

No Influence of the *MDR-1* C3435T Polymorphism or a *CYP3A4* Promoter Polymorphism (*CYP3A4-V* Allele) on Dose-adjusted Cyclosporin A Trough Concentrations or Rejection Incidence in Stable Renal Transplant Recipients

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Background: A substantial proportion of the variability in the absorption and clearance of cyclosporin A (CsA) after oral administration has been attributed to variability in liver cytochrome P-450 3A4 (*CYP3A4*) activity and intestinal P-glycoprotein (P-gp) concentration. A polymorphism in the *CYP3A4* promoter region, termed "variant" allele *CYP3A4-V*, was postulated to be associated with altered *CYP3A4* enzyme activity. A polymorphism in exon 26 (C3435T) of the multidrug resistance-1 (*MDR-1*) gene was correlated with intestinal expression and *in vivo* activity of P-gp.

Methods: We investigated the occurrence of both polymorphisms in 124 stable Caucasian renal transplant recipients (>6 months after transplantation) on CsA as the primary immunosuppressant. Real-time, rapid-cycle PCR methods were developed and used for genotyping.

Results: The estimated allele frequencies for the *MDR-1* C3435T allele (54%) and the *CYP3A4-V* allele (4.8%) were similar to those reported for Caucasian populations. No significant differences were found for the CsA doses needed to maintain similar CsA trough concentrations in patients with and without the *CYP3A4-V* allele or in patients with different *MDR-1* C3435T genotypes. Furthermore, neither of the polymorphisms investigated was associated with renal function as assessed by creat-

inine plasma concentration or, in a retrospective analysis, the incidence of acute rejection.

Conclusions: These findings suggest that the *MDR-1* C3435T mutation and the *CYP3A4-V* variant are not major determinants of CsA efficacy in renal transplant recipients.

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Cyclosporin A (CsA)⁴ is a critical-dose immunosuppressive drug with a narrow therapeutic range and wide interindividual variation in its pharmacokinetics. Monitoring CsA whole-blood concentrations is therefore essential to optimize the therapeutic dose in patients receiving this drug for the prophylaxis of acute rejection after organ transplantation (1). Monitoring trough CsA concentrations, determined immediately before the next dose, is performed by most centers to adjust CsA dose and is widely accepted as a useful and practical monitoring procedure.

Cytochrome P-450 3A4 (*CYP3A4*) (2), the most abundant CYP enzyme in the human liver and intestine with wide intra- and interindividual differences in activity [for a review see Ref. (3)], makes a substantial contribution to the metabolism of CsA. This remarkable variation in drug metabolism may be caused by genetic polymorphisms, induction or inhibition of enzyme activity, physiological status, and concomitant diseases (4). CsA is also a substrate for intestinal P-glycoprotein (P-gp), the product of the multidrug resistance (*MDR*) gene (5). As with

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⁴Nonstandard abbreviations: CsA, cyclosporin A; *CYP3A4*, cytochrome P-450 3A4; P-gp, P-glycoprotein; and *MDR*, multidrug resistance.

CYP3A4, significant interindividual variation has been observed in P-gp (6). It is therefore not surprising that a substantial proportion of the variability in the oral clearance of CsA could be attributed to variability in liver CYP3A4 activity and intestinal P-gp concentration (6).

Functional polymorphisms have been described for both of these pharmacogenetically interesting genes. A polymorphism in the CYP3A4 promoter region was identified and termed "variant" allele CYP3A4-V (7). The mutation is an A→G transition at position -290 and is situated in the nifedipine-specific element of the gene. The presence of the CYP3A4-V allele was associated with higher grade prostate cancer (7), and it was proposed that people with the CYP3A4-V genotype may have decreased CYP3A4 protein activity. However, in an in vitro system, higher expression of a reporter gene assay for the CYP3A4-V allele was observed as compared with the -W allele (8). The induction of P-gp by rifampin decreases the area under the curve of orally administered digoxin, another substrate of P-gp (9). Recently, Hoffmeyer et al. (10) found that a polymorphism in exon 26 (C3435T) of the MDR-1 gene was correlated with intestinal expression and in vivo activity of P-gp.

