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Association between the methylene tetrahydrofolate reductase gene C677T mutation and colchicine unresponsiveness in Behcet's disease

Nevin Karakus,[1,2] Serbulent Yigit,[2] Goknur Kalkan,[3] Aydin Rustemoglu,[2] Ahmet Inanir,[4] Ulker Gul,[5] Gunseli Sefika Pancar,[6] Songul Akkanet,[2] Omer Ates[2]

[1]Ondokuz Mayis University, Faculty of Medicine, Department of Medical Biology, Samsun, Turkey; [2]Gaziosmanpasa University, Faculty of Medicine, Department of Medical Biology, Tokat, Turkey; [3]Department of Dermatology, Tokat, Turkey; [4]Department of Physical Therapy and Rehabilitation, Tokat, Turkey; [5]Ankara Numune Training and Research Hospital, Second Dermatology Clinics, Ankara, Turkey; [6]Tokat State Hospital, Department of Dermatology, Tokat, Turkey

Purpose: Behcet's disease (BD) is a multisystemic immunoinflammatory disorder characterized by mucocutaneous, ocular, vascular, and central nervous system manifestations. The common methylene tetrahydrofolate reductase (*MTHFR*) gene C677T mutation is a known risk factor for thrombosis. The aim of this study was to investigate the *MTHFR* gene C677 mutation in patients with BD and evaluate if there was an association with clinical features, especially thrombosis, in a relatively large cohort of patients with BD.
Methods: The study included 318 patients with BD and 207 healthy controls. Genomic DNA was isolated and genotyped using PCR-based restriction fragment length polymorphism assay for the *MTHFR* gene C677T mutation.
Results: The genotype and allele frequencies of the C677T mutation showed a statistically significant difference between BD patients and controls (p=0.003 and p=0.001, respectively). There was also a significant association between C677T alteration and response to colchicine in BD patients (p=0.046).
Conclusions: The results of this study showed that there was a high association between the *MTHFR* gene C677T mutation and BD. Stratification analysis according to clinical features for this disease did not reveal an association except response to colchicine that was shown to be influenced by the *MTHFR* C677T mutation.

Behcet's disease (BD), first described in 1937 by dermatologist Dr. Hulusi Behçet from Istanbul, 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 inflammation. 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]. Colchicine is commonly used in the treatment of Behçet's disease; however, some patients are unresponsive to colchicine treatment.

BD is endemically higher in Turkey and Japan. These countries have populations derived historically from the ancient Silk Road and have a prevalence of 80 to 370 cases per 100,000 [4]. BD occurs more commonly in men than in women and affects primarily individuals between the second and fourth decades of life, with a more aggressive course in young male adults. The leading cause of chronic morbidity is high, especially with ophthalmic inflammation, which can eventually result in blindness.

Although the pathogenesis of BD remains poorly understood, certain infectious and environmental factors are able to trigger symptomatology in individuals with particular genetic variants [5]. In common with ankylosing spondylitis and psoriatic arthropathy, BD has major histocompatibility complex (MHC) class I associations. Human leukocyte antigen HLA-B51 is the most strongly associated known genetic factor to BD [6]; however, it accounts for less than 20% of the genetic risk, even in familial cases (less than 5%), which indicates that other genetic factors exist.

Methylene tetrahydrofolate reductase (MTHFR) is the enzyme that catalyzes the transformation of homocysteine (Hcy) to methionine via the remethylation pathway (gene located in 1p36) [7]. Hyperhomocysteinemia (HHC), a known prothrombotic condition, is the consequence of decreased activity of MTHFR [8]. The C677T mutation of the *MTHFR* gene, which causes an amino acid change from alanine to valine, is associated with reduced activity and increased thermolability of the enzyme [9]. This mutation is considered the most common genetic cause of elevated Hcy levels [10, 11] and could slightly increase the risk of arterial or venous thrombosis [12,13]. HHC was repeatedly observed in BD [14-17], sometimes with a positive correlation with

Correspondence to: Dr. Nevin Karakus, Gaziosmanpasa University, Faculty of Medicine, Department of Medical Biology, 60100, Tokat, Turkey; Phone: +90 3562129500/7353; FAX: +90 356 2133179; email: nevinbalci@hotmail.com

thrombosis [14,16,18] and ocular involvement [15,17], which is usually accompanied by retinal vaso-occlusive disease.

Our aim in this study was to investigate the *MTHFR* gene C677T mutation in patients with BD and evaluate if there was an association with clinical features, especially thrombosis, in a relatively large cohort of patients with BD.

METHODS

Subjects: The study group consisted of 318 unrelated patients with BD (160 male and 158 female; mean age and standard deviation [SD] 36.57±9.485 years) and 207 unrelated healthy controls (82 male and 125 female; mean age and SD 36.31±11.978 years). BD patients were gathered from different clinics (Ankara Numune Training and Research Hospital Second Dermatology Clinic, Ankara, Turkey Department of Dermatology and Department of Physical Therapy and Rehabilitation of Gaziosmanpasa University, Tokat, Turkey) and fulfilled the International Criteria of Behcet's Disease for classification [19]. All participants were of Turkish origin from the central region of Turkey. The healthy controls were matched in age and geographic area with BD patients. The study protocol was approved by the Local Ethics Committee of Gaziosmanpasa University Faculty of Medicine and written informed consent was obtained from the study participants.

Genotyping of MTHFR gene mutation: Genomic DNA was extracted from EDTA-treated whole venous blood samples, using a commercial DNA isolation kit (Sigma-Aldrich, Taufkirchen, Germany). The *MTHFR* C677T mutation was analyzed by PCR-based restriction fragment length polymorphism (RFLP) methods as described previously [8]. The PCR protocol consisted of an initial melting step of 5 min at 94 °C; followed by 35 cycles of 30 s at 94 °C, 30 s at 61 °C, and 30 s at 72 °C; and a final elongation step of 5 min at 72 °C. PCR primers (5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ and 5′-AGG ACG GTG CGG TGA GAG TG-3′) were used to amplify a portion of the *MTHFR* gene from 100 ng of genomic DNA in a 25-µl reaction containing 2.5 µl of 10× PCR buffer (Fermentas, Shenzhen, China), 200 µM dNTP (Fermentas), 10 pM each of primers (MWG-Biotech AG, Ebersberg, Germany), and one unit of Taq DNA polymerase (Fermentas). After amplification, the 198-bp PCR product was digested with HinfI (Fermentas) in a 15-µl reaction solution containing 10 µl of PCR product, 1.5 µl of 10× buffer, and two units of HinfI at 37 °C overnight. The digestion products were separated on 3% agarose gels, and fragments stained with ethidium bromide were photographed on an ultraviolet transilluminator. Wild-type individuals were identified by only a 198-bp fragment, heterozygotes by both the 175/23-bp fragments, and homozygote variants (TT) by the 175-bp fragment.

Statistical analysis: Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 13.0, SPSS Inc., Chicago, IL) and the OpenEpi Info software package version 2.2. Results were given as mean±standard deviation (SD). The χ^2 test was used to evaluate the Hardy–Weinberg equilibrium for the distribution of the genotypes of the patients and the controls. The relationships between the C677T mutation and the clinical and demographics features were analyzed by using the χ^2 test or ANOVA (ANOVA) statistics. The χ^2 test and Fisher's exact test were used to compare categorical variables appropriately, and odds ratio (OR) and 95% confidence interval (CI) were used for the assessment of risk factors. All p values were 2-tailed, and CIs were set at 95%. A p value less than 0.05 was considered significant.

RESULTS

The baseline clinical and demographics features of the study patients with BD are shown in Table 1. With one exception, there was no statistically significant association observed between clinical and demographic features of BD patients (gender, age, disease duration, treatment duration, oral ulcers, genital ulcers, ocular inflammation, deep venous thrombosis, skin lesions, colchicine use, papulopustule, erythema nodosum) and the *MTHFR* gene C677T mutation. There was a statistically significant association between response to colchicine and the *MTHFR* C677T mutation in BD patients (p=0.046; data not shown). Because BD is common in males, we compared colchicine responsiveness with gender and no association was found. The response to treatment was higher in homozygous normal BD patients than heterozygous BD patients (65.5% versus 29.1%). Allelic and genotypic distributions of the C677T mutation are shown in Table 2. The observed and expected frequencies of the mutation in both patient and control groups were in Hardy–Weinberg equilibrium. The genotype and allele frequencies of C677T mutation showed a statistically significant difference between BD patients and controls (p=0.003 and p=0.001, respectively; OR 1.7, 95% CI 1.23–2.35). Although the frequency of heterozygosity of C677T was nearly similar between two groups, the homozygosity of C677T was significantly higher in BD patients than healthy controls (p=0.004; OR 5.05, 95% CI: 1.49–17.11).

DISCUSSION

In our study we found a high association between BD and the *MTHFR* gene C677T mutation. In other studies of the *MTHFR* C677T mutation and BD in the Turkish population, such a significant association has not been reported [20,21]. Ozkul et al. found equal frequencies of *MTHFR* C677T polymorphism overall in 59 patients and 42 healthy controls [21]. Although in their study the frequency of the TT homozygous genotype was higher in patients than in controls, the difference was statistically not significant. Canataroglu et al. reported the frequencies of homozygosity for the *MTHFR* C677T mutation as 7.5% and 10% in BD patient and

Table 1. Baseline clinical and demographics features of the study patients with BD.

Characteristic	Study group
Gender, number male/female (male % /female %)	160/158 (50.3/49.7)
Age, mean±SD (range) years	36.57±9.485
Disease duration, mean±SD (range) years	7.09±6.601
Treatment duration, mean±SD (range) years	5.64±6.588
Oral ulcers, n (%)	216/218 (99.1)
Genital ulcers, n (%)	164/218 (75.2)
Ocular inflammation, n (%)	89/210 (42.4)
Deep venous thrombosis (DVT), n (%)	41/210 (19.5)
Skin lesions, n (%)	96/210 (45.7)
Colchicine use, n (%)	216/218 (99.1)
Response to colchicine, n (%)	165/215 (76.7)
Papulopustule, n (%)	125/218 (57.3)
Erythema nodosum, n (%)	84/218 (38.5)

Table 2. Genotype and allele frequencies of *MTHFR* gene C677T polymorphisms in patient and control groups.

MTHFR C677T	BD patients n (%)	Healthy controls n (%)	p	OR (CI 95%)
Genotypes				
CC	189 (59.4)	146 (70.5)	0.003	
CT	107 (33.6)	58 (28.0)		
TT	22 (6.9)	3 (1.4)		
CC+CT: TT	296: 22	204: 3	0.004	5.05 (1.49–17.11)
CC: CT+TT	189: 129	146: 61	0.009	1.63 (1.13–2.38)
Alleles				
C	485 (76.3)	350 (84.5)	0.001	1.70 (1.23–2.35)
T	151 (23.7)	64 (15.5)		

control groups (p>0.05), respectively, from the southern region of Turkey (40 BD patients and 60 healthy controls) [20]. There was no concordance between our results and the results of other studies of the Turkish population, perhaps because of the low patient and control populations of these studies compared to ours. No relation was found between the *MTHFR* C677T mutation and BD in reports of small study populations from Spain [22,23]. In concordance with our study, homozygosity of the T allele of the *MTHFR* gene was found to be prevalent in BD patients in a Tunisian population (35 BD patients were compared to 39 healthy volunteers) [24]. Our study is the first study that has investigated the association between the *MTHFR* C677T mutation and BD in a large study population.

With one exception, no statistically significant association was observed in our study between clinical and demographic features of BD patients (gender, age, disease duration, treatment duration, oral ulcers, genital ulcers, ocular inflammation, DVT [deep venous thrombosis], skin lesions, colchicine use, papulopustule, erythema nodosum) and the *MTHFR* gene C677T mutation. Many previous studies focused on the genotype distribution of *MTHFR* C677T of BD patients with and without DVT. While one study reported an association between thrombosis and the *MTHFR* C677T mutation in BD patients [20], no association was found in the others [22-28]. The *MTHFR* C677T mutation was also shown to have an association with ocular involvement in BD patients [21], but this was not confirmed by another study [29].

In our study, we found a significant association between response to colchicine and the *MTHFR* C677T mutation in BD patients (p=0.046); this has not been observed in other studies. Colchicine, commonly used in the treatment of Behçet's disease and other inflammatory diseases, inhibits neutrophil chemotaxis, resulting in a decreased number in the inflamed area, thereby preventing exacerbation of the disease [30]. However, some patients are unresponsive to colchicine treatment in BD and other inflammatory diseases. Colchicine unresponsiveness was studied in Familial Mediterranean Fever (FMF) patients with different genes. For example, C3435T polymorphism in exon 26 of multpile drug resistance 1 *(MDR1)* gene was associated with colchicine resistance in nonresponder FMF patients during the common therapy protocol [31]. Soylemezoglu et al. [32] reported that the M694V/M694V mutation of the Mediterranean fever *(MEFV)* gene is associated with a lower response to colchicine treatment. In the literature, colchicine efficacy was not found to be the same for female and male patients [33], but in our

study there was no difference in colchicine responsiveness between gender.

In patients with *MTHFR* mutations, folate metabolism is affected in several ways. Specifically, the methylation cycle seems to be impaired; reduced enzyme activity and decreased remethylation of Hcy to methionine lead to elevated total Hcy and reduced de novo methyl group supply for transmethylation reactions. Moreover, altered Th1/Th2 (T helper 1/2) balance resulting from inhibition of the remethylation cycle is speculated to cause abnormal cellular immune response in relevant patients [34]. Because of this effect on immune response, unresponsiveness to colchicine may occur in *MTHFR* mutation carriers.

Although in this study we hoped to find an association between DVT in BD patients and the *MTHFR* C677T mutation, surprisingly we found an association between response to colchicine treatment of BD patients and the *MTHFR* C677T mutation. This is an important result that must be confirmed by other studies not only in BD but also in other inflammatory disorders using colchicine for treatment. Last but not least, if our observation can be substantiated with further studies, evaluation for MTHFR mutations and perhaps folate supplementation may become necessary in selected patients.

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