1P_1 | P_1P_2 | P_2P_2 | P value |
|--------------------------|--------|--------|--------|---------|
| Oral ulcers              | yes    | 9(3.81)| 45(19.06)| 182(77.11)| p>0.05 |
|                          | no     | -      | 1(50)  | 1(50)   |         |
| Genital ulcers           | yes    | 4(2.4) | 34(20.3)| 129(77.2) | p>0.05 |
|                          | no     | 5(7.04)| 11(15.49)| 55(77.46) |         |
| Ocular involvement       | yes    | 4(3.88)| 32(31.06)| 67(65.04) | p>0.05 |
|                          | no     | 4(2.96)| 30(22.22)| 101(78.81)|         |
| Deep venous thrombosis   | yes    | 4(9.75)| 14(34.14)| 23(56.09) | p=0.01  |
|                          | no     | 4(2.03)| 48(24.36)| 145(73.60)|         |
| Skin lesions             | yes    | 3(2.88)| 31(29.80)| 70(67.30) | p>0.05 |
|                          | no     | 5(3.73)| 31(23.13)| 98(73.13) |         |
| Response to colchicine   | yes    | 7(3.44)| 37(18.22)| 159(78.32)| p>0.05 |
|                          | no     | 2(5.71)| 9(25.71) | 24(68.57) |         |
| Papulopustule            | yes    | 4(4.16)| 21(21.87)| 71(73.95) | p>0.05 |
|                          | no     | 5(3.52)| 25(17.60)| 112(78.87)|         |
| Erythema.nodusum         | yes    | 9(3.84)| 43(18.37)| 182(77.77)| p>0.05 |
|                          | no     | -      | 2(50)  | 2(50)   |         |

The results that are statistically significant are typed in bold. Wild type allele (P_1) mutant type allele (P_2).

### Table 5. IL-4 allele frequencies according to the clinical characteristics in BD patients (n=238).

| Clinical Characteristics | P_1 | P_2 | P value |
|--------------------------|-----|-----|---------|
| Oral ulcers              | yes | 63(13.34)| 409(86.65)| p=0.008|
|                          | no  | 1(25) | 3(75)   |         |
| Genital ulcers           | yes | 42(12.57)| 292(87.42)| p>0.05 |
|                          | no  | 21(14.78)| 121(85.21)|         |
| Ocular involvement       | yes | 40(19.41)| 166(80.58)| p>0.05 |
|                          | no  | 38(14.07)| 232(85.92)|         |
| Deep venous thrombosis   | yes | 22(26.82)| 60(73.17)| p=0.008|
|                          | no  | 56(14.21)| 336(85.78)|         |
| Skin lesions             | yes | 37(17.78)| 171(82.21)| p>0.05 |
|                          | no  | 41(15.29)| 227(84.70)|         |
| Response to colchicine   | yes | 51(12.56)| 355(87.43)| p>0.05 |
|                          | no  | 13(18.57)| 57(81.42) |         |
| Papulopustule            | yes | 29(15.10)| 163(84.89)| p>0.05 |
|                          | no  | 35(12.32)| 249(87.67)|         |
| Erythema.nodusum         | yes | 61(13.03)| 407(86.96)| p>0.05 |
|                          | no  | 2(25) | 6(75)   |         |

The results that are statistically significant are typed in bold. Wild type allele (P_1) mutant type allele (P_2).
Several previous studies have shown that cytokines play critical roles in the pathogenesis of BD, because cytokines mediate many of the effector and regulatory functions of immune and inflammatory responses [24]. Single nucleotide polymorphisms and a VNTR are the common polymorphisms found in the human genome and cause various disorders [31]. Oller et al. wrote that variation in cytokine gene polymorphisms to determine whether a genetic basis for cytokine dysregulation is associated with disease. The genetic markers used most often in studies are either microsatellite repeat polymorphisms [33] or single nucleotide polymorphisms (SNPs) [34]. SNPs located in the intronic regions of genes can have no effect on either the level or quality of the protein produced, or, if positioned within an area influencing messenger ribonucleic acid splicing, lead to different splice variants. An IL-4 gene polymorphism has been reported for its association with several diseases such as Graves disease [1], subacute sclerosing panencephalitis [2], rheumatoid arthritis [3-4], end-stage renal disease [5], idiopathic thrombocytopenic purpura [6], chronic polyarthritis [7], fibromyalgia [8], malaria [9], transitional cell carcinoma of the urinary bladder [10], oral cancer [11], and gastric cancer [12,35]. Several studies have investigated VNTR polymorphisms in different diseases [31,35-39]. Based on these findings, we decided to investigate the effect of the 70 bp VNTR polymorphism on the third intron of the IL-4 gene in Behçet’s disease.

In this study, the distribution of the IL-4 gene polymorphic genotypes was analyzed in patients with BD in a Turkish population to assess the possible role of these genotypes in the pathogenesis of BD. The present study indicates that the percentage of the IL-4 polymorphism allele and the distribution of genotypes differed significantly between the patient group and the control group. When we examined IL-4 genotype frequencies according to the clinical characteristics, we found a statistically significant association between the P2P2 genotype and deep venous thrombosis. Venous involvement is observed in 25% of patients with BD. Vascular lesions include arterial aneurysms, small-vessel vasculitis, and arterial and venous thrombosis. Venous thrombosis is more common than arterial thrombosis, deep vein thrombosis being the most frequent type of venous thrombosis [40]. The mechanism of the thrombosis in BD is not yet clearly understood. Venous thrombosis and inflammation are two closely related entities [41]. Previous studies have shown that levels of inflammatory substances known as cytokines are raised around the time of a thrombosis [42-44]. In addition, specific polymorphisms in cytokine genes are risk factors for venous thrombosis [42-44]. In this study, the deep venous thrombosis ratio was 29.8%. Men are more severely affected than women. In the present study, the male:female ratio was 1:1.1. The incidence rate of BD is higher in patients’ family members than in the general population to assess the possible role of these genotypes in the pathogenesis of BD.

### Table 6. Ocular involvement distribution according to deep venous thrombosis.

| Clinical status (n,% | Deep venous thrombosis (+) | Deep venous thrombosis (-) | Total (n,% | \(\chi^2\) | P value |
|---------------------|----------------------------|-----------------------------|------------|----------|---------|
| Ocular involvement (+) | 92 (38.65%) | 11 (4.62%) | 103 (43.27%) | 5.459 | 0.014 |
| Ocular involvement (-) | 105 (38.65%) | 30 (12.60%) | 135 (56.72%) |          |         |
| Total (n,% | 197 (82.77%) | 41 (17.22%) | 238 (100%) |          |         |

The results that are statistically significant are typed in bold.

### Table 7. Distribution of IL-4 gene 70 bp VNTR genotype and allele frequencies according to clinical status.

| Clinical status | Genotype (n,% | \(\chi^2\) | P value | Allele (n,% | \(\chi^2\) | P value |
|-----------------|---------------|----------|--------|-------------|----------|--------|
| Ocular involvement (+) Deep venous thrombosis (+) | P\(_{1}\) \_\_ | 6 (2.6) | 2.018 | 0.365 | P\(_{2}\) | 16 (3.4) | 41.34 | <0.001 |
| Ocular involvement (-) Deep venous thrombosis (-) | P\(_{1}\) \_\_ | 58 (24.4) | 7 (2.9) | 382 (80.2) | 72 (15.2) |        |        |        |

The results that are statistically significant are typed in bold. Homozygous wild type (P\(_{1}\) P\(_{1}\)) heterozygous mutant type (P\(_{1}\) P\(_{2}\)) homozygous mutation type (P\(_{2}\) P\(_{2}\)). Wild type allele (P\(_{1}\)) mutant type allele (P\(_{2}\)).
population [4]. Therefore, genetic analysis is important to elucidate the pathogenic mechanism.

In previous studies, overexpression of proinflammatory cytokines from various cellular sources seemed to be responsible for the enhanced inflammatory reaction in BD, and this may be associated with the genetic susceptibility [40]. Oral et al. investigated IL-4 and IL-4Ra gene polymorphisms, which are different from our polymorphic region, and found that the frequency of IL-4 −1098 TG and 590 CT genotypes was higher in the patients with BD compared to healthy controls [11]. Analysis of allele frequencies showed that IL-4 −1098 G and IL-4 590 T alleles were more common in patients with BD when compared to healthy controls.

They also reported that the IL-4RA gene polymorphism seems to confer pathergy test positivity in patients with BD, whereas none of the IL-4 gene polymorphisms were associated with clinical findings and specific diagnostic tests for BD [11]. Kurata et al. reported that SNPs rs9261365 and rs2074474 were associated with BD independently of HLA-B51 and - A26 [45]. Akman et al. showed that the tumor necrosis factor-α −1031C allele is associated with susceptibility to BD in the Turkish population [46]. Kim et al. indicated that the interaction of specific rs2275913 in IL-17A, IL-23R, and rs7574865/rs11889341/rs1685878 in STAT-4 SNPs modulate susceptibility to intestinal BD in the Korean population, suggesting that the IL-17/23 axis plays a significant role in disease pathogenesis [47]. Recently, genome-wide association studies revealed that variants rs12119179/rs1554286 in IL-10 and rs1495965 IL-23R-IL-12RB2 are associated with BD [48,49]. Other studies also identified a strong relationship between the polymorphisms of rs1735018 IL-23R and IL-17 and BD [50,51]. Ozçimen et al. performed a study in Turkish patients with BD to determine the influence of single nucleotide polymorphisms in IL-1A, IL-1B, IL-1R, and IL-1RA on disease susceptibility [10]. The authors demonstrated that the IL-1b +3962 gene polymorphism seems to be associated with the presence of erythema nodosum in patients with BD. To the best of our knowledge, no reports have been published regarding the role of the IL-4 70 bp VNTR polymorphism in BD. Chen et al. reported that the plasma soluble endothelial protein C receptor (sEPCR) level was associated with the polymorphism of EPCR gene 6936A/G. The plasma sEPCR level in patients with deep venous thrombosis was higher than that in healthy control subjects [52]. Shahram et al. examined the IL-2 (−330, +166), IL-4 (−1098, −590, −33), IL-10 (−1082, −819, −592), IL-12 (−1188), interferon-γ (5644), transforming growth factor (TGF)-β (codon 10, 25), and IL-4RA (+1902) polymorphisms, and reported a significantly increased frequency of IL-2 (−330) GG genotype (p<0.001), IL-4 (−33) CC genotype (p<0.001), and TGF-β (codon 10) CC genotype (p=0.004). A significant decrease in the frequency of the IL-4 (−33) TC genotype (p<0.001) was reported in the patient group compared with healthy controls. The genotype CC of TGF-β at codon 10 was also significantly overrepresented in the patient group (p=0.004) [53]. Recent studies have suggested that the IL-23/IL-17 axis may be crucial to BD development. A study showed that the expression of IL-23p19 messenger ribonucleic acid, IL-23, IL-17, and interferon-κ was markedly elevated in patients with BD with active uveitis [54]. In another study, nuclear factor xB (NF-xB) essential modulator (NEMO), heterozygous (1217A>T, D406V) NEMO mutation is a cause of familial occurrence of Behcet’s disease in female patients [55]. Ghioni et al. investigated potential associations between A-13G and G79A polymorphisms of the protein Z gene and venous thrombosis and other clinical manifestations in Italian patients with BD. However, no associations were found [56]. Cho et al. demonstrated that the heterogeneous nuclear ribonucleoprotein A2/B1 is a target protein of serum antiendothelial cell immunoglobulin A antibody in patients with BD. Reactivity of serum immunoglobulin A against human recombinant heterogeneous nuclear ribonucleoprotein A2/B1 was detected in 83.3% of patients with BD, whereas it was detected in 0% to 30% of healthy people and disease controls [57]. In a study performed in Turkey, the possible roles of methylenetetrahydrofolate reductase gene C677T, factor V gene G1691A (Leiden), and prothrombin gene G20210A polymorphisms in venous thrombogenesis were evaluated in patients with BD. No association was found among these three thrombogenetic mutations and patients with BD with thrombosis [39]. Several studies have shown that elevated levels of coagulation factors increase the thrombotic risk [58,59]. Increased procoagulant levels might be acquired. Experimental studies in human volunteers injected with low-dose endotoxin provide credence to this possibility, as they showed increases in procoagulant protein levels in parallel with an inflammatory response [60]. Increased levels of inflammatory markers were also found in patients who had had venous thrombotic disease. Inflammation might increase procoagulant protein levels and thus increase the prothrombotic state of the blood [60]. In addition, inflammation may promote tissue factor expression of white blood cells and endothelial cells, thus providing a trigger that may lead to thrombotic disease [60].

In conclusion, our results suggest that possession of the P, allele of the IL-4 gene 70 bp VNTR polymorphism may constitute a risk for developing BD. Several studies have demonstrated that different genes mutations might play an important role in the etiology of BD. Due to limited research on the IL-4 gene in BD, the present study makes an important
contribution to the literature. Our study demonstrates that polymorphisms in the IL-4 gene seem to be involved in the susceptibility to BD. Further work is required to confirm these findings in different study groups.

ACKNOWLEDGMENTS
We thank Gorkem Kismali for editing the manuscript.

REFERENCES
1. Sakane T, Takeno M, Suzuki N, Inaba G. Behcet’s disease. N Engl J Med 1999; 341:1284-91. [PMID: 10528040].
2. Kuzu MA, Ozaslan C, Koksoy C, Gurler A, Tuzuner A. Vascular involvement in Behcet’s disease: 8 year audit. World J Surg 1994; 18:948-53. [PMID: 8746925].
3. Gül A, Ozbek U, Ozturk C, Inanc M, Konice M, Ozcetik L. Congulation factor V gene mutation increases the risk of venous thrombosis in Behcet’s disease. Br J Rheumatol 1996; 35:1178-80. [PMID: 8948311].
4. Muftuolu A, Yurdakul S, Yazici H. Vascular involvement in Behcet’s disease. A review of 129 cases. J Rheumatol 1992; 19:402-10. [PMID: 1578454].
5. Koç Y, Gullu I, Akpek G, Akpolat T, Kansu E, Kiraz S, Batman F, Kansu T, Balkanci F, Akkaya S. Vascular involvement in Behcet’s disease. J Rheumatol 1992; 19:402-10. [PMID: 1578454].
6. Hoving MN, Ben Ghorbel I, Khiari Ben Salah I, Lam loum M, Ben Ahmed M, Miled M. Deep vein thrombosis in Behcet’s disease. Clin Exp Rheumatol 2001; 19:S48-50. [PMID: 11760399].
7. Yurdakul S, Guınaydin I, Tüzün Y, Tankurt N, Pazarli H, Ozyazgan Y, Yazici H. The prevalence of Behcet’s syndrome in a rural area in northern Turkey. J Rheumatol 1988; 15:820-2. [PMID: 3172095].
8. Azizlerli G, Kose AA, Sarica R, Gul A, Tutkun IT, Kulaç M, Tunc R, Urgancioglu M, Dişiç R. Prevalence of Behcet’s disease in Istanbul, Turkey. Int J Dermatol 2003; 42:803-6. [PMID: 14521694].
9. Gül A, Tugal-Tutkun I, Dinarello CA, Reznikov L, Esen BA, Mirza A, Scanlon P, Solinger A. Interleukin-1β-regulating antibody XOMA 052 (gevokizumab) in the treatment of acute exacerbations of resistant uveitis of Behcet’s disease: an open-label pilot study. Ann Rheum Dis 2012; 71:S63-6. [PMID: 22084392].
10. Ozcımen AA, Dilek K, Bingu U, Sarıca ogu H, Sarando A, Taskapılıoğlu O, Yurtkuran M, Yurtkuran MA, Oral B. IL-1 cluster gene polymorphisms in Turkish patients with Behcet’s disease. Int J Immunogenet 2011; 38:295-301. [PMID: 21418526].
11. Oral HB, Dilek K, Ozcımen AA, Taskapılıoğlu O, Bingu U, Sarando A, Sarıca ogu H, Yurtkuran M, Yurtkuran A. Interleukin-4 Gene Polymorphisms Confer Behcet’s Disease in Turkish Population. Scand J Immunol 2011; 73:594-601. [PMID: 21323696].
12. Negro K, Kinouchi Y, Hiwata shi N, Takahashi S, Takagi S, Satoh J, Shimosegawa T, Toyota T. Crohn’s disease is draining region of the associated with novel polymorphisms in the tumor necrosis factor gene. Gastroenterology 1999; 117:1062-8. [PMID: 10535868].
13. Elkarim RA, Mustafa M, Kivisakk P, Link H, Bakhiet M. Cytokine autoantibodies in multiple sclerosis, aseptic meningitis and stroke. Eur J Clin Invest 1998; 28:295-9. [PMID: 9615907].
14. Sobti RC, Maithil N, Thakur H, Sharma Y, Talwar KK. VEGF and IL-4 gene variability and its association with the risk of coronary heart disease in north Indian population. Mol Cell Biochem 2010; 341:139-48. [PMID: 20364398].
15. International Study Group for Behcet’s Disease. Criteria for diagnosis of Behcet’s disease. Lancet 1990; 335:1078-80. [PMID: 1970380].
16. Mout R, Willemze R, Landegent JE. Repeat polymorphisms in the interleukin-4 gene. Nucleic Acids Res 1991; 19:3763-7. [PMID: 1804125].
17. Dean AG, Sullivan KM, Soe MM. “Open Source Epidemiologic Statistics for Public Health” Version 2.3.1. www.OpenEpi.com, updated 2010/09/09, accessed 2011/02/21.
18. Michealson JB, Chisari FV. Behçet’s disease. Surv Ophthalmol 1982; 20:189-20. [PMID: 1970380].
19. Al-Mutawa SA, Hegab SM. Behçet’s disease. Clin Exp Med 2004; 4:103-31. [PMID: 15599660].
20. Opremcak EM. Uveitis. A clinical manual for ocular inflammation. Springer Verlag, New York Berlin Heidelberg, 1994; pp 200–202.
21. George RK, Chan CC, Whitecup SM, Nussenblatt RB. Ocular immunopathology of Behçet’s disease. Surv Ophthalmol 1997; 42:157-62. [PMID: 9381369].
22. Sakane T, Takeno M, Suzuki N, Inaba G. Behcet’s disease. N Engl J Med 1999; 341:1284-91. [PMID: 10528040].
23. Tursen U, Pıksın G, Lotti T, Davatchi F. Pathological and Immunological Developments in Behçet’s Disease Hindawi Publishing Corporation Pathology Research International Volume 2012, Article ID 305780, 2 pages.
24. Marshall SE. Behcet’s disease. Best Pract Res Clin Rheumatol 2004; 18:291-311. [PMID: 15158742].
25. Ohno S, Ohguchi M, Hirole S, Matsuda H, Wakisaka A, Aizawa M. Close association of HLA-Bw51 with Behcet’s disease. Arch Ophthalmol 1982; 100:1455-8. [PMID: 6956266].
26. Gül A. Behcet’s disease: an update on the pathogenesis. Clin Exp Rheumatol 2001; 19:S6-12. [PMID: 11760403].
27. Ohno S, Asanuma T, Sugiyama S, Wakisaka A, Aizawa M, Itakura K. HLA-Bw51 and Behcet’s disease. JAMA 1978; 240:529. [PMID: 671660],