Because these two polymorphisms might be expected to have an impact on the metabolism and therapeutic efficacy of CsA, we undertook a study to determine their occurrence in 124 stable renal transplant recipients and to establish whether the presence of either polymorphism was correlated with the following: (a) an altered CsA dose requirement to achieve the target therapeutic range; (b) acute rejection as determined by biopsy; or (c) renal function as assessed by plasma creatinine concentrations.

Materials and Methods

SUBJECTS

The present study was approved by the ethics committee of the Medical Faculty, Georg-August-University (Göttingen, Germany). One hundred twenty-four unrelated Caucasian renal transplant recipients (71 men and 53 women) were recruited, and all patients gave informed consent. Only renal transplant recipients who had received a graft at least 6 months before the study and who had been on CsA immunosuppression (Neoral formulation) for 6 months or longer were eligible for participation in the study.

ROUTINE ANALYSES

During a visit to the transplant outpatient clinic, blood samples were drawn for the determination of CsA trough concentrations, as well as for routine laboratory analyses. Plasma creatinine concentrations were measured on a Hitachi 917 analyzer using reagents from Roche Diagnostics. CsA concentrations were determined using the Emit assay (Dade Behring) on a Cobas Mira analyzer (Roche) (11). Dose-adjusted trough concentrations were calculated by dividing CsA trough concentrations by the corresponding 12-h dose on a mg/kg basis.

GENOTYPE DETERMINATION

Genotyping for the MDR-1 C3435T and CYP3A4-V polymorphisms was performed using homogeneous hybridization probe assays on the LightCycler™ (Roche Biochemica).

MDR-1 C3435T PRIMERS AND PROBES

A 252-bp fragment of the MDR-1 gene (GenBank Accession No. AC005068) was generated by PCR amplification with the primers (forward, 0.5 μM) 5'-TGT TTT CAG CTG CTT GAT GG-3' and (reverse, 0.5 μM) 5'-CAT GCT CCC AGG CTG TTT AT-3'. The reaction mixture also included the wild-type probe (0.1 μM) 5'-AAG AGA TCG TGA GGG CA-3'-fluorescein and the anchor probe (0.3 μM) 5'-Cy5.5-AAG GAG GCC AAC ATA CAT GCC-3'-phosphate. The anchor and probe are derived from the sequence of the sense strand. The underlined position in the probe defines a C→T polymorphism designated C3435T. The resulting C:A mismatch is very unstable and destabilizes the probe. Heterozygous samples are therefore well resolved (Fig. 1A). The designation MDR-1 wild type is used throughout this report to designate the allele that contains no 3435T mutation. The presence of the expected mutation was confirmed by sequencing the products (sequenase cycle sequencing reagent set; Amersham) on an automated DNA sequencer (Licor 4200; Licor). The resulting characterized samples were used as controls in the assay.

CYP3A4-V ALLELE PRIMERS AND PROBES

A 427-bp fragment from the CYP3A4 gene promoter (GenBank Accession No. D11131) was amplified by PCR with the primers (forward, 0.5 μM) (12) 5'-AAC AGG CGT GGA AAC ACA AT-3' and (reverse, 0.5 μM) 5'-CCA CTC ACT GAC CTC CTT TGA-3'. The wild-type probe (0.1 μM) 5'-AGG GCA AGA GAG AGG C-3'-fluorescein and the anchor probe (0.3 μM) 5'-LC-Red640-TTA ATA GAT TTT ATG CCA ATG GCT CCA CTT GAG-3'-phosphate were included in the reaction mixture. The probes were modified as indicated by the addition of fluorescein, the LC-Red640 dye (Roche Biochemica), or phosphorylation. The probe and anchor bind to the antisense strand. The probe is specific for the sequence of the CYP3A4 gene and has no hybridization site on the related CYP3A5 and CYP3A7 genes. The CYP3A4-V allele is characterized by an A→G polymorphism at the underlined position. The resulting A:C mismatch significantly destabilizes the probe and allows for a good assignment of heterozygous samples (Fig. 1B). The presence of the expected mutation was also confirmed by the sequencing of PCR products. Resulting characterized samples were used as controls in the assay.

