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A New Functional *CYP3A4* Intron 6 Polymorphism Significantly Affects Tacrolimus Pharmacokinetics in Kidney Transplant Recipients

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BACKGROUND: Tacrolimus (Tac) is a potent immunosuppressant with considerable toxicity. Tac pharmacokinetics varies between individuals and thus complicates its use in preventing rejection after kidney transplantation. This variability might be caused by genetic polymorphisms in metabolizing enzymes.

METHODS: We used TaqMan analyses to evaluate the impact of a newly discovered *CYP3A4* (cytochrome P450, family 3, subfamily A, polypeptide 4) single-nucleotide polymorphism (SNP) (rs35599367C>T; *CYP3A4*22*) on Tac pharmacokinetics in 185 renal transplant recipients who participated in an international randomized controlled clinical trial (fixed-dose, concentration-controlled study).

RESULTS: The overall mean daily-dose requirement to reach the same predose Tac blood concentration was 33% lower for carriers of the T variant allele than for rs35599367CC patients (95% CI, −46% to −20%; $P = 0.018$). When combined with the **3* genotype of the *CYP3A5* (cytochrome P450, family 3, subfamily A, polypeptide 5) gene, the rs35599367C>T SNP was also associated with a risk of supratherapeutic Tac concentrations (>15 μg/L) during the first 3 days after surgery, with an odds ratio of 8.7 for carriers of the *CYP3A4* T allele plus *CYP3A5*3/*3* ($P = 0.027$) and 4.2 for the *CYP3A4* CC homozygotes plus *CYP3A5*3/*3* ($P = 0.002$), compared with *CYP3A4* CC homozygotes having 1 or 2 *CYP3A5*1* alleles. The overall increase in the Tac dose-adjusted trough blood concentration was +179% for carriers of the *CYP3A4* T allele with *CYP3A5*3/*3* ($P < 0.001$), +101% for *CYP3A4* CC homozygotes with *CYP3A5*3/*3* ($P < 0.001$), and +64% for *CYP3A4* T allele carriers with *CYP3A5*1* ($P = 0.020$), compared with *CYP3A4* CC homozygotes with *CYP3A5*1*.

CONCLUSIONS: The *CYP3A4* rs35599367C>T polymorphism is associated with a significantly altered Tac metabolism and therefore increases the risk of supratherapeutic Tac concentrations early after transplantation. Analysis of this *CYP3A4*22* SNP may help in identifying patients at risk of Tac overexposure.

CYP3A4 is the most abundant cytochrome P450 enzyme in the human liver and is responsible for the metabolism of 45%–60% of prescribed drugs *(1)*. CYP3A4 activity varies widely, with 10- to 100-fold variation between individuals *(2–5)*. A recent study identified a functional single-nucleotide polymorphism (SNP)[5] in intron 6 (*CYP3A4*22*) that was associated with decreased CYP3A4 production and activity and that was correlated with the statin dose requirement for lipid concentration control *(6)*.

The immunosuppressive drug tacrolimus (Tac) is extensively metabolized by CYP3A4 and CYP3A5 *(7–9)*. The **3* allele of the *CYP3A5*[6] (cytochrome P450, family 3, subfamily A, polypeptide 5) gene, which codes for the absence of CYP3A5 *(10)*, was previously associated with the Tac predose concentration (C_0) and Tac dose requirements. The *CYP3A5* genotype explains a major portion of the interindividual variation in Tac pharmacokinetics: carriers of 2 *CYP3A5*3* nonfunctional alleles require substantially less Tac (about 50%

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[5] Nonstandard abbreviations: SNP; single-nucleotide polymorphism; Tac, tacrolimus; C_0, Tac predose concentration; FDCC, fixed-dose, concentration-controlled; DGF, delayed graft function; BPAR, biopsy-proven acute rejection.

[6] Human genes: *CYP3A5*, cytochrome P450, family 3, subfamily A, polypeptide 5; *ABCB1*, ATP-binding cassette, sub-family B (MDR/TAP), member 1; *CYP3A4*, cytochrome P450, family 3, subfamily A, polypeptide 4; *UGT1A9*, UDP glucuronosyltransferase 1 family, polypeptide A9; *UGT2B7*, UDP glucuronosyltransferase 2 family, polypeptide B7.

less) to reach an identical C_0 concentration *(11, 12)* than kidney, liver, lung, and heart transplant recipients carrying a *CYP3A5*1* active allele. The clinical benefit of *CYP3A5*-based Tac dosing remains debatable, however *(13, 14)*. The drug transporter encoded by the *ABCB1* [ATP-binding cassette, sub-family B (MDR/TAP), member 1] gene is also involved in Tac disposition. Genetic variants have been associated with Tac drug disposition, although contradictory results have been published *(11, 12)*.

No studies to date have been able to identify SNPs in *CYP3A4* (cytochrome P450, family 3, subfamily A, polypeptide 4) that could account for the interindividual variation in CYP3A4 activity. A newly discovered SNP in intron 6 (rs35599367C>T) may now explain this variability *(6)*. The purpose of our study was to test whether this new *CYP3A4* SNP correlates with increased Tac exposure on standard dosages and thus might predict lower dose requirements.