28. Pirim I, Atasoy M, Ikal M, Erdem T, Aliagaoglu C. HLA class I and class II genotyping in patients with Behcet’s disease: a regional study of eastern part of Turkey. Tissue Antigens 2004; 64:293-7. [PMID: 15304011],

29. Lazić R, Boras I, Vlasić M, Gabrić N, Tomić Z. Anti-VEGF in treatment of central retinal vein occlusion. Coll Antropol 2010; 34:Suppl 269-72. [PMID: 21305727],

30. Germain M, Saut N, Greliche N, Dina C, Lambert J-C, Perret C, Cohen W, Oudot-Mellakh T, Antoni G, Alessi M-C, Zelenika D, Cambien F, Tiret L, Bertrand M, Dupuy A-M, Letenneur L, Lathrop M, Emmerich J, Amouyel P, Tregouet D-A, Morange P-E. Genetics of Venous Thrombosis: Insights from a New Genome Wide Association Study. PLoS ONE 2011; 6:e25581- [PMID: 21980494],

31. Vasudevan R, Norhasniza MN, Patimah I. Association of variable number of tandem repeats polymorphism in the IL-4 gene with end-stage renal disease in Malaysian patients. Genet Mol Res 2011; 10:943-7. [PMID: 21644211],

32. Oliver WER. Cytokine genes and disease susceptibility. Cytokine 2004; 28:174-8. [PMID: 15588692],

33. Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 1989; 44:388-96. [PMID: 2916582],

34. Kruglyak L. Prospects for whole-genome linkage disequilibrium mapping of common disease genes. Nat Genet 1999; 22:139-44. [PMID: 10369254],

35. Konwar R, Bid HK. Location of the 70bp VNTR polymorphic site is in third intron of IL-4 gene. Indian J Clin Biochem. 2008; 23:204-5. [PMID: 23105754].

