Brazilian Journal of Medical and Biological Research (2008) 41: 769-772
ISSN 0100-879X Short Communication

ABCB1 C1236T, G2677T/A and C3435T polymorphisms in systemic lupus erythematosus patients

T.P. Gonzalez[1], T. Mucenic[2], J.C.T. Brenol[2], R.M. Xavier[2], M. Schiengold[1] and J.A.B. Chies[1]

[1]Departamento de Genética, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil
[2]Divisão de Reumatologia, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brasil

Correspondence to: J.A.B. Chies, Departamento de Genética, UFRGS, Av. Bento Gonçalves, 9500, Caixa Postal 15053, 91501-970 Porto Alegre, RS, Brasil
Fax: +55-51-3308-7311. E-mail: jabchies@terra.com.br

P-glycoprotein (Pgp), the *ABCB1* gene product, acts as an efflux pump that transports a large variety of substrates and is a mechanism of cell protection against xenobiotics. An increasing number of studies have shown that some *ABCB1* polymorphisms may affect Pgp expression and activity, as well as affecting the development and susceptibility to diseases and pharmacological response. High activity of Pgp has been detected in systemic lupus erythematosus (SLE) patients. The C1236T, G2677T/A, and C3435T are the most commonly studied single nucleotide polymorphisms in the *ABCB1* gene. Therefore, their frequencies were determined in Brazilian individuals with European ancestry (N = 143) and in SLE patients (N = 137). Genotyping was performed by PCR-RFLP analysis using specific primers followed by incubation with the appropriate restriction enzymes. The resulting DNA fragments were visualized on agarose or polyacrylamide gels. No statistically significant differences were observed in allelic and genotypic frequencies between SLE and healthy subjects (Fisher exact test). Nevertheless, the 2677A allelic frequency was lower in SLE patients with malar rash (0.007) compared with patients without this feature (0.04; P = 0.0054), while the frequency of this variant was higher in SLE patients with pleuritis (0.07) compared with patients without this feature (0.01; P = 0.0156). We suggest that although the *ABCB1* polymorphisms do not directly interfere in SLE susceptibility, their evaluation, especially the 2677A allele, in other immunological processes may be interesting since they can interfere in clinical features of this disease.

Key words: *ABCB1*; *MDR1*; P-glycoprotein; Polymorphism; Systemic lupus erythematosus

Research partially supported by CNPq (#132310/2004-0) and FAPERGS (#05/1932-5).

Received January 22, 2008. Accepted August 26, 2008

Introduction

The *ABCB1* (also called *MDR1*) gene product P-glycoprotein (Pgp) is an ATP-dependent drug efflux pump. Although its physiological role is not completely understood, this molecule is important for the protection of the cell against xenobiotics, and its expression is detected in several tissues (1).

ABCB1 is a highly polymorphic gene and, to date, 28 single nucleotide polymorphisms (SNPs) have been described at 27 positions (2). Three SNPs (synonymous C1236T and C3435T, and nonsynonymous G2677T/A) have been studied particularly in healthy populations as well as in different clinical conditions. It has been suggested that the *ABCB1* mRNA stability is decreased for the 3435T allele, which leads to low steady-state *ABCB1* mRNA levels (3). Nevertheless, other studies report conflicting results. It has been reported that high *ABCB1* mRNA levels are associated with 3435T allele (4), while others (5) failed to show any correlation between C3435T alleles and *ABCB1* mRNA expression levels. The same conflicting situation is observed with respect to Pgp activity

and expression. The effects of the G2677T/A SNP in *ABCB1* mRNA and Pgp expression levels are also the subject of controversy. While some studies reported higher expression levels in 2677TT/A subjects (6), others showed no differences between genotypes (5). The frequencies of *ABCB1* allelic variants differ among different ethnic groups and linkage disequilibrium is reported between the alleles at positions 1236, 2677, and 3435 (6).

High Pgp activity has been detected in some autoimmune diseases, such as systemic lupus erythematosus (SLE) (7,8), and therapy for SLE includes the use of corticosteroids and immunosuppressants (9) that are substrates of Pgp. Therefore, in order to evaluate a possible role of the *ABCB1* gene as well as of its polymorphisms in SLE, we analyzed the allelic and genotypic frequencies of C1236T, G2677T/A and C3435T polymorphisms in healthy and SLE Brazilian subjects. We also analyzed clinical features present in the SLE patients.

Material and Methods

One hundred and thirty-seven patients (16 men and 121 women; 102 of European ancestry and 35 of African ancestry) aged 1-63 years at diagnosis (mean age ± SD = 29.9 ± 14.17) with SLE and 143 healthy subjects (blood donors) of European ancestry (102 men and 41 women) aged 27-59 years (44.7 ± 6.86) were studied. Patients were diagnosed according to the criteria of the American College of Rheumatology and were recruited in the Rheumatology Division, Hospital de Clínicas de Porto Alegre (HCPA), in Porto Alegre, RS, the southernmost State of Brazil. This study was approved by the HCPA Ethics Committee and written informed consent was obtained from all subjects. The most common clinical manifestations in this sample were malar rash (present in 50.4% of the patients), photosensitivity (62.0%), arthritis (78.8%) and hematological and immunological abnormalities (68.6 and 59.9%, respectively).

Genomic DNA was extracted from venous blood using a salting-out method and was screened for the SNPs using PCR-RFLP analysis. Genotyping was performed by PCR-RFLP using specific primers followed by incubation with the appropriate restriction enzymes as previously described for C1236T and G2677T/A (10) and C3435T (11) polymorphisms. The resulting DNA fragments were visualized on agarose or polyacrylamide gels.

Genotypic distribution and allelic frequencies were estimated by gene counting. Deviation from Hardy-Weinberg equilibrium was determined by the chi-square test and linkage disequilibrium and haplotype frequencies were estimated by use of the Arlequin software (version 2.000). The determination of statistically significant differences in genotypic and allelic frequencies between groups was assessed by the chi-square test or, when appropriate, by the Fisher exact test using WINPEPI (12). A significance level of 0.05 was used and statistical power was calculated for P values <0.05 (13).

Results

The frequencies of *ABCB1* C1236T, G2677T/A, and C3435T polymorphic alleles and genotypic distribution frequencies in patients and control group are shown in Table 1. The genotypic frequencies for all three polymorphisms in both groups were consistent with Hardy-Weinberg equilibrium (P > 0.5). No statistically significant differ-

Table 1. Distribution of *ABCB1* C1236T, G2677T/A and C3435T genotypes and their allele frequencies in patients with systemic lupus erythematosus (SLE) and in healthy controls.

SNP	Genotypes						Allele frequencies		
C1236T	CC		CT		TT		*C*	*T*	
SLE	51 (37.2%)		69 (50.4%)		17 (12.4%)		0.63	0.37	
Control	56 (39.2%)		67 (46.8%)		20 (14.0%)		0.62	0.38	
G2677T/A	GG	GT	TT	GA	TA	AA	*G*	*T*	*A*
SLE	53 (38.7%)	65 (47.4%)	12 (8.8%)	3 (2.2%)	4 (2.9%)	0 (0.0%)	0.62	0.36	0.02
Control	57 (39.9%)	58 (40.5%)	22 (15.4%)	4 (2.8%)	2 (1.4%)	0 (0.0%)	0.63	0.34	0.03
C3435T	CC		CT		TT		*C*	*T*	
SLE	38 (27.7%)		76 (55.5%)		23 (16.8%)		0.55	0.45	
Control	47 (32.9%)		61 (42.6%)		35 (24.5%)		0.55	0.45	

Data are reported as number of subjects with percent in parentheses for 137 SLE patients and 143 healthy controls. SNP = single nucleotide polymorphism. No statistically significant differences were observed in allelic or genotypic frequencies from SLE patients and controls (Fisher exact test).

ences were observed in allelic distribution between men and women in either groups (data not shown); therefore, all analyses were performed without separation of gender. No statistically significant differences were observed in genotypic or allelic frequencies between patients and controls. Allelic frequencies for C3435T were significantly different between patients with African and European ancestry (P = 0.0430, statistical power = 0.6486) but no statistically significant differences were observed in allelic distribution when only patients with European ancestry were compared to the control group (data not shown).

Haplotypic frequencies were estimated by a maximum-likelihood method for both patients and control group, and for all subgroups of patients. The most frequently observed haplotypes were 1236C/2677G/3435C and 1236T/2677T/3435T, accounting, respectively, for 44.9 and 28.2% of all haplotypes in SLE and for 44.8 and 27.0% in the control group.

Discussion

In this study, we presented data relative to the frequencies of the three more commonly studied *ABCB1* SNPs in a sample of healthy and SLE Brazilian individuals. No differences were observed for allelic frequencies among Brazilians with European ancestry and published data from Italian (14), Spanish (15), or German (5) individuals. However, C3435T allelic frequency in our European ancestry individuals differed from that observed in Portugal (16) (P = 0.013, statistical power = 0.7318; data not shown). Patients of African ancestry included in our study presented the 3435C allele at a higher frequency (0.67) than patients of European ancestry (0.51; P = 0.043; Fisher exact test). Higher frequencies of the 3435C allele are a constant feature in populations from Africa or with African ancestry (16).

The genotypic, allelic and haplotypic distributions in SLE patients did not differ from those observed in healthy subjects in the present study. The polymorphism C3435T has been analyzed in rheumatoid arthritis in Polish individuals (17) and no differences were observed in genotypic or allelic frequencies compared to healthy subjects. These data suggest that these *ABCB1* polymorphisms are not risk factors for the development of these diseases, although more research is necessary to clearly establish whether this is also true for other autoimmune diseases.

It has been suggested that Pgp may be involved in immunological processes. For instance, cytokines may be transported by Pgp, particularly IL-2, IL-4 and INF-γ (18), and pro-inflammatory cytokines seem to increase *ABCB1* mRNA expression.

In our patients, we compared allelic and genotypic frequencies of the different polymorphic sites with clinical features and manifestations. We observed that the 2677A allelic frequency was decreased in patients with malar rash and was increased in patients presenting pleuritis (Table 2). These observations are intriguing since malar rash presents features of an allergic immune response although pleuritis involves an inflammatory process. In addition, Pgp activity has been reported to be increased in lymphocytes

Table 2. G2677T/A genotypes and their allele frequencies in systemic lupus erythematosus (SLE) patients with and without malar rash and pleuritis.

	Malar rash		Pleuritis	
	Positive (N = 69)	Negative (N = 68)	Positive (N = 34)	Negative (N = 103)
2677				
GG (N = 53)	28 (40.5%)	25 (36.9%)	15 (44.1%)	38 (36.9%)
GT (N = 65)	36 (52.2%)	29 (42.5%)	13 (38.2%)	52 (50.5%)
TT (N = 12)	4 (5.8%)	8 (11.8%)	1 (2.95%)	11 (10.7%)
GA (N = 3)	0 (0.0%)	3 (4.4%)	1 (2.95%)	2 (1.9%)
TA (N = 4)	1 (1.5%)	3 (4.4%)	4 (11.8%)	0 (0.0%)*
P value	0.2004		0.0071[a]	
G	0.667	0.53*	0.65	0.63
T	0.336	0.43*	0.28	0.36
A	0.007	0.04*	0.07	0.01*
P value	0.0054[b]		0.0156[c]	

Data are reported as number with percent in parentheses or frequency. Allelic and genotypic frequencies were compared in SLE patients grouped according to the presence or absence of specific clinical features. *P < 0.05 compared to number of positive patients (Fisher exact test). Statistical power: [a]0.89, [b]0.81, [c]0.78.

from SLE patients (7) and also in other autoimmune disorders such as rheumatoid arthritis (19) and thrombocytopenic purpura (20). In all cases, patients with active disease or patients who were refractory to treatment presented the highest levels of Pgp activity.

Thus, although the *ABCB1* polymorphisms are not associated with SLE, the evaluation of *ABCB1* polymorphisms, especially the 2677A allele, in other immunological processes may be relevant since our data suggest that they can affect clinical features of this disease.

