BJCP British Journal of Clinical Pharmacology

DOI:10.1111/bcp.12345

Relationship of *CYP3A5* genotype and *ABCB1* diplotype to tacrolimus disposition in Brazilian kidney transplant patients

Diego Alberto C. Cusinato,[1] Riccardo Lacchini,[1] Elen A. Romao,[2] Miguel Moysés-Neto[2] & Eduardo B. Coelho[1,2]

[1]Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Av. Bandeirantes, 3900, 14049-900, Ribeirão Preto, São Paulo and [2]Department of Internal Medicine. Faculty of Medicine of Ribeirão Preto, University of São Paulo, Av. Bandeirantes, 3900, 14049-900, Ribeirão Preto, São Paulo, Brazil

Correspondence
Professor Eduardo Barbosa Coelho MD PhD, Department of Internal Medicine, Faculty of Medicine of Ribeirao Preto, University of São Paulo, Av. Bandeirantes, 3900, 14049-900 Ribeirão Preto, SP, Brazil.
Tel.: +5516 3602 2543
Fax: +5516 3633 6695
E-mail: ebcoelho@fmrp.usp.br

Keywords
ABCB1 diplotype, cytochrome P450, kidney transplantation, pharmacogenetics, tacrolimus pharmacokinetics

Received
12 August 2013

Accepted
24 January 2014

Accepted Article Published Online
17 February 2014

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Tacrolimus disposition results largely from the actions of CYP3A5 enzymes and P-glycoprotein (PGP1). Several clinical studies have reported that homozygous carriers of the *CYP3A5**3 allele require lower doses of tacrolimus to achieve similar target blood concentrations. In contrast, *ABCB1* polymorphism studies have shown controversial results, and few studies have addressed the interaction between *ABCB1* diplotype and tacrolimus (TAC) pharmacokinetics.

WHAT THIS STUDY ADDS

- This study shows that individuals carrying the homozygote variant diplotype (TTT/TTT) present a higher TAC dose-normalized concentration. We also show that the effects of *CYP3A5* polymorphism and *ABCB1* diplotype on TAC dose-normalized concentration are independent and additive.

AIMS

Tacrolimus (TAC) is one of the most successful immunosuppressive drugs in transplantation. Its pharmacokinetics (PK) and pharmacogenetics (PG) have been extensively studied, with many studies showing the influence of CYP3A5 on TAC metabolism and bioavailability. However, data concerning the functional significance of *ABCB1* polymorphisms are uncertain due to inconsistent results. We evaluated the association between *ABCB1* diplotypes, *CYP3A5* polymorphisms and TAC disposition in a cohort of Brazilian transplant recipients.

METHODS

Individuals were genotyped for the *CYP3A5**3 allele and *ABCB1* polymorphisms (2677G>A/T, 1236C>T, 3435C/T) using a TaqMan® PCR technique. Diplotypes were analyzed for correlation with the TAC dose-normalized ratio (Co : dose).

RESULTS

We genotyped 108 Brazilian kidney recipients for CYP3A5 (11% CYP3A5*1/*1; 31% CYP3A5*1/*3 and 58% CYP3A5*3/*3) and ABCB1 haplotypes (42% CGC/CGC; 41% GCG/TTT and 17% TTT/TTT). Homozygous subjects for the *CYP3A5**3 allele or carriers of the *ABCB1* TTT/TTT diplotype showed a higher Co : dose ratio compared with wild type subjects [median (interquartile range) 130.2 (97.5–175.4) *vs.* 71.3 (45.6–109.0), $P < 0.0001$ and 151.8 (112.1–205.6) *vs.* 109.6 (58.1–132.9), $P = 0.01$, respectively]. When stratified for the *CYP3A5**3 group, *ABCB1* TTT/TTT individuals showed a higher Co : dose ratio compared with non-TTT/TTT individuals [167.8 (130.4–218.0) *vs.* 119.4 (100.2–166.3), $P = 0.04$]. Multivariate linear regression analysis showed that the effects of *CYP3A5* polymorphisms and *ABCB1* diplotypes remained significant after correction for confounding factors.

CONCLUSIONS

CYP3A5 is the major enzyme responsible for the marked interindividual variability in TAC PK, but it cannot be considered alone when predicting dose adjustment because *ABCB1* diplotypes also affect TAC disposition, showing independent and additive effects on the TAC dose-normalized concentration.

Introduction

The administration of the calcineurin inhibitor tacrolimus (TAC) is an important cornerstone of immunosuppressive therapy after kidney transplantation. Currently, TAC is one of the most successful immunosuppressive drugs in transplantation, but its clinical use is hampered by a narrow therapeutic window and large intra- and interindividual variability in pharmacokinetics (PK), which can result in either under-immunosuppression, leading to increased risk of allograft rejection, or over-immunosuppression and increased incidence of adverse effects, such as nephrotoxicity and neurotoxicity [1]. Consequently, therapeutic drug monitoring is currently regarded as a vital part of clinical practice in kidney transplantation.

TAC is metabolized by the cytochrome P450 (CYP) enzymes 3A4 and 3A5. The intrinsic clearance of TAC by CYP3A5 is approximately two-fold higher than that by CYP3A4, limiting the influence of this enzyme in TAC PK [2]. Tacrolimus is also a substrate of P-glycoprotein (PGP1), the product of the ATP-binding cassette transporter (*ABCB1*) gene, which acts as an efflux transporter that limits a drug's oral absorption [3–5]. The different degrees of intestinal and hepatic expression of CYP3A5, CYP3A4 and PGP1 regulate the absorption and hepatic clearance of TAC, leading to variable drug concentration within the systemic circulation and low oral bioavailability, both of which can influence drug efficacy or toxicity [5–7].

One of the causes that may lead to the differences in the functions of these proteins is the presence of genetic polymorphisms (SNPs) that may have functional effects on gene expression or protein structure. Several studies have shown an association between SNPs in genes encoding enzymes or transporters and differences in dosing, toxicity and drug interactions [8, 9].

*CYP3A5*3* is one of the most important SNPs for TAC disposition. Homozygous carriers of the *CYP3A5*3* variant allele have higher TAC concentrations due to lower expression of CYP3A5 [8, 10]. Individuals carrying the *CYP3A5*1* allele, known as 'CYP expressers', exhibit 25–40% increased TAC clearance and two–three-fold lower dose-corrected trough TAC concentrations, suggesting the value of *CYP3A5* genetic information in initial drug dosing [11].

The functional consequences of the most common and extensively studied *ABCB1* polymorphisms (3435C>T, 1236C>T and 2677G>T/A) on TAC disposition are not completely understood and are still controversial [8, 9]. The majority of the studies have failed to find an association between *ABCB1* SNPs and TAC PK. Those that found an association suggested that the observed effect could, in fact, be related to *CYP3A5* polymorphism [9, 12–14]. The *ABCB1* 3435T variant allele occurs more frequently in conjunction with the *CYP3A5*3* allele and also with the *ABCB1* 1236C>T and 2677G>T/A SNPs [15–17].

Individual SNPs may cause changes in gene expression or in protein function, either of which can have a physiological effect on the organism. However, SNPs are not inherited individually but in linkage disequilibrium, in which certain alleles of several contiguous polymorphisms are found together. The combined influence of SNPs may have stronger physiological effects than any single SNP alone [8, 9]. Indeed, the *ABCB1* 1236T/2677T/3435T (TTT) variant haplotype significantly minimizes PGP1 activity (0–28% activity) compared with *ABCB1* wild-type activity in transepithelial cells and is relatively frequent in several populations: approximately 32% of Caucasians, 5% of African-Americans, 27% of Asian-Americans and 35% of Mexican-Americans [17, 18].

Similar to studies on *ABCB1* genotype, most clinical studies have not detected any association between *ABCB1* haplotypes and TAC PK [13, 19, 20], but others have indicated that the presence of three or more variant *ABCB1* alleles is associated with lower dose-adjusted blood concentrations of TAC [14, 16, 21, 22]. However, to the best of our knowledge, few studies have analyzed the effects of *ABCB1* diplotypes (i.e. pairs of haplotypes) on TAC disposition and their interaction with the *CYP3A5*3* genotype [21, 23].

In this context, the aim of this study was to investigate the impact of *ABCB1* diplotypes and *CYP3A5* polymorphism on TAC dose-normalized concentration (*C*o : dose).

Methods

Subjects

This retrospective study was conducted on kidney transplant recipients at the Clinical Hospital of the Faculty of Medicine of Ribeirão Preto, Brazil. For the purpose of this study, 108 kidney recipients of any age and gender were included. Eligible patients were those with single kidney transplantation and administration of a TAC-based immunosuppressive regimen for at least 12 months [The study involves a period from 3 up to 12 months after transplant (follow-up period 9 months, 95% CI 8.9, 9.0)]. Patients receiving chronic therapy with drugs that could potentially interfere with the transport and metabolism of immunosuppressive drugs (e.g. macrolides, rifampicin, phenytoin, carbamazepine), those who underwent combined organ transplantations, those who had any known drug or alcohol addiction and those who started TAC immunosuppressive therapy more than 2 months after transplantation were excluded. The study was approved by the Institutional Review Board at the Clinical Hospital of the Faculty of Medicine of Ribeirão Preto (no. 13464/2010), and each included patient provided informed consent.

The initial TAC (Prograf®, Janssen-Cilag, Brasil) dosage was 0.05–0.2 mg kg^{-1} day^{-1} for all patients, and it was subsequently adjusted according to blood concentration or any toxicity sign. Target concentrations were 8–10 ng ml^{-1}

during the first 3 months post-transplantation and were then adjusted to 3–7 ng ml^{-1} until the end of the first year. Tacrolimus was given twice a day in combination with sodium mycophenolate (720–1440 mg day^{-1}) or mofetil (1–2 g day^{-1}) and a tapering schedule of steroids (1000 mg i.v. methylprednisolone before the surgery and 0.5 mg kg^{-1} orally, which was progressively tapered to 5 mg once a day at 6 months after transplantation).

Data collection

TAC trough concentrations (*Co*, ng ml^{-1}) were routinely obtained at steady-state from TAC whole blood concentrations measured immediately before the morning doses of TAC using an Abbott ARCHITECT TAC immunoassay (Abbott Diagnostics, IL, USA). Daily doses (mg kg^{-1}) were retrieved from medical records from the third month up to 1 year post-transplantation. Dose-normalized TAC concentrations (*Co* : dose, ng ml^{-1} per mg day^{-1} per kg body weight) were calculated by dividing the drug trough concentration (*Co*, ng ml^{-1}) by the daily dose adjusted for body weight (mg day^{-1} kg^{-1}). Once the patients had more than one measurement in the study period, the median value was considered to represent *Co* : dose (median 5.0, range 4.0–8.0, number of blood samples collected/patient).

Genotype determination

Blood samples were drawn from each subject and immediately stored at −80°C until genotype analysis. Genomic DNA was isolated from peripheral blood leukocytes using a salting-out procedure [24]. Subjects were genotyped for the *CYP3A5* SNP (rs776746) and the three major *ABCB1* SNPs, 1236C>T (rs1128503), 2677 G > T/A (rs2032582) and 3435C>T (rs1045642). Genotypes were determined using a pre-designed TaqMan® Allele Discrimination assay (Applied Biosystems, Foster City, CA, USA): C_26201809_30 (*CYP3A5* 6986A>G), C_7586657_20 (*ABCB1*, 3435C>T), C_7586662_10 (*ABCB1*, 1236C>T), C_11711720D_40 (*ABCB1*, 2677G>A), and C_11711720C_30 (2677G>T).

TaqMan® polymerase chain reaction (PCR) amplification was performed in a total volume of 10 µl as follows: 30 ng of template DNA, 1× TaqMan® master mix and 1× drug metabolism assay. Fluorescence from PCR amplification was detected using a Roche LightCycler®480 and analyzed with the associated software version 1.5 (Roche Diagnostics GmbH, Mannheim, Germany). The PCR assay was carried out following the manufacturer's instructions.

Statistical analysis

The Power and Sample Calculation program (PS version 2.1.30, Vanderbilt University, USA) was used to estimate the number of individuals for this study. Santoro *et al.* reported a *Co* : dose value for the *ABCB1* wild-type diplotype (CGC/CGC) of 1.92 ± 0.94 ng ml^{-1} mg^{-1}. Considering an increase of 50% in *Co* : dose, a TTT variant haplotype frequency of 26%, a power of 0.80 and a type 1 error rate of 0.05, we calculated a sample size of 90 transplant recipients [23].

Clinical characteristics and pharmacokinetic data are expressed as median and interquartile range. Genotype groups were compared using non-parametric tests. The distribution of genotypes for each polymorphism was assessed for deviation from Hardy–Weinberg equilibrium, and differences in genotype frequency and in allele frequency between the groups were assessed using the χ^2 test.

Linear regression analysis and non-linear fitting routines were performed to assess univariate relationships between variables (software JMP 5.0.1a; SAS Institute). In addition, a bivariate analysis was used to assess the potential confounding influence of each covariate on the association between TAC concentration, *CYP3A5* polymorphism, and *ABCB1* polymorphism. The final multivariate linear regression model considered TAC blood concentration as the dependent variable, whereas gender, age, corticoid dosage, haematocrit and *CYP3A5* polymorphisms and *ABCB1* diplotypes were used as independent variables. A value of $P < 0.05$ was considered statistically significant. Other statistical analyses were performed using GraphPad Prism version 5.1 for Windows (GraphPad Software, San Diego, CA, USA, [http://www.graphpad.com]). PHASE software version 2.1 was used to estimate the haplotype frequencies in each group.

Results

A total of 108 renal transplant recipients (48 women) with a median age of 52 (41–58) years at the time of transplantation were recruited between March and November 2011 and were included in this study. Demographic and clinical characteristics are listed in Table 1.

Allele, genotype and haplotype frequencies in transplanted subjects are shown in Tables 2 and 3. Allele frequencies did not deviate from Hardy–Weinberg equilibrium. The wild-type haplotype 1236C/2677G/3435C was the most common (49%), followed by the variant (TTT) haplotype (30%) (Table 3). Diplotype analysis showed 30 individuals carrying the wild-type CGC/CGC, 30 heterozygous CGC/TTT or TTT/CGC and 12 TTT/TTT. We observed that individuals carrying at least one wild-type haplotype, CGC/CGC or CGC/TTT, were responsible for the wild-type effect and thus were combined in a single group called non-TTT/TTT (n = 60), whereas individuals homozygous for the variant haplotype formed another group called TTT/TTT (n = 12).

The presence of the *CYP3A5*1* allele was strongly associated with lower dose-normalised concentration (*Co* : dose) up to 12 months post-transplant. Furthermore, we observed that *CYP3A5*1* carriers required a higher TAC

Table 1

Clinical and demographic characteristics of renal transplant recipients up to 12 months post-tranplantation (n = 108)

Parameters	Median (interquatile range) or number (%)
Co (ng ml^{-1})	6.8 (5.3–10.0)
Dose (mg kg^{-1} day^{-1})	0.07 (0.05–0.10)
Body weight (kg)	64.8 (58.5–77.6)
Co : dose (ng ml^{-1} per mg day^{-1} per kg body weight)	109.3 (70.3–145.9)
Serum creatinine (μmol l^{-1})	124.2 (95.5–143.2)
Creatinine clearance* (ml min^{-1} 1.73 m^{-2})	55.7 (46.8–70.5)
Gender (male/female)	60/48
Receptor's age (years)	52 (41–58)
Donor type (living/deceased)	18/90
Number of transplant (first/second[1])	95/13
Induction therapy (*n*, %)	
Basiliximab usage	65 (60.2)
ATG usage	06 (5.6)
Duration of the dialysis before Tx (months)	39 (24–70)
CMV infection (*n*, %)	24 (22.2)
Cold ischaemia (h)	25 (20–31)

*Creatinine clearance was estimated using MDRD equation. Data are expressed as median and interquartile ranges, or absolute numbers and percentiles. ATG, antithymocyte globulin; CMV, cytomegalovirus; *Co*, Tacrolimus trough concentration; Tx, transplant.

Table 2

Allelic frequency of 108 renal Tx recipients according to CYP3A5 and ABCB1 genotype

Gene	SNP	Genotype frequency	Allelic frequency	HWE*
CYP3A5	6986A>G	AA = 0.11 AG = 0.31 GG = 0.58	A = 0.26 G = 0.74	Yes
ABCB1	1236C>T	CC = 0.38 CT = 0.47 TT = 0.15	C = 0.60 T = 0.40	Yes
	3435C>T	CC = 0.38 CT = 0.45 TT = 0.17	C = 0.60 T = 0.40	Yes
	2677G>A/T	GG = 0.44 GT = 0.38 GA = 0.03 AT = 0.02 TT = 0.13 AA = 0.0	G = 0.65 T = 0.33 A = 0.02	Yes

*Hardy–Weinberg equilibrium (HWE): Chi-squaredb test P value <0.05.

dose [0.10 mg kg^{-1} day^{-1} (0.07–0.12) *vs.* 0.06 mg kg^{-1} day^{-1} (0.04–0.08), $P < 0.0001$] but showed reduced blood TAC (*Co*) (Table 4, Figure 1A).

Diplotype analysis showed that patients carrying the homozygous diplotype variant (TTT/TTT) had a higher TAC dose-normalized concentration [151.8 ng ml^{-1} per mg day^{-1} per kg body weight (112.1–205.6) *vs.* 109.6 ng ml^{-1} per mg day^{-1} per kg body weight (58.1–132.9), $P = 0.01$) compared with individuals carrying the wild-type diplotype (non-TTT/TTT) (Table 4). On the other hand, no association was found between *ABCB1* diplotype and either TAC dose requirement or TAC blood concentration.