Materials and Methods

PATIENTS AND STUDY DESIGN

Patients were de novo kidney transplant recipients participating in a phase IV, open, prospective, randomized controlled, international multicenter trial comparing fixed-dose (FD) with concentration-controlled (CC) mycophenolate mofetil treatment (FDCC study) *(15)*. Randomization to a fixed-dose or concentration-controlled regimen was done in blocks of 8 patients per center. Patients were randomized centrally through an automated telephone system, in a 1:1 ratio. A pharmacogenetic substudy was started in parallel. Findings on the roles of genetic polymorphisms in the *UGT1A9* (UDP glucuronosyltransferase 1 family, polypeptide A9) gene for mycophenolate mofetil *(16)*, the *UGT2B7* (UDP glucuronosyltransferase 2 family, polypeptide B7) gene for acyl-glucuronide mycophenolic acid *(17)*, and *CYP3A5*/*ABCB1* for Tac exposure and acute rejection *(18)* in the FDCC study have previously been published. Patients provided separate written informed consent for the substudy. The protocol was approved by the ethics committees of all participating centers and the relevant authorities in participating countries.

Immunosuppressive therapy consisted of calcineurin inhibitor and corticosteroids. The choice of Tac or cyclosporine and the target blood concentrations for each drug were in accordance with each center's protocol. Oral Tac treatment began within 48 h before transplantation. Therapeutic drug monitoring was performed routinely, and centers were free to aim for the target concentrations they considered appropriate. A retrospective analysis showed that all centers started Tac with an aim of whole-blood concentrations of 7–15 μg/L, tapering to 5–12 μg/L at month 3 and to 4–10 μg/L at month 12. Corticosteroid tapering was recommended but not mandatory, and tapering regimens were left to the discretion of the investigators. In general, centers used higher doses in the first 2 weeks (20–25 mg of prednisolone equivalent daily), lower doses thereafter (15 mg on week 4, 5 mg at month 3), and low-dose or no prednisolone during months 6–12. More details can be found in the original FDCC study publication *(15)*. Genetic data were available for 185 kidney transplant recipients treated with Tac. Pharmacokinetic data were not always available for all patients at all time points. The C_0 was measured on days 3 and 10, at months 1, 3, 6, and 12 after transplantation, and whenever deemed necessary by the attending physician. Donor DNA was not collected, and no kidney biopsies were performed. Delayed graft function (DGF) was defined as a need for dialysis within the first week after transplantation. Biopsy-proven acute rejection (BPAR) was defined as any histologically confirmed episode with a Banff score ≥1. All biopsy samples were assessed locally by a pathologist.

DRUG CONCENTRATION MEASUREMENT

The C_0 was measured in whole blood in laboratories in each participating center by either the Tac II microparticulate enzyme immunoassay (Abbott Laboratories) or the enzyme-multiplied immunoassay technique (EMIT 2000; Syva Company/Dade Behring). The specificities of the 2 assays are comparable, and high correlations exist between the immunoassay and HPLC results *(19, 20)*. Although immunoassays overestimate Tac concentrations by up to 20% because of concurrent measurement of metabolites, this methodology has proved feasible for assessing differences in Tac concentrations with respect to the *CYP3A5* genotype *(21)*. A limited number of centers used liquid chromatography–tandem mass spectrometry to measure Tac concentrations; this method was used for 30 (16%) of the 185 patients. Proficiency testing was performed by participation of all centers in the UK Quality Assessment Scheme. Dose-adjusted predose concentrations were calculated by dividing the C_0 by the corresponding 24-h dose on a milligram-per-kilogram basis.

GENOTYPE ANALYSIS

The MagnaPure LC System (Roche Diagnostics) was used to isolate genomic DNA from 200 μL EDTA-treated whole blood. The *CYP3A4* intron 6 C>T genotype was determined with 50 ng genomic DNA in the allelic discrimination reaction performed with TaqMan® (Applied Biosystems) genotyping assays (C_59013445 10) on an ABI PRISM 7500® Fast Real-Time PCR System (Applied Biosystems). *CYP3A5*3* analysis and *ABCB1* 1236C>T, 2677G>T/A, and 3435C>T

analyses were performed as described previously *(18, 22)*.

STATISTICAL ANALYSIS

Statistical analyses were performed with Predictive Analytics Software (PASW) statistics (version 17.0 for Windows; SPSS/IBM). C_0 and dose-adjusted C_0 values were normalized by logarithmic transformation. Kolmogorov–Smirnov tests confirmed that log-transformed data were normally distributed. For comparisons of 2 genotype groups, Student independent *t*-tests were used to compare the means at single time points. With >2 groups, ANOVAs were performed under the null hypothesis that the means of the compared groups were equal. When the differences between means were significant, we carried out a post hoc analysis consisting of an a priori polynomial linear contrast test to assess any potential linear trend according to genotype classification. The corresponding linear contrast does test the probability of a positive linear trend of the dependent variable across the ordered level of genotype classifications. Differences between groups were assumed statistically significant for *P* values <0.05. For univariate analyses of associations between categorical data (e.g., incidence of acute rejection), we used the Fisher exact test or the Pearson χ^2 test. The Tac daily dose and the dose-adjusted C_0 of different genotypes were compared with a mixed-model analysis, which was based on the maximum likelihood ratio, with patient genotype status as the fixed factor and time after transplantation as the repeated measurement. The sex, ethnicity, and age of the patients were introduced as random effects to adjust for these covariables. No structure was imposed on the variances and covariances between and within the times of follow-up of the repeated Tac measurements. We assumed levels of covariables (sex, ethnicity, and age) to be uncorrelated and to have a constant variance across the time of follow-up. Coefficients estimated from mixed-model ANOVA were back-transformed by taking their antilogarithm so that the data could become interpretable as percentage differences in geometric mean values of untransformed outcomes. Multiple logistic regression analysis was performed according to criteria defined by McMaster et al. *(23)*, with a fixed Tac supratherapeutic threshold set at 15 μg/L. We computed genotype-specific odds ratios and 95% CIs by using backward stepwise analysis based on maximum likelihood ratios to assess the impact of genotype on the risk of Tac plasma concentrations >15 μg/L. *P* values <0.05 were considered statistically significant for entry, and *P* values <0.10 were required for staying in the model. For these analyses, each genotype was coded as a "dummy variable."

Results

CYP3A4 INTRON 6 GENOTYPE AND Tac EXPOSURE

Table 1 summarizes the patient characteristics. Overall, 173 patients were homozygous for the *CYP3A4* intron 6 wild type (rs35599367CC), 11 patients were heterozygous (rs35599367CT), and 1 patient was homozygous for the T variant (rs35599367TT), resulting in a minor-allele (T) frequency of 3.5%. The observed genotype distribution was in accordance with the Hardy–Weinberg equilibrium ($P = 0.25$, χ^2 test). Heterozygous CT and homozygous TT variants were grouped and analyzed together as carriers of the rs35599367 T allele, against the patients homozygous for the wild type (rs35599367CC). We observed no linkage disequilibrium between the *CYP3A4* intron 6 SNP and either the *CYP3A5*3* or *CYP3A4*1B* allele [χ^2 (2) = 0.24 ($P = 1.0$) and 1.36 ($P = 0.46$), respectively].

The 2 *CYP3A4* intron 6 genotype groups were comparable with respect to the Tac daily dose on day 3 after transplantation: 13.3 mg/day for the wild-type CC patients vs 13.0 mg/day for the carriers of 1 or 2 T alleles ($P = 0.84$, Table 2; see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue11). With these comparable dosages, the C_0 was higher for carriers of the T variant than for CC patients: 20.5 μg/L vs 14.9 μg/L ($P = 0.05$, Table 2; see Fig. 1 in the online Data Supplement). The differences between the 2 groups in C_0 were not observed at later time points ($P > 0.05$, Table 2; see Fig. 1 in the online Data Supplement), but carriers of the T allele required significantly lower Tac doses than CC patients to reach this C_0, from day 10 to month 6 (Table 2; see Fig. 1 in the online Data Supplement). Identical trends were observed when dose was adjusted for weight (Table 2). Analysis of repeated measurement in a mixed model demonstrated overall mean daily Tac dose requirements (adjusted for covariates age, sex, and ethnicity) to be 33% lower for carriers of the T allele (95% CI, −46% to −20%; $P = 0.018$) than for CC patients. Consequently, the calculated dose-adjusted C_0 was lower for CC patients than for CT/TT patients. These differences were significantly different at day 10 and month 1 after transplantation (Table 2; see Fig. 1 in the online Data Supplement), but not at later time points, a result that might be explained by a decrease in the number of participants, thereby yielding a larger 95% CI. The mixed model for repeated measurements showed that the overall mean dose-adjusted C_0 (adjusted for the covariates of age, sex, and ethnicity) was 47% higher in carriers of the T variant (95% CI, 8%–100%; $P = 0.001$) than the wild-type CC patients. As stated earlier, immunoassays overestimate Tac concentrations in blood by up to 20%, which may have influenced our results;

Table 1. Patient demographics.

	All patients	*CYP3A4* intron 6 CC homozygote	*CYP3A4* intron 6 allele T carriers (CT plus TT)	*P*
Patients, n	185	173	12	—
Male/female sex, n	112 (61%)/73 (40%)	108 (62%)/65 (38%)	4 (33%)/8 (67%)	0.07
Age, years[a]	47.9 (13.8)	47.7 (14.0)	51.8 (11.3)	0.32
Weight, kg[a]	72.6 (14.0)	72.8 (14.3)	71.1 (10.4)	0.69
Transplantation no., n				0.80
First	156 (84%)	145 (84%)	11 (92%)	—
Second	19 (10%)	18 (10%)	1 (8.3%)	—
Third or more	5 (2.7%)	5 (2.9%)	0 (0.0%)	—
Missing information	5 (2.7%)	5 (2.9%)	0 (0.0%)	—
Living/deceased donor, n	71 (38%)/114 (62%)	66 (38%)/107 (62%)	5 (42%)/7 (58%)	0.81
FDCC MMF therapy, n	98 (53%)/87 (47%)	92 (53%)/81 (47%)	6 (50%)/6 (50%)	0.83
Induction therapy, n[b]	67 (36%)	62 (36%)	5 (42%)	0.69
Primary kidney disease, n				0.29
Diabetic nephropathy	16 (8.6%)	15 (8.7%)	1 (8.3%)	—
Glomerulonephritis	51 (28%)	48 (28%)	3 (25%)	—
Hypertensive nephropathy	18 (9.7%)	18 (10.4%)	0 (0.0%)	—
Obstructive/reflux nephropathy	9 (4.9%)	8 (4.6%)	1 (8.3%)	—
Other	42 (23%)	42 (24%)	0 (0.0%)	—
Polycystic kidney disease	29 (16%)	24 (14%)	5 (42%)	—
Pyelonephritis/interstitial nephritis	9 (4.9%)	8 (4.6%)	1 (8.3%)	—
Unknown	9 (4.9%)	8 (4.6%)	1 (8.3%)	—
HLA mismatches, n[c]	2.9 (3.0%)	2.9 (3.0%)	3.2 (3.0%)	0.55
Panel reactive antibodies, n				
<10%/≥10%	167 (90%)/18 (9.7%)	155 (90%)/18 (10.4%)	12 (100.0%)/0 (0.0%)	0.24
Ethnicity, n				0.65
Asian	9 (4.9%)	9 (5.2%)	0 (0.0%)	—
Black	8 (4.3%)	8 (4.6%)	0 (0.0%)	—
Caucasian	164 (89%)	152 (88%)	12 (100%)	—
Other	4 (2.2%)	4 (2.3%)	0 (0.0%)	—

[a] Data are presented as the mean (SD).
[b] All patients who received induction therapy were treated with antibody against the interleukin-2 receptor; none were treated with antithymocyte globulin.
[c] Data are presented as the mean (% of total).

however, the proportions of T variant carriers in the immunoassay group and the HPLC group were the same (6.5% and 6.7%, respectively; $P = 1.0$, Fisher exact test). Moreover, when the analytical method for measuring Tac concentration was introduced as a random effect, carriers of the T allele still showed a higher overall dose-adjusted C_0 (+37% for T allele carriers, $P < 0.001$) and a lower Tac daily dose (−20% for T allele carriers, $P = 0.007$) than the wild-type CC patients. For the *ABCB1* gene, neither 2677G>T/A nor 1236C>T was associated with differences in Tac pharmacokinetics during the entire study. Also *ABCB1* 3435C>T was not significantly associated with Tac dose and dose-adjusted Tac exposure in a linear mixed model when the *CYP3A4* genotype was not taken into account as previously reported *(18)*. When *ABCB1* haplotypes were generated, no differences between the *ABCB1* CGC (n = 26) and TTT haplotype groups (n = 20) were observed with respect to Tac dose and dose-adjusted Tac exposure (data not shown).

COMBINED EFFECTS OF *CYP3A4* INTRON 6 GENOTYPE, *CYP3A5*3*, AND *ABCB1* 3435C>T

We investigated the effects of *CYP3A4* intron 6, *ABCB1* 3435C>T, and *CYP3A5*3* genotypes in combination. Patients carrying 1 or 2 *CYP3A5*1* alleles (CYP3A5 expressers) were compared with *CYP3A5*3/*3* nonexpressers. In the mixed-model analysis adjusted for the

Table 2. Tac dose, C_0, and dose-adjusted C_0 (C_0/dose) according to CYP3A4 intron 6 C>T SNP genotype.

	CYP3A4 intron 6 CC homozygote	n	*CYP3A4* intron 6 allele T carriers (CT plus TT)	n	*P*[a]
Tac dose, mg/day					
Day 3	13.3 (12.6–13.9)	136	13.0 (11.7–14.3)	9	0.84
Day 10	12.9 (11.9–13.9)	134	9.2 (7.2–11.7)	10	**0.05**
Month 1	11.2 (10.4–11.9)	137	6.6 (5.5–7.7)	10	**<0.001**
Month 3	7.5 (6.8–8.2)	131	5.2 (4.3–6.1)	11	**<0.001**
Month 6	6.2 (5.5–6.8)	120	4.5 (3.7–5.3)	10	**0.004**
Month 12	5.2 (4.7–5.8)	112	4.6 (3.1–6.1)	9	0.55
Weight-adjusted Tac dose, mg · day^{-1} · (kg body weight)$^{-1}$					
Day 3	0.181 (0.174–0.189)	135	0.193 (0.178–0.207)	9	0.45
Day 10	0.176 (0.162–0.190)	133	0.134 (0.101–0.168)	10	0.10
Month 1	0.156 (0.15–0.166)	136	0.097 (0.078–0.116)	10	**0.004**
Month 3	0.105 (0.095–0.115)	130	0.076 (0.061–0.091)	11	0.10
Month 6	0.087 (0.078–0.097)	119	0.066 (0.054–0.079)	10	0.25
Month 12	0.072 (0.063–0.081)	111	0.069 (0.041–0.097)	9	0.84
C_0, μg/L[b]					
Day 3	14.9 (13.8–16.0)	144	20.5 (15.2–27.7)	8	**0.05**
Day 10	11.5 (10.9–12.1)	133	13.2 (11.3–15.5)	9	0.21
Month 1	12.5 (11.8–13.2)	145	12.8 (10.9–15.0)	11	0.81
Month 3	10.2 (9.8–10.8)	145	11.0 (9.7–12.4)	11	0.47
Month 6	8.6 (8.1–9.2)	125	9.1 (7.6–11.0)	10	0.63
Month 12	7.2 (6.5–7.9)	110	9.5 (6.9–13.1)	8	0.12
C_0/dose, μg/L per mg/kg[b]					
Day 3	84.7 (78.2–91.8)	125	108.7 (82.9–142.6)	8	0.14
Day 10	74.3 (67.3–82.0)	120	101.3 (86.5–118.6)	8	**0.006**
Month 1	87.5 (80.3–95.4)	128	136.6 (107.9–173.1)	10	**0.006**
Month 3	112.2 (101.0–124.6)	124	154.2 (117.6–202.3)	10	0.10
Month 6	121.3 (106.4–138.3)	105	144.3 (111.7–186.3)	9	0.26
Month 12	114.7 (100.7–130.5)	98	171.8 (120.2–245.5)	8	0.09
Creatinine clearance, mL/min					
Day 3	35.8 (31.7–39.9)	151	42.0 (20.1–63.8)	10	0.47
Day 10	45.1 (41.2–49.0)	148	47.4 (29.0–65.7)	11	0.77
Month 1	55.6 (52.0–59.3)	148	57.3 (42.1–72.5)	11	0.82
Month 3	60.1 (56.3–63.9)	144	66.2 (50.9–81.5)	11	0.40
Month 6	63.7 (59.8–67.7)	130	66.1 (50.0–82.3)	10	0.74
Month 12	65.6 (61.5–69.6)	114	59.1 (41.3–76.9)	9	0.40

[a] Statistically significant results ($P < 0.05$) are highlighted in boldface.
[b] Values are expressed as the geometric mean (95% CI).

covariates of age, sex, and ethnicity and including *ABCB1* 3435C>T, *CYP3A5**3, and *CYP3A4* intron 6 genotype status as fixed effects, all investigated SNPs were significantly correlated with dose-adjusted Tac exposure. Overall, the dose-adjusted C_0 was 43% higher among patients with *CYP3A4* intron 6 CT/TT (95% CI, 13%–88%; $P < 0.001$) than among CC patients and was 43.3% lower among CYP3A5 expressers than among nonexpressers (95% CI, −52.7% to −32.1%; $P = 0.001$). Regarding *ABCB1* 3435TT individuals, patients with *ABCB1* 3435CT and 3435CC genotypes had a lower overall dose-adjusted C_0: −14.3%

Table 3. *CYP3A4/CYP3A5* genotype cluster classification.

	CYP3A4 intron 6 CT or TT	*CYP3A4* intron 6 CC
***CYP3A5*1* noncarriers**	Group 1, poor metabolizers	Group 2, intermediate-1 metabolizers
	n = 10 (5.4%)	n = 142 (76.8%)
***CYP3A5*1* carriers**	Group 3, intermediate-2 metabolizers	Group 4, extensive metabolizers
	n = 2 (1.1%)	n = 31 (16.8%)

(95% CI, −26.2% to −0.5%; $P = 0.042$) and −20.9% (95% CI, −32.7% to −7.1%; $P = 0.003$), respectively. Only *CYP3A4* intron 6 and *CYP3A5* genotypes correlated significantly with the Tac dose requirement, because *ABCB1* 3435C>T genotype status was no longer a significant fixed effect in the mixed model. In this final model, the Tac dose requirement was 25% lower for carriers of the T allele (95% CI, −43% to −7%; $P = 0.04$) than for CC patients and was 63.7% higher for patients who expressed CYP3A5 than for nonexpressers (95% CI, 39.1%–88.2%; $P < 0.001$).

Because the effects of the *CYP3A4* intron 6 and *CYP3A5*3* SNPs appeared independent, we combined genotype groups. Group 1 contained CYP3A5 nonexpressers and carriers of the *CYP3A4* intron 6 T variant (poor metabolizers); group 2 contained CYP3A5 nonexpressers and *CYP3A4* intron 6 CC patients (intermediate-1 metabolizers); group 3 clustered CYP3A5 expressers carrying the *CYP3A4* intron 6 T allele (intermediate-2 metabolizers); and group 4 merged CYP3A5 expressers with individuals with the *CYP3A4* intron 6 CC wild type (extensive metabolizers) (Table 3). The C_0 values of the groups were significantly different at the first visits (Table 4). The Tac daily-dose requirements, which were based on reaching the target C_0 by therapeutic drug monitoring, were significantly different from day 10 and remained so (Table 4). Identical significant differences were observed when dose was adjusted for patient weight (Table 4). The dose-adjusted C_0 was significantly different among groups at all time points (Table 4; see Fig. 1 in the online Data Supplement). This trend was linear and was a function of genotype category classification, either for the C_0 at day 3 (group 1 > group 2 > group 3 > group 4; $P < 0.004$; Table 4) or for the Tac dose requirement from day 10 to month 12 (group 1 < group 2 < group 3 < group 4; $P = 0.001$; Table 4). This trend was also observed for the dose-adjusted C_0 and was highly significant at all investigated time points (group 1 > group 2 > group 3 > group 4; $P = 0.006$; Table 4). The mixed-model analysis revealed an overall increase in the Tac dose-adjusted trough blood concentration of +179.3% for the poor metabolizer cluster ($P < 0.001$), +101.4% for the intermediate-1 metabolizer cluster ($P < 0.001$), and +64.4% for the intermediate-2 metabolizer cluster ($P = 0.020$), compared with the extensive metabolizers.

Patients from groups 1 and 2 had a day 3 C_0 geometric mean above the consensus supratherapeutic threshold (15 μg/L): 21.5 μg/L for group 1 and 15.8 μg/L for group 2. Logistic regression models showed that the risk of presenting a supratherapeutic C_0 at day 3 was significantly higher for group 1 (odds ratio, 8.3; 95% CI, 1.3–57.0; $P = 0.027$) and group 2 (odds ratio, 4.7; 95% CI, 1.9–13.4; $P = 0.002$), compared with group 4 (Fig. 1). Group 3 was excluded from the analysis because C_0 data were available for only a single patient (C_0 = 14.9 μg/L). No significant differences were observed across the different genotype clusters with respect to the risk of a C_0 <10 μg/L (data not shown).

CYP3A4 GENOTYPE, DGF, CREATININE CLEARANCE, AND ACUTE REJECTION

Of the 185 patients, DGF was observed in 38 patients, 2 of whom carried the *CYP3A4* intron 6 T allele. No significant differences were observed in DGF incidence [χ^2 (1) = 0.12; $P = 0.72$] or in creatinine clearance (Table 2) between T variant carriers and CC patients. Similarly, we observed no differences between groups of combined genotypes for *CYP3A4* and *CYP3A5* SNPs, in creatinine clearance (Table 4). BPAR occurred in 37 of the 185 patients, 4 of whom carried *CYP3A4* intron 6 variant T, but no significant differences in BPAR incidence were observed between carriers of the T variant and CC patients [χ^2 (1) = 1.42; $P = 0.23$]. Similarly, we did not find any significant difference in the incidence of either DGF or BPAR among different clusters of combined genotypes with respect to *CYP3A4* intron 6 and *CYP3A5*3* allelic status [χ^2 (3) = 0.66 ($P = 0.89$) and 4.52 ($P = 0.18$), respectively].

Discussion

We show for the first time that the new *CYP3A4* intron 6 C>T SNP is associated with lower Tac dose requirements, in agreement with the reduced function of this

Table 4. Tac dose, C_0, and dose-adjusted C_0 according to the combined CYP3A4 intron 6 C>T SNP and CYP3A5 genotype.

	Group 1	n	Group 2	n	Group 3	n	Group 4	n	*P*, ANOVA[a]	*P*, polynomial[a,b]
Tac dose, mg/day										
Day 3	13.0 (10.9–15.1)	7	13.1 (12.4–13.8)	111	13.0 (0.3–25.7)	3	14.0 (12.0–15.9)	25	0.78	—
Day 10	8.3 (5.9–10.6)	8	11.8 (10.8–12.8)	109	13.0 (0.3–25.7)	2	17.6 (15.0–20.2)	25	**<0.001**	**<0.001**
Month 1	6.5 (5.0–8.0)	8	10.3 (9.5–11.0)	112	7.0 (−5.7 to 19.7)	2	15.2 (13.1–17.2)	25	**<0.001**	**<0.001**
Month 3	5.0 (3.8–6.2)	9	6.7 (6.2–7.3)	105	6.0 (6.0–6.0)	2	10.5 (8.5–12.6)	26	**<0.001**	**0.001**
Month 6	4.3 (3.2–5.3)	8	5.3 (4.8–5.9)	97	5.5 (−0.9 to 11.9)	2	9.7 (7.9–11.6)	23	**<0.001**	**<0.001**
Month 12	3.9 (2.9–4.9)	8	4.6 (4.1–5.1)	91	10.0	1	7.9 (6.2–9.6)	21	**<0.001**	**<0.001**
Weight-adjusted Tac dose, mg · day^{-1} · (kg body weight)$^{-1}$										
Day 3	0.190 (0.173–0.207)	7	0.178 (0.171–0.185)	110	0.201 (0.012–0.222)	2	0.197 (0.172–0.222)	25	0.18	—
Day 10	0.118 (0.089–0.146)	8	0.160 (0.147–0.174)	108	0.201 (0.012–0.390)	2	0.245 (0.212–0.277)	25	**<0.001**	**<0.001**
Month 1	0.094 (0.070–0.118)	8	0.143 (0.132–0.154)	111	0.108 (−0.084 to 0.300)	2	0.212 (0.189–0.235)	25	**<0.001**	**<0.001**
Month 3	0.072 (0.054–0.089)	9	0.093 (0.084–0.102)	104	0.093 (0.089–0.096)	2	0.152 (0.122–0.183)	26	**<0.001**	**0.001**
Month 6	0.062 (0.048–0.075)	8	0.074 (0.065–0.082)	96	0.085 (−0.010 to 0.180)	2	0.142 (0.112–0.173)	23	**<0.001**	**<0.001**
Month 12	0.058 (0.042–0.074)	8	0.062 (0.054–0.070)	90	0.155	1	0.114 (0.088–0.140)	21	**<0.001**	**<0.001**
C_0, μg/L[c]										
Day 3	21.5 (14.2–32.5)	7	15.8 (14.7–17.1)	118	14.9	1	11.2 (9.1–13.7)	26	**<0.001**	**0.004**
Day 10	13.0 (10.5–16.0)	8	11.7 (11.0–12.4)	110	15.3	1	10.8 (9.3–12.5)	23	0.40	—
Month 1	12.8 (10.2–16.2)	9	12.7 (12.0–13.4)	120	12.4 (7.9–19.7)	2	11.5 (9.7–13.6)	25	0.62	—
Month 3	11.1 (8.9–13.0)	10	10.4 (9.9–11.0)	120	9.3	1	9.4 (8.4–10.5)	25	0.36	—
Month 6	9.1 (7.1–11.7)	9	8.7 (8.1–9.3)	105	9.0	1	8.2 (6.9–9.9)	20	0.90	—
Month 12	9.5 (6.4–14.0)	8	7.2 (6.6–8.0)	91	—	0	6.8 (5.1–9.0)	19	0.27	—
C_0/dose, μg/L per mg/kg[c]										
Day 3	113.6 (78.4–164.7)	7	91.7 (83.9–100.3)	100	80.1	1	61.6 (53.3–71.1)	25	**<0.001**	**0.006**
Day 10	104.3 (84.5–128.9)	7	83.0 (74.6–92.3)	97	82.2	1	46.6 (39.8–54.4)	23	**<0.001**	**0.003**
Month 1	142.3 (100.9–200.6)	8	97.5 (89.3–106.3)	103	116.2 (12.3–1100.0)	2	56.2 (46.8–67.4)	25	**<0.001**	**<0.001**
Month 3	123.3 (115.9–226.2)	9	127.9 (114.9–142.3)	100	100.0	1	65.0 (52.3–80.7)	24	**<0.001**	**<0.001**
Month 6	148.3 (105.3–208.7)	8	140.2 (123.0–159.9)	86	116.1	1	62.8 (46.4–85.0)	19	**<0.001**	**0.001**
Month 12	171.8 (111.7–276.8)	8	131.2 (116.4–147.8)	80	—	0	63.0 (42.3–93.9)	18	**<0.001**	**<0.001**
Creatinine clearance, mL/min										
Day 3	43.9 (16.4–71.3)	8	36.1 (31.6–40.6)	125	34.3 (−202.9 to 271.4)	2	34.6 (23.6–45.2)	26	0.85	—
Day 10	49.0 (25.9–72.0)	9	44.1 (39.8–48.3)	123	40.2 (−64.8 to 145.2)	2	50.0 (39.4–60.7)	25	0.67	—
Month 1	59.1 (40.0–78.2)	9	55.2 (51.1–59.2)	123	49.2 (19.5–78.9)	2	58.0 (48.8–67.2)	25	0.88	—
Month 3	67.6 (50.8–84.4)	10	60.0 (56.0–63.9)	118	52.5	1	60.9 (49.1–72.7)	26	0.77	—
Month 6	68.1 (50.5–85.8)	9	63.5 (59.2–67.7)	107	48.5	1	64.9 (53.6–76.1)	23	0.84	—
Month 12	60.9 (40.8–81.0)	8	65.4 (61.1–69.8)	94	45.0	1	66.1 (55.1–77.1)	20	0.75	—

[a] Statistically significant results ($P < 0.05$) are highlighted in boldface.
[b] The corresponding linear contrast tested the probability of a positive linear trend of the dependent variable across the ordered level of the genotype classification (a priori polynomial linear contrast test).
[c] Values are expressed as the geometric mean, (95% CI).

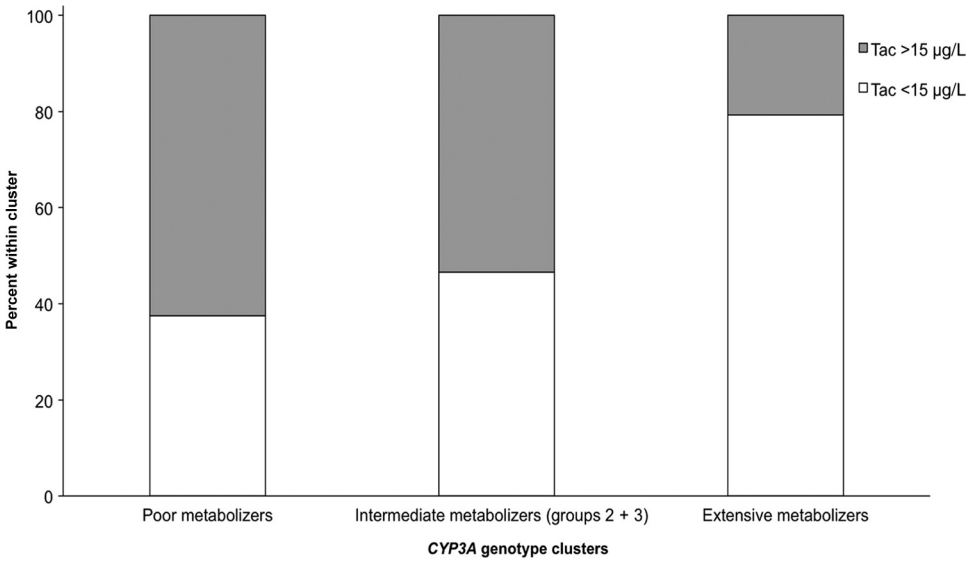

Fig. 1. Percentage of patients within each *CYP3A* metabolizer cluster stratified by day 3 values of C_0 below or above the 15-μg/L supratherapeutic threshold.

CYP3A4 variant and the expected reduced clearance of Tac *(6)*. We have demonstrated that de novo kidney transplant recipients who carry 1 or 2 T alleles require significantly lower Tac doses to reach the target C_0 than wild-type CC patients. During the first year after transplantation, carriers of the T allele required a 33% lower mean Tac dose compared with the wild-type patients.

Our findings are in agreement with those of Wang et al., who addressed the functional defect caused by this SNP *(6)* and showed that this SNP is significantly linked to reductions in *CYP3A4* mRNA production and enzyme activity in human livers. Thus far, this *CYP3A4* SNP is the only one that has a relatively high allele frequency in Caucasians (2.5%–6.9%, http://www.ncbi.nlm.nih.gov/projects/SNP) and that shows such a large effect. A recent report by Jacobson et al. *(24)* described 3 other *CYP3A4* polymorphisms with respect to Tac pharmacokinetics, but these were observed only in Africans. In our study, only 8 of the patients were of African origin, which is why we did not include these SNPs.

Combining *CYP3A4* and *CYP3A5* genotypes revealed an increased significance of the observed effects on Tac pharmacokinetics compared with the *CYP3A4* or *CYP3A5* genotype alone. This effect was allele-dose dependent and was influenced quantitatively by genotype classification: at all time points, the Tac dose requirement was lowest for CYP3A poor metabolizers, followed by intermediate-1 metabolizers, intermediate-2 metabolizers, and, finally, extensive metabolizers. All groups were significantly different ($P = 0.001$), except for day 3 ($P = 0.78$). This latter observation reflects the fact that at this time point no dose adjustments could have been made on the basis of therapeutic drug monitoring, and dosing thus was independent of genotype or metabolizer status. Similarly, the dose-adjusted C_0 was affected significantly by the *CYP3A4* and *CYP3A5* combined genotype: extensive metabolizers < intermediate-2 metabolizers < intermediate-1 metabolizers < poor metabolizers—demonstrating that poor metabolizers require lower doses to achieve a target C_0 at all time points (including at day 3 after transplantation) than the other groups. Therefore, genotype classification might lead to a better prediction of the optimal Tac starting dose.

The risk of a supratherapeutic C_0 (>15 μg/L) on day 3 was significantly higher for poor and intermediate-1 metabolizers than for extensive metabolizers. This risk was even more pronounced among poor metabolizers than among intermediate-1 metabolizers. We observed that both poor and intermediate-1 metabolizers had a mean C_0 at day 3 of >15 μg/L (21.5 μg/L and 15.8 μg/L, respectively). We reported earlier that a significantly larger proportion of patients carrying the *CYP3A5*1* allele had a C_0 <10 μg/L *(18)*. When the genetic status for the *CYP3A4* intron 6 SNP was taken into account, no significant differences were observed with respect to the risk of presenting a Tac concentration below this threshold (data not shown). As was recently suggested, it is likely that clinicians are able to target the C_0 above this threshold rapidly after transplantation by performing

simple concentration-controlled Tac dose adjustments without consideration of *CYP3A5* status *(13)*. In the present study, 15% of patients had a C_0 <10 μg/L at day 3, and 2 patients had a C_0 <5 μg/L. Approximately 50% of the patients had a C_0 >15 μg/L. Neither a subtherapeutic C_0 at day 3 nor the *CYP3A5*1* allele was associated with BPAR within 1 month after surgery (data not shown), a result in accordance with previous studies *(18, 21, 25–27)*. We found that 50% of patients did overshoot the upper limit of Tac exposure, whereas only 15% had Tac exposures of <10 μg/L. This result indicates that overexposure is a problem more frequently encountered than underexposure. It may be especially relevant in patients experiencing DGF. Regarding *ABCB1*, we found the 3435C>T SNP to be independently associated with the dose-adjusted C_0. The influence of this SNP (14.3% and 20.9% lower dose-adjusted C_0 for heterozygotes and homozygotes, respectively) was modest compared with the effects of the *CYP3A4* and *CYP3A5* polymorphisms and disappeared in the mixed-model analysis in which the *CYP3A4* and *CYP3A5* genotype were included. The relatively minor contribution of the *ABCB1* polymorphism to Tac pharmacokinetics is in line with previous investigations *(28–30)*.

The present study has limitations. Although most participating centers have used immunoassays to measure Tac concentrations, some centers applied a liquid chromatography–tandem mass spectrometry approach. In an additional mixed-model analysis in which we adjusted for Tac assay by introducing a dummy variable as a random effect, the effect of *CYP3A4* intron 6 genotype was still significant, both for the Tac daily dose (−20% for carriers of the T allele, $P = 0.007$) and for the dose-adjusted C_0 (+37% for T allele carriers, $P < 0.001$). Second, corticosteroids are known to influence Tac exposure *(31, 32)*. Given that corticosteroid tapering was recommended but not mandatory, the different centers may have used different corticosteroid regimens, which we cannot exclude from having influenced the analysis. If all patients had been treated with the same dose, the influence of genotype might have been stronger by reducing the uncontrolled variation generated by different tapering regimens. Unfortunately, the corticosteroid dose could not be included in the mixed-model analysis because different formulations with different immunosuppressive potencies were used. Third, diabetic gastrointestinal-motility disorders can affect Tac pharmacokinetics. Although diabetic gastropathy may alter the curve of the Tac area under the ROC curve during a dosing interval, C_0 values are generally less affected *(33)*, and we therefore believe that the influence of diabetes on the outcomes of the present study was limited. Fourth, our set contained some missing data points. To overcome this limitation, we performed mixed-model analysis, which compensates for missing records. The correct use of mixed-model analysis requires that data be missing at random. We investigated this criterion, and, indeed, the proportion of missing data for carriers of the *CYP3A4* intron 6 T allele was not significantly different from that of noncarriers with respect to C_0 ($P = 0.71$), Tac dose ($P = 0.14$), C_0/dose ($P = 0.28$), and creatinine clearance ($P = 0.24$). Finally, we realize that our findings are significant at a 95% CI. Therefore, our results need to be confirmed with independent cohorts.

In conclusion, we have shown that the new genetic *CYP3A4* intron 6 polymorphism was associated with reduced Tac clearance in our patient cohort. Therefore, pretransplantation genotyping of the *CYP3A4* intron 6 C>T SNP, along with *CYP3A5*3*, could potentially benefit patients by reducing initial Tac doses among CYP3A poor metabolizers and thereby reduce the risk of reaching supratherapeutic Tac concentrations.

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