Genotype-guided tacrolimus dosing in African-American kidney transplant recipients

K Sanghavi1, RC Brundage1, MB Miller2, DP Schladt3, AK Israni4, W Guan5, WS Oetting1, RB Mannon6, RP Remmel7, AJ Matas4, PA Jacobson1 for the DEKAF Investigators8

Tacrolimus is dependent on CYP3A5 enzyme for metabolism. Expression of the CYP3A5 enzyme is controlled by several alleles including CYP3A5*1, CYP3A5*3, CYP3A5*6 and CYP3A5*7. African Americans (AAs) have on average higher tacrolimus dose requirements than Caucasians; however, some have requirements similar to Caucasians. Studies in AAs have primarily evaluated the CYP3A5*3 variant; however, there are other common nonfunctional variants in AAs (CYP3A5*6 and CYP3A5*7) that do not occur in Caucasians. These variants are associated with lower dose requirements and may explain why some AAs are metabolically similar to Caucasians. We created a tacrolimus clearance model in 354 AAs using a development and validation cohort. Time after transplant, steroid and antiviral use, age and CYP3A5*1, *3, *6 and *7 alleles were significant toward clearance. This study is the first to develop an AA-specific genotype-guided tacrolimus dosing model to personalize therapy.

The Pharmacogenomics Journal (2017) 17, 61–68; doi:10.1038/tpj.2015.87; published online 15 December 2015

INTRODUCTION

Kidney transplantation is a common and effective treatment for end-stage renal disease. African Americans (AAs) represent ~ 34% of the candidates in the kidney transplant waiting list.1,2 Long-term graft survival rates are lower and all-cause mortality rates are higher in AAs than in Caucasians or Asians.3–6 There are several reasons cited for poor outcomes including greater variation in human leukocyte antigen, immunological differences, higher medical nonadherence, socioeconomic barriers and pharmacokinetic differences of the immunosuppressive agents including tacrolimus.7,8 Tacrolimus has a narrow therapeutic index9–13 with wide interindividual variability in pharmacokinetics resulting in unpredictable blood concentrations.14–16 This necessitates therapeutic drug monitoring to avoid subtherapeutic and supratherapeutic concentrations that place the recipient at risk of rejection and toxicity, respectively.17,18 There is a significant difference in tacrolimus pharmacokinetics by race where AAs have 20–50% lower bioavailability, higher clearance and lower blood concentrations as compared with Caucasians.19–23 To achieve target tacrolimus trough concentrations some AAs require ~ 1.5 to 2 times higher doses than Caucasians.24–29 However, not all AAs will require a higher dose and these individuals may have nonfunctional genetic variants that lead to reduced metabolic capacity similar to Caucasians. Tacrolimus is metabolized by hepatic and intestinal CYP3A4 and CYP3A5 enzymes.14,30 CYP3A5 is a more efficient catalyst of tacrolimus metabolism as compared with CYP3A4.31 Tacrolimus is also a substrate of P-glycoprotein that is an efflux transporter expressed on enterocytes.32,33 Genetic variants associated with CYP3A5, CYP3A4, P450 (cytochrome) oxidoreductase (POR) and P-glycoprotein have been studied for their influence on tacrolimus clearance, although CYP3A5 variants have demonstrated major clinical relevance.23,30,34–44

CYP3A5*3 is an intronic variant that generates a cryptic splice site resulting in a nonfunctional enzyme.45–47 The presence of the CYP3A5*3 allele is associated with lower oral tacrolimus clearance (Cl/F), whereas the CYP3A5*1 allele is associated with high Cl/F (CYP3A5*1/*1 individuals ~ 1 h⁻¹ kg⁻¹ CYP3A5*1/*3 ~ 0.81 h⁻¹ kg⁻¹ vs CYP3A5*3/*3 ~ 0.51 h⁻¹ kg⁻¹).14,48,49 Therefore, the dose requirements for CYP3A5*1/*1 or *1/*3 carriers are ~ 1.5–1.7-fold higher than CYP3A5*3/*3 carriers.23,40,42,50,51 These genotypes are also associated with delays in achieving therapeutic concentrations.32,52

CYP3A5*6 is a missense mutation that codes for a splicing defect, deleting exon 7 resulting in absence of CYP3A5 enzyme and activity.52 CYP3A5*7 is a frameshift mutation due to an insertion within codon 346 and termination of protein synthesis.56,57 Few studies have evaluated the association between CYP3A5*6 and *7 alleles and tacrolimus pharmacokinetics.58–59 Brazilian transplant recipients carrying two CYP3A5 variant alleles (*3, *6 or *7) had higher tacrolimus trough concentrations compared with those who did not (P < 0.0001).57 However, no clearance models with dosing algorithms have been developed to account for these common AA variants. Algorithms that do not account for these alleles may incorrectly approximate clearance and dosing requirements. The objective of this study was to develop an AA dosing model that comprehensively includes the common AA-specific CYP3A5 variants.

1Department of Experimental and Clinical Pharmacology, College of Pharmacy, University of Minnesota, Minneapolis, MN, USA; 2Department of Psychology, University of Minnesota, Minneapolis, MN, USA; 3Department of Nephrology and Chronic Disease Research Group, Minneapolis Medical Research Foundation, Hennepin County Medical Center, Minneapolis, MN, USA; 4Department of Surgery, University of Minnesota, Minneapolis, MN, USA; 5Department of Biostatistics, University of Minnesota, Minneapolis, MN, USA; 6Department of Nephrology, University of Alabama, Birmingham, AL, USA and 7Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN, USA. Correspondence: Dr PA Jacobson, Department of Experimental and Clinical Pharmacology, Weaver Densford Hall, 7-151, 308 Harvard Street SE, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455, USA.
E-mail: jacob117@umn.edu

8The DEKAF Investigators are listed before References.

Received 28 April 2015; revised 7 October 2015; accepted 2 November 2015; published online 15 December 2015
MATERIALS AND METHODS

Subjects

The data for this analysis were obtained from our multicenter observational trial (DEKAF Genomics, clinicaltrials.gov NCT00270712). The study was approved by institutional review board and an informed consent was obtained from each subject before the study. AA kidney transplant recipients (n = 354) ≥ 18 years old who received tacrolimus maintenance immunosuppression from 6 centers in the United States and Canada were studied. Tacrolimus was administered orally once or twice daily. The initial dose was based on weight and doses adjusted to achieve each institution’s target trough concentrations. Trough blood concentrations (n = 6037) were measured at each center and, in general, concentrations of 8–12 ng ml⁻¹ were targeted for the first 3 months and 6–10 ng ml⁻¹ for 3–6 months after transplant. A median (range) of 18 (1–24) concentrations were obtained from each subject in the first 6 months after transplant and, if available, concentrations were obtained twice each week for the first 2 months, and then twice in each month up to 6 months. The concentrations were quantified in each center by their standard analysis technique. The majority (92.9%) of concentrations were measured by liquid chromatography with mass spectroscopy in CLIA (Clinical Laboratory Improvement Amendments)-certified labs.

Genotypes

Genotyping was performed on recipient DNA isolated from peripheral blood. Single-nucleotide polymorphisms CYP3A4*22 (rs776746, g.6986A>g), CYP3A5*6 (rs102612472, g.14690 G>A) and CYP3A5*7 (rs41303343, g.27131-27132insT) were found to be significant in our previous genome-wide association study analysis and therefore were chosen for this analysis. The allele frequency of CYP3A4*3 (G allele), CYP3A4*6 (T allele), CYP3A5*7 (T allele) and CYP3A4*22 (A allele) were 29.0%, 12.3%, 8.8%, 19.0% and 2.4%, respectively.

Population modeling of trough concentrations

The 354 subjects were randomly divided into a development (60%) and a validation cohort (40%). The data from the development cohort (212 subjects with 3704 troughs) was used to evaluate the developed model. To assess differences in demographics, clinical and genotype distributions, a two-sample t-test (for continuous factors) and sample proportion test (for categorical factors) were performed using R software (R Core Development Team, Vienna, Austria) package. Nonlinear mixed effect modeling was used to develop the CI/F model with NONMEM (version 7.2, ICON, Development Solutions, Hanover, MD, USA) software. The NONMEM execution, model diagnostics, covariate testing and bootstrapping were conducted with Perl Speaks NONMEM (PsN) toolkit and the Xpose4 package through Pirana workbench (version 2.7.2, Amsterdam, The Netherlands). R studio 0.98.501 was used for predictive performance checks. A steady-state infusion model was used to develop the pharmacokinetic base model using SPRED library in NONMEM. In the absence of intravenous data for the tacrolimus, it was not possible to calculate oral bioavailability. Therefore, tacrolimus apparent oral clearance (CI/F), which is the ratio of total clearance (Cl) to the bioavailability (F), was used to regress steady-state tacrolimus concentrations (Css, av) to the administered dose. CI/F was related to tacrolimus trough concentrations by the following equation:

\[ C_{ij} = C_{pred,i} + \epsilon_i \]  

(3)

where, \( C_{ij} \) is the \( j^{th} \) observed tacrolimus trough concentrations in the \( i^{th} \) individual, \( C_{pred,i} \) is the \( i^{th} \) predicted tacrolimus trough concentrations in the \( i^{th} \) individual and \( \epsilon_i \) is the residual unexplained variability and where \( \epsilon \sim N(0, \sigma^2) \). FOCE interaction was used as the NONMEM estimation method.

Covariate analysis

Clinical factors and genotypes were tested for their influence on tacrolimus TCV/F. Covariates tested were recipient and donor age, gender, days after transplant, steroid use (prednisone, methylprednisolone) at each trough measurement, calcium channel blocker use at each trough measurement, angiotensin-converting enzyme inhibitor use at each trough measurement, cytomegalovirus serostatus at the time of transplant (antibody positive or negative), anti-cytomegalovirus viral drug (as prophylaxis) use at each trough measurement, diabetes diagnosis at the time of transplant, glomerular filtration rate calculated by the Modification of Diet in Renal Disease equation as a time-varying covariate, body mass index (kg m⁻²), actual body weight (kg) at baseline (time of transplant) and actual body weight (kg) at the time of trough measurement as a time-varying covariate. Alleles tested were CYP3A4*3, CYP3A5*6, CYP3A5*7, POR*28 and CYP3A4*22. Recipients who did not carry any CYP3A4*3, *6 or *7 alleles were designated as CYP3A5*1/*1 genotype and those who carried one CYP3A4*3, *6 or *7 alleles were designated CYP3A5*1/*1, *1/*6 or *1/*7 genotype, respectively. Recipients were classified into one of nine CYP3A45 genotypes (CYP3A45 *3/*3, *3/*6, *3/*7, *6/*6, *7/*7, *6/*13, *13/*13, *13/*6, *13/*7 and *17/*17). Recipients were also classified based on POR (POR*1/*1, *1/*28 or *28/*28) and CYP3A4 (CYP3A4*1/*1 or *1/*22) genotype. No subjects had the CYP3A5*7/*7 or CYP3A4*22/*22 genotype. Recipient age, donor age and days after transplant were tested both as continuous (using linear, exponential and power models) and categorical covariates. All other clinical factors were tested as categorical covariates. A strategy of forward inclusion and backward elimination was tested for inclusion of the covariates. In NONMEM, minimization of –2 log likelihood is used as a model statistic and is given by the objective function value; measure of goodness of fit similar to sum of squares. The significance of inclusion of each covariate was tested based on likelihood ratio test that follows the \( \chi^2 \) distribution. A lower objective function value is considered to be a better fit and a decrease in the objective function value by ≥ 3.8 (P < 0.05) was considered significant for forward inclusion and an increase in objective function value by 6.6 (P < 0.01) was chosen for backward elimination.

Model evaluation

To evaluate the precision of the parameter estimates, a nonparametric bootstrap approach was performed using the development cohort. The method used random sampling with replacement to generate 1000 bootstrapped data sets using PsN toolkit. The final model developed with NONMEM was fit to each of the bootstrapped data sets and the parameters were obtained with their 5th and 95th prediction intervals. The model was also validated by using subjects in the validation cohort. The final model parameters were fixed in NONMEM (the estimation method was set to MAXEVAL = 0 with the POSTHOC option) and were used to predict trough concentrations in validation cohort subjects. Population-predicted trough concentrations (PRED) were obtained for each observed concentration (the dependent variable (DV)) given their actual administered dose, the time after transplant, significant clinical covariates and genotypes (those identified from the development model). Median prediction error (MPE) and median percentage prediction error (MPPE) was then used to calculate the bias in model predictions and median absolute prediction error (MAPE) was used to calculate the imprecision.

The following equations were used:

\[ MPE = \text{Median} ([\text{PRED} - \text{DV}] / \text{DV} \times 100) \]

\[ MAPE = \text{Median} ([|\text{PRED} - \text{DV}|]) \]

RESULTS

Characteristics of the subjects in the development and validation cohorts are shown in Table 1. The median (range) daily dose and
Tacrolimus trough concentrations did not differ between the cohorts. The median tacrolimus concentrations were low during the first week after transplant and slowly increased over time until month 2 (2.8, 5.3, 6.3, 6.9, 6.9, 7 and 7.1 ng ml\(^{-1}\) in weeks 1–8 and 7.4, 7.2, 6.9 and 7 ng ml\(^{-1}\) in months 3–6, respectively). Tacrolimus TVCl/F was 54.6 l h\(^{-1}\) and was significantly influenced by recipient age, steroid and antiviral coadministration, days after transplant and CYP3A5*1/*3, *3/*3, *1/*6, *1/*7, *3/*6, *6/*6, *6/*7 and *3/*7 genotypes. All other tested covariates were not significant. The effect of genotypes and clinical covariates on tacrolimus TVCl/F and final parameter estimates in the model development cohort and in the bootstrap analysis are shown in Table 2. The interindividual variability in TVCl/F after inclusion of covariates was 48.6%. Days after transplant was the most important covariate where TVCl/F was 33% higher in the first 9 days after transplant compared with after 9 days. Days after transplant was first tested as continuous covariate but the model failed to converge and hence was modeled as a categorical covariate. The plot of dose-normalized trough concentrations over time showed a general increase in concentrations early after transplant and hence was modeled as a continuous covariate but the model failed to converge and hence was only categorized as a bivariate. Tacrolimus TVCl/F increased by 23% with concomitant steroid

---

**Table 1.** Patient demographics

|                           | All subjects | Development cohort subjects | Validation cohort subjects | P-value* |
|---------------------------|--------------|-----------------------------|---------------------------|----------|
| No. of subjects           | 354          | 212                         | 142                       |          |
| No. of male subjects (%)  | 227 (64)     | 140 (63)                    | 87 (61)                   | 0.35     |
| Daily dose (mg)\(^b\)     | 8 (0.50–36)  | 8 (0.5–36)                  | 8 (1–30)                  | 0.17     |
| No. of troughs            | 3637         | 3704                        | 2333                      | 0.09     |
| Tacrolimus trough (ng ml\(^{-1}\))\(^b\) | 6.50 (0.10–65.60) | 6.50 (0.10–65.60) | 6.40 (0.70–50.00) | 0.34     |
| Weight at baseline (kg)\(^b\) | 85 (42–140)  | 85 (42–140)                 | 83 (47–137)               | 0.34     |
| GFR by MDRD ml min\(^{-1}\) per 1.73 m\(^2\)\(^bc\) | 55.89 (6.18–168.28) | 55.88 (6.18–168.28) | 55.24 (14.25–122.71) | 0.08     |
| No. recipients in age category (%) |  |  |  |  |
| 18–34 years               | 66 (19)      | 36 (17)                     | 30 (21)                   | 0.32     |
| 35–64 years               | 268 (76)     | 163 (77)                    | 105 (74)                  | 0.52     |
| >64 years                 | 20 (6)       | 13 (6)                      | 7 (5)                     | 0.63     |
| Age at transplant\(^b\)   | 48 (20–73)   | 47 (20–73)                  | 49 (21–72)                | 0.57     |
| No. receiving dialysis at time of transplant (%) | 56 (16) | 34 (16) | 22 (15) | 0.50 |
| No. with diabetes at transplant (%) | 129 (36) | 79 (37) | 50 (35) | 0.69 |
| No. of troughs with calcium channel blocker (%) | 2944 (49) | 1838 (50) | 1106 (53) | 0.01 |
| No. of troughs with ACE inhibitor (%) | 905 (15) | 522 (14) | 383 (16) | 0.01 |
| No. of troughs with antiviral drug (%) | 3441 (57) | 2128 (57) | 1313 (56) | 0.001 |
| No. of troughs with steroid (%) | 3283 (54) | 1941 (52) | 1342 (58) | 0.46 |
| Simultaneous pancreas and kidney transplant (%) | 16 (5) | 11 (5) | 5 (4) | 0.64 |
| No. with living donor (%) | 172 (31) | 108 (30) | 64 (31) | 0.27 |
| No. with prior transplant (%) | 34 (10) | 22 (10) | 12 (8) | 0.54 |
| Primary cause of kidney disease (%) |  |  |  |  |
| Diabetes                  | 94 (27)      | 58 (27)                     | 36 (25)                   | 0.67     |
| Glomerular nephritis      | 50 (14)      | 28 (13)                     | 22 (15)                   | 0.54     |
| Hypertension              | 148 (42)     | 93 (44)                     | 55 (39)                   | 0.34     |
| Polycystic kidney disease | 11 (3)       | 4 (2)                       | 7 (5)                     | 0.1      |
| Other                     | 44 (12)      | 26 (12)                     | 18 (13)                   | 0.91     |
| Unknown                   | 7 (2)        | 3 (1)                       | 4 (3)                     | 0.35     |
| No. of individuals with genotype (%) |  |  |  |  |
| CYP3A5*1/*3               | 96 (27)      | 65 (31)                     | 31 (22)                   | 0.07     |
| CYP3A5*3/*3               | 34 (10)      | 20 (9)                      | 14 (10)                   | 0.89     |
| CYP3A5*1/*7               | 36 (10)      | 14 (7)                      | 22 (15)                   | 0.006    |
| CYP3A5*7/*7               | 0            | 0                           | 0                         | 0.55     |
| CYP3A5*1/*6               | 47 (13)      | 30 (14)                     | 17 (12)                   | 0.15     |
| CYP3A5*6/*6               | 4 (1)        | 1 (0.5)                     | 3 (2)                     | 0.26     |
| CYP3A5*3/*6               | 21           | 15 (7)                      | 6 (4)                     | 0.59     |
| CYP3A5*3/*7               | 15 (4)       | 8 (4)                       | 7 (5)                     | 0.32     |
| CYP3A5*6/*7               | 11 (3)       | 5 (2)                       | 6 (4)                     | 0.77     |
| CYP3A5*1/*1               | 89 (23)      | 49 (23)                     | 31 (21)                   | 0.007    |
| CYP not determined\(^d\)  | 7 (2)        | 3 (1)                       | 4 (3)                     | 0.35     |

**Abbreviations:** ACE, angiotensin-converting enzyme; GFR, glomerular filtration rate; MDRD, Modification of Diet in Renal Disease. *P*-value is the comparison of model development and validation cohorts. \(^b\)Data are median (range). \(^c\)GFR is glomerular filtration rate calculated by MDRD equation. \(^d\)These individuals did not have one or more of the CYP3A5 genotypes available and were excluded from all analyses.
use and reduced by 8% with concomitant antiviral use. Tacrolimus TVCI/F was 24% greater in subjects under the age of 34 years vs older subjects. Similar to days after transplant, age as a continuous covariate had problems with model convergence, giving unrealistic parameter estimates. Hence, age was categorized based on clinical definition of young age (18–34 years), middle age (35–64 years) and older age (>64 years). In the current study, only 6% of AA patients were >64 years old, and therefore we were unable to test the effect of the older age group and therefore was combined with age group 35–64 years.

In subjects with CYP3A5*1/*3, *1/*6 or *1/*7 genotypes, the tacrolimus TVCI/F decreased by 16.2%, 8.2% and 24.1%, respectively, compared with CYP3A5*1/*1 genotype. For CYP3A5*3/*3, *3/*6, *3/*7 or *6/*7, the TVCI/F declined by 51%, 36.5%, 54.5% and 44.2%, respectively, relative to CYP3A5*1/*1. Only one subject had *6/*6 genotype in the development cohort and therefore *6/*6 was not evaluated independently. To build a parsimonious model and to improve the power, we combined the genotypes with similar effect sizes and overlapping confidence intervals on tacrolimus TVCI/F and reran the model. The tacrolimus TVCI/F decreased by 47% in subjects carrying two loss-of-function alleles (CYP3A5*3/*3 or *3/*6 or *3/*7 or *6/*7) and by 15% in subjects carrying one loss-of-function allele (CYP3A5*1/*3, *1/*6 or *1/*7) compared with the CYP3A5*1/*1. The POR*28 and CYP3A4*22 genotypes did not influence TVCI/F.

To examine the goodness of fit, diagnostic plots were assessed during model development (Figure 1). Histograms of \( \eta_1 \) and CI/F satisfied conditions of normal and log-normal distribution, respectively. Figures 1a and b show the plots of observed concentration vs population-predicted concentration and observed concentrations vs individual-predicted concentrations. Figures 1c and d show the conditional weighted residuals (CWRES) vs independent variables, population-predicted concentration and time. Although the model underpredicted slightly at higher concentrations, most of the data are evenly distributed across the line of unity. In addition, the CWRES do not show any specific trends of model misspecification. Thus, the model adequately explains the observed data. The final tacrolimus TVCI/F model with clinical factors and genotypes is as follows:

\[
\text{Tacrolimus TVCI/F (l h}^{-1}) = 54.61 \text{h}^{-1} \times (1.33, \text{if days} < 9\text{ after transplant}) \times (0.53, \text{if CYP3A5*3/*3 or CYP3A5*3/*7 or CYP3A5*3/*6 or CYP3A5*6/*7 or CYP3A5*6/*6}) \times (0.85, \text{if CYP3A5*1/*3 or CYP3A5*1/*6 or CYP3A5*1/*7} \times (1.23, \text{if receiving a steroid}) \times (0.92, \text{if receiving an anti-cytomegalovirus viral drug}) \times (1.24, \text{if recipient age 18–34 years}).
\]

Model evaluation using bootstrap

Table 2 shows the median of the parameter estimates and their 95% prediction intervals obtained from 1000 bootstrap runs. Out of 1000 runs, 991 runs minimized successfully and the estimates from each bootstrap run were used to calculate the median and 95% interval. Parameter estimates for fixed and random effects obtained from the original data set fell within the prediction interval of the estimates obtained from bootstrap, therefore indicating that the model is robust and reproducible.

Table 2: The effect of genotypes and clinical covariates on tacrolimus clearance (Cl/F) and final parameters estimates

| Parameter/covariate | Model development cohort. Estimate (%RSE) of the effect on TVCI/F | Bootstrap analysis. Median (95% confidence interval) |
|---------------------|--------------------------------------------------------------------|---------------------------------------------------|
| Typical value of Cl/F (TVCI/F) in 1h\(^{-1}\) | 54.60 (10.0%) | 54.48 (44.51–66.63) |
| Two loss-of-function alleles (CYP3A5*3/*3 or *3/*7 or CYP3A5*3/*6 or *6/*7) | 0.53 (10.9%) | 0.53 (0.43–0.66) |
| One loss-of-function allele (CYP3A5*1/*3 or CYP3A5*1/*6 or CYP3A5*1/*7) | 0.85 (9.7%) | 0.85 (0.70–1.04) |
| Less than day 9 after transplant | 1.33 (4.2%) | 1.33 (1.23–1.45) |
| Steroid drug use | 1.23 (6.9%) | 1.24 (1.07–1.42) |
| Antiviral drug use | 0.92 (2.9%) | 0.91 (0.87–0.97) |
| Recipient age (18–34 years) | 1.24 (7.8%) | 1.24 (1.07–1.47) |
| Between-subject variability* | 0.21 (18.1%) (CV% = 48.6%) | 0.21 (0.14–0.28) (CV% = 46.7% (38.76–56.84%)) |
| Residual unexplained variability in trough (ng ml\(^{-1}\)) | 2.76 (7.5%) | 2.75 (2.55–2.96) ng ml\(^{-1}\) |

Abbreviations: Cl/F, ratio of total clearance (Cl) to the bioavailability (F); RSE, relative standard error. *0.21 Represents the estimate of the variance of individual (\( \eta_1 \)), CV% is the coefficient of variance and represents interindividual variability in the population. CV% = \sqrt{\text{exp (variance)}} – 1.

Using the TVCI/F calculated using the model above and a desired target tacrolimus trough concentration, the daily tacrolimus dose can be calculated by:

\[
\text{Daily dose (mg/day)} = \frac{\text{TVCI/F} \times \text{target tacrolimus trough concentration (ng/ml)} \times 24\text{hrs}}{1000}
\]

DISCUSSION

AAs have poorer outcomes after transplantation and a possible contributory factor is high pharmacokinetic variability in immunosuppression leading to multiple dose changes and longer periods of time out of the therapeutic range.\(^ {22,26} \) On average, AAs require higher tacrolimus doses than Caucasians to achieve the same target blood concentration and most centers administer higher initial doses to AAs. However, not all individuals require higher doses and therefore some may have elevated concentrations. Median absolute prediction error was 2.32 (2.21–2.44) ng ml\(^{-1}\).
CYP3A5*6 and/or *7 alleles that also encode for low activity or nonfunctional enzyme that have not been accounted for in most studies. CYP3A5*6 and *7 are common in AAs with a minor allele frequency of 16–18% and 10–12%, respectively, but absent in Caucasians.47,63,64,66,67 We found that AAs who carry two nonfunctional alleles (*3, *6 or *7) have a tacrolimus clearance similar to Caucasians, whereas those who carry no nonfunctional alleles have high clearance. Therefore, AAs have a broad range of CYP3A5 metabolism phenotypes. To develop personalized strategies to reduce pharmacokinetic variability, we evaluated the effect of these variants on tacrolimus clearance and developