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CYP3A5 and *ABCB1* Polymorphisms and Tacrolimus Pharmacokinetics in Renal Transplant Candidates: Guidelines from an Experimental Study

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Genetic polymorphisms in biotransformation enzyme *CYP3A5* (6986G > A, *CYP3A5*3*; 14690A > G, *CYP3A5*6*) and drug transporter *ABCB1* (1236C > T; 2677G > T/A; 3435C > T) are known to influence tacrolimus (Tac) dose requirements and trough blood levels in stable transplant patients. In a group of 19 volunteers selected with relevant genotypes among a list of 221 adult renal transplant candidates, we evaluated whether consideration of *CYP3A5* and *ABCB1* genetic polymorphisms could explain the interindividual variability in Tac pharmacokinetics after the first administration of a standard dose (0.1 mg/kg body weight twice a day). Lower area under the time versus blood concentration curves (AUC) or lower trough concentrations were observed among CYP3A5 expressors (n = 9) than among nonexpressors (n = 10) using two different analytical methods for Tac determination (liquid chromatography with tandem mass spectrometry (LC-MS/MS) and immunoassay). The median $AUC_{0-\infty}$ was 2.6- and 2.1-fold higher in nonexpressors for LC-MS/MS and immunologic methods, respectively. No difference was observed in Tac pharmacokinetic parameters in relation to *ABCB1* polymorphisms. In conclusion, our study confirms the very significant effect of *CYP3A5* polymorphism early after the first administration of Tac. It also provides a strong argument for a doubling of the loading dose in patients early identified *a priori* on the transplantation list as possessing at least one *CYP3A5*1* allele.

Key words: ABCB1 (MDR1), CYP3A5, polymorphisms, tacrolimus, transplantation

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Introduction

Tacrolimus (Tac, FK506) is widely used to prevent acute rejection following solid-organ transplantation. Like cyclosporin (CsA), this drug is characterized by a narrow therapeutic index, and close drug monitoring programs are required both to optimize efficacy and to limit toxicity. Achieving therapeutic trough levels is of critical importance, especially during the initial period after transplantation which is characterized by the highest risk of organ rejection. Practically, daily oral doses are adjusted according to the whole-blood trough concentrations measured 12 h postdose, just before the next dose (C_0). The daily practice of drug monitoring reveals a large interindividual variability in Tac pharmacokinetics and particularly in the dose required to achieve target blood concentrations (1). Among several factors investigated, polymorphisms in genes coding for biotransformation enzymes (CYP isoenzymes 3A4 and 3A5) and drug transporters (ABCB1, previously known as MDR1) have received much attention and it becomes increasingly clear that a significant part of the interindividual variability in Tac pharmacokinetics is explained by the presence of a single nucleotide polymorphism (SNP) within intron 3 of *CYP3A5* (6986G > A, *CYP3A5*3* allele) resulting in the absence of the functional CYP3A5 protein in homozygous carriers (*CYP3A5*3/*3*). Studies in kidney (2–5), liver (6), lung (7) and heart (8) transplant recipients have demonstrated that patients who do not express functional CYP3A5 (individuals homozygous for *CYP3A5*3*, representing 80% of the Caucasian population (9)) require significantly less Tac to reach target concentrations compared to patients who do express CYP3A5 (*CYP3A5*1* allele carriers, requiring 30–50% higher Tac doses) (for a comprehensive review, see (10)). In contrast, SNPs in *ABCB1* appear to contribute little, if at all, to the interindividual variability in Tac pharmacokinetics (10). In relation to transplantation outcome, MacPhee and colleagues (11) have assessed the time taken to achieve Tac target concentrations in renal transplant recipients. In their study, despite the use of therapeutic drug monitoring (TDM), patients who expressed CYP3A5 had significantly lower mean Tac C_0 during the first 2 weeks after transplantation and experienced a delay in achieving target concentrations. Acute rejection episodes occurred earlier in CYP3A5 expressors compared with nonexpressors (median of day 8 vs. day 13), although there was no statistically significant difference in

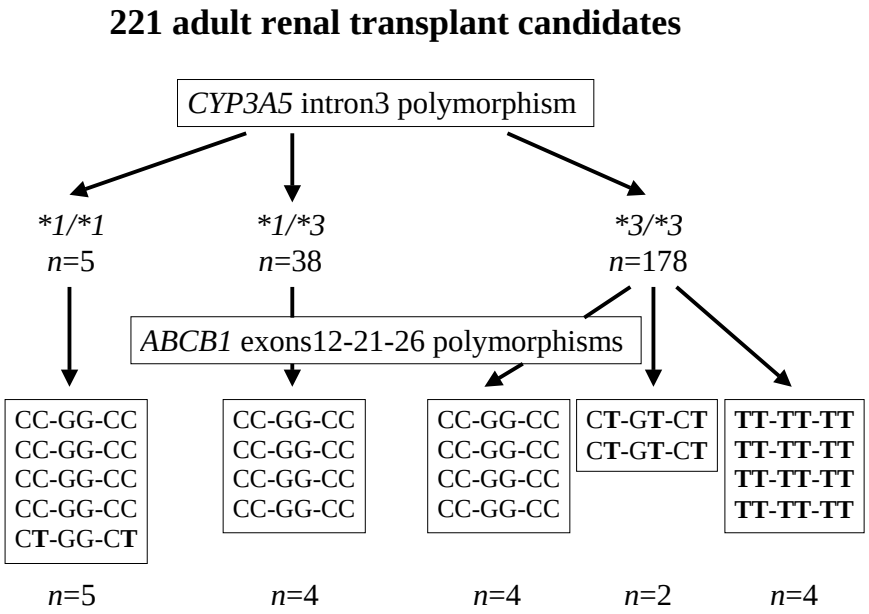

Figure 1: Genotype screening of patients included in a list of adult renal transplant candidates and selection of 19 volunteers.

the overall rate of biopsy-confirmed acute rejection (11). While *ABCB1* SNPs do not appear to contribute much to the interindividual variability in Tac pharmacokinetics, it is not excluded that they could be associated with the occurrence of Tac-related nephrotoxicity, as recently reported for CsA (12).

As suggested by Thummel (13), *CYP3A5* genotyping is considered to prospectively adjust the initial Tac dose for 'rapid metabolizers' in order to reach earlier a steady state of target blood concentration. Although some transplant centers have already adopted the strategy to give a 2-fold higher Tac dose to CYP3A5 expressors (10), well-controlled studies are still needed to support practices. We therefore investigated under strict experimental conditions the impact of *CYP3A5* polymorphisms and *ABCB1* genotype/haplotype on Tac pharmacokinetics in a group of volunteers selected on the basis of their genotype. This study would also contribute to verify whether significant genotype-phenotype associations, previously observed after restoration of homeostasis and achievement of steady-state blood concentrations, also apply to the first Tac dose (13).

Materials and Methods

Selection of volunteers and genotyping analysis

For the initial genotype screening, 221 patients from different ethnic groups, between 18 and 70 years of age, were recruited among a list of adult renal transplant candidates. They were asked to provide a blood sample for genotyping of *CYP3A5* and *ABCB1*. Two *CYP3A5* variant alleles, i.e. *CYP3A5*3* and *CYP3A5*6* (http://www.imm.ki.se/CYPalleles) and three *ABCB1* SNPs, i.e. 1236C > T, 2677G > T/A and 3435C > T were determined by restriction fragment length polymorphism (RFLP) analysis, as described elsewhere (3). Based on previous results from our cross-sectional study, which pointed to CYP3A5*1/*3 polymorphism as the main determinant of variability in Tac dose requirement (3), volunteers were first classified according to their *CYP3A5*3* status. Then *ABCB1* and *CYP3A5*6* status were taken into account for further selection. Based on the results of this initial screening, 19 volunteers were finally selected to obtain a balanced distribution between the variant alleles of *CYP3A5* (homozygous 'wild-type' *CYP3A5*1/*1* (n = 5), heterozygous *CYP3A5*1/*3* (n = 4) and homozygous mutant *CYP3A5*3/*3* (n = 10)). We also aimed to assess the independent effect of *ABCB1* polymorphisms in the subgroup of CYP3A5 nonexpressors volunteers (*CYP3A5*3/*3*). In this subgroup, volunteers were therefore selected to obtain a balanced distribution between *ABCB1* haplotype (*ABCB1* exons 12–21–26; CC-GG-CC (n = 4), TT-TT-TT (n = 4) and CT-GT-CT (n = 2)) (Figure 1). The main characteristics of the final study population are summarized in Table 1. The absence of medication known to interact with calcineurin inhibitors, such as calcium channel blockers (diltiazem, nicardipine and verapamil), antiepileptics (phenytoin and carbamazepine), antimycotics (fluconazole and ketoconazole), macrolide antibiotics (erythromycin and clarithromycin) and antiretrovirals (ritonavir and saquinavir) was checked for each of the 19 volunteers.

Exposure of volunteers

The 19 volunteers were admitted, between two dialysis periods, for a 24-h hospitalization and were given an oral dose of Tac of 0.1 mg/kg body weight twice a day. After overnight fasting, and 1 h before the first meal, the volunteers were orally administered the first dose of Tac. The second dose was administered exactly 12 h after the first dose and 1 h before the supper. The study protocol was approved by the local Ethical Committee and all patients provided their informed consent to participate in the study. Patients were kept under continuous medical surveillance during the hospitalization period.

Sample collection and analysis

Blood samples were respectively collected before and 1, 2, 4, 8 and 12 h after the administration of the first Tac dose (t_0, t_1, t_2, t_4, t_8 and t_{12}). The second dose of Tac was administered just after t_{12} and further blood samples were respectively collected 1, 2, 4, 8 and 12 h after the administration of the second Tac dose (t_{13}, t_{14}, t_{16}, t_{20} and t_{24}). Tac whole blood concentrations were determined both by liquid chromatography with tandem mass

Table 1: Main characteristics of the final study population according to *CYP3A5* and *ABCB1* genotype

	Age (years)	Weight (kg)	Tac dose (mg)	Tac dose/weight (mg/kg)	*CYP3A5* intron 3 6986 G > A status	*ABCB1* exon 12 1236 C > T status	*ABCB1* exon 21 2677 G > T/A status	*ABCB1* exon 26 3435 C > T status
CYP3A5 expressors								
Volunteers 1–5 (5 males)	**60** (47–69)	**80** (55–80)	**8** (6–8)	**0.100** (0.100–0.109)	CYP3A5*1/*1	C/C[1]	G/G	C/C[1]
Volunteers 6–9 (2 females, 2 males)	**59** (48–66)	**69** (55–84)	**7** (6–8)	**0.103** (0.095–0.109)	CYP3A5*1/*3	C/C	G/G	C/C
CYP3A5 nonexpressors								
Volunteers 10–13 (4 males)	**41** (26–46)	**85** (82–96)	**8.5** (8–10)	**0.101** (0.096–0.105)	CYP3A5*3/*3	C/C	G/G	C/C
Volunteers 14–17 (4 males)	**54** (33–65)	**70** (59–78)	**6.5** (6–8)	**0.101** (0.099–0.103)	CYP3A5*3/*3	T/T	T/T	T/T
volunteers 18–19 (1 female, 1 male)	**45** (34–56)	**63** (50–76)	**6.5** (5–8)	**0.103** (0.100–0.105)	CYP3A5*3/*3	C/T	G/T	C/T

Values are given as **median** and range. Presence of *CYP3A5*6* allele was also checked for all volunteers: only volunteer 5 possessed one *CYP3A5*6* allele (*1/*6).
[1]Volunteer 4 possessed C/T genotype.

spectrometry (LC-MS/MS) and by immunoassay. The liquid chromatography assay was developed on a LC-MS/MS MicroQuattro system from Waters-Micromass Ltd and the analytical procedure has been described elsewhere (14). Briefly, chromatographic conditions include a cartridge column C18 Phenomenex 4 × 3 mm maintained at 55°C, a 0.3 mL/min flow rate of a mobile phase (30% buffer ammonium acetate 2 mM/70% methanol-ammonium acetate 2 mM). Tac and ascomycin (internal standard) are monitored by detecting specific product ions resulting from the fragmentation of their precursor ions using MRM acquisition mode (821.4 > 768.2 m/z and 809.6 > 756.6 m/z, respectively). Immunoassays were performed by MEIA (microparticle enzyme immunoassay) on the IMx analyzer (Abbott Diagnostics Laboratories, Abbott-Park, IL). Tac analytical performances displayed a between-day coefficient of variation of <11% with the IMx analyzer. Among Tac metabolites, only MII presents an activity close to the parent compound (approximately 100%). The other metabolites (MI, MIII, MIV, etc.) display an activity of <7%. With the IMx analyzer, MI, MII, MIII and MIV display a cross-reactivity with the antibody of <1%, 109%, 90.5% and 8.8%, respectively (15). The laboratory successfully participates in international proficiency testing schemes (UK, David Holt).