GENERAL PCR PROCEDURE

Genomic DNA was isolated from the EDTA-anticoagulated whole blood not required for CsA measurements using a rapid method based on alkaline lysis (13). DNA

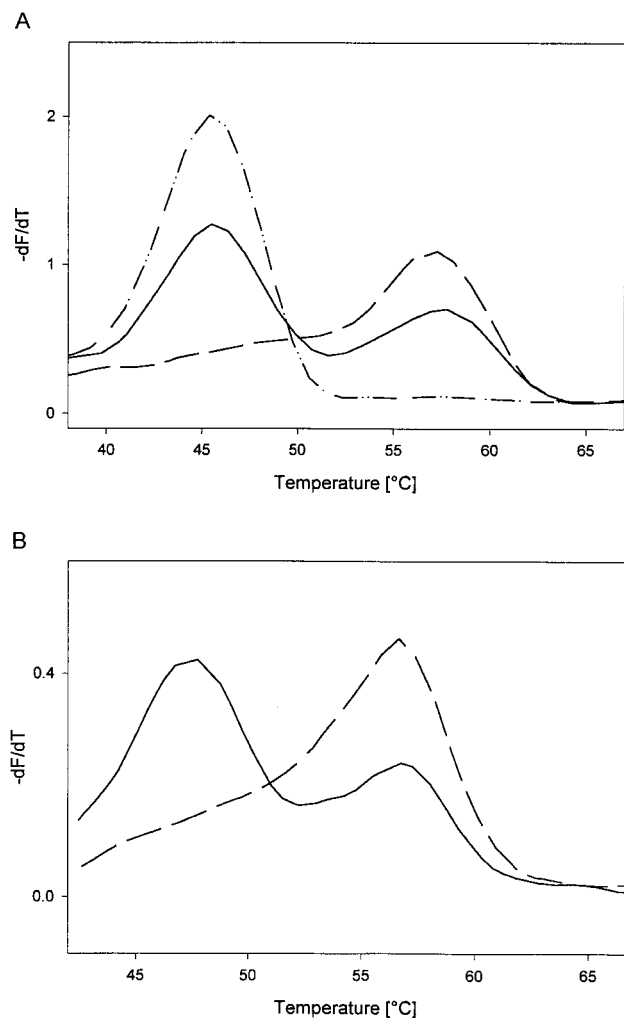


Fig. 1. Typical genotyping results for the *MDR-1* C3435T genotype (A) and *CYP3A4-V* allele (B) using the LightCycler.

The wild type allele (---), heterozygous sample (—), and homozygous mutation (- · -; only in A) are shown.

prepared by alternative methods is equally suitable. We do not routinely quantify DNA before the assay because the assay is very robust at different concentrations (13). The reaction mixture for PCR amplification consisted of 1 μ L of genomic DNA solution, primers and probes at the concentrations indicated above, 0.5 U of Taq DNA polymerase (Life Technologies), 1 μ L of 10 \times PCR buffer (Life Technologies), 0.2 mM each dNTP (Roche Biochemica), 2.5 mM MgCl₂, 500 mg/L bovine serum albumin (New England BioLabs), and 50 mL/L dimethyl sulfoxide (Sigma). PCR-grade water was added to a final volume of 10 μ L. The cycling program consisted of 30 s of initial denaturation at 95 $^{\circ}$ C and 45 cycles of the following with the maximum ramp rate: 95 $^{\circ}$ C for 0 s; 55 $^{\circ}$ C for 5 s; and 72 $^{\circ}$ C for 10 s. Melting curve acquisition was from 40 to 65 $^{\circ}$ C at 0.2 $^{\circ}$ C in channel two (LC-Red640) or channel three (Cy5.5), respectively. For the *MDR-1* C3435T polymorphism, the melting point of the detection probe was 57.0 $^{\circ}$ C when hybridized to the wild-type allele and

45.5 $^{\circ}$ C when hybridized to the mutant allele. For the *CYP3A4* allele, the melting point of the detection probe was 56.5 $^{\circ}$ C when hybridized to the wild-type allele and 47.5 $^{\circ}$ C when hybridized to the mutant allele (Fig. 1).

STATISTICAL ANALYSIS

All calculations were performed with SPSS for Windows. Between-group comparisons were done with the Mann-Whitney *U*-test for the mutant *CYP3A4* allele. Between-group frequencies of alleles were compared by Fisher's exact test or Pearson's χ^2 , as appropriate. Between-group comparisons for the *MDR-1* genotypes were performed by one-way ANOVA.

Results

Of the 124 transplant recipients, the *MDR-1* wild-type genotype (3435 CC) was observed 31 patients, whereas 52 patients were heterozygous (3435 CT) and 41 patients were homozygous (3435 TT) for the mutation. There was no gender-related difference for the prevalence of the mutation ($P = 0.628$); therefore, male and female patients were combined for further evaluation. No differences in medication with respect to calcium antagonists and diuretic drugs were found among patients with different genotypes (data not shown). The population was in Hardy-Weinberg equilibrium ($\chi^2=3.01$; $P = 0.08$). As can be seen from Table 1, the current total CsA doses and weight-adjusted doses were similar, irrespective of the *MDR-1* genotype. Patients who were homozygous for the *MDR-1* wild-type genotype (3435 CC) were maintained at CsA trough concentrations similar to those for patients who were homozygous for the *MDR-1* 3435 TT mutation. Consequently, there was no significant difference in the dose-adjusted trough concentrations. Neither the incidence of acute rejection ($P = 0.602$) nor the plasma creatinine concentrations ($P = 0.238$) differed between the groups with different *MDR-1* 3435 alleles. Acute rejection was confirmed by biopsy.

Table 1. Comparison of patients with different *MDR-1* C3435T genotypes.^a

	<i>MDR-1</i>		
	3435 CC	3435 CT	3435 TT
n (%) ^b	31 (25%)	52 (42%)	41 (33%)
CsA dose, mg/day	250 \pm 60	246 \pm 62	247 \pm 82
CsA trough concentration, μ g/L	137 \pm 23	143 \pm 23	140 \pm 29
CsA 24-h dose/weight, mg/kg	3.4 \pm 0.8	3.6 \pm 1.1	3.6 \pm 1.2
Dose-adjusted trough concentration, μ g/L	85 \pm 26	88 \pm 30	85 \pm 33
Plasma creatinine, mg/L	16 \pm 6	19 \pm 8	17 \pm 9
Time since transplantation, years	7 \pm 4	7 \pm 4	10 \pm 14
Prednisolone dose, mg/day	6.3 \pm 2.2	5.7 \pm 2.5	6.4 \pm 1.8
No. of rejections (%) ^c	13 (42%)	17 (33%)	17 (41%)

^a Data are mean \pm SD. No significant differences for between-group comparison by one-way ANOVA.

^b Percentage refers to the total number of cases.

^c Percentage refers to the number of cases in the respective groups.

The variant *CYP3A4-V* allele was found in 12 of 124 transplant recipients. All 24 subjects were heterozygous for the mutation. There was no gender-related difference for the prevalence of the mutation ($P = 0.232$) and the population was in Hardy–Weinberg equilibrium ($\chi^2=0.32$; $P = 0.57$). Patients who were heterozygous for the *CYP3A4-V* allele were maintained at CsA doses and trough CsA concentrations similar to those for patients who were homozygous for the wild-type allele (Table 2). The incidence of acute rejection was similar ($P = 0.533$) between the two groups and the plasma creatinine concentrations did not differ ($P = 0.842$). To investigate a possible interaction between the *CYP3A4-V* allele and the *MDR-1* 3435 genotype, we compared eight patients carrying the heterozygous *CYP3A4-V* allele, as well as a heterozygous ($n = 5$) or a homozygous ($n = 3$) *MDR-1* 3435 genotype. These cases were not different from those having both wild-type alleles ($n = 27$) with respect to the CsA daily dose ($P = 0.862$), the CsA dose per kg ($P = 0.269$), the dose-adjusted trough concentration ($P = 0.499$), or acute rejections ($P = 0.236$).

Discussion

Hepatic CYP3A4 activity and intestinal P-gp content are important determinants that explain up to three-fourths of CsA variation after oral administration (6, 14). In an investigation of 25 stable renal transplant recipients receiving the Sandimmun formulation (6), hepatic CYP3A4 activity accounted for 56% of the variability in the apparent oral clearance of CsA, whereas enterocyte content of P-gp accounted for an additional 17% of the variation. For the peak CsA blood concentration, hepatic CYP3A4 activity was responsible for 32%, and P-gp for an additional 30%, of the variability (6). Allelic variants in the *CYP3A4* (7) and *P-gp* genes (10) that lead to altered metabolism are, therefore, potential candidates for the explanation of at least some of this variation.

The recently reported functional polymorphism of the *MDR-1* gene (C3435T) correlated with *MDR-1* in vivo

expression and activity (10). The mutant allele, although caused by a wobble base, was associated with significantly decreased intestinal P-gp expression, higher peak digoxin concentrations, and, in a small group of individuals, a higher digoxin area under the concentration–time curve after rifampin induction of P-gp. This mutation appears to be fairly common with an estimated allele frequency of 48% in a Caucasian population (10). In our collective of 124 Caucasian renal transplant recipients, the estimated allele frequency of the *MDR-1* C3435T allele of 54% was similar to that reported by Hoffmeyer et al. (10). However, no association was observed between the occurrence of this mutation and the dose-adjusted CsA trough concentration. Thus, the CsA dose required to maintain CsA whole-blood trough concentrations within the therapeutic range (100–150 $\mu\text{g/L}$) at our institution for renal transplant recipients was independent of the *MDR-1* genotype. Because trough CsA concentrations are a product of absorption, distribution, and elimination, it may be possible that the *MDR-1* C3435T mutation is associated with higher peak CsA concentrations during the early postabsorptive phase leading to increased exposure to CsA. A retrospective analysis revealed no correlation between the *MDR-1* genotype and the incidence of acute rejection in this collective. The rejection incidence in the group of patients who were homozygous for the *MDR-1* C3435T genotype was almost identical to that found in patients who were homozygous for the wild-type allele (41% vs 42%, respectively). Furthermore, plasma creatinine concentrations did not differ between patients who were homozygous for the *MDR-1* C3435T genotype and those who were homozygous for the wild-type genotype. Because the nephrotoxicity of CsA is associated with increased exposure to the drug, these findings suggest that the *MDR-1* C3435T mutation may not be an important determinant for CsA concentrations in renal transplant recipients receiving the microemulsion formulation Neoral. Because we investigated only stable renal transplant patients who received transplants at least 6 months before this study, the possibility of a selection bias must be considered. However, the allele frequencies in this transplant collective for both *MDR-1* and *CYP3A4* were in Hardy–Weinberg equilibrium and were also in agreement with published allele frequencies for healthy controls (10). This argues against a major selection bias.

It can be concluded that any influence of the *MDR-1* polymorphism on CsA exposure is not sufficient to have a major effect on the clinical outcome of patients whose CsA dosage is monitored according to trough CsA concentrations. The possibility must be considered that the microemulsion formulation could potentially prevent the interaction of CsA with P-gp. Choc et al. (15) investigated the influence of a CYP3A/P-gp inhibitor on the bioavailability of CsA and concluded that for Neoral and Sandimmun, there was no discriminating formulation-dependent effect on the CYP3A and P-gp systems.

