Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Investigation of ABCB1 gene polymorphism with colchicine response in Behçet's disease

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Genet. Mol. Res. 10 (1): 1-6 (2011)
Received November 11, 2010
Accepted December 12, 2010
Published January 4, 2011
DOI 10.4238/vol10-1gmr824

ABSTRACT. Colchicine is commonly used in the treatment of Behçet's disease. However, some patients are unresponsive to colchicine treatment. Adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1) transports colchicine out of cells. We investigated a possible association of C3435T polymorphism of the ABCB1 (MDR1) gene with colchicine response in patients with Behçet's disease. We randomly selected 97 patients with Behçet's disease, examined ABCB1 (MDR1) gene C3435T polymorphisms, and evaluated patient responses to colchicine. Forty-three patients were colchicine responsive, while the remaining 54 patients were unresponsive. No significant difference was found between genotypic and allelic frequencies of the ABCB1 C3435T polymorphisms in patients with Behçet's disease and healthy volunteers. Also, there was no significant difference among responsive and nonresponsive patients. We concluded that ABCB1 C3435T polymorphism is not associated with a colchicine response in patients with Behçet's disease.

Key words: Behçet's disease; Colchicine; ABCB1 gene; Polymorphism

INTRODUCTION

Behçet's disease is a chronic, relapsing, multisystem vasculitis, which was first described in 1937 by Hulusi Behçet (Behçet, 1937). The disease is characterized by mucocutaneous symptoms such as oral aphthae, genital ulceration, papulopustule, erythema nodosum, and inflammatory lesions of the eyes and joints. Central nervous system and gastrointestinal system involvement as well as trombo-occlusive events are also observed.

Etiopathogenesis of the disease is unknown, but environmental factors are likely to be triggers in genetically predisposed patients. Affected organs show a significant neutrophilic and lymphocyte infiltration. Colchicine, commonly used in inflammatory diseases, inhibits neutrophil chemotaxis, decreases their number in the inflamed area, thereby preventing exacerbation of the disease (Ehrenfeld et al., 1980).

Some patients do not respond to regular use of colchicine. In diseases, which are treated with colchicine, polymorphisms of the adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1) gene have been investigated for clinical drug response (Tufan et al., 2007). P-glycoprotein (P-gp) encoded by the ABCB1 (MDR1) gene, is an integral membrane protein that serves as a cellular efflux pump (Ben-Chetrit and Levy, 1998). P-gp transports a variety of drugs including colchicine out of cells (Kim, 2002). To date, many single-nucleotide polymorphisms (SNP) of the ABCB1 gene have been identified (Hoffmeyer et al., 2000). Depending on the type of SNP, MDR1 genetic variants can impact target cell concentrations of P-gp/MDR1 substrates, either increasing or decreasing therapy resistance (Rund et al., 1999). The C3435T polymorphism, which was found significantly in correlation with the function of MDR1 and the expression of P-gp, also affects the clinical drug response and results of the treatment (Illmer et al., 2002; Marzolini et al., 2004; Babaoglu et al., 2005).

In this study, we investigated the C3435T polymorphism of the ABCB1 (MDR1) gene for colchicine response in patients with Behçet's disease.

MATERIAL AND METHODS

Patients

Patients (N = 97) who fulfilled International Study Group criteria (International Study Group for Behçet's Disease Contributors, 1991) for diagnosis of Behçet's disease were included in the study. All patients were under supervision at the multidisciplinary Behçet's syndrome center in Uludag University Medical Faculty and were randomly selected. The control group consisted of 47 healthy subjects who had no oral aphthae history. The Ethics Committee of Uludağ University approved the present study and all subjects gave written informed consent for the study.

Inclusion criteria for the study were regular use of colchicines, used at a dosage of >1 mg/day for at least 6 months, with no additional treatment. Colchicine response was defined as completely improvement in mucocutaneous lesions or a 50% decrease in frequency of occurrence of symptoms. It was observed that 43 patients were colchicine resistant while 54 patients responded to colchicine therapy.

For the study, which included 97 (62 females/35 males) patients and 47 (28 females/19 males) controls, C3435T polymorphisms were studied.

Analysis of the ABCB1 C3435T polymorphism

Peripheral blood (2 mL) was collected in tubes containing EDTA for DNA extraction. DNA was extracted by a DZ DNA isolation kit. After DNA extraction, polymerase chain reaction (PCR)-restriction fragment length polymorphism was used for the detection of C3435T SNP (Miao et al., 2008). PCR was performed in a total volume of 25 mL, using 100 ng genomic DNA with 20 pmoL forward primer (5'-GAT CTG TGA ACT CTT GTT TTC A-3') and reverse primer (5'-GAA GAG AGA CTT ACA TTA GGC-3'), 0.2 mM each dNTP, 1X buffer, 2 mM $MgCl_2$ and 1 U Taq DNA polymerase (Bioron, Germany). Cycling was performed in a GenAmp PCR System 9700 (Applied Biosystems) as follows: initial denaturation at 94°C for 5 min and 35 cycles of the following: 94°C for 30 s, 60°C for 30 s, 72°C for 30 s, and a final step at 72°C for 10 min. The PCR products were then submitted to *Mbo*I (Bioron, Germany) digestion at 37°C for 16 h. Fragments obtained were 244 bp for the T/T genotype, 174 and 70 bp for the C/C genotype, and 244, 174 and 70 bp for the C/T genotype. Reaction products were separated by 2% agarose gel electrophoresis (Figure 1).

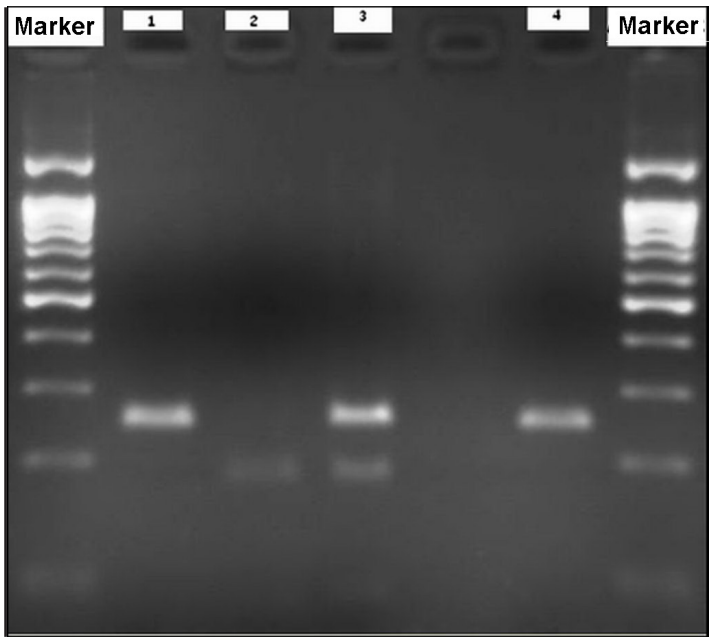

Figure 1. Agarose gel showing results of the ABCB1 C3435T polymorphism in some individuals. *Lane 1* = samples of the homozygous T/T genotype. *Lane 2* = samples of the homozygous C/C genotype. *Lane 3* = samples of the heterozygous C/T genotype. *Lane 4* = uncut product.

Statistical analysis

Allelic frequencies and genotype distributions among groups were compared by the chi-square test. Mann-Whitney U-tests were used for comparison of numerical variables among groups. The significance of the differences was defined as $P < 0.05$. All values are reported as means ± standard deviation.

RESULTS

The colchicine treatment dosages were either 1 or 1.5 mg/day. When the responsive and nonresponsive groups were compared, no difference was observed in terms of demographic and clinical features such as age, disease duration, colchicine dosage, treatment duration, HLA-B5, and pathergy phenomenon positivity. However, in the nonresponsive group, female patients were significantly more common when compared to the responsive group (P = 0.019). Colchicine-resistant patients were similar to the responsive group for the clinical manifestations including oral aphthae, genital ulceration, papulopustule, erythema nodosum, uveitis, thrombophlebitis, and arthritis (Table 1). Liver function abnormality was observed in 9 patients, diarrhea in 3 patients, gastric disturbance in 5 patients, and leukocytopenia in 1 patient. In three patients, the treatment was discontinued because of side effects, with elevated transaminases in two patients and diarrhea in one patient. Among 18 patients in which side effects were observed, 11 patients were responsive and 7 were nonresponsive.

Table 1. Demographic and clinical features of colchicine-responsive and -nonresponsive patients.

Feature	Responsive	Nonresponsive	P
Age (years)	37.1 ± 10.3	37.8 ± 9.1	NS
Gender (female/male)	29/25	33/10	0.019
Disease duration (years)	6.6 ± 6.1	7.8 ± 5.5	NS
Colchicine dosage	1.3 ± 0.2	1.3 ± 0.2	NS
Treatment duration (years)	4.9 ± 4.6	6.0 ± 5.1	NS
Oral aphthae	100.0%	100.0%	NS
Genital ulceration	81.5%	79.1%	NS
Papulopustule	61.1%	65.1%	NS
Erythema nodosum	46.3%	55.8%	NS
HLA-B5	20.4%	27.9%	NS
Pathergy phenomenon	53.7%	52.4%	NS
Uveitis	33.3%	16.7%	NS
Thrombophlebitis	11.1%	4.8%	NS
Arthritis	5.6%	4.7%	NS

Data are reported as means ± SD or percent. NS = nonsignificant (P > 0.05).

After measuring the frequency of ABCB1 genotypes of the 47 healthy volunteers in the study, 5 were found to be ABCB1 CC genotype carriers, while 33 were ABCB1 CT carriers and the remaining 9 were ABCB1 TT carriers. Among the 97 patients suffering from Behçet's disease, 16 were ABCB1 CC carriers, 60 were ABCB1 CT carriers and the remaining 21 were ABCB1 TT carriers. The ABCB1 C3435T gene frequency was determined to be 52.0% for healthy volunteers and 53.0% for patients with Behçet's disease (Table 2), which showed no significant difference (P > 0.05).

Table 2. Allele frequency and genotype distribution among Behçet's patients and controls.

	Behçet's patients	Controls	P
CC genotype	16 (16.49%)	5 (10.63%)	
CT genotype	60 (61.85%)	33 (70.21%)	P > 0.05
TT genotype	21 (12.64%)	9 (19.14%)	
C allele frequency (%)	47%	46%	
T allele frequency (%)	53%	52%	

Data are reported as number with percent in parentheses, unless otherwise indicated.

Among the 97 patients with Behçet's disease, who were examined for colchicine treatment response, distributions of all genotypes were not significantly different between the responsive and nonresponsive groups (P > 0.05) (Table 3).

Table 3. Distribution of C3435T genotypes by colchicine response.

	Responsive	Nonresponsive	P
CC genotype	9 (16.66%)	7 (16.27%)	
CT genotype	33 (61.11%)	27 (62.79%)	P > 0.05
TT genotype	12 (22.22%)	9 (20.93%)	

Data are reported as number with percent in parentheses.

DISCUSSION

Colchicine accumulates in white blood cells and has a relatively high affinity to granulocytes (Ertel and Wallace, 1971; Ben-Cherit and Levy, 1998). Colchicine affinity to granulocytes may explain its beneficial effect in colchicine-responsive inflammatory diseases. P-gp, one of the most clinically important transmembrane transporters in humans, discharges colchicine out of cells thus playing an important role in resistance to the drug (Kim, 2002; Zhou, 2008). The referred to transporter is encoded by the ABCB1/MDR1 gene. Quite a number of SNP have been found for the MDR1 gene. These polymorphisms are associated with drug bioavailability and resistance and a susceptibility to some inflammatory diseases (Zhou, 2008)

An association of the ABCB1 C3435T polymorphism with colchicine response in familial Mediterranean fever has been reported. There were two reports about the effects of the C3435T polymorphism on colchicine response in familial Mediterranean fever (Gershoni-Baruch et al., 2005; Tufan et al., 2007). Tufan et al. (2007) observed that patients with the TT genotype had better treatment outcome, requiring fewer colchicine dosages for remission. Gershoni-Baruch et al. (2005) reported an opposite result in favor of the CC genotype for responsiveness and for drug concentrations in lymphocytes. Tufan et al. (2007) suggested that inter-ethnic variability may be the cause of different results.