Table 3

Haplotype frequency of 108 renal transplant recipients according to ABCB1 SNPs

Haplotype	SNP 1236C>T	SNP 2677G/T>A	SNP 3435C>T	Haplotype frequency
CGC	C	G	C	0.49
TTT	T	T	T	0.30
CGT	C	G	T	0.08
TGC	T	G	C	0.06
TTC	T	T	C	0.02
CAC	C	A	C	0.02
TGT	T	G	T	0.01
CAT	C	A	T	0.01
CTT	C	T	T	0.01

When we restricted our analysis to CYP3A5 non-expressers (*CYP3A5*3/*3*) we found a higher *Co* : dose ratio in those patients carrying the *ABCB1* variant diplotype (*3-TTT/TTT, n = 10) compared with *3-non-TTT/TTT (n = 34) [167.8 ng ml^{-1} per mg day^{-1} per kg body weight (130.4–218.0) *vs.* 119.4 ng ml^{-1} per mg day^{-1} per kg body weight (100.2–166.3), $P = 0.04$] (Table 4, Figure 1C).

Multivariate analysis showed that the effects of *CYP3A5* polymorphisms and *ABCB1* diplotypes remained significant even after adjusting the model for gender, age, haematocrit and corticoid dose (Table 5) and the effect was independent for each gene.

Discussion

Intestinal CYP3A and PGP1 are co-expressed and may act synergistically as a barrier to oral TAC absorption. Therefore, polymorphisms in these genes may play an important role in TAC bioavailability. SNPs in the *CYP3A5* gene explain part of the between patient variability in the PK of TAC. However, the effects of *ABCB1* gene polymorphisms are still unclear, as several contradictory results have been published [8]. Nonetheless, few studies have addressed the effect of the *ABCB1* diplotype on TAC disposition and its interaction with the *CYP3A5* genotype, and they have reported inconsistent results. Studies that performed haplotype analysis did not include all three *ABCB1* SNPs in the same analysis, and others analyzed haplotypes rather than diplotypes [25–29].

The frequencies of SNPs and haplotypes in the *CYP3A5* and *ABCB1* genes, respectively, vary largely, so it is not uncommon to find some peculiarities in different populations. The Brazilian population is one of the most hetero-

Table 4

PK parameters of renal Tx recipients according to their CYP3A5 and ABCB1 genotypes up to 12 months post-transplant

PK parameters	CYP3A5 *1 (*n* = 45)	CYP3A5*3 (*n* = 63)	*P* value
Co (ng ml^{-1})	6.0 (4.7–8.2)	7.3 (6.2–10.8)	0.01*
Dose (mg kg^{-1} day^{-1})	0.10 (0.07–0.12)	0.06 (0.04–0.08)	<0.0001*
Co : dose†	71.3 (45.6–109.0)	130.2 (97.5–175.4)	<0.0001*
	Non-TTT/TTT (*n* = 60)	**TTT/TTT (*n* = 12)**	***P* value**
Co (ng ml^{-1})	6.7 (5.2–8.2)	8.21 (6.3–11.2)	0.08
Dose (mg kg^{-1} day^{-1})	0.07 (0.05–0.1)	0.05 (0.04–0.08)	0.16
Co : dose†	109.6 (58.1–132.9)	151.8 (112.1–205.6)	0.01*
	(*3-non-TTT/TTT) (*n* = 34)	**(*3 TTT/TTT) (*n* = 10)**	***P* value**
Co (ng ml^{-1})	7.20 (6.1–10.8)	9.7 (6.3–11.3)	0.37
Dose (mg kg^{-1} day^{-1})	0.06 (0.04–0.07)	0.05 (0.04–0.06)	0.29
Co : dose†	119.4 (100.2–166.3)	167.8 (130.4–218.0)	0.04*

*Statistically significant. †ng ml^{-1} per mg day^{-1} per kg body weight. Co, tacrolimus trough concentration; PK, pharmacokinetics; Tx, transplant.

geneous in the world, being a mixture of different ethnic groups, especially Europeans, Africans and Amerindians, which contributes to the formation of a multi-ethnic and highly admixed population [30, 31]. As in other studies, we found the *CYP3A5* to be an unusual gene in that the frequency of the variant allele (G) was higher (74%) than the wild-type (A) frequency. For the *ABCB1* gene, the alleles were evenly distributed between the groups, with the wild-type allele having a slight higher frequency in European Caucasians and Black Africans. These results are consistent with frequencies recently reported in Brazilian and other populations and support the fact that the Brazilian population is special in terms of the distribution of genetic polymorphisms [23, 32–35].

The frequency of CYP3A5 non-expressers and ABCB1 TTT/TTT is higher in White Brazilians than the frequency observed in European Caucasians, reflecting the admixture of Brazilian population. MacPhee *et al*. reported a lower *Co* : dose of TAC in Black kidney-transplanted patients, reflecting higher frequencies of CYP3A non-expressers in this ethnicity. Moreover, White patients carrying CYP3A5 non-expressers alleles showed TAC Co : dose similar to Black patients. Thus, they concluded that the main factor affecting TAC PK is related to White genetic background instead of ethnicity. These data support the clinical relevance of genotyping to CYP3A5 and PGP1 polymorphisms in populations with high frequency of non-expressers, like Brazilians, to avoid under-immunosuppression and risk of acute rejection of kidney transplant [36].