36. Chen X, Xu J, Chen Z, Zhou Z, Feng X, Zhou Y, Ren Q, Yang R, Han ZC. Interferon-γ +874A/T and interleukin-4 intron3 VNTR gene polymorphisms in Chinese patients with idiopathic thrombocytopenic purpura. Eur J Haematol 2007; 79:191-7. [PMID: 17655693].

37. Tsai FJ, Chang CH, Chen CC, Hsia TC, Chen HY, Chen WC. Interleukin-4 gene intron-3 polymorphism is associated with transitional cell carcinoma of the urinary bladder. BJU Int 2005; 95:432-5. [PMID: 15679809],

38. Buchs N, Silvestrini T, di Giovasse F, Chabaud M, Vannier E, Duff GW, Miossec P. IL-4 VNTR gene polymorphism in chronic polyarthritis. The rare allele is associated with protection against destruction. Rheumatol 2000; 39:1126-31.

39. Toydemir PB, Elhan AH, Tüken A, Toydemir R, Güler A, Tüzünler A, Böksøy I. Effects of Factor V Gene G1691A, Methylenetetrahydrofolate Reductase Gene C677T, and Prothrombin Gene G20210A Mutations on Deep Venous Thrombogenesis in Behcet’s Disease. J Rheumatol 2000; 27:2849-54. [PMID: 11128675].

40. Pieroni F, Dayse M, Lourenço, Morelli VM, Maffei FH, Zago MA, Franco RF. Cytokine gene variants and venous thrombotic riskin the Bratros (BRAZILIAN THROMBOSIS STUDY) Thromb Res 2007; 120:221-9. [PMID: 17113632].

41. Koster T, Blann AD, Bier E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet 1995; 345:152-5. [PMID: 7823669].

42. Meijers JC, Tekelenburg WL, Bouma BN, Bertina RM, Rosendaal FR. High levels of coagulation factor XI as a risk factor for venous thrombosis. N Engl J Med 2000; 342:696-701. [PMID: 10706899].

43. van Hylckama Vlieg A, van der Linden IK, Bertina RM, Rosendaal FR. High levels of factor IX increase the risk of venous thrombosis. Blood 2000; 95:3678-82. [PMID: 10845896].

44. Abdelrahman MH, Mahdy S, Khanjar IA, Siam AM, Malallah HA, Al-Emadi SA, Sarakbi HA, Hammoudeh M. Prevalence of HLA-B27 in Patients with Ankylosing Spondylitis in Qatar. Int J Rheumatol 2012; 2012:6860213-[PMID: 22548073].

45. Kurata R, Nakaoka H, Tajima A, Hosomichi K, Shinta T, Meguro A, Mizuki N, Ohono S, Inoue I, Inoko H. TRIM39 and RNFL39 are associated with Behçet’s disease independently of HLA-B51 and -A26. Biochem Biophys Res Commun 2010; 401:533-7. [PMID: 20875797].

46. Akman A, Sallakci N, Coskun M, Baciarı A, Yavuzer U, Alpsoy E, Yegin O. TNF-a gene 1031 T/C polymorphism in Turkish patients with Behçet’s disease. J Dermatol 2006; 33:350-6. [PMID: 16882174].

47. Kim ES, Kim SW, Moon CM, Park JJ, Kim TI, Kim WH, Cheon HJ. Interactions between IL17A, IL23R, and STAT4 polymorphisms confer susceptibility to intestinal Behcet’s disease in Korean population. Life Sci 2012; 90:740-6. [PMID: 22483685].

48. Mizuki N, Meguro A, Ota M, Ohno S, Shiota T, Kawagoe T, Ito N, Kera J, Okada E, Yatsu K, Song YW, Lee EB, Kitaiichi N, Namba K, Horie Y, Takento M, Sugita S, Mochizuki M, Bahram S, Ishigatsubo Y, Inoko H. Genome-wide association studies identify IL23R–IL12RB2 and IL10 as Behcet’s disease independently of HLA-B51 and -A26. Biochem Biophys Res Commun 2010; 401:533-7. [PMID: 20875797].

