International Journal of Dermatology

Medical genetics

MDR1 C3435T polymorphism associated with the development of clinical features in Behçet's disease in Iranian Azeri Turkish patients

Mortaza Bonyadi[1,2], PhD, Monireh Gholizadeh[1], MSc, and Mahboob Soltan-Ali[3], PhD

[1]Center of Excellence for Biodiversity, Faculty of Natural Sciences, University of Tabriz, [2]Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences, and [3]Department of Community and Family Medicine, School of Medicine, National Public Health Management Centre, Tabriz University of Medical Sciences, Tabriz, Iran

Correspondence
Mortaza Bonyadi, PhD
Center of Excellence for Biodiversity
Faculty of Natural Sciences
University of Tabriz
Tabriz 51656-39758
Iran
E-mail: jabbarpour@tabrizu.ac.ir

Conflicts of interest: None.

Abstract

Background Behçet's disease (BD) is a systemic vasculitis of unknown cause with a higher prevalence along the ancient Silk Road. Behçet's occasional familial aggregation and its close association with genes of major histocompatibility complexes justify that genetic factors play an important role in the development of the disease. In this study, we evaluated the association of multidrug resistance (*MDR1*) C3435T polymorphism with the severity of BD.

Method We investigated the distribution of *MDR1* C3435T polymorphism in 69 patients from the Iranian Azeri Turks group with BD and 92 ethnically sex-matched healthy controls, via the polymerase chain reaction–restriction fragment length polymorphism technique.

Result Although there was no significant association of *MDR1* C3435T polymorphism between two groups of patients and healthy controls, our data showed a substantial association of CC genotype with the development of several clinical features, including erythema nodosum ($P = 0.001$, OR = 2.686, 95%), pseudofolliculitis ($P = 0.002$, OR = 2.812, 95%), and skin lesions ($P = 0.040$, OR = 1.934, 95%).

Conclusion These results suggest that CC genotype is a risk factor for the development of some clinical features of BD in patients from the Iranian Azeri Turk ethnic group.

Introduction

Behçet's disease (BD) is a systemic vasculitis.[1–3] It is an autoinflammatory disease of unknown cause. In this disease, the immune system of the human body increases the inflammatory response.[4] Patients with BD may manifest all or only some of the following clinical features: oral and/or genital ulcers, skin lesions such as erythema nodosum, uveitis, and/or arthritis. This disease can also involve the central nervous system and gastrointestinal tract.[5,6] Distribution of BD along the old Silk Road (Mediterranean, Middle East, and Asia, including countries such as Turkey, China, Japan, and Iran), as well as its familial aggregation and the strong association of the HLA-B51 gene with this disease strongly support the contribution of genetic factors to the pathogenesis of BD.[4,7,8] Furthermore, environmental factors and extrinsic triggering factors such as bacterial and viral infections are also suggested to be important in the pathogenesis of BD.[9] *MDR1* encodes P-glycoprotein (P-gp), which is a 170 kDa member of the adenosine triphosphate binding cassette superfamily of membrane transporters.[10] Although this protein was first identified in cancer cells as responsible for resistance to many drugs, observation of its expression in normal tissues suggests its possible role in regulating the access of toxic compounds to cells.[11]

Up to now, several mutations were found in the *MDR1* gene, and in recent studies, more than 20 different exonic and intronic single nucleotide polymorphisms (SNPs) have been identified, some of which may change the amino acid sequence of the gene's protein. Among these SNPs, more attention has been focused on the C3435T polymorphism in exon 26, and in spite of the fact, it was a silent polymorphism, this SNP alters the expression and function of P-gp. Subjects homozygous for the T allele have twofold lower P-gp expression than subjects with CC genotype.[12–14] Latest studies exposed a substantial difference between an African and Chinese population in C allele frequencies, as the mutant T allele has rather higher frequencies in Chinese Han populations than African populations. Several studies have shown that the frequency of T and C alleles in different populations is different.[15–17] The results of some studies support the role of *MDR1* as a candidate gene for ulcerative colitis.[18,19] In this study, we investigated the possible association of the *MDR1* gene with BD in our population. The positive association

of several different genes, including *HLA-B51*,[20] *MEFV*,[21] and *TNF-α*[22] with BD in previous studies has been shown.