The presence of only one wild-type allele (*1) is enough to express CYP3A5 proteins, so individuals with at least one *CYP3A5*1* allele have higher expression of these enzymes and therefore higher TAC metabolism and lower blood TAC concentration [23, 27, 37, 38]. We found that participants with the *CYP3A5*1* allele had significantly lower tacrolimus dose-normalized (*Co* : dose) post-transplantation compared with *CYP3A5*3* homozygotes. Moreover, *CYP3A5*1* carriers required a higher dose to reach the target concentration. Overall, the mean *Co* : dose ratio in CYP3A5 non-expressers (*CYP3A5*3*) was nearly two-fold higher even though their dose requirements were approximately half of those expressing this protein. These findings are consistent with previous studies and indicate lower metabolic capacity in patients with the variant allele [29, 39, 40]. Thervet *et al*., in a prospective clinical trial involving 280 renal transplant recipients, demonstrated that pretransplant TAC dose adaptation according to *CYP3A5* genotype resulted in more rapid achievement of target *Co* and fewer dose modifications [11]. These findings suggest that the *CYP3A5* genotype is an important factor in determining the dose requirement for TAC and that genotyping patients for this polymorphism may be useful in early prediction of the optimal dose.

The majority of studies reviewed by Staatz *et al*. found no evidence that *ABCB1* polymorphisms play a significant role in TAC PK but suggested that the combined influence of all three *ABCB1* polymorphisms could be more effective in predicting any influence than studying single SNPs alone [8]. Consistent with these findings, we found no association between TAC PK and *ABCB1* polymorphisms individually (data not shown).

Patients carrying the wild-type *ABCB1* haplotype (CGC) might have more active PGP1 pumps and would, therefore, extrude intracellular TAC more efficiently than individuals with the variant haplotype (TTT) [8]. Anglicheau *et al*. reported that TAC blood concentrations in renal transplant recipients carrying the variant TTT haplotype were significantly higher than those carrying the wild-type CGC [16]. In another study involving renal recipients, Roy *et al*. showed that patients wild-type for the *ABCB1*

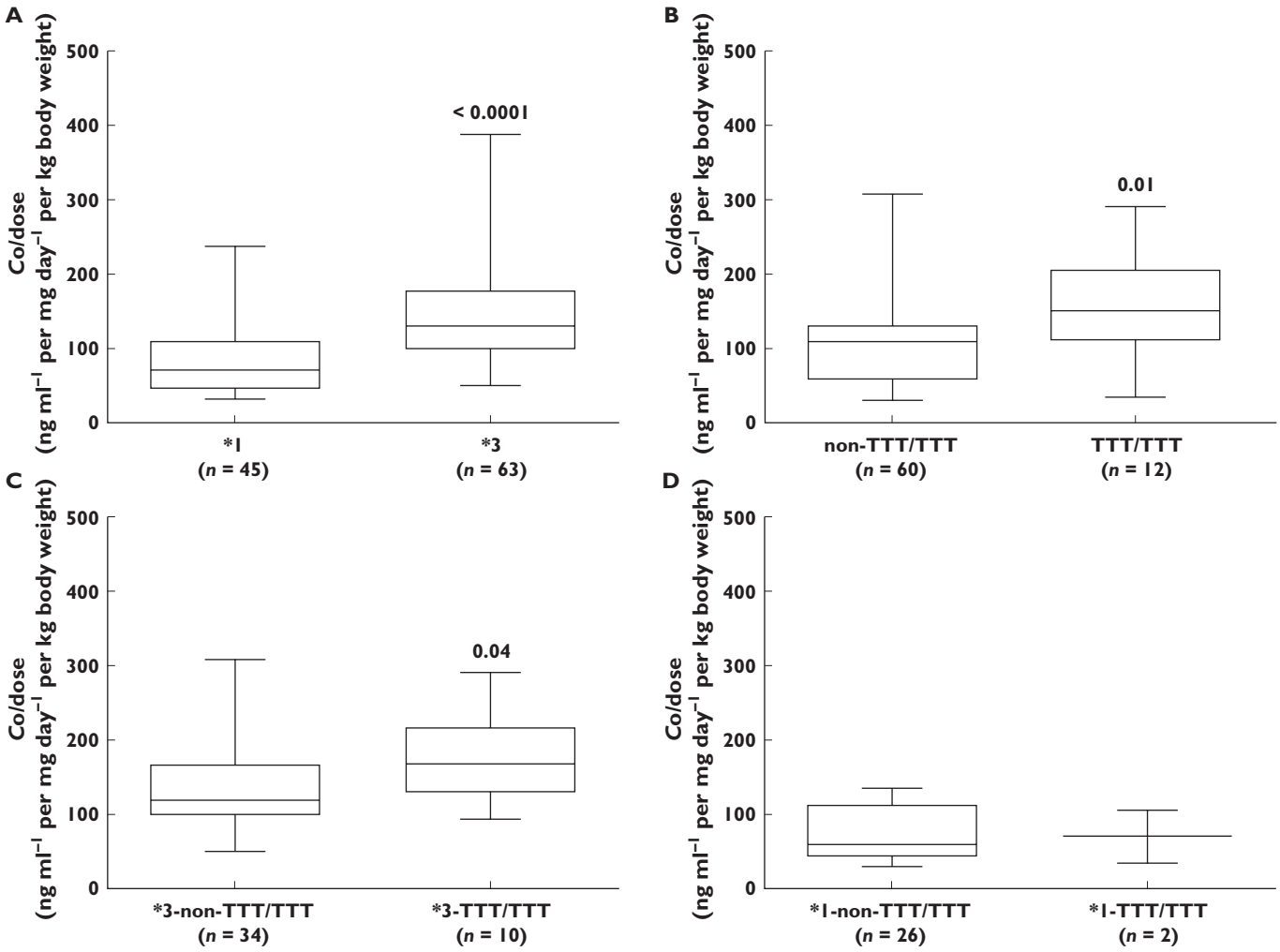

Figure 1

Tacrolimus dose-normalized concentration (*Co* : dose) up to 12 months post-transplantation of 108 renal transplant recipients according to their genotype. (A) Patients carrying CYP3A5*1 ($n = 45$) showed lower *Co* : dose when compared with patients homozygous CYP3A5*3 ($n = 63$), $P < 0.0001$. (B) Patients with non-TTT/TTT diplotype ($n = 60$) also showed lower *Co* :dose when compared with patients TTT/TTT ($n = 12$), $P = 0.01$. (C) Analysis considering CYP3A5 group stratified according to the ABCB1 genotype, patients carrying the *3-non-TTT/TTT diplotype ($n = 34$) showed lower *Co* : dose when compared with patients carrying *3-TTT/TTT diplotype ($n = 10$), $P = 0.04$. (D) Analysis considering CYP3A5 group stratified according to the ABCB1 genotype, patients carrying the *1-non-TTT/TTT diplotype ($n = 26$) showed higher *Co* : dose when compared with patients carrying *1-TTT/TTT diplotype ($n = 2$). The data, however, could neither be analyzed nor taken into consideration, due to the insufficient number of individuals. All data are expressed as median and percentiles

