ABCB1 polymorphisms may have a minor effect on ciclosporin blood concentrations in myasthenia gravis patients

Ya-tong Zhang,^{1,2} Li-ping Yang,² Hong Shao,¹ Ke-xin Li,²
Chun-hua Sun² & Lu-wen Shi¹

¹Department of Pharmacy Administration and Clinical Pharmacy, School of Pharmaceutical Science, Peking University and ²Department of Pharmacy, Beijing Hospital, Beijing, China

Correspondence

Professor Hong Shao, Department of Pharmacy Administration and Clinical Pharmacy, School of Pharmaceutical Science, Peking University, Beijing 100083, China.

Tel: +86-10-06209-1701 Ext. 237

Fax: +86-10-6209-1701 Ext. 211

E-mail: h_shao@163.com

Ya-tong Zhang, Department of Pharmacy Administration and Clinical Pharmacy, School of Pharmaceutical Science, Peking University, Beijing 100083, China.

E-mail: zyt2002888@gmail.com

Keywords

ABCB1, myasthenia gravis, ciclosporin, pharmacogenomics, single nucleotide polymorphism, blood concentrations

Received

17 May 2007

Accepted

10 Mar 2008

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Numerous studies have shown that MDR1 polymorphisms in the form of single nucleotide polymorphisms or haplotype affect ciclosporin pharmacokinetics or blood concentrations in organ transplantation patients, but some results conflict with others.

WHAT THIS STUDY ADDS

- We had thought that the disease condition might conceal the minor effect of MDR1 polymorphisms.
- We chose myasthenia gravis patients as a population in which disease conditions were less severe.
- We also used different pharmacokinetics indices, such as dose-adjusted trough blood concentrations, dose-adjusted peak blood concentrations and trough blood concentrations under the same ciclosporin regimen.

AIMS

Ciclosporin (CsA), which is widely used in autoimmune disease and transplantation, has a narrow therapeutic index. It also shows considerable interindividual variability in its pharmacokinetics, which may be attributable to polymorphisms of the multidrug efflux pump P-glycoprotein, encoded by MDR-1. The aim was to determine the role of genetic polymorphisms in MDR-1 with respect to interindividual variability of CsA blood concentrations in myasthenia gravis (MG) patients.

METHODS

MG patients ($n = 129$) receiving CsA were genotyped for MDR-1 1236C→T (exon 12), 2677G→T (exon 21) and 3435C→T (exon 26). Trough blood and peak blood concentrations were determined to see if there was correlation with the corresponding genotype.

RESULTS

We observed a trend for CsA blood concentrations, especially peak blood concentrations, to be higher with the wild-type allele compared with minor alleles in genotype and haplotype. Furthermore, under the same CsA regimen, it was found that the trough concentrations of variant genotype (ABCB1 1236TT or ABCB1 2677TT) were significant greater than those of wild-type (ABCB1 1236CC or ABCB1 2677GG, respectively) ($P = 0.0222$ and 0.0081). The trough concentrations of wild-type haplotype pair group were significantly lower than those of the mutant type pair group (TT-TT-TT) ($P = 0.007$).

CONCLUSIONS

ABCB1 polymorphisms in both genotype and haplotype may have a minor effect on the CsA blood concentrations.

Introduction

Ciclosporin A (CsA) is frequently used as an immunosuppressant in the prevention of allograft rejection after kidney, liver, heart and bone marrow transplantation as well as in the treatment of various autoimmune diseases, such as rheumatoid arthritis, psoriasis, myasthenia gravis (MG), systemic lupus erythematosus and diabetes mellitus. [1] CsA has a narrow therapeutic range and wide interindividual variation in pharmacokinetics. Monitoring CsA whole-blood concentrations is therefore essential to optimize the therapeutic dose in patients receiving this drug and prevent acute drug reactions associated with CsA. Monitoring trough CsA concentrations (C₀) determined immediately before the next dose, or peak CsA concentrations assayed 2 h after the CsA dosing (C₂) is performed by most centres to adjust CsA dose and is widely accepted as a useful and practical monitoring procedure [2].

CsA is a substrate for intestinal P-glycoprotein (P-gp), the product of the multidrug resistance 1 (ABCB1) gene [3]. P-gp is an integral membrane glycoprotein, a member of the ATP-binding cassette transporter family, which is responsible for the multidrug resistant phenotype of several tumour types. P-gp exists as two homologous halves, and each half is composed of six transmembrane loops and an ATP-binding site [4]. P-gp is constitutively expressed in healthy tissue, where it functions as an energy-dependent efflux transporter that exports a number of substrates, drugs or physiological molecules out of cells and the body. Recently, it was reported that several single nucleotide polymorphisms (SNPs) exist in the ABCB1 gene that have contributory roles in the bioavailability of drugs which are P-gp substrates. Lown *et al.* [5] have shown that 30% of the variability in oral C_{max} and 17% of the variability in oral clearance of CsA in humans can be accounted for by the individual variation in P-gp concentrations. Hoffmeyer *et al.* [6] performed systematic screening of the entire ABCB1 gene, and 15 SNPs were detected. Of these, only the SNP in exon 26 (3435C→T) of the ABCB1 gene, which occurred at a wobble position, was found to be of functional importance. Individuals homozygous for the T allele showed significantly lower duodenal P-gp expression and higher digoxin plasma concentrations. Further studies have revealed that the 3435C→T SNP was associated with enhanced uptake of P-gp substrates and varied significantly amongst different ethnic populations. Because the exon 26 SNP is in a nonpromoter region of the ABCB1 gene and does not alter its protein sequence, it was hypothesized that this SNP was linked to an as yet unidentified region(s) of the ABCB1 gene controlling expression. In the study by Kim *et al.* [7], allelic variations in exons 12, 21 and 26 of the ABCB1 gene were found to be associated with altered P-gp function. The two synonymous SNPs in exons 12 (1236C→T) and 26 (3435C→T) and a nonsynonymous SNP in exon 21 (2677G→T/A) were found to be in linkage disequilibrium. Strong associations

between the 3435C→T and 2677G→T/A alleles have also been found by Tanabe *et al.* [8] in human placental samples.

In this study, we performed a pharmacogenetic analysis of ABCB1 in MG patients admitted to Beijing Hospital between January 2000 and November 2004. We investigated for genetic polymorphisms in exons 12, 21 and 26 of ABCB1 and the haplotype frequencies. We also determined the relationship between the ABCB1 polymorphisms and the trough or peak blood concentrations of oral CsA given as Neoral (Novartis Pharmaceuticals Co., Eberbach, Germany), a microemulsion formulation, to MG patients.

Materials and methods

Patients

MG patients who were admitted to our hospital between 2000 and 2004, given CsA immunosuppressive treatment and received CsA blood concentration monitoring were invited to participate in this study. All gave written informed consent. A total of 129 MG patients (66 men and 63 women) were recruited. Mean age was 40.6 ± 10.6 years, mean body weight was 64.0 ± 15.2 kg, and mean serum creatinine was 0.96 ± 1.0 mg dl⁻¹. Twenty-seven of these patients satisfied the following conditions: (i) age 18–50 years, (ii) normal hepatic function, (iii) not in the acute episode of MG, (iv) no known medicine or clinical measures which interfere with CsA metabolism administered, and were selected to receive C₂ monitoring.

For all patients, the initial dosage of CsA was 50 mg twice daily, which was increased according to the clinical situation and CsA whole blood trough concentrations, whose target was 100–250 ng ml⁻¹. In addition to CsA, all patients received the cholinesterase inhibitor, pyridostigmine. Some patients took corticosteroids. Data assayed when the corticosteroid dose was <50 mg day⁻¹ were excluded. Apart from steroids, any medication known to interfere with P-gp function (inhibitors or inducers) was checked for all patients. However, no patient was excluded from the study for taking such medication.

The study was approved by the ethics committee of Beijing Hospital, the Ministry of Health, and all patients gave their written informed consent.

Therapeutic drug monitoring

Ethylenediamine tetraacetic acid anticoagulated blood was collected just before the morning CsA dosing to assay CsA trough blood concentrations and 2 h after the morning CsA dosing to assay CsA peak blood concentrations. CsA whole-blood concentrations were measured using Fluorescence Polarization Immunoassay on a TDXFLX analyser (Abbott Laboratory, Abbott, Campoverde di Aprilia, Italy) using the CsA monoclonal whole blood kit. The assay was calibrated with manufacturer-supplied reagents and verified for performance daily by assaying

quality control samples with expected concentrations in low (120–180 $\mu\text{g l}^{-1}$), medium (340–460 $\mu\text{g l}^{-1}$) or high (680–920 $\mu\text{g l}^{-1}$) ranges. The between-day and within-day variability was <4% in a concentration range between 150 and 800 $\mu\text{g l}^{-1}$. The assay detection limit was 25 $\mu\text{g l}^{-1}$.

Genotype and haplotype determination

Purified genomic DNA was extracted from peripheral blood leucocytes (5 ml) using the phenol–chloroform extraction method [9]. A total of 129 MG patients (north Chinese) were genotyped for the ABCB1 gene. A polymerase chain reaction (PCR)-restriction fragment length polymorphism was used for the detection of 1236C→T (exon 12), 2677G→T (exon 21) and 3435C→T (exon 26) SNPs.

Fragments containing each SNP were amplified in the presence of 1× PCR buffer, 0.2 mmol l^{-1} dNTPs, 0.2 $\mu\text{mol l}^{-1}$ of each specific primer, 1.5 mmol l^{-1} MgCl_2 , 1 U HotStarTaq Polymerase (Promega, Madison, WI, USA) and 50 ng genomic DNA template in a 25 μl reaction. PCR was carried out in a Thermocycler (Takara, Shiga, Japan) with an initial denaturation at 94°C for 15 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56–60°C for 30 s for the different pairs of primers, and extension at 72°C for 30–60 s for the different lengths of target sequences. A final termination elongation was performed at 72°C for 2 min. The primer sequences are as described previously [10]. The amplified reaction products were digested with specific restriction enzymes: Eco01091 for exon 12, BanI and RsaI for exon 21, which was tri-allelic, and DdnII for exon 26. The digested PCR products were analysed by electrophoretic separation on agarose gels, followed by direct visualization under ultraviolet light.

Pairwise linkage analysis on the basis of Expectation–Maximization was to identify linkages between any two loci in order to find the most common haplotypes [11].

Statistical analysis

For the analyses of CsA blood trough concentrations, CsA blood peak concentrations and dose-adjusted CsA blood concentrations, groups were compared using nonparametric tests. Student's *t*-test was used for comparisons between two groups and the analysis of variance (ANOVA) test for

comparisons among several groups. Statistical analysis was carried out by using the SPSS package (version 11.0; SPSS Inc., Chicago, IL, USA). The parameters were assumed to be normally distributed; no transformation was applied, and allele and genotype frequencies for the various SNPs were assessed for deviation from Hardy–Weinberg equilibrium using Fisher's exact test. $P < 0.05$ was considered to be significant. Haplotype frequencies were estimated on the basis of the Expectation–Maximization algorithm using the population genetics data analysis program Arlequin (<http://www.lgb.unige.ch/arlequin/>).

Results

Frequency of ABCB1 variants in MG patients

On the basis of allele frequency, genotype frequencies were not significantly different from those predicted by the Hardy–Weinberg equation (Table 1). In our population, the presence of the 2677A variant in exon 21 was not observed. A high frequency was observed of variants leading to an amino acid modification in exon 21 2677G→T (Ala 893Ser). A little less than 50% of patients exhibited a variant nucleotide at position 2677, and 29% were homozygous for the minor allele. The other exonic variants, 1236C→T in exon 12 and 3435C→T in exon 26, which do not affect the amino acid sequence, were also frequently observed. The mutations were presented as homozygous in 51% and 22.5% of patients for exons 12 and 26, respectively.

Linkage disequilibrium study

The four SNPs were investigated for any linkage disequilibrium to determine whether they were randomly associated in the same patient. Pairwise linkages between SNPs were not randomly distributed, and only a few major linkages, defined as >10% occurrence, were observed. Significant linkage disequilibrium was observed between the exon 12, 21 and 26 SNPs. Most of the wild-type alleles at any one position were associated with the wild-type allele at the following position. Conversely, a minor allele in the exon 12 SNP was usually associated with minor alleles in the exon

Table 1

Allele and genotype frequency in the MDR1 gene by exons

Exon	% Allele frequency			% Genotype frequency (n)			HWE P-value
12	T	C		TT	CT	CC	0.87
	71.32	28.68		51.16 (66)	40.31 (52)	8.53 (11)	
21	G	T	A	GG	TG	TT	0.96
	52.71	47.29	0	27.90 (36)	49.61 (64)	28.84 (29)	
26	C	T		CC	CT	TT	0.58
	53.87	46.12		30.23 (39)	47.28 (61)	22.48 (29)	

The number in parentheses indicates the number of patients. HWE, Hardy–Weinberg equilibrium.

Table 2

Influence of MDR1 genotypes on ciclosporin trough blood concentrations adjusted by dosage and body weight

Genotype	Allelic status (n)	[cyclo]	[cyclo]/Ldose	[cyclo]/Ddose	[cyclo]/Ldose.wt	[cyclo]/Ddose.wt
MDR1 exon 12 C1236T	TT (66)	138.7 ± 64.63	1.481 ± 0.6734	0.6317 ± 0.2464	94.49 ± 46.75	40.91 ± 18.34
	CT (52)	147.7 ± 79.11	1.614 ± 0.8282	0.6973 ± 0.3599	104.6 ± 62.92	45.22 ± 26.95
	CC (11)	122.3 ± 53.3	1.493 ± 0.5488	0.5582 ± 0.1564	94.40 ± 45.66	35.24 ± 14.32
MDR1 exon 21 G2677T/A	GG (36)	137.2 ± 71.1	1.640 ± 0.7902	0.6714 ± 0.3489	107.7 ± 60.10	44.13 ± 26.13
	TG (64)	144.0 ± 75.15	1.395 ± 0.6363	0.6210 ± 0.2702	89.44 ± 49.16	40.03 ± 21.17
	TT (29)	144.1 ± 62.02	1.780 ± 0.8171	0.7426 ± 0.2943	112.4 ± 54.35	47.21 ± 20.60
MDR1 exon 26 C3435T	CC (39)	138.1 ± 78.13	1.575 ± 0.7705	0.6490 ± 0.3262	102.9 ± 57.70	42.50 ± 24.34
	CT (61)	140.6 ± 71.57	1.465 ± 0.6763	0.6422 ± 0.2918	94.47 ± 52.66	41.69 ± 22.95
	TT (29)	144.3 ± 61.51	1.633 ± 0.8353	0.7066 ± 0.3106	101.9 ± 52.15	44.30 ± 19.34
MDR1 exons 12-21-26	CC-GG-CC (8)	143.7 ± 99.98	1.571 ± 0.5698	0.5613 ± 0.1669	97.27 ± 49.93	34.49 ± 15.59
	CT-GT-CT (25)	154.4 ± 81.49	1.618 ± 0.7636	0.6963 ± 0.3303	100.8 ± 58.71	43.31 ± 24.41
	TT-GT-GT (25)	127.9 ± 64.43	1.220 ± 0.5244	0.5444 ± 0.2193	77.82 ± 40.71	35.19 ± 18.63
	TT-TT-TT (23)	135.3 ± 57.38	1.695 ± 0.8417	0.7082 ± 0.2749	105.0 ± 52.62	44.18 ± 18.10

Values are given as arithmetic mean ± SD, the number in parentheses indicates the number of patients, [cyclo] indicates the unadjusted ciclosporin trough blood concentration (ng ml⁻¹). Ldose, last dose (mg); Ddose, daily dose (mg); wt, body weight (kg). No statistical difference was found between genotype groups or haplotype groups with ANOVA test.

21 and 26 SNPs. Interestingly, a minor allele in the exon 12 SNP was also associated with wild-type allele in exon 21 (frequency = 0.254). Accordingly, the most common haplotype pairs were CC-GG-CC, TT-TT-TT and CT-GT-CT.

Effect of ABCB1 genotype/haplotype on CsA trough and peak blood concentrations

No statistically significant association was observed between CsA dose-adjusted trough blood concentrations and polymorphisms in exon 12, 21 or 26 (Table 2). Figure 1 appeared to show that patients homozygous for the CC-GG-CC genotypes (C-G-C haplotypes) had lower trough CsA concentrations compared with patients carrying genotypes with minor alleles at all three points (TT-TT-TT), and patients with heterozygote genotype (CT-GTCT) had intermediate values, although without statistical significance.

The same phenomenon occurred for the CsA dose-adjusted peak blood concentrations. Patients with wild-type genotype in the three loci generally had lower values than those with minor alleles (Table 3), but statistical significance was not reached.

CsA concentrations were also compared in patients on the same CsA medication regimen between different genotype groups for the three ABCB1 SNPs and different haplotypes (Table 4). For the regimen of 100 mg twice daily, a significant difference in blood concentrations was observed among ABCB1 1236C→T genotype groups ($P = 0.0272$). The blood concentrations in variant genotype (ABCB1 1236TT) was greater than that of wild-type (ABCB1 1236CC) ($P = 0.0222$). Significant difference was also observed among ABCB1 2677G→T genotype groups ($P = 0.0445$), and the blood concentrations of variant genotype (ABCB1 2677TT) was greater than that of wild-type (ABCB1 2677GG) ($P = 0.0081$).

Under the CsA regimen of 100 mg twice daily, the trough concentrations of wild-type haplotype (CC-GG-CC)

Figure 1

Dose-adjusted trough blood concentrations of ciclosporin A (CsA) (ng ml⁻¹ per mg kg⁻¹ body weight) according to MDR1 exons 12-21-26 haplotype, CC-GG-CC ($n = 8$), CT-GT-CT ($n = 25$) and TT-TT-TT ($n = 23$). Blood concentrations are adjusted to the last or daily dose. The mean values are indicated

were significantly lower than those of the mutant type (TT-TT-TT) ($P = 0.007$) (Table 4), and for the CsA regimen of 50 mg three times daily or 100 mg twice daily, there was a trend for CsA trough blood concentrations to increase from the wild-type allele to the minor alleles (Figure 2).

Discussion

Pharmacogenetic evaluation of several genes implicated in drug pharmacokinetics should, in theory, provide effi-

Table 3

Influence of MDR1 genotypes on ciclosporin peak blood concentrations adjusted by dosage and body weight

Genotype	Allelic status (n)	[cyclo]	[cyclo]/Ldose	[cyclo]/Ddose	[cyclo]/Ldose.wt	[cyclo]/Ddose.wt
MDR1 exon 12 C1236T	TT (9)	477.8 ± 255.5	5.683 ± 3.367	2.540 ± 1.341	360.6 ± 206.4	163.7 ± 94.46
	CT (14)	431.9 ± 225.4	5.505 ± 2.787	2.401 ± 1.030	358.3 ± 179.0	159.4 ± 80.79
	CC (4)	443.7 ± 246.7	6.080 ± 2.186	2.509 ± 1.084	306.5 ± 129.6	124.0 ± 52.45
MDR1 exon 21 G2677T/A	GG (12)	425.6 ± 215.1	5.791 ± 2.640	2.417 ± 1.018	341.7 ± 151.4	146.3 ± 75.93
	TG (10)	447.3 ± 265.3	5.156 ± 2.641	2.379 ± 1.194	324.3 ± 187.9	147.9 ± 79.87
	TT (5)	508.1 ± 229.6	6.297 ± 3.963	2.743 ± 1.333	428.8 ± 228.6	193.3 ± 99.59
MDR1 exon 26 C3435T	CC (12)	397.9 ± 238.8	5.634 ± 3.078	2.310 ± 1.171	320.7 ± 177.2	132.0 ± 72.61
	CT (11)	505.5 ± 234.2	5.545 ± 2.035	2.623 ± 1.019	374.7 ± 170.7	178.6 ± 87.66
	TT (4)	446.4 ± 211.9	5.984 ± 4.504	2.485 ± 1.387	379.3 ± 230.9	163.3 ± 84.99
MDR1 exons 12-21-26	CC-GG-CC (4)	443.7 ± 246.7	6.080 ± 2.186	2.509 ± 1.084	306.5 ± 129.6	124.0 ± 52.45
	CT-GT-CT (5)	498.3 ± 227.9	5.490 ± 1.837	2.606 ± 0.8309	354.6 ± 137.9	168.4 ± 64.52
	TT-TT-TT (4)	446.4 ± 211.9	5.984 ± 4.504	2.485 ± 1.387	379.3 ± 230.9	163.3 ± 84.99

Values are given as arithmetic mean ± SD, the number in parentheses indicates the number of patients, [cyclo] indicates the unadjusted ciclosporin peak blood concentration (ng ml⁻¹). Ldose, last dose (mg); Ddose, daily dose (mg); wt, body weight (kg). No statistical difference was found between genotype groups or haplotype groups with ANOVA test.

cient tools for individualizing drug dosage. This would both improve drug efficacy and prevent adverse effects [12]. Such evaluation may be particularly useful for drugs characterized by a narrow therapeutic index and/or significant toxicity, such as CsA.

MG patients from northern China in our study exhibited genotype frequencies of the different SNPs in accordance with those already described in Chinese populations, except the exon 21 SNP. For example, Balram Chowbay *et al.* [13], I, in a genetic analysis of 96 Chinese, have reported the allele frequency for 2677A to be 12.5%, which was not found in our population. This may in part be attributed to the heterogeneity of the Chinese population.

The linkage that we found between the SNPs in exons 12, 21 and 26 has been described previously. Kim *et al.* [14] reported co-segregation of the exon 26 minor allele with the T allele of the non-synonymous exon 21 SNP 2677G→T and with the T allele of the synonymous exon 12 SNP 1236C→T. These three SNPs in exons 12, 21 and 26 were closely linked at high frequency and occurred in 62% of European Americans and 13% of African-Americans. Anglicheau *et al.* have reported that the 1236C→T SNP correlates with the 2677G→T/A and 3435C→T SNP (the correlation coefficient $\Delta = 0.79$ and 0.66 , respectively), and the 2677G→T/A SNP correlates with the 3435C→T SNP ($\Delta = 0.66$) [15]. This high level of incomplete disequilibrium was the origin of the haplotype approach. Haplotypes generally contained more information than did individual SNPs.

Many factors affect CsA blood concentrations, such as diet, age, liver function and disease condition. Most previous studies have been of organ transplantation patients who had poor and changeable metabolizing organ function (liver and kidney transplantation) or unpredicted pharmacokinetic changes (such as heart transplantation). Our study was the first to investigate the pharmacogenetic effect of ABCB1 on CsA blood concentrations in MG

patients who were relatively stable. Furthermore, given the complexity of CsA's pharmacokinetics, the way that concentrations were adjusted by CsA dosage and the patients' body weight with '[cyclo]/(dose.wt)' may introduce more errors. Therefore, we compared blood concentrations on the same CsA regimen in patients with stable weight. Based on the dose- and body weight-adjusted trough blood concentrations or peak blood concentrations, we did not detect a notable effect of ABCB1 genotype or haplotype in our study, whereas comparison of blood trough concentrations on the same CsA regimen suggested a minor effect of ABCB1 genotype or haplotype, consistent with the results of previous studies [16].

One study investigating the association between haplotypes of the ABCB1 gene and the steady-state kinetics of digoxin has shown that ABCB1 polymorphisms were significantly associated with the absorption, but not with the elimination of the drug, suggesting that the SNP action occurred mainly in the gut [17]. However, for drugs whose half-life is significantly smaller than the dosing interval (e.g. CsA), trough concentrations predict systemic drug exposure poorly. Pharmacokinetic analysis shows that the greatest variability occurs during the absorption phase (up to 4 h after a dose of CsA), where C₂ is the best predictor of AUC₀₋₄ and adequate absorption is crucial to ensure effective immunosuppression. Although in our study no significant difference was observed in the dose-adjusted C₂ between different genotype groups, this might be attributed to the small number studied.

There are two ways to obtain efficient treatment with CsA. One is dose adjustment using sensitive pharmacokinetic indices such as AUC₀₋₄ or C₂. The other is to gather enough information to predict the effect of drug on patients, such as sex, age, co-medication, physical status, food and genotypes, and interpret this information rationally. To do so, large-scale trials in diverse populations are required.

Comparison of ciclosporin (CsA) trough blood concentration of different genotypes and haplotypes under the same regimen

Values are given as arithmetic mean $\pm$ SD. The number in parentheses indicates the number of patients. C0, CsA trough concentrations in ng ml⁻¹.

REFERENCES

- 1 Gaston RS. Maintenance immunosuppression in the renal transplant recipient: an overview. *Am J Kidney Dis* 2001; 38: S25–35.
- 2 Clase CM, Mahalati K, Kiberd BA, Lawen JG, West KA, Fraser AD, Belitsky P. Adequate early cyclosporin exposure is critical to prevent renal allograft rejection: patients monitored by absorption profiling. *Am J Transpl* 2002; 2: 789–95.
- 3 Saeki T, Ueda K, Tanigawara Y, Hori R, Komano T. Human P-glycoprotein transports cyclosporin A and FK506. *J Biol Chem* 1993; 268: 6077–80.
- 4 Fromm MF. Genetically determined differences in P-glycoprotein function: implications for disease risk. *Toxicology* 2002; 181–182: 299–303.
- 5 Lown KS, Mayo RR, Leichtman AB, Hsiao HL, Turgeon DK, Schmiedlin-Ren P, Brown MB, Guo W, Rossi SJ, Benet LZ, Watkins PB. Role of intestinal P-glycoprotein (mdr) in interpatient variation in the oral bioavailability of cyclosporine. *Clin Pharmacol Ther* 1997; 62: 248–60.
- 6 Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, John A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, Brinkmann U. 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 USA* 2000; 97: 3473–8.
- 7 Kim RB. MDR1 single nucleotide polymorphisms: multiplicity of haplotypes and functional consequences. *Pharmacogenetics* 2002; 12: 425–7.

- 8** Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, Takahashi M, Kurata Y, Kigawa J, Higuchi S, Terakawa N, Otsubo K. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J Pharmacol Exp Ther* 2001; 297: 1137–43.
- 9** Hänni C, Brousseau T, Laudet V, Stehelin D. Isopropanol precipitation removes PCR inhibitors from ancient bone extracts. *Nucleic Acids Res* 1995; 23: 881–2.
- 10** Tang K, Ngoi SM, Gwee PC, Chua JM, Lee EJ, Chong SS, Lee CG. Distinct haplotype profiles and strong linkage disequilibrium at the MDR1 multidrug transporter gene locus in three ethnic Asian populations. *Pharmacogenetics* 2002; 12: 437–50.
- 11** John A, Kopke K, Gerloff T, Mai I, Rietbrock S, Meisel C, Hoffmeyer S, Kerb R, Fromm MF, Brinkmann U, Eichelbaum M, Brockmoller J, Cascorbi I, Roots I. Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein MDR1 gene. *Clin Pharmacol Ther* 2002; 72: 584–94.
- 12** Evans WE, Relling MV. Pharmacogenomics. Translating functional genomics into rational therapeutics. *Science* 1999; 286: 487–91.
- 13** Balram C, Sharma A, Sivathasan C, Lee EJ. Frequency of C3435T single nucleotide MDR1 genetic polymorphism in an Asian population: phenotypic–genotypic correlates. *Br J Clin Pharmacol* 2003; 56: 78–83.
- 14** Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, Taylor A, Xie HG, McKinsey J, Zhou S, Lan LB, Schuetz JD, Schuetz EG, Wilkinson GR. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 2001; 70: 189–99.
- 15** Anglicheau D, Verstuyft C, Laurent-Puig P, Becquemont L, Schlageter MH, Cassinat B, Beaune P, Legendre C, Thervet E. Association of the multidrug resistance-1 gene single-nucleotide polymorphisms with the tacrolimus dose requirements in renal transplant recipients. *J Am Soc Nephrol* 2003; 14: 1889.
- 16** Balram C, Sharma A, Sivathasan C, Lee EJ. Frequency of C3435T single nucleotide MDR1 genetic polymorphism in an Asian population: phenotypic–genotypic correlates. *Br J Clin Pharmacol* 2003; 56: 78–83.
- 17** John A, Kopke K, Gerloff T, Mai I, Rietbrock S, Meisel C, Hoffmeyer S, Kerb R, Fromm MF, Brinkmann U, Eichelbaum M, Brockmoller J, Cascorbi I, Roots I. Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein MDR1 gene. *Clin Pharmacol Ther* 2002; 72: 584–94.