Materials and Methods

In this study, we investigated 69 Iranian Azeri Turkish patients with BD who had no symptoms or family history of familial Mediterranean fever (FMF). All patients were diagnosed according to the diagnostic criteria proposed by the international study group for BD[6] and referred to the Molecular-Medical Genetic Center of Tabriz. All patients were of Azeri Turkish origin between the ages of 17 and 50 years. In addition, 92 unrelated ethnically matched healthy controls without BD and other inflammatory diseases were studied.

Written consent was obtained from each participant after an explanation of the purpose of the study. Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols.[23] Each sample was tested for the C3435T polymorphism in the *MDR1* gene by a polymerase chain reaction (PCR)–restriction fragment length polymorphism assay.

PCR amplified the 248 bp region of the *MDR1* gene, including the C3435T polymorphism site, using the forward 5′-TGCTGGTCCTGAAGTTGATCTGTGAAC-3′ and reverse 5′-ACATTAGGCAGTGACTCGATGAAGGCA-3′ primer pair. The PCR reaction was subjected for initial denaturation at 94 °C for five minutes, followed by 35 cycles (30 s at 94 °C for denaturation, 30 s at 58 °C for annealing, and 30 s at 72 °C for extension). At the end of the 35 cycles at 72 °C for five minutes, final extension was applied. The PCR products were digested by restriction enzyme *Mbo*I at 37 °C for overnight. The fragments of digested PCR were separated in an 8% polyacrylamide gel electrophoresis and visualized by ethidium bromide staining.

The chi-squared test or Fisher's exact test were used for comparison of alleles and genotype frequencies between patients and healthy controls. Alleles and genotype frequencies were also compared between patients with/without clinical features. $P \leq 0.05$ was regarded as statistically significant. The odds ratios (OR) and 95% confidence intervals (CI) were calculated for all data. A chi-squared test of goodness-of-fit was applied to test for Hardy–Weinberg equilibrium.

Results

We analyzed allelic and genotypic frequencies of *MDR1* C3435T polymorphism in 69 patients with BD with/without particular symptoms and 92 unrelated ethnically matched healthy controls. Of these 69 referred patients, 44 were men and 25 were women (male-to-female ratio was estimated as 1.76). The age range of patients was 17–50 years (mean age 33.88 years). Mean age of men was 34 and women was 33.42.

Table 1 Genotype and allele distribution of *MDR1* C3435T polymorphism between patients and controls

Genotypes and alleles	Patient group $n = 69$ 100%)	Control group $n = 92$ (100%)	*P* value	OR (95% CI)
TC	32 (46.37)	49 (53.26)	0.386	0.75 (0.40–1.41)
TT	24 (34.78)	25 (27.17)	0.298	1.42 (0.72–2.80)
CC	13 (18.84)	18 (19.56)	0.920	0.95 (0.43–2.11)
C	58 (42.07)	85 (46.19)	0.458	0.84 (0.54–1.31)
T	80 (57.97)	99 (53.80)	0.458	1.18 (0.75–1.84)

OR, odds ratio.

Comparison of the allele and genotype distributions of *MDR1* C3435T polymorphisms showed no significant difference between patients with BD and healthy controls in this cohort (Table 1). The frequencies of the *MDR1* T/T, T/C, and C/C genotypes were 0.347, 0.463, and 0.188 in patients with BD, and 0.271, 0.532, and 0.195 in healthy controls, respectively. The allele frequencies of *MDR1* 3435C were 0.420 and 0.461 in patients with BD and healthy controls, respectively. To study the possible association between *MDR1* C3435T genotypes and clinical features, genotype frequencies in patients with/without specific clinical features were compared (Table 2). Based on these data, it seems that the CC genotype could be a risk factor in the pathogenesis of some clinical manifestations, including skin lesions (CC, $P = 0.040$; TT, $P = 0.005$), pseudofolliculitis (CC, $P = 0.002$; TT, $P = 0.008$), erythema nodosum (CC, $P = 0.001$; TT, $P = 0.000$), and the positive pathergy test (CC, $P = 0.011$; TT, $P = 0.000$). Significant difference was also observed in the allelic frequencies between patients with/without skin lesions ($P = 0.031$), pseudofolliculitis ($P = 0.018$), erythema nodosum ($P = 0.002$), arthritis ($P = 0.010$), neurological symptoms ($P = 0.001$), and positive pathergy test ($P = 0.002$). This shows that the C allele could be acting as a risk factor for developing the above-mentioned clinical features. A comparison between controls and patients with/without specific features regarding *MDR1* genotypes and alleles was performed separately; there was a significant difference in genotype distribution and allelic frequency among some groups (Table 3).

Discussion

Behçet's disease is a systemic vasculitis and inflammatory disease, and the etiology remains to be discovered.[21,22] Genetic and environmental risk factors appear to trigger BD. Interestingly, in recent years, the possible association between BD and some gene mutations, including FMF gene (*MEFV*), autoinflammatory gene mutations[4,8,24–29]

Table 2 Genotype and allele distribution of *MDR1 C3435T* polymorphism between patients with Behçet's disease with/without specific features

Features		CC	CT	TT	C	T
Oral ulcer (n = 53)	With	12 (0.2307)	26 (0.5)	14 (0.2692)	50 (0.4807)	54 (0.5192)
	Without	0 (0)	1 (1)	0 (0)	1 (0.5)	1 (0.5)
	P value				0.440	0.448
Genital ulcer (n = 53)	With	11 (0.3142)	14 (0.4)	10 (0.2857)	36 (0.5142)	34 (0.4857)
	Without	1 (0.0555)	13 (0.7222)	4 (0.2222)	15 (0.4166)	21 (0.5833)
	P value	0.000*	0.000*	0.156	0.166	0.166
Skin lesion (n = 51)	With	7 (0.2692)	14 (0.5384)	5 (0.192)	28 (0.5384)	24 (0.4615)
	Without	4 (0.16)	12 (0.48)	9 (0.36)	20 (0.4)	30 (0.6)
	P value	0.040*	0.409	0.005*	0.050*	0.50*
Pseudofolliculitis (n = 48)	With	5 (0.294)	9 (0.529)	3 (0.176)	19 (0.5588)	15 (0.4411)
	Without	4 (0.129)	17 (0.548)	10 (0.322)	25 (0.4032)	37 (0.5967)
	P value	0.002*	0.788	0.008*	0.028*	0.028*
Erythema nodosum (n = 51)	With	4 (0.363)	6 (0.545)	1 (0.090)	14 (0.6363)	8 (0.3636)
	Without	7 (0.175)	20 (0.5)	12 (0.3)	34 (0.4358)	44 (0.5641)
	P value	0.001*	0.524	0.000*	0.004*	0.004*
Ocular involvement (n = 52)	With	6 (0.176)	17 (0.5)	11 (0.323)	29 (0.4264)	39 (0.5735)
	Without	6 (0.3333)	9 (0.5)	3 (0.1666)	21 (0.5833)	15 (0.4166)
	P value	0.011*	1	0.004*	0.026*	0.026*
Anterior uveitis (n = 33)	With	2 (0.095)	12 (0.571)	7 (0.3333)	16 (0.3809)	26 (0.6190)
	Without	3 (0.25)	5 (0.4166)	4 (0.3333)	11 (0.4583)	13 (0.5416)
	P value	0.002*	0.010*	0.520	0.267	0.267
Posterior uveitis (n = 33)	With	5 (0.192)	15 (0.576)	6 (0.230)	25 (0.4807)	27 (0.5192)
	Without	0 (0)	2 (0.285)	5 (0.714)	2 (0.1428)	12 (0.8571)
	P value		0.000*	0.000*	0.000*	0.000*
Arthritis (n = 53)	With	1 (0.25)	3 (0.75)	0 (0)	5 (0.625)	3 (0.375)
	Without	11 (0.224)	24 (0.489)	14 (0.285)	46 (0.4693)	52 (0.5306)
	P value	0.394	0.000*		0.010*	0.013*
Epididymitis (n = 52)	With	0 (0)	1 (0.5)	1 (0.5)	1 (0.25)	3 (0.75)
	Without	10 (0.2)	26 (0.52)	14 (0.28)	46 (0.46)	54 (0.54)
	P value		0.444	0.001*	0.001*	0.001*
Gastrointestinal involvement (n = 51)	With	1 (0.5)	0 (0)	1 (0.5)	2 (0.5)	2 (0.5)
	Without	10 (0.204)	26 (0.530)	13 (0.265)	46 (0.4693)	52 (0.5306)
	P value	0.000*		0.001*	0.385	0.289
Neurological involvement (n = 48)	With	2 (0.6666)	0 (0)	1 (0.3333)	4 (0.6666)	2 (0.3333)
	Without	8 (0.1777)	25 (0.5555)	12 (0.2666)	41 (0.4555)	49 (0.5444)
	P value	0.000*		0.140	0.001*	0.001*
Positive pathergy test (n = 39)	With	6 (0.260)	15 (0.652)	2 (0.086)	27 (0.5869)	19 (0.4130)
	Without	2 (0.125)	8 (0.5)	6 (0.375)	12 (0.375)	20 (0.625)
	P value	0.011*	0.030*	0.000*	0.003*	0.003*
C-reactive protein (n = 29)	With	0 (0)	11 (0.6111)	7 (0.3888)	11 (0.3055)	25 (0.6944)
	Without	5 (0.454)	6 (0.545)	0 (0)	16 (0.7272)	6 (0.2727)
	P value		0.344		0.000*	0.000*
HLA-B51 (n = 21)	With	4 (0.2)	12 (0.6)	4 (0.2)	20 (0.5)	20 (0.5)
	Without	0 (0)	0 (0)	1 (1)	0 (0)	2 (1)
	P value					

*Significant P value.

has been investigated. In addition, HLA-B51 and other gene(s) located near HLA-B51 are found commonly in patients from the Old Silk Route.[20,30]

The *MDR1* gene encodes P-gp, an ATP-dependent efflux pump that transports inflammatory material and xenobiotic toxins from the intracellular to the extracellular region. The correlation of *MDR1* polymorphisms and their haplotypes with several different diseases, including cancer, inflammatory bowel disease, Parkinson's disease, and FMF are shown.[31–35]

The present study addresses the distribution of alleles and genotypes of *MDR1* gene C3435T polymorphism and their associations with BD in patients from Azeri Turks living in northwestern Iran. This ethnic group, constituting 25% of the Iranian population, is ethnically identical to Azeri and closely related to Turks. The C3435T silent poly-

Table 3 Genotype and allele distribution of *MDR1* C3435T polymorphism between controls and patients with/without features

Feature		CC	TC	TT	C	CC
Erythema nodosum (n = 51)	With	4 (0.363)	6 (0.545)	1 (0.090)	14 (0.6363)	8 (0.3636)
	Controls	18 (0.1956)	49 (0.5326)	25 (0.2717)	85 (0.4619)	99 (0.5380)
	P value	0.003*	0.860	0.001*	0.008*	0.007*
	OR	2.344	1.051	0.265	2.038	0.491
	Without	7 (0.175)	20 (0.51)	12 (0.3)	34 (0.4358)	44 (0.5641)
	Controls	18 (0.1956)	49 (0.5326)	25 (0.2717)	85 (0.4619)	99 (0.5380)
	P value	0.708	0.645	0.658	0.347	0.324
	OR	0.872	0.878	1.149	0.900	1.111
Posterior uveitis (n = 33)	With	5 (0.192)	15 (0.576)	6 (0.230)	25 (0.4807)	27 (0.5192)
	Controls	18 (0.1956)	49 (0.5326)	25 (0.2717)	85 (0.4619)	99 (0.5380)
	P value	0.517	0.537	0.496	0.349	0.428
	OR	0.977	1.192	0.801	1.078	0.927
	Without	0 (0)	2 (0.285)	5 (0.714)	2 (0.1428)	12 (0.8571)
	Controls	18 (0.1956)	49 (0.5326)	25 (0.2717)	85 (0.4619)	99 (0.5380)
	P value	0.000	0.000*	0.000*	0.000*	0.000*
	OR	0.000	0.350	6.692	0.194	5.151
Arthritis (n = 53)	With	1 (0.25)	3 (0.75)	0 (0)	5 (0.625)	3 (0.375)
	Controls	18 (0.1956)	49 (0.5326)	25 (0.2717)	85 (0.4619)	99 (0.5380)
	P value	0.221	0.001*	0.000	0.013*	0.010*
	OR	1.371	2.633	0.000	1.942	0.515
	Without	11 (0.224)	24 (0.489)	14 (0.285)	46 (0.4693)	52 (0.5306)
	Controls	18 (0.1956)	49 (0.5326)	25 (0.2717)	85 (0.4619)	99 (0.5380)
	P value	0.622	0.537	0.834	0.507	0.492
	OR	1.187	0.840	1.068	1.030	0.971
Epididymitis (n = 52)	With	0 (0)	1 (0.5)	1 (0.5)	1 (0.25)	3 (0.75)
	Controls	18 (0.1956)	49 (0.5326)	25 (0.2717)	85 (0.4619)	99 (0.5380)
	P value	0.000	0.000	0.001*	0.001*	0.001*
	OR	0.000	0.000	2.681	0.388	2.576
	Without	10 (0.2)	26 (0.52)	14 (0.28)	46 (0.46)	54 (0.54)
	Controls	18 (0.1956)	49 (0.5326)	25 (0.2717)	85 (0.4619)	99 (0.5380)
	P value	0.938	0.858	0.896	0.546	0.455
	OR	1.028	0.951	1.042	0.992	1.008