In this study, no significant difference was found between genotypic and allelic frequencies of ABCB1 C3435T polymorphisms in patients with Behçet's disease and healthy volunteers. Also there was no significant difference among responsive and nonresponsive patients. To our knowledge, no study conducted has evaluated the association of the ABCB1 C3435T polymorphism with colchicine response in Behçet's disease.

In the literature, colchicine efficacy was not found to be the same for female and male patients (Yurdakul et al., 2001). We also observed that the number of female patients was higher in the nonresponsive group. But there was no difference in genotypic frequencies between both genders.

Our results demonstrate that ABCB1 C3435T polymorphisms are not associated with colchicine response in Behçet's disease. However, it would be desirable to confirm the study in different cohorts with Behçet's disease.

REFERENCES

Babaoglu MO, Bayar B, Aynacioglu AS, Kerb R, et al. (2005). Association of the ABCB1 3435C>T polymorphism with antiemetic efficacy of 5-hydroxytryptamine type 3 antagonists. *Clin. Pharmacol. Ther.* 78: 619-626.

Behçet H (1937). Uber rezidivierende aphthöse, durch ein Virus verursachte Geschwure, am Mund, am Auge, und an den

Genitalien. *Dermatol. Wschr.* 105: 1152-1157.
Ben-Chetrit E and Levy M (1998). Does the lack of the P-glycoprotein efflux pump in neutrophils explain the efficacy of colchicine in familial Mediterranean fever and other inflammatory diseases? *Med. Hypotheses* 51: 377-380.
Ehrenfeld M, Levy M, Bar EM, Gallily R, et al. (1980). Effect of colchicine on polymorphonuclear leucocyte chemotaxis in human volunteers. *Br. J. Clin. Pharmacol.* 10: 297-300.
Ertel NH and Wallace SL (1971). Measurement of colchicine in urine and peripheral leucocytes. *Clin. Res.* 19: 348.
Gershoni-Baruch R, Peretz Y, Merav L, Dagan E, et al. (2005). The Influence of Polymorphisms in MDR1 on Colchicine Unresponsiveness in Familial Mediterranean Fever. In: The Fourth International Congress on Systemic Autoinflammatory Diseases, November 6-10, Bethesda, 25.
Hoffmeyer S, Burk O, von Richter O, Arnold HP, et al. (2000). Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 97: 3473-3478.
Illmer T, Schuler US, Thiede C, Schwarz UI, et al. (2002). MDR1 gene polymorphisms affect therapy outcome in acute myeloid leukemia patients. *Cancer Res.* 62: 4955-4962.
International Study Group for Behçet's Disease Contributors (1991). Evaluation of Diagnostic ("Classification") Criteria in Behçet's Disease: Toward Internationally Agreed Criteria. In: Behçet's Disease: Basic and Clinical Aspects (O' Duffy and Kökmen B, eds.). Marcel Dekker, New York, 11-39.
Kim RB (2002). Drugs as P-glycoprotein substrates, inhibitors, and inducers. *Drug Metab. Rev.* 34: 47-54.
Marzolini C, Paus E, Buclin T and Kim RB (2004). Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin. Pharmacol. Ther.* 75: 13-33.
Miao LY, Huang CR, Hou JQ and Qian MY (2008). Association study of ABCB1 and CYP3A5 gene polymorphisms with sirolimus trough concentration and dose requirements in Chinese renal transplant recipients. *Biopharm. Drug Dispos.* 29: 1-5.
Rund D, Azar I and Shperling O (1999). A mutation in the promoter of the multidrug resistance gene (MDR1) in human hematological malignancies may contribute to the pathogenesis of resistant disease. *Adv. Exp. Med. Biol.* 457: 71-75.
Tufan A, Babaoglu MO, Akdogan A, Yasar U, et al. (2007). Association of drug transporter gene ABCB1 (MDR1) 3435C to T polymorphism with colchicine response in familial Mediterranean fever. *J. Rheumatol.* 34: 1540-1544.
Yurdakul S, Mat C, Tuzun Y, Ozyazgan Y, et al. (2001). A double-blind trial of colchicine in Behcet's syndrome. *Arthritis Rheum.* 44: 2686-2692.
Zhou SF (2008). Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition. *Xenobiotica* 38: 802-832.