Association of Drug Transporter Gene *ABCB1 (MDR1)* 3435C to T Polymorphism with Colchicine Response in Familial Mediterranean Fever

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ABSTRACT. ***Objective.*** Colchicine is a mainstay of treatment in familial Mediterranean fever (FMF); however, 5%–10% of patients do not respond to colchicine. Adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1 or MDR1) is a drug transporter that extrudes colchicine out of cells. *ABCB1* gene 3435C to T polymorphism has been demonstrated to alter MDR1 expression in mononuclear cells. Thus, the amount of MDR1 in mononuclear cells may alter response to colchicine. We investigated the association between *MDR1* 3435C to T polymorphism and colchicine response in patients with FMF.

Methods. Patients (n = 120) were examined for colchicine responses. *ABCB1* gene 3435C to T genotypes were determined to analyze associations with colchicine resistance.

Results. Ninety-eight patients were evaluated as responders and 22 as nonresponders. The distributions of *ABCB1* CC, CT, and TT genotypes were significantly different between responsive and nonresponsive groups (chi-square = 6.86, p = 0.032). Colchicine resistance was significantly higher in patients harboring the C allele than in patients with TT genotype (odds ratio 9.71, 95% CI 1.58–58.76). Similarly, the mean colchicine dose to prevent remission was significantly lower in the TT group compared with subjects with the C allele (p = 0.014).

Conclusion. Our study revealed an association between 3435C to T polymorphism and colchicine response in patients with FMF. Patients with the TT genotype for the *ABCB1* 3435C to T variant responded better to colchicine in terms of treatment efficacy and colchicine dose requirements. (First Release June 15 2007; J Rheumatol 2007;34:1540–4)

Key Indexing Terms:
FAMILIAL MEDITERRANEAN FEVER — COLCHICINE
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Familial Mediterranean fever (FMF) is characterized by recurrent inflammatory febrile attacks of peritonitis, pleuritis, arthritis, and erysipelas-like erythema[1,2]. FMF is caused by mutations in Mediterranean fever gene (MEFV) that code for pyrin, but its exact molecular function has not been clarified[3]. It has been proposed that numerous cytokines[4-6] and apoptotic[3] and cellular[3,7] pathways are involved in the pathogenesis of FMF.

Colchicine is the mainstay of FMF treatment; it reduces frequency and duration of attacks and also prevents development of amyloidosis[8,9]. Despite regular use, 5%–10% of patients with FMF do not respond to colchicine[9]. Although alternative agents such as interferon-α and infliximab have been tried[10,11], currently no standard alternative drug is available for the disease.

Lidar, *et al* defined clinical features of patients nonresponsive to colchicine: these patients had more severe disease and more prevalent abdominal, pleural, articular, and scrotal attacks and erysipeloid eruptions[12]. They found 2-fold greater colchicine concentration in the mononuclear cells of responders compared with nonresponders and they suggested that colchicine treatment failure is related to a genetic defect distinct from that of FMF[12].

Adenosine triphosphate-binding cassette subfamily B member 1 (ABCB1, also known as MDR1, P-glycoprotein) serves as a cellular efflux transporter and extrudes a variety of drugs including colchicine out of cells[13]. Many single-

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nucleotide polymorphisms of ABCB1 have been identified; among these 3435C to T has been reported to be associated with the function and amount of expression of ABCB1[14,15]. The difference in transporter function among genotypes may presumably be due to changes in *MDR1* mRNA stability during its translation. It has been reported that corresponding mRNA of CC wild-genotype causes more efficient expression of P-glycoprotein than the TT variant[16]. Recent studies have shown that 3435C to T polymorphism was associated with clinical drug responses such as efficacy of antiemetics[17] and with treatment outcomes in certain malignancies[18], epilepsy[19], human immunodeficiency virus (HIV) infection[20], and rheumatoid arthritis[21].

We investigated the clinical relevance of 3435C to T polymorphism of *ABCB1* gene for colchicine efficacy in patients with FMF.