Neurological involvement (n = 48)	With	2 (0.6666)	0 (0)	1 (0.3333)	4 (0.6666)	2 (0.3333)
	Controls	18 (0.1956)	49 (0.5326)	25 (0.2717)	85 (0.4619)	99 (0.5380)
	P value	0.000*	0.000	0.159	0.002*	0.002*
	OR	8.222	0.000	1.340	2.329	0.429
	Without	8 (0.1777)	25 (0.5555)	12 (0.2666)	41 (0.4555)	49 (0.5444)
	Controls	18 (0.1956)	49 (0.5326)	25 (0.2717)	85 (0.4619)	99 (0.5380)
	P value	0.745	0.745	0.935	0.458	0.430
	OR	0.889	1.097	0.974	0.975	1.026
C-reactive protein (n = 29)	With	0 (0)	11 (0.6111)	7 (0.3888)	11 (0.3055)	25 (0.6944)
	Controls	18 (0.1956)	49 (0.5326)	25 (0.2717)	85 (0.4619)	99 (0.5380)
	P value	0.000	0.262	0.078	0.011*	0.009*
	OR	0.000	1.379	1.705	0.512	1.951
	Without	5 (0.454)	6 (0.545)	0 (0)	16 (0.7272)	6 (0.2727)
	Controls	18 (0.1956)	49 (0.5326)	25 (0.2717)	85 (0.4619)	99 (0.5380)
	P value	0.000*	0.800	0.000	0.000*	0.000*
	OR	3.420	1.051	0.000	3.105	0.322

OR, odds ratio.

morphism of *MDR1* was associated with altered P-gp expression and function. In some previous studies, association between *MDR1* C3435T polymorphisms and BD has been reported. However, consistent with other results reported in the Turkish population,[36] our results show no association between genotypic and allelic frequencies of the *MDR1* C3435T polymorphisms in patients with BD and the healthy control group ($P > 0.05$).

Interestingly, comparison of clinical symptoms and *MDR1* C3435T polymorphism among patients with BD showed substantial association between special features and the frequency of different genotypes in patients.

Based on these results, the clinical features – skin lesion, pseudofolliculitis, erythema nodosum, and positive pathergy test – showed a significant association with the CC genotype, and it seems that the CC genotype could be a risk factor in developing these clinical features in patients with BD.

Consistent with these results, some other studies have shown that the CC genotype acts as a risk factor for some diseases in different populations such as ulcerative colitis in a German[37] and Polish population,[38] Crohn's disease in a Spanish[39] and Polish population,[38] and acute lymphoblastic leukemia in a Polish population.[40]

A previous study has shown there is an association between the C-allele and high expression of P-gp.[38] The *MDR1* 3435 TT homozygosity is associated with low P-gp expression in the duodenum and high plasma concentrations of digoxin[13] and low P-gp expression in CD56 natural killer cells.[41] Therefore, this high-level expression of *MDR1* 3435 CC homozygosity could explain our results; however, the mechanism that *MDR1* C3435T polymorphism may affect the outcome of clinical features in patients with BD remains to be elucidated.

In conclusion, our results show a significant association between clinical symptoms and *MDR1* C3435T polymorphism among patients with BD from the Iranian Azeri Turk ethnic group. To confirm the observed association, we suggest further study of this polymorphism among patients with BD in other ethnic groups.

Acknowledgments

The authors would like to thank all participating patients. This project was financially supported by the Center of Excellence for Biodiversity (University of Tabriz).

References

1 Wallace GR, Kondeatis E, Vaughan RW, *et al.* IL-10 genotype analysis in patients with Behçet's disease. *Hum Immunol* 2007; **68**: 122–127.
2 Harmouche H, Maamar M, Sahnoune I, *et al.* Fever revealing Behçet's disease: two new cases. *Eur J Intern Med* 2007; **18**: 146–147.
3 Schwartz T, Langevitz P, Zemer D, *et al.* Behçet's disease in familial Mediterranean fever: characterization of the association between the two diseases. *Semin Arthritis Rheum* 2000; **29**: 286–295.
4 Touitou I, Kone-Paut I. Autoinflammatory diseases. *Best Pract Res Clin Rheumatol* 2008; **22**: 811–829.
5 Sakane T, Takeno M, Suzuki N, *et al.* Behçet's disease. *N Engl J Med* 1999; **341**: 1284–1291.
6 Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's disease. *Lancet* 1990; **335**: 1078–1080.
7 Verity DH, Marr JE, Ohno S, *et al.* Behçet's disease, the silk road and HLA-B51: historical and geographical perspectives. *Tissue Antigens* 1999; **54**: 213–220.
8 Ben-Chetrit E, Cohen R, Chajek-Shaul T. Familial Mediterranean fever and Behçet's disease – are they associated? *J Rheumatol* 2002; **29**: 530–534.
9 Durrani K, Papaliodis GN. The genetics of Adamantiades – Behçet's disease. *Semin Ophthalmol* 2008; **23**: 73–79.
10 Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 1976; **455**: 152–162.
11 Ling V. Multidrug resistance: molecular mechanisms and clinical relevance. *Cancer Chemother Pharmacol* 1997; **40**(Suppl.): S3–S8.
12 Decleves X, Chevillard S, Charpentier C, *et al.* A new polymorphism (N21D) in the exon 2 of the human MDR1 gene encoding the Pglycoprotein. *Hum Mutat* 2000; **15**: 486.
13 Hoffmeyer S, Burk O, von Richter O, *et al.* 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* 2000; **97**: 3473–3478.
14 Ito S, Ieiri I, Tanabe M, *et al.* Polymorphism of the ABC transporter genes, MDR1, MRP1 and MRP2/cMOAT in healthy Japanese subjects. *Pharmacogenetics* 2001; **11**: 175–184.
15 Ameyaw MM, Regateiro F, Li T, *et al.* MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. *Pharmacogenetics* 2001; **11**: 7–21.
16 Nakamura T, Sakaeda T, Horinouchi M, *et al.* Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. *Clin Pharmacol Ther* 2002; **71**: 297–303.
17 Sakaeda T, Nakkamura T, Horinouchi M, *et al.* MDR1 genotyperelated pharmacokinetics of digoxin after single oral administration in healthy Japanese subjects. *Pharm Res* 2001; **18**: 1400–1404.
18 Huebner C, Browning B, Petermann I, *et al.* Genetic analysis of MDR1 and inflammatory bowel disease reveals protective effect of heterozygous variants for ulcerative colitis. *Inflamm Bowel Dis* 2009; **15**: 1784–1793.
19 Ardizzone S, Maconi G, Bianchi V, *et al.* Multidrug resistance 1 gene polymorphism and susceptibility to inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 516–523.
20 Ahmad T, Wallace GR, James T, *et al.* Mapping the HLA association in Behçet's disease: a role for tumor necrosis factor polymorphisms? *Arthritis Rheum* 2003; **48**: 807–813.
21 Esmaeili M, Bonyadi M, Khabbazi A, *et al.* Common MEFV mutations in Iranian Azeri Turkish patients with Behçet's disease. *Scand J Rheumatol* 2011; **40**: 383–386.
22 Bonyadi M, Jahanafrooz Z, Esmaeili M, *et al.* TNF-α gene polymorphisms in Iranian Azeri Turkish patients

with Behçet's Disease. *Rheumatol Int* 2009; **30**: 285–289.
23 Miller SA, Dynes DD, Polesky F. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215.
24 Kone-Paut I, Sanchez E, Le Quellec A, *et al.* Autoinflammatory gene mutations in Behçet's disease. *Ann Rheum Dis* 2007; **66**: 832–834.
25 Touitou I, Magne X, Molinari N, *et al. MEFV* mutations in Behçet's disease. *Hum Mutat* 2000; **16**: 271–272.
26 Atagunduz P, Ergun T, Direskeneli H. *MEFV* mutations are increased in Behçet's disease (BD) and are associated with vascular involvement. *Clin Exp Rheumatol* 2003; **21**: S35–S37.
27 Imirzalioglu N, Dursun A, Tastan B, *et al. MEFV* gene is a probable susceptibility gene for Behçet's disease. *Scand J Rheumatol* 2005; **34**: 56–58.
28 Rabinovich E, Shinar Y, Leiba M, *et al.* Common FMF alleles may predispose to development of Behçet's disease with increased risk for venous thrombosis. *Scand J Rheumatol* 2007; **36**: 48–52.
29 Ayesh S, Abu-Rmaileh H, Nassar S, *et al.* Molecular analysis of *MEFV* gene mutations among Palestinian patients with Behçet's disease. *Scand J Rheumatol* 2008; **37**: 370–374.
30 Mizuki N, Ohno S, Sato T, *et al.* Microsatellite polymorphism located between the TNF and HLA-B genes in Behçet's disease. *Hum Immunol* 1995; **43**: 129–135.
31 Chen G, Quan S, Hu Q, *et al.* Lack of association between MDR1 C3435T polymorphism and chemotherapy response in advanced breast cancer patients: evidence from current studies. *Mol Biol Rep* 2012; **39**: 5161–5168.
32 Sheng X, Zhang L, Tong N, *et al.* MDR1 C3435T polymorphism and cancer risk: a meta-analysis based on 39 case–control studies. *Mol Biol Rep* 2012; **39**: 7237–7249.
33 Dudarewicz M, Barańska M, Rychlik Sych M, *et al.* C3435T Polymorphism of the *ABCB1/MDR1* gene encoding P-glycoprotein in patients with inflammatory bowel disease in a Polish population. *Pharmacol Rep* 2012; **64**: 343–350.
34 Droździk M, Białecka M, Myśliwiec K, *et al.* Polymorphism in the P-glycoprotein drug transporter MDR1 gene: a possible link between environmental and genetic factors in Parkinson's disease. *Pharmacogenetics* 2003; **13**: 259–263.
35 Tufan A, Babaoglu MO, Akdogan A, *et al.* Association of drug transporter gene ABCB1 (MDR1) 3435C to T polymorphism with colchicine response in familial Mediterranean fever. *J Rheumatol* 2007; **7**: 1540–1544.
36 Rustemoglu A, Gül Ü, Gümüş-Akay G, *et al.* MDR1 gene polymorphisms may be associated with Behçet's disease and its colchicum treatment response. *Gene* 2012; **505**: 333–339.
37 Fiedler T, Büning C, Reuter W, *et al.* Possible role of *MDR1* two-locus genotypes for young-age onset ulcerative colitis but not Crohn's disease. *Eur J Clin Pharmacol* 2007; **63**: 917–925.
38 Jamroziak K, Balcerczak E, Mlynarski W, *et al.* Distribution of allelic variants of functional C3435T polymorphism of drug transporter MDR1 gene in a sample of Polish population. *Pol J Pharmacol* 2002; **54**: 495–500.
39 Urcelay E, Mendoza JL, Martín MC, *et al.* MDR1 gene: susceptibility in Spanish Crohn's disease and ulcerative colitis patients. *Inflamm Bowel Dis* 2006; **12**: 33–37.
40 Jamroziak K, Młynarski W, Balcerczak E, *et al.* Functional C3435T polymorphism of MDR1 gene: an impact on genetic susceptibility and clinical outcome of childhood acute lymphoblastic leukemia. *Eur J Haematol* 2004; **72**: 314–321.
41 Tanabe M, Ieiri I, Nagata N, *et al.* Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J Pharmacol Exp Ther* 2001; **297**: 1137–1143.