Pharmacokinetic analysis

C_{12} and C_{24} are defined as the trough levels obtained after the first and the second dose of Tac, respectively. Area under the time versus blood concentration curve (AUC) was evaluated using the linear trapezoidal rule. Total Tac $AUC_{0-\infty}$ was determined by the summation of AUC_{0-12}, AUC_{12-24} and $AUC_{24-\infty}$. $AUC_{24-\infty}$ was determined from the ratio of the last Tac blood concentration (C_{24}) to the elimination rate constant (ke expressed in h^{-1}) calculated from the second dosing interval. Apparent clearance of Tac (Cl) was determined from the ratio of the total administered dose, normalized for body weight, to the total Tac $AUC_{0-\infty}$. Apparent volume of distribution of Tac (Vd) was determined by the following equation: Vd = Cl/ke (ke calculated from the second dosing interval). The limited sampling strategy does not allow accurate determination of C_{max} and t_{max} (five values for both dosing intervals among which only two during the early phase).

Statistical analysis

Statistical analysis was carried out using the SPSS package (version 11.0, SPSS, Chicago, IL). Groups were compared using nonparametric tests. To compare two groups, we used the Mann-Whitney U-test, and to compare several groups, the Kruskal-Wallis test. p values less than 0.05 were considered statistically significant. All values are expressed as median and range unless otherwise stated. Multiple regression analysis models were used to assess the contribution of genotypes and other covariates (age, gender, body weight, Tac dose) to the interindividual variability of Tac PK parameters. For these multivariate regression analyses, each genotype was coded with a distinct dummy variable set at 0 (presence of at least one functional allele for *CYP3A5* or double presence of 'wild-type' allele for *ABCB1* genotypes), at 1 (double presence of *CYP3A5*3* allele (i.e. absence of CYP3A5 activity) or heterozygous status for *ABCB1*) or at 2 (double presence of 'mutant' allele for *ABCB1* genotypes). When appropriate, significant covariates of Tac PK parameters were traced by a stepwise regression procedure using a significance level of 0.10 for entry and 0.05 for staying in the model.

Results

Influence of CYP3A5 genotype on Tac pharmacokinetics

As shown in Table 2, lower AUCs or trough levels (C_{12} and C_{24}) and higher Cl or Vd were observed among CYP3A5 expressors (n = 9) than among nonexpressors (n = 10). Similar results were observed with the immunoassay (data not

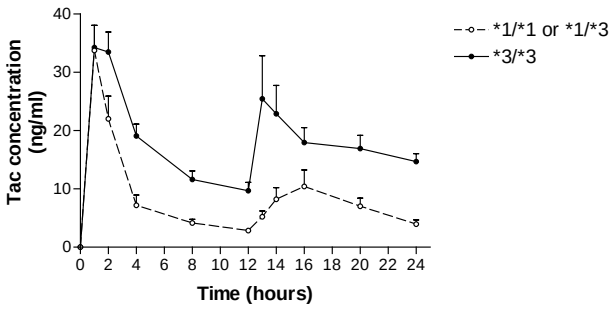

Figure 2: Whole blood Tac concentration measured by LC-MS/MS (ng/mL) according to *CYP3A5* genotype (*CYP3A5*1/*1* or **1/*3*, n = 9 and *CYP3A5*3/*3*, n = 10). The mean values and SEM are indicated.

Table 2: *CYP3A5* genotype and Tac pharmacokinetics (LC-MS/MS)

	C_1 ng/mL	C_2 ng/mL	C_4 ng/mL	C_8 ng/mL	C_{12} ng/mL	AUC_{0-12} ng.h/mL
*1/*1 or *1/*3 (*n* = 9)	**32.4** (14.9–59.2)	**19.6*** (6.7–43.4)	**5.5**** (3.0–19.1)	**3.0***** (2.2–8.5)	**2.5***** (2.0–4.4)	**100.2**** (64.4–224.4)
*3/*3 (*n* = 10)	**33.5** (10.0–49.7)	**31.3** (16.9–51.8)	**18.0** (8.2–32.0)	**9.0** (7.4–20.2)	**7.5** (5.5–20.2)	**197.1** (110.8–323.6)
	C_{13} ng/mL	C_{14} ng/mL	C_{16} ng/mL	C_{20} ng/mL	C_{24} ng/mL	AUC_{0-24} ng.h/mL	$AUC_{0-\infty}$ ng.h/mL	CL mL/min/kg	Vd L/kg
*1/*1 or *1/*3	**4.3****	**5.7****	**7.2***	**7.4****	**3.0*****	**156.2****	**189.9****	**17.60****	**7.54****
(*n* = 9)	(2.0–10.1)	(2.5–19.4)	(2.6–28.0)	(2.4–15.8)	(2.0–7.6)	(103.3–428.2)	(133.9–474.8)	(7.20–26.35)	(2.65–22.84)
*3/*3	**20.6**	**20.8**	**16.9**	**18.2**	**15.2**	**387.0**	**495.5**	**6.63**	**3.61**
(*n* = 10)	(6.5–83.5)	(7.9–58.5)	(9.1–32.1)	(4.5–26.1)	(8.9–23.1)	(261.1–751.9)	(393.0–1034.6)	(3.37–8.62)	(2.48–4.81)

C_1, C_2, … C_{24} represent the blood Tac concentration found 1, 2, … 24 h after the first oral administration.
Values are given as **median** and range, *$p < 0.05$; **$p < 0.01$; *** $p < 0.001$ (2-tailed, Mann-Whitney U-test).

shown). In contrast, no difference in C_1 was observed between expressors and nonexpressors volunteers. A graph depicting the average curve according to *CYP3A5* genotype is presented in Figure 2. Body weight and Tac dose were not different between expressors and nonexpressors. It should, however, be noted that age was different between both groups (60 years (range: 47–69) for expressors vs. 44.5 years (range: 26–65) for nonexpressors, $p < 0.05$). The median $AUC_{0-\infty}$ was 2.6- and 2.1-fold higher in nonexpressors with LC-MS/MS and MEIA methods, respectively. This difference was more striking when considering C_{24} (5.1- and 2.9-fold, respectively). A very small overlap was observed between CYP3A5 expressors and nonexpressors for $AUC_{0-\infty}$ (Figure 3A) and involved an individual possessing one *CYP3A5*6* allele. The separation was clear-cut when considering C_{24} (Figure 3B). To indirectly assess the potential impact of Tac metabolites, we also analyzed the ratio of selected pharmacokinetic parameters calculated using both analytical methods (ratio expressed as (PK parameter MEIA/PK parameter LC-MS/MS)*100) in relation to CYP3A5 expressor status. Interestingly, significant differences between groups were observed for $AUC_{0-\infty}$ (160% for expressors vs. 116.5% for nonexpressors, $p < 0.05$) and C_{24} (211% vs. 119.5%, $p < 0.05$).

Influence of ABCB1 genotype/haplotype on Tac pharmacokinetics

When considering the whole group of volunteers, no statistically significant association was observed between Tac PK parameters and polymorphisms in *ABCB1* exon 12, 21 or 26. In order to assess a possible independent effect of *ABCB1* polymorphism on Tac PK parameters, the statistical analysis was performed on the subgroup of CYP3A5 nonexpressors (n = 10, volunteers 10–19), selected *a priori* to obtain a balanced distribution between *ABCB1* haplotypes (*ABCB1* exons 12–21–26; CC-GG-CC (n = 4), TT-TT-TT (n = 4) and CT-GT-CT (n = 2)) (see Table 1). No effect of *ABCB1* haplotype was observed (Table 3 and Figure 4).

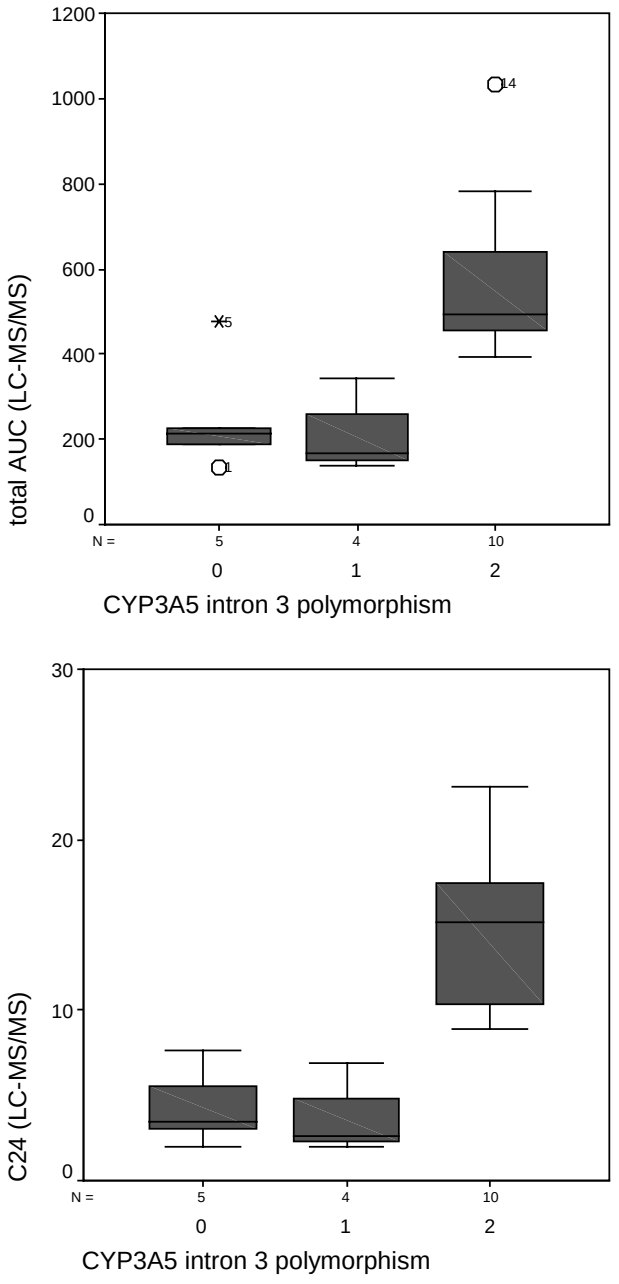

Figure 3: (A) Total $AUC_{0-\infty}$ (ng h/mL) and (B) C_{24} (ng/mL) determined by LC-MS/MS according to *CYP3A5* intron 3 polymorphism, G6986A (*CYP3A5* 1/* 1*, n = 5; *CYP3A5* 1/* 3*, n = 4 and *CYP3A5* 3/* 3*, n = 10). Data are shown by box-and-whisker plots. Subdivisions of the boxes and the top and bottom lines on the boxes represent median values and the upper and lower quartiles, respectively.

Multivariate regression analyses

Stepwise multiple regression analyses were also performed to trace the contribution of genotypes and other covariates (gender, body weight, Tac dose) to the interindividual variability in Tac PK parameters. With the only

Table 3: *ABCB1* haplotype (exons 12–21–26) and Tac pharmacokinetics (LC-MS/MS)

	C_1 ng/mL	C_2 ng/mL	C_4 ng/mL	C_8 ng/mL	C_{12} ng/mL	AUC_{0-12} ng.h/mL
CC-GG-CC ($n = 4$)	**29.1** (10.0–49.7)	**29.8** (16.9–51.8)	**20.2** (8.2–32.0)	**14.8** (8.9–20.2)	**9.9** (7.1–20.2)	**223.4** (110.8–323.6)
CT-GT-CT ($n = 2$)	**37.8** (33.1–42.4)	**34.7** (30.8–38.5)	**17.0** (15.6–18.4)	**9.0** (9.0–9.0)	**6.0** (5.5–6.4)	**188.6** (182.4–194.9)
TT-TT-TT ($n = 4$)	**39.4** (24.5–45.9)	**32.9** (24.8–46.8)	**19.3** (14.0–23.7)	**9.0** (7.4–14.0)	**8.9** (6.4–13.5)	**202.2** (160.3–252.0)
	C_{13} ng/mL	C_{14} ng/mL	C_{16} ng/mL	C_{20} ng/mL	C_{24} ng/mL	AUC_{0-24} ng.h/mL	$AUC_{0-\infty}$ ng.h/mL	CL mL/min/kg	Vd L/kg
CC-GG-CC	**28.6**	**22.5**	**22.2**	**18.2**	**16.8**	**452.0**	**651.9**	**5.28**	**4.38**
($n = 4$)	(6.9–83.5)	(9.2–58.5)	(10.7–32.1)	(12.8–26.1)	(10.0–23.1)	(261.1–751.9)	(424.9–1034.6)	(3.37–7.65)	(2.48–4.81)
CT-GT-CT	**19.7**	**22.2**	**13.2**	(9.5–22.6)	**(11.7)**	**(371.4)**	**(461.6)**	**(7.42)**	**(3.47)**
($n = 2$)	(6.5–32.8)	(7.9–36.4)	(9.4–16.9)	(9.5–22.6)	(10.3–13.0)	(361.0–381.9)	(455.0–468.2)	(7.12–7.71)	(3.35–3.58)
TT-TT-TT	**17.6**	**17.4**	**17.4**	**17.0**	**15.2**	**411.2**	**531.3**	**6.34**	**3.56**
($n = 4$)	(7.2–25.1)	(10.2–26.8)	(9.1–22.1)	(4.5–23.2)	(8.9–17.5)	(269.9–476.9)	(393.0–639.4)	(5.35–8.62)	(3.36–4.05)

C_1, C_2, … C_{24} represent the blood Tac concentration found 1, 2, … 24 h after the first oral administration.
Values are given as **median** and range (Kruskal-Wallis test).