MATERIALS AND METHODS

Patients and controls. The study was approved by the ethics committee of Hacettepe University and all patients gave written informed consent. Consecutive FMF patients (n = 133) fulfilling the criteria for FMF[22] and using colchicine compliantly were included in the study. All patients were recruited through the same FMF clinic at Hacettepe University, between December 2004 and December 2005. Inclusion criteria for the study were age > 18 years, having a regular followup, having no comorbid disease that might mimic FMF attacks, and taking colchicine therapy for a minimum of 1 year. A detailed physical examination and a clinical interview including attack sites, patterns, frequencies, and colchicine side effects were performed for each patient. Disease severity was assessed by the FMF severity scores 1 and 2[23].

Resistance to colchicine was defined as the occurrence of more than one typical attack at any site within a 3 month period while compliantly using colchicine ≥ 2 mg/day[12].

Eight patients were excluded because of psychiatric disturbances and noncompliance with the colchicine therapy or absence in followup visits. In 5 patients, polymerase chain reaction (PCR) analysis was unsuccessful. Finally, 120 patients (68 women, 52 men) were evaluated for associations between the polymorphism and drug response. The control group consisted of a historical sample of 84 healthy subjects in which the 3435C to T polymorphism had been studied[24].

Analysis of ABCB1 3435C to T polymorphism. Blood samples (5 ml in tubes containing EDTA) were collected from each patient and stored at –80°C until studied. Deoxyribonucleic acid was extracted from blood samples using a commercial kit (Qiagen GmbH, Hilden, Germany). 3435C to T polymorphism was analyzed using a validated PCR-based endonuclease digestion method[17,25]. Clinical evaluation and genotyping were conducted by 2 separate departments (Rheumatology and Pharmacology) in a blinded fashion.

Statistical analysis. Allelic frequencies and genotype distributions among groups were compared by chi-square test with or without Yates' correction where applicable. Mann-Whitney U tests or t tests were used for comparison of numerical variables among groups. All reported p values are 2-sided; $p < 0.05$ was considered to indicate statistical significance. For post-hoc power analysis, PS power and sample size calculation software v.2.1.31 was used (available from: http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize)

RESULTS

Of 120 patients studied, 98 were evaluated as responders and the remaining 22 as nonresponders according to their clinical attack patterns. Eight patients (4 consanguineous pairs) were first-degree relatives and one of these 8 subjects was a nonresponder. All patients were highly compliant as they reported a dose omission of less than 3 tablets per month. Colchicine dosages of patients were 1 to 2 mg/day and no patient was receiving more than 2 mg/day.

Demographic and clinical features of patients. Nonresponder patients were similar to the responder group for various demographic features including sex, age at onset of disease, and age at diagnosis (Table 1). Joint attacks were found to be significantly more common in patients nonresponsive to colchicine (Table 1). Severe disease, evaluated by FMF severity scores 1 and 2, was significantly more prevalent in the nonresponsive group. Both groups were comparable with respect to other clinical presentations of FMF.

3435C to T genotypes of patients and controls. Within 120 patients studied, 3435CC, 3435CT, and 3435TT genotypes were found at frequencies of 25.8%, 47.5%, and 26.7%, respectively. Overall, no difference was found for distribution of genotypes or allelic frequencies between the patients with FMF and controls as reported[24] (Table 2).

Association between 3435C to T genotypes and colchicine response. Among 120 FMF patients examined for colchicine treatment response, distributions of ABCB1 3435C to T genotypes were significantly different between the responsive and nonresponsive groups (chi-square = 6.86, p = 0.032; Table 3). Except for one patient, all the colchicine nonresponsive patients had either the CC or CT genotype (Table 3). The risk of having resistance to colchicine was significantly higher in patients harboring the C allele (odds ratio between TT and non-TT genotypes 9.71, 95% CI 1.58–58.76). A post-hoc power calculation revealed a power of 0.82 for the distribution of genotypes within responsive and nonresponsive groups.

Similarly, the dose of colchicine required for patients to obtain remission was smaller in the TT group (mean 1.22 ± SEM 0.058) as compared to patients with a C allele (1.40 ± 0.038) (p = 0.014; Figure 1).

3435C to T genotypes and colchicine side effects. Side effects of colchicine were evaluated only by examining laboratory measures, i.e., cytopenias, liver function test abnormalities, and elevations in creatine kinase. Overall, these side effects were very rare. Cytopenia, liver function abnormality, and elevation in creatine kinase were observed in 9, 24, and 13 cases, respectively. All side effects were mild and no patient needed to discontinue their drug.

DISCUSSION

We observed an association between drug response and *ABCB1* 3435C to T polymorphism in patients with FMF treated with colchicine. In patients with TT genotype, the treatment outcome was significantly better and the dose required for remission was significantly smaller.

Although colchicine treatment is highly effective in FMF, it is evident that 5%–10% of patients do not respond and continue to experience FMF attacks[9]. The mechanism of

Table 1. Demographic and clinical features of colchicine responsive and nonresponsive patients. Results are given as mean (SD) unless otherwise stated.

Feature	Responsive	Nonresponsive	p
Female/male	56/42	12/10	NS
Age, yrs	31.5 (9.6)	33.3 (9.8)	NS
Age at onset of FMF, yrs	13.6 (9.0)	13 (9.9)	NS
Age at FMF diagnosis, yrs	23.8 (10.4)	24.7 (11.1)	NS
Treatment duration, yrs	6.3 (5.9)	8.5 (7.3)	NS
Fever (%)	96.9	100	NS
Peritonitis (%)	95.9	100	NS
Pleuritis (%)	70.4	77.3	NS
Arthritis (%)	40.8	81.8	< 0.001
Erysipelas-like erythema (%)	22.4	40.9	0.07
Family history of FMF (%)	58.2	40.9	NS
Severe disease with FSS1, n (%)	39.8	81.8	< 0.001
Severe disease with FSS2 (%)	42.9	86.4	< 0.001
ESR*, mm/h	12.8 (16.1)	30.9 (24.6)	< 0.001
CRP*, mg/dl	0.54 (0.66)	1.20 (1.65)	0.004
Fibrinogen*, mg/dl	351 (104)	427 (130)	0.006

* As measured in attack free period; FMF: familial Mediterranean fever, FSS1/2: FMF severity score 1/2, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein.

Table 2. Allele and genotype frequencies for *ABCB1* 3435C to T variant in FMF patients and in healthy subjects.

Genetic Feature	FMF	Controls	p
C allele frequency	0.50	0.49	
T allele frequency	0.50	0.51	
CC genotype, % (n)	25.8 (31)	25 (21)	NS
CT genotype, % (n)	47.5 (57)	49 (41)	NS
TT genotype, % (n)	26.7 (32)	26 (22)	NS

Table 3. Distribution of C3435T genotypes with respect to colchicine response.

ABCB1 3435 Genotypes	Colchicine Response N, %	
	Responsive	Nonresponsive
CC	23 (74.2)	8 (25.8)
CT	44 (77.2)	13 (22.8)
TT	31 (96.9)	1 (3.1)

Overall p: 0.032; chi-square: 6.862. Yates P value for non-TT vs TT: 0.009; odds ratio: 9.71 (95% CI = 1.58–58.76)

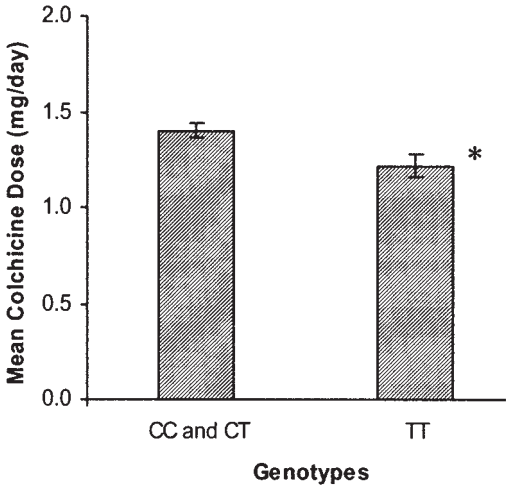

Figure 1. Colchicine dose requirements to obtain remission with respect to ABCB1 3435C to T genotypes in responsive patients. The dose requirement in the TT group was lower than those in the CC and CT groups. *p = 0.014.

colchicine resistance is unknown. In a recent study, Lidar, *et al* reported that plasma and polymorphonuclear leukocyte concentrations of colchicine were similar in responders and nonresponders[12]. However, mononuclear cell (i.e., lymphocytes and monocytes) colchicine concentrations of responders were more than twice those of nonresponders[12]. Lidar, *et al* suggested that colchicine nonresponsiveness is distinct from the genetic defect of FMF, which may be associated with *ABCB1* encoding MDR1[12].

MDR1 is a cellular efflux pump that exports numerous drugs including colchicine out of cells[13,15]. The importance of MDR1 as a mechanism of drug resistance has been well characterized in various forms of cancer[26], rheumatoid arthritis[27], systemic lupus erythematosus[28], and HIV infection[20]. Genetic heterogeneity in terms of single-nucleotide polymorphisms in *ABCB1* gene, particularly variations in position 3435, has received significant attention as a potential determinant of variability in drug efficacy[15,29].

Our results suggest that FMF patients with the 3435TT genotype for the ABCB1 3435 C to T polymorphism respond better to colchicine treatment, in terms of both clinical efficacy and colchicine dose requirements (Tables 2 and 3). There is only one study describing effects of 3435C to T polymorphism on colchicine response, in an Israeli population, which

reported an opposite result in favor of the CC genotype for responsiveness and for drug concentrations in lymphocytes[30]. Because MDR1 polymorphism is subject to inter-ethnic variability, diverse results can be observed — contradictory results in studies examining drug response have been obtained between Caucasian populations and some Asian populations[15,29].

Ueda, *et al* observed a 6 to 15-fold increased resistance to colchicine in carcinoma cells overexpressing MDR1[31]. Desrayaud, *et al* demonstrated that an MDR1 inhibitor, SDZ PSC 833, increased brain penetration of colchicine by 10-fold in rats[32]. MDR1 is expressed in many tissues and in peripheral blood mononuclear cells, particularly in lymphocytes and CD56+ cells. Although MDR1 is expresssed in granulocytes, a significant drug-transport function has not been demonstrated in this type of cell[33-35]. It has been shown that 3435TT genotype is associated with low levels of MDR1, and conversely, CC genotype was associated with high levels of MDR1 in mononuclear cells in Caucasian subjects[20]. High MDR1 activity may inhibit accumulation of drugs within cells, thereby reducing their efficacy.

The clinical and demographic features of our nonresponder patients were similar to those in the study by Lidar, *et al*[12]. In our study, disease severity was not affected by 3435C to T polymorphism (results not shown). However, the levels of acute-phase reactants, erythrocyte sedimentation rate, C-reactive protein, and fibrinogen measured during attack-free periods were higher in nonresponders compared with the responders (Table 1).

It has been suggested that ABCB1 3435C to T polymorphism could be a risk factor for the development of drug side effects[15,36]. Since MDR1 is expressed in enterocytes, hepatocytes, and bone marrow cells[31-33], side effects of colchicine may have an association with the expressed amount of the transporter. In our study, since side effects of colchicine were observed rarely, any association with the 3435C to T polymorphism could not be determined.

A main limitation of this study is that attack frequencies and characteristics were obtained mainly by self-report by patients and thus the results were prone to a degree of subjective error. Because of technical limitations it was not possible to study cellular and plasma concentrations of colchicine. The number of controls was smaller than the number of FMF patients. However, our results were similar to the frequency and genotype distributions for 3435C to T polymorphism reported in a larger population of healthy Caucasians[37].

Our study suggested an association between 3435C to T polymorphism of the drug transporter gene *ABCB1* and patients' response to colchicine. Our results present new evidence for the role of MDR1 in resistance to colchicine in patients with FMF.

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