References

1. Schiengold M, Schwantes L, Schwartsmann G, Chies JA, Nardi NB. Multidrug resistance gene expression during the murine ontogeny. *Mech Ageing Dev* 2001; 122: 255-270.
2. Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther* 2004; 75: 13-33.
3. Wang D, Sadee W. Searching for polymorphisms that affect gene expression and mRNA processing: example ABCB1 (MDR1). *AAPS J* 2006; 8: E515-E520.
4. Nakamura T, Sakaeda T, Horinouchi M, Tamura T, Aoyama N, Shirakawa T, 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.
5. Siegmund W, Ludwig K, Giessmann T, Dazert P, Schroeder E, Sperker B, et al. The effects of the human MDR1 genotype on the expression of duodenal P-glycoprotein and disposition of the probe drug talinolol. *Clin Pharmacol Ther* 2002; 72: 572-583.
6. Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 2001; 70: 189-199.
7. Diaz-Borjon A, Richaud-Patin Y, Alvarado de la Barrera C, Jakez-Ocampo J, Ruiz-Arguelles A, Llorente L. Multidrug resistance-1 (MDR-1) in rheumatic autoimmune disorders. Part II: Increased P-glycoprotein activity in lymphocytes from systemic lupus erythematosus patients might affect steroid requirements for disease control. *Joint Bone Spine* 2000; 67: 40-48.
8. Tsujimura S, Saito K, Nakayamada S, Tanaka Y. Relevance of multidrug resistance 1 and P-glycoprotein to drug resistance in patients with systemic lupus erythematosus. *Histol Histopathol* 2007; 22: 465-468.
9. Vasoo S, Hughes GR. Theory, targets and therapy in systemic lupus erythematosus. *Lupus* 2005; 14: 181-188.
10. Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M, et al. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin Pharmacol Ther* 2001; 69: 169-174.
11. Roberts RL, Joyce PR, Mulder RT, Begg EJ, Kennedy MA. A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression. *Pharmacogenomics J* 2002; 2: 191-196.
12. Abramson JH. WINPEPI (PEPI-for-Windows) computer programs for epidemiologists. *Epidemiol Perspect Innov* 2004; 1: 6.
13. Lenth R. *Java applets for power and sample size*. [Computer program]. http://www.stat.uiowa.edu/~rlenth/. 2006.
14. Furuno T, Landi MT, Ceroni M, Caporaso N, Bernucci I, Nappi G, et al. Expression polymorphism of the blood-brain barrier component P-glycoprotein (MDR1) in relation to Parkinson's disease. *Pharmacogenetics* 2002; 12: 529-534.
15. Bernal ML, Sinues B, Fanlo A, Mayayo E. Frequency distribution of C3435T mutation in exon 26 of the MDR1 gene in a Spanish population. *Ther Drug Monit* 2003; 25: 107-111.
16. Ameyaw MM, Regateiro F, Li T, Liu X, Tariq M, Mobarek A, et al. MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. *Pharmacogenetics* 2001; 11: 217-221.
17. Pawlik A, Wrzesniewska J, Fiedorowicz-Fabrycy I, Gawronska-Szklarz B. The MDR1 3435 polymorphism in patients with rheumatoid arthritis. *Int J Clin Pharmacol Ther* 2004; 42: 496-503.
18. Park SW, Lomri N, Simeoni LA, Fruehauf JP, Mechetner E. Analysis of P-glycoprotein-mediated membrane transport in human peripheral blood lymphocytes using the UIC2 shift assay. *Cytometry A* 2003; 53: 67-78.
19. Llorente L, Richaud-Patin Y, Diaz-Borjon A, Alvarado de la Barrera C, Jakez-Ocampo J, De La Fuente H, et al. Multidrug resistance-1 (MDR-1) in rheumatic autoimmune disorders. Part I: Increased P-glycoprotein activity in lymphocytes from rheumatoid arthritis patients might influence disease outcome. *Joint Bone Spine* 2000; 67: 30-39.
20. Ruiz-Soto R, Richaud-Patin Y, Lopez-Karpovitch X, Llorente L. Multidrug resistance-1 (MDR-1) in autoimmune disorders III: increased P-glycoprotein activity in lymphocytes from immune thrombocytopenic purpura patients. *Exp Hematol* 2003; 31: 483-487.