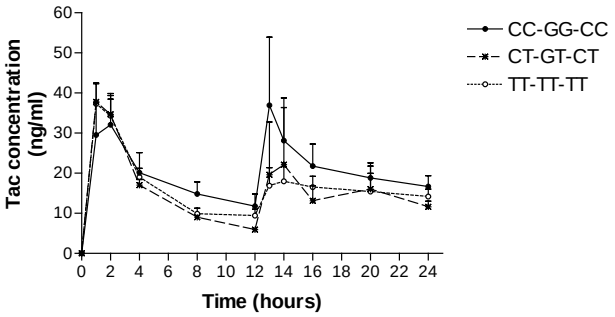

Figure 4: Whole blood Tac concentration measured by LC-MS/MS (ng/mL) according to *ABCB1* haplotype in the subgroup of CYP3A5 nonexpressors (*ABCB1* exons 12–21–26; CC-GG-CC, n = 4; TT-TT-TT, n = 4 and CT-GT-CT, n = 2). The mean values and SEM are indicated.

exception of models considering C_1 as the dependent variable, the *CYP3A5* intron 3 polymorphism was the most significant independent variable, followed by Tac dose (Table 4). When considering $AUC_{0-\infty}$ as the dependent variable, *CYP3A5* intron 3 polymorphism was associated with a positive slope, because CYP3A5 expressors (presence of at least one functional allele, *1/*1 and *1/*3) were coded as '0' and CYP3A5 nonexpressors (*3/*3) were coded as '1'. The resulting models explained 73% of the total variance for $AUC_{0-\infty}$ and up to 80% for C_{24} calculated with LC-MS/MS. Similar results were observed for MEIA (data not shown).

Discussion

The selection of the volunteers has been performed among a list of 221 patients to meet some important criteria among them, a good balance between *CYP3A5* and *ABCB1* genotypes. Homozygous carriers *CYP3A5*1/*1* are rare in the Caucasian population and those included in this study were all from African origin (all *CYP3A5*1/*1* individuals detected in the initial genotype screening were included in the final study). After selection of volunteers, primarily based on genotype criteria, nongenetic parameters were compared among the groups. Body weight and Tac dose to be administered did not differ among *CYP3A5* and *ABCB1* genotype groups. The only difference observed was related to the age of the volunteers which was significantly higher in CYP3A5 expressors compared to nonexpressors. To the best of our knowledge, and according to a very recent review on this topic (16), among all population pharmacokinetic studies that have investigated age as a covariate, none has found a significant influence on Tac bioavailability, volume of distribution or clearance. Based on these data, we decided to include the 19 selected volunteers in the experimental protocol.

The effect of *CYP3A5* genotype on Tac pharmacokinetics is significant early after the first administration of the drug. Indeed, in our study, volunteers were recruited from a list of adult renal transplant candidates and had never been exposed to Tac before the study. This information is of primary importance as the first days after transplantation are generally characterized by the highest risk of acute organ rejection. Our data confirm that pharmacogenetic analysis before transplantation may assist in guiding individual Tac dosing (13). In this respect, the PK parameter giving the most realistic idea of the global exposure to Tac is the total $AUC_{0-\infty}$. For this parameter, we did not observe any difference between *CYP3A5*1/*1* and *CYP3A5*1/*3* volunteers confirming previous observations that the presence of at least one functional *CYP3A5* allele is sufficient for optimal CYP3A5 activity (17,18). However, the median $AUC_{0-\infty}$ was 2.6- and 2.1-fold higher in nonexpressors with the LC-MS/MS and MEIA methods, respectively. Based on these data, a minimal 2-fold higher Tac loading dose could theoretically be administered to CYP3A5 expressors. This information provides a strong argument for transplant centers that already have adopted the strategy to give a 2-fold higher Tac dose in CYP3A5 expressors (MacPhee IA, personal communication in (10)). Furthermore, the high values observed for r^2 in the multivariate models make unlikely that other genetic parameters contribute very

Table 4: Determinants of tacrolimus pharmacokinetic parameters (LC-MS/MS)

Dependent variables	Independent variables	Partial r^2	Slope	p Value[1]
$AUC_{0-\infty}$	*CYP3A5* intron 3	0.56	positive	<0.001
	Tac dose	0.17	positive	0.007
		Model r^2: 0.73		
C_{12}	*CYP3A5* intron 3	0.53	positive	<0.001
	Tac dose	0.14	positive	0.019
		Model r^2: 0.67		
C_{24}	*CYP3A5* intron 3	0.73	positive	<0.001
	Tac dose	0.07	positive	0.029
		Model r^2: 0.80		

Tested independent variables were *CYP3A5* intron 3,[2] the 3 *ABCB1* genotypes, gender, body weight and Tac dose.
[1]Partial r^2 p value.
[2]Presence of at least one functional allele (*1/*1 and *1/*3 coded as '0' and *3/*3 coded as '1').

significantly to the interindividual variability in Tac $AUC_{0-\infty}$ or C_{12} and C_{24}. Considering $AUC_{0-\infty}$ and C_{24}, the ratio calculated using MEIA and LC-MS/MS is significantly higher in CYP3A5 expressors and the effect is more pronounced for C_{24} than for total $AUC_{0-\infty}$. The higher value observed for CYP3A5 expressors probably reflects a higher concentration of metabolites (MII or MIII) showing a cross-reactivity with the Tac antibody. In this context, trough level such as C_{24} appears logically more affected by the production of metabolites than total $AUC_{0-\infty}$ integrating concentrations over 24 h provided the half-life of the metabolites is not significantly shorter than the parent compound. A direct quantification of Tac metabolites would definitely confirm this observation. In the nonexpressors group, the value observed for total $AUC_{0-\infty}$ ratio suggests a mean overestimation of 16.5% using MEIA, a value in agreement with several data in the literature (19,20) and that could reflect an additional metabolite production resulting from the activity of other CYP isoforms, among which CYP3A4 (21).

Besides the need for doubling the loading dose in CYP3A5 expressors, based on $AUC_{0-\infty}$ values (Figure 2), the analysis of the raw data (not shown) corresponding to trough values (C_{24}) shows that seven or five out of the ten CYP3A5 nonexpressors presented Tac blood concentrations higher than 15 ng/mL using MEIA or LC-MS/MS methods, respectively. As this value is generally considered as the highest concentration of the therapeutic range for Tac, we also propose to reduce the loading dose in CYP3A5 nonexpressors. Therefore a loading dose of 0.075 mg/kg body weight twice a day should be given to these patients while a double dose of 0.150 mg/kg body weight twice a day should be administered to CYP3A5 expressors. It should be noted, however, that after several days of treatment, the known inductive effect of steroids comedication would most probably be minimized by the simultaneous occurrence of the steady state for Tac.

Our data also confirm that *ABCB1* genotype/haplotype contributes little, if at all, to the interindividual variability in Tac pharmacokinetics early after the first doses of the immunosuppressant (10,22). The study was designed to explore an independent effect of *ABCB1* genotype/haplotype in CYP3A5 nonexpressors. Even after 'standardization' for CYP3A5 activity, we were unable to observe any effect of *ABCB1* genotype/haplotype on Tac PK parameters, particularly on C_1 and total $AUC_{0-\infty}$ that should be affected by any variation in the activity of intestinal P-gp (23).

In conclusion, our study confirms the very significant effect of *CYP3A5* polymorphism early after the first administration of Tac. It also provides a strong argument for doubling the loading dose in patients early identified *a priori* on the transplantation list as possessing at least one functional *CYP3A5* allele (*CYP3A5*1/*1, *1/*3, *1/*6*). For these patients a loading dose of 0.150 mg/kg body weight twice a day is proposed, while a moderate reduction of the loading dose (i.e. 0.075 mg/kg body weight twice a day) should allow CYP3A5 nonexpressors to reach more rapidly efficient and nontoxic trough concentrations. However, as corticosteroids administration is expected to independently affect Tac clearance *in vivo*, it remains important to assess in longitudinal studies the beneficial effect of the loading dose adjustment, in association with TDM, on transplantation outcomes.

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