49. Remmers EF, Cosan F, Kirino Y, Ombrello MJ, Abaci N, Satorius C, Le JM, Yang B, Korman BD, Cakiris A, Aglar O, Emrence Z, Azakli H, Ustek D, Tugal-Tutkun I, Akman-Demir G, Chen W, Amos CI, Dizon MB, Kose AA, Azizlerli G, Erer B, Brand OJ, Kaklamani VG, Kaklamani P, Bencherit E, Stanford M, Fortune F, Ghabra M, Olifer WE, Cho YH, Bang D, O’Shea J, Wallace GR, Gadina M, Kastner DL, Gül A. Genome-wide association study identifies IL23R–IL12RB2 and IL10 as Behcet’s disease susceptibility loci. Nat Genet 2010; 42:703-6. [PMID: 20622879].

50. Jang WC, Nam YH, Ahn YC, Lee SH, Park SH, Choe JY, Lee SS, Kim SK. Interleukin-17F gene polymorphisms in Korean patients with Behcet’s disease. Rheumatol Int 2008; 29:173-8. [PMID: 18769923].
51. Jiang Z, Yang P, Hou S, Du L, Xie L, Zhou H, Kijlstra A. IL-23R gene confers susceptibility to Behcet’s disease in a Chinese Han population. Ann Rheum Dis 2010; 69:1325-8. [PMID: 20375120].

52. Chen XD, Tian L, Ming LI, Jin W, Zhang H, Zheng C. Relationship between endothelial cell protein C receptor gene 6936A/G polymorphisms and deep venous thrombosis Chin Med J (Engl) 2011; 124:72-5. [PMID: 21362311].

53. Shahram F, Nikoopour E, Rezaei N, Saeedfar K, Ziaei N, Davatchi F, Amirzargar A. Association of interleukin-2, interleukin-4 and transforming growth factor-beta gene polymorphisms with Behcet’s disease. Clin Exp Rheumatol 2011; 29:Suppl 67S28-31. [PMID: 21640045].

54. Chi W, Zhu X, Yang P, Liu X, Lin X, Zhou H, Huang X, Kijlstra A. Upregulated IL-23 and IL-17 in Behcet patients with active uveitis. Invest Ophthalmol Vis Sci 2008; 49:3058-64. [PMID: 18579762].

55. Takada H, Nomura A, Ishimura M, Ichiyama M, Ohga S, Hara T. NEMO mutation as a cause of familial occurrence of Behcet’s disease in female patients. Clin Genet 2010; 78:575-9. [PMID: 20412081].

56. Ghinoi A, Boiardi L, Atzeni F, Casali B, Farnetti E, Nicoli D, Pipitone N, Olivieri I, Cantini F, Salvì F, La Corte R, Triolo G, Filippini D, Paolazzi G, Salvarani C. Protein Z G79A and A-13G gene polymorphisms in Italian patients with Behçet’s disease. Clin Exp Rheumatol 2009; 27:Suppl 53S23-8. [PMID: 19796528].

57. Cho SB, Ahn KJ. Kim do H, Zheng Z, Cho S, Kang SW, Lee JH, Park YB, Lee KH, Bang D. Identification of HnRNP-A2/BI as a target antigen of anti-endothelial cell IgA antibody in Behçet’s disease. J Invest Dermatol 2012; 132:601-8. [PMID: 22205302].

58. Roumen-Klappe EM, Den Heijer M, Janssen MC, Van der Vleuten C, Thien T, Wollersheim H. The postthrombotic syndrome: Incidence and prognostic value of non-invasive venous examinations in a six-year follow-up study. Thromb Haemost 2005; 94:825-30. [PMID: 16270638].

59. Stain M, Schonauer V, Minar E, Bialonczyk C, Hirschl M. The postthrombotic syndrome: Risk factors and impact on the course of thrombotic disease. J Thromb Haemost 2005; 3:2671-6. [PMID: 16359506].

60. Christiansen SC, Ness IA, Cannegieter SC, Hammerstrom J, Rosendaal FR. Inflammatory Cytokines as Risk Factors for a First Venous Thrombosis: A Prospective Population-Based StudyPieter H. Reitsma 2006; 3:334-5. .