haplotype had lower *Co* : dose compared with patients carrying the variant copy of this gene. However, none of these assays considered the individual's pair of haplotypes, which could result in even stronger differences [21]. Based on the three most clinically relevant exonic sites for the *ABCB1* gene, we detected nine haplotypes. Two of them, the wild-type 1236C/2677G/3435C and the variant 1236T/2677T/3435T, accounted for 79% of the overall genetic variability, which is in agreement with reports of other groups in the Brazilian population [23, 32]. In our comparison of TAC PK parameters, we found that patients carrying the variant *ABCB1* diplotype (TTT/TTT) showed a higher *Co* : dose ratio than patients carrying the wild-type diplotype, which could support the hypothesis of increased absorption and reduced drug excretion due to lower functional activity of PGP1.

In the only other study that has analyzed *ABCB1* diplotypes in the Brazilian population, Santoro *et al*., similar to our study, found a 71% increase in *Co* : dose in the TTT/TTT diplotype *vs.* non-TTT/TTT, but this difference was not statistically significant [23]. This could partially be because in their study, *Co* : dose was not normalized to dose per day or body weight, which could have resulted in higher sample variability. It is important to note that in our study, TAC blood target concentration were between 3 and 7 ng ml^{-1}, and the observed trough concentration (*Co*) median was 6.8 (5.3–10.0) ng ml^{-1}. A 71% increase in *Co* : dose, as we observed, is clinically relevant because it

Table 5

Effects of CYP3A5 genotypes and ABCB1diplotypes on tacrolimus dose-normalized concentration

Source	Estimate	*P* value
ABCB1 diplotypes (non-TTT/TTT)	–16.13	0.047*
CYP3A5 (*1)	–30.73	<0.001*
Gender (male)	–4.97	0.413
Age (years)	0.91	0.111
Corticoid dose (mg kg^{-1} day^{-1})	–149.71	0.348
Haematocrit (%)	–0.21	0.894
Model		
R^2	0.42	
RMSE	48.66	

*Statistically significant. Were included in this model only carriers of *1 and *3 alleles for CYP3A5 and non-TTT/TTT and TTT/TTT diplotypes for ABCB1 (n = 72). R^2, proportion of the variance around the mean of tacrolimus concentrations that is explained by the present model; RMSE, root mean square error. The final multivariate linear regression model was adjusted for gender, age, corticoid dose and haematocrit.

directly affects trough doses and could expose patients to TAC nephrotoxicity.

Authors who observed an association between *ABCB1* genotype and/or haplotype and TAC PK suggested that the observed effect on TAC PK might, in fact, be the effect of *CYP3A* SNPs, as these have a major influence over TAC PK. Indeed, many studies have observed that the impact of *ABCB1* SNPs is lost after eliminating the confounder *CYP3A5* genotype [12–14, 27]. To assess this hypothesis, we stratified CYP3A5 non-expressers (*CYP3A5*3/*3* carriers), who lack CYP3A5 activity, into two groups according to their *ABCB1* diplotype and observed that in the absence of CYP3A5, patients carrying the variant TTT/TTT diplotype (and thus having lower PGP1 activity) had a higher dose-normalized TAC concentration. This result shows that *ABCB1* diplotype also plays a role in TAC PK and supports the hypothesis that the combination of multiple *ABCB1* polymorphisms has a stronger effect on CNI disposition than any single polymorphism.

We also performed a multivariate analysis considering *CYP3A5* polymorphism as a confounding variable and observed the maintenance of the *ABCB1* genotype effect. Roy *et al*. had already considered this effect, studying 44 renal transplant recipients and analyzing the *ABCB1* haplotype frequency in the CYP non-expressers group, but they did not detect a statistically significant effect and therefore did not include a diplotype analysis [21]. These results suggest that PGP1 expression could compensate for the lack of CYP3A5 expression and play a role in individuals expressing CYP3A5 enzymes. Thus, it is likely that these polymorphisms in the *ABCB1* gene have an independent effect on TAC bioavailability.

In addition to genetic factors, clinical variables have been associated with TAC PK [28, 41, 42]. Corticoid drugs and TAC share the same metabolic pathway, so they could exhibit a drug–drug interaction in which corticoid induces CYP expression and consequently increasing TAC metabolism [43, 44]. Several authors suggest that age affects metabolism in different ways, such as altering hepatic flow, CYP activity and liver size. Others showed that TAC binds to red blood cells and suggested that individuals showing high haematocrit could bind TAC more efficiently, reducing its metabolism [45]. To address these associations, we performed a multivariate regression analysis considering age, gender, corticoid dose and haematocrit as co-variables and observed that the effect of genetic variables on TAC PK remained even after considering them. These data are consistent with data from Cho *et al*. but do not support the findings in other populations, although none of these studies performed a diplotype analysis [28, 39, 41, 42].

In conclusion, this study demonstrates that the *CYP3A5*3* allele is highly associated with higher TAC bioavailability in renal transplant patients, confirming previous findings and supporting the idea that CYP3A5 is the major enzyme responsible for the marked interindividual variability in TAC PK. In addition, this study confirms that the combination of multiple *ABCB1* polymorphisms has a stronger effect on CNI disposition. Finally, this study shows that the effects of *CYP3A5* and *ABCB1* on TAC bioavailability are independent and additive. The inclusion of CYP3A5 and ABCB1 genetic factors in an algorithm which takes into account clinical factors that may modify TAC PK could make it possible to calculate more precisely the initial TAC dosage in order to prevent drug toxicity and improve therapy towards individualized therapy.