The *CYP3A4-V* allele has an estimated frequency of

Table 2. Comparison of patients with and without the variant *CYP3A4-V* allele.^a

	<i>CYP3A4</i> wild-type allele	<i>CYP3A4-V</i> allele
n (% ^b)	112 (90%)	12 (10%)
CsA dose, mg/day	246 \pm 68	260 \pm 76
CsA trough concentration, $\mu\text{g/L}$	141 \pm 26	134 \pm 15
CsA 24-h dose/weight, mg/kg	3.6 \pm 1.1	3.2 \pm 0.8
Dose-adjusted trough concentration, $\mu\text{g/L}$	86 \pm 31	87 \pm 23
Plasma creatinine, mg/L	17 \pm 8	16 \pm 6
Time since transplantation, years	7 \pm 4	6 \pm 5
Prednisolone dose, mg/day	6.1 \pm 2.4	6.0 \pm 1.4
No. of rejections (% ^c)	44 (39%)	3 (25%)

^a Data are mean \pm SD. No significant differences for between-group comparisons.

^b Percentage refers to the total number of cases.

^c Percentage refers to the number of cases in the respective groups.

3.6–9.6% in the Caucasian population (7, 17, 18). The observed frequency of 4.8% in our collective of renal transplant recipients is in accordance with these previous findings. On the basis of clinical studies (7, 12), it was postulated that the *CYP3A4-V* allele may be associated with decreased *CYP3A4* expression or decreased enzymatic activity. However, in vitro studies do not support this hypothesis (8, 16–18). Our results are in agreement with these latter studies and show a lack of functional significance of the *CYP3A4-V* allele for the CsA dose required to achieve therapeutic concentrations of CsA, as well as for the incidence of acute rejection in renal transplant recipients on CsA immunosuppression. While this report was in preparation, the investigation of Rivory et al. (19) was published, reporting an incidence for the *CYP3A4-V* allele of 3% among 91 renal transplant recipients. The polymorphism did not influence the CsA pseudoclearance, which is in agreement with our findings. Data on rejection incidence or renal function were not reported in this study. From a comparison of patients carrying the heterozygous *CYP3A4-V* allele and also a heterozygous or homozygous *MDR-1* 3435 genotype with those having both wild-type alleles, we found no evidence for possible interactions between these genotypes.

In conclusion, our results support the notion that the investigated polymorphisms are not major determinants of interindividual CsA variability. We found no significant differences in the CsA doses needed for the maintenance of similar CsA trough concentrations in stable renal transplant recipients with and without the *CYP3A4-V* allele or in patients with different *MDR-1* C3435T genotypes. Furthermore, after stratification according to genotype, we found no significant differences with respect to renal function as assessed by creatinine plasma concentration or with respect to the incidence of acute rejection. If there is a functional relevance of the *CYP3A4-V* allele, then this would only lead to minor changes in *CYP3A4* expression. The manifestation of diseases eventually caused by this polymorphism might only be expected in the long term. In the case of the *MDR-1* C3435T polymorphism, other factors may be necessary for the genotype to gain clinically functional relevance, such as induction of enzyme activity, physiological status, or concomitant disease. The genotyping of these polymorphisms is unlikely to be useful for the optimization of CsA drug therapy in the clinical setting.

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References

- Oellerich M, Armstrong VW, Schütz E, Shaw LM. Therapeutic drug monitoring of cyclosporine and tacrolimus. Update on Lake Louise Consensus Conference on cyclosporin and tacrolimus. *Clin Biochem* 1998;31:309–16.
- Kronbach T, Fischer V, Meyer UA. Cyclosporine metabolism in human liver: identification of a cytochrome P-450III gene family as the major cyclosporine-metabolizing enzyme explains interactions of cyclosporine with other drugs. *Clin Pharmacol Ther* 1988;43:630–5.
- Thummel KE, Wilkinson GR. In vitro and in vivo drug interactions involving human CYP3A. *Annu Rev Pharmacol Toxicol* 1998;38:389–430.
- Ingelman-Sundberg M, Oscarson M, McLellan RA. Polymorphic human cytochrome P450 enzymes: an opportunity for individualized drug treatment. *Trends Pharmacol Sci* 1999;20:342–9.
- 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.
- Lown KS, Mayo RR, Leichtman AB, Hsiao HL, Turgeon DK, Schmiedlin-Ren P, et al. Role of intestinal P-glycoprotein (*mdr1*) in interpatient variation in the oral bioavailability of cyclosporine. *Clin Pharmacol Ther* 1997;62:248–60.
- Rebeck TR, Jaffe JM, Walker AH, Wein AJ, Malkowicz SB. Modification of clinical presentation of prostate tumors by a novel genetic variant in *CYP3A4*. *J Natl Cancer Inst* 1998;90:1225–9.
- Amirimani B, Walker AH, Weber BL, Rebeck TR. Re: modification of clinical presentation of prostate tumors by a novel genetic variant in *CYP3A4* [Response]. *J Natl Cancer Inst* 1999;91:1588–90.
- Greiner B, Eichelbaum M, Fritz P, Kreichgauer HP, von Richter O, Zundler J, et al. The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J Clin Invest* 1999;104:147–53.
- Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmüller J, John A, 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;97:3473–8.
- Schütz E, Svinarov D, Shipkova M, Niedmann PD, Armstrong VW, Wieland E, et al. Cyclosporin whole blood immunoassays (AxSYM, CEDIA, and Emit): a critical overview of performance characteristics and comparison with HPLC. *Clin Chem* 1998;44:2158–64.
- Felix CA, Walker AH, Lange BJ, Williams TM, Winick NJ, Cheung NK, et al. Association of *CYP3A4* genotype with treatment-related leukemia. *Proc Natl Acad Sci U S A* 1998;95:13176–81.
- von Ahsen N, Oellerich M, Schütz E. Use of two reporter dyes without interference in a single-tube rapid-cycle PCR: α_1 -antitrypsin genotyping by multiplex real-time fluorescence PCR with the LightCycler. *Clin Chem* 2000;46:156–61.
- Hall SD, Thummel KE, Watkins PB, Lown KS, Benet LZ, Paine MF, et al. Molecular and physical mechanisms of first-pass extraction. *Drug Metab Dispos* 1999;27:161–6.
- Choc MG, Mueller EA, Robinson WT, Kumle A, Smith HT, Charnick SB. Relative bioavailability of Neoral versus Sandimmune in the presence of a P450IIIa and P-glycoprotein inhibitor. *Transplant Proc* 1998;30:1664–5.
- Ando Y, Tateishi T, Sekido Y, Yamamoto T, Satoh T, Hasegawa Y, et al. Re: modification of clinical presentation of prostate tumors by a novel genetic variant in *CYP3A4* [Letter]. *J Natl Cancer Inst* 1999;91:1587–90.
- Westlind A, Lofberg L, Tindberg N, Andersson TB, Ingelman-Sundberg M. Interindividual differences in hepatic expression of *CYP3A4*: relationship to genetic polymorphism in the 5'-upstream regulatory region. *Biochem Biophys Res Commun* 1999;259:201–5.
- Ball SE, Scatina J, Kao J, Ferron GM, Fruncillo R, Mayer P, et al. Population distribution and effects on drug metabolism of a genetic variant in the 5' promoter region of *CYP3A4*. *Clin Pharmacol Ther* 1999;66:288–94.
- Rivory LP, Qin H, Clarke SJ, Eris J, Duggin G, Ray E, et al. Frequency of cytochrome P450 3A4 variant genotype in transplant population and lack of association with cyclosporin clearance. *Eur J Clin Pharmacol* 2000;56:395–8.