Competing Interests

All authors have completed the Unified Competing Interest form and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

We thank Mariana A. T de Oliveira and Renata H. C. Pocente for assisting with the PCR analysis, Juliana T. Abumansur for assisting with DNA sample preparation and Neuza L. de Almeida for help in including the patients.

We also would like to thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support.

REFERENCES

1 Laskow DA, Vincenti F, Neylan JF, Mendez R, Matas AJ. An open-label, concentration-ranging trial of FK506 in primary

kidney transplantation: a report of the United States Multicenter FK506 Kidney Transplant Group. Transplantation 1996; 62: 900–5.

2 Dai Y, Hebert MF, Isoherranen N, Davis CL, Marsh C, Shen DD, Thummel KE. Effect of CYP3A5 polymorphism on tacrolimus metabolic clearance *in vitro*. Drug Metab Dispos 2006; 34: 836–47.

3 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.

4 Sattler M, Guengerich FP, Yun CH, Christians U, Sewing KF. Cytochrome-P-450 3a enzymes are responsible for biotransformation of fk506 and rapamycin in man and rat. Drug Metab Dispos 1992; 20: 753–61.

5 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.

6 Zhang YC, Benet LZ. The gut as a barrier to drug absorption – combined role of cytochrome P450 3A and P-glycoprotein. Clin Pharmacokinet 2001; 40: 159–68.

7 Thervet E, Anglicheau D, Legendre C, Beaune P. Role of pharmacogenetics of immunosuppressive drugs in organ transplantation. Ther Drug Monit 2008; 30: 143–50.

8 Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part I. Clin Pharmacokinet 2010; 49: 141–75.

9 Knops N, Levtchenko E, van den Heuvel B, Kuypers D. From gut to kidney: transporting and metabolizing calcineurin-inhibitors in solid organ transplantation. Int J Pharm 2013; 452: 14–35.

10 de Jonge H, Kuypers DR. Pharmacogenetics in solid organ transplantation: current status and future directions. Transplant Rev (Orlando) 2008; 22: 6–20.

11 Thervet E, Loriot MA, Barbier S, Buchler M, Ficheux M, Choukroun G, Toupance O, Touchard G, Alberti C, Le Pogamp P, Moulin B, Le Meur Y, Heng AE, Subra JF, Beaune P, Legendre C. Optimization of initial tacrolimus dose using pharmacogenetic testing. Clin Pharmacol Ther 2010; 87: 721–6.

12 Cheung CY, Op den Buijsch RA, Wong KM, Chan HW, Chau KF, Li CS, Leung KT, Kwan TH, de Vrie JE, Wijnen PA, van Dieijen-Visser MP, Bekers O. Influence of different allelic variants of the CYP3A and ABCB1 genes on the tacrolimus pharmacokinetic profile of Chinese renal transplant recipients. Pharmacogenomics 2006; 7: 563–74.

13 Fredericks S, Moreton M, Reboux S, Carter ND, Goldberg L, Holt DW, MacPhee IA. Multidrug resistance gene-1 (MDR-1) haplotypes have a minor influence on tacrolimus dose requirements. Transplantation 2006; 82: 705–8.

14 Loh PT, Lou HX, Zhao Y, Chin YM, Vathsala A. Significant impact of gene polymorphisms on tacrolimus but not cyclosporine dosing in Asian renal transplant recipients. Transplant Proc 2008; 40: 1690–5.

15 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.

16 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: 1889–96.

17 Kroetz DL, Pauli-Magnus C, Hodges LM, Huang CC, Kawamoto M, Johns SJ, Stryke D, Ferrin TE, DeYoung J, Taylor T, Carlson EJ, Herskowitz I, Giacomini KM, Clark AG, Pharmacogenetics Membrane T. Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. Pharmacogenetics 2003; 13: 481–94.

18 Salama NN, Yang Z, Bui T, Ho RJY. MDR1 haplotypes significantly minimize intracellular uptake and transcellular P-gp substrate transport in recombinant LLC-PK1 cells. J Pharm Sci 2006; 95: 2293–308.

19 Haufroid V, Mourad M, Van Kerckhove V, Wawrzyniak J, De Meyer M, Eddour DC, Malaise J, Lison D, Squifflet JP, Wallemacq P. The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. Pharmacogenetics 2004; 14: 147–54.

20 Tsuchiya N, Satoh S, Tada H, Li Z, Ohyama C, Sato K, Suzuki T, Habuchi T, Kato T. Influence of CYP3A5 and MDR1 (ABCB1) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. Transplantation 2004; 78: 1182–7.

21 Roy JN, Barama A, Poirier C, Vinet B, Roger M. Cyp3A4, Cyp3A5, and MDR-1 genetic influences on tacrolimus pharmacokinetics in renal transplant recipients. Pharmacogenet Genomics 2006; 16: 659–65.

22 Chen JS, Li LS, Cheng DR, Ji SM, Sun QQ, Cheng Z, Wen JQ, Sha GZ, Liu ZH. Effect of CYP3A5 genotype on renal allograft recipients treated with tacrolimus. Transplant Proc 2009; 41: 1557–61.

23 Santoro A, Felipe CR, Tedesco-Silva H, Medina-Pestana JO, Struchiner CJ, Ojopi EB, Suarez-Kurtz G. Pharmacogenetics of calcineurin inhibitors in Brazilian renal transplant patients. Pharmacogenomics 2011; 12: 1293–303.

24 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215.

25 Provenzani A, Notarbartolo M, Labbozzetta M, Poma P, Vizzini G, Salis P, Caccamo C, Bertani T, Palazzo U, Polidori P, Gridelli B, D'Alessandro N. Influence of CYP3A5 and ABCB1 gene polymorphisms and other factors on tacrolimus dosing in Caucasian liver and kidney transplant patients. Int J Mol Med 2011; 28: 1093–102.

26 Wu P, Ni X, Wang M, Xu X, Luo G, Jiang Y. Polymorphisms in CYP3A5*3 and MDR1, and haplotype modulate response to

plasma levels of tacrolimus in Chinese renal transplant patients. Ann Transplant 2011; 16: 54–60.

27 Gervasini G, Garcia M, Macias RM, Cubero JJ, Caravaca F, Benitez J. Impact of genetic polymorphisms on tacrolimus pharmacokinetics and the clinical outcome of renal transplantation. Transpl Int 2012; 25: 471–80.

28 Stratta P, Quaglia M, Cena T, Antoniotti R, Fenoglio R, Menegotto A, Ferrante D, Genazzani A, Terrazzino S, Magnani C. The interactions of age, sex, body mass index, genetics, and steroid weight-based doses on tacrolimus dosing requirement after adult kidney transplantation. Eur J Clin Pharmacol 2011; 68: 671–80.

29 Yoon S-H, Cho J-H, Kwon O, Choi J-Y, Park S-H, Kim Y-L, Yoon Y-R, Won D-I, Kim C-D. CYP3A and ABCB1 genetic polymorphisms on the pharmacokinetics and pharmacodynamics of tacrolimus and its metabolites (M-I and M-III). Transplantation 2013; 95: 828–34.

30 Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. Color and genomic ancestry in Brazilians. Proc Natl Acad Sci U S A 2003; 100: 177–82.

31 Suarez-Kurtz G, Pena SD, Struchiner CJ, Hutz MH. Pharmacogenomic diversity among brazilians: influence of ancestry, self-reported color, and geographical origin. Front Pharmacol 2012; 3: 191.

32 Suarez-Kurtz G, Perini JA, Bastos-Rodrigues L, Pena SD, Struchiner C. Impact of population admixture on the distribution of the CYP3A5*3 polymorphism. Pharmacogenomics 2007; 8: 1299–306.

33 Estrela RC, Ribeiro FS, Carvalho RS, Gregorio SP, Dias-Neto E, Struchiner CJ, Suarez-Kurtz G. Distribution of ABCB1 polymorphisms among Brazilians: impact of population admixture. Pharmacogenomics 2008; 9: 267–76.

34 Ferreira PE, Veiga MI, Cavaco I, Martins JP, Andersson B, Mushin S, Ali AS, Bhattarai A, Ribeiro V, Bjorkman A, Gil JP. Polymorphism of antimalaria drug metabolizing, nuclear receptor, and drug transport genes among malaria patients in Zanzibar, East Africa. Ther Drug Monit 2008; 30: 10–5.

35 Turolo S, Tirelli AS, Ferraresso M, Ghio L, Belingheri M, Groppali E, Torresani E, Edefonti A. Frequencies and roles of CYP3A5, CYP3A4 and ABCB1 single nucleotide polymorphisms in Italian teenagers after kidney transplantation. Pharmacol Rep 2010; 62: 1159–69.

36 Macphee IA, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, Goldberg L, Holt DW. Tacrolimus pharmacogenetics: polymorphisms associated with expression of cytochrome p4503A5 and P-glycoprotein correlate with dose requirement. Transplantation 2002; 74: 1486–9.

37 MacPhee IA, Fredericks S, Mohamed M, Moreton M, Carter ND, Johnston A, Goldberg L, Holt DW. Tacrolimus pharmacogenetics: the CYP3A5*1 allele predicts low dose-normalized tacrolimus blood concentrations in Whites and South Asians. Transplantation 2005; 79: 499–502.

38 Li L, Li CJ, Zheng L, Zhang YJ, Jiang HX, Si-Tu B, Li ZH. Tacrolimus dosing in Chinese renal transplant recipients: a population-based pharmacogenetics study. Eur J Clin Pharmacol 2011; 67: 787–95.

39 Cho JH, Yoon YD, Park JY, Song EJ, Choi JY, Yoon SH, Park SH, Kim YL, Kim CD. Impact of cytochrome P450 3A and ATP-binding cassette subfamily B member 1 polymorphisms on tacrolimus dose-adjusted trough concentrations among korean renal transplant recipients. Transplant Proc 2012; 44: 109–14.

40 Shi Y, Li Y, Tang J, Zhang J, Zou Y, Cai B, Wang L. Influence of CYP3A4, CYP3A5 and MDR-1 polymorphisms on tacrolimus pharmacokinetics and early renal dysfunction in liver transplant recipients. Gene 2013; 512: 226–31.

41 Kim JS, Aviles DH, Silverstein DM, LeBlanc PL, Vehaskari VM. Effect of age, ethnicity, and glucocorticoid use on tacrolimus pharmacokinetics in pediatric renal transplant patients. Pediatr Transplant 2005; 9: 162–69.

42 Ferraris JR, Argibay PF, Costa L, Jimenez G, Coccia PA, Ghezzi LF, Ferraris V, Belloso WH, Redal MA, Larriba JM. Influence of CYP3A5 polymorphism on tacrolimus maintenance doses and serum levels after renal transplantation: age dependency and pharmacological interaction with steroids. Pediatr Transplant 2011; 15: 525–32.

43 Zhao JY, Ikeguchi M, Eckersberg T, Kuo MT. Modulation of multidrug-resistance gene-expression by dexamethasone in cultured hepatoma-cells. Endocrinology 1993; 133: 521–28.

44 McCune JS, Hawke RL, LeCluyse EL, Gillenwater HH, Hamilton G, Ritchie J, Lindley C. *In vivo* and *in vitro* induction of human cytochrome P4503A4 by dexamethasone. Clin Pharmacol Ther 2000; 68: 356–66.

45 Kim IW, Moon YJ, Ji E, Kim KI, Han N, Kim SJ, Shin WG, Ha J, Yoon JH, Lee HS, Oh JM. Clinical and genetic factors affecting tacrolimus trough levels and drug-related outcomes in Korean kidney transplant recipients. Eur J Clin Pharmacol 2012; 68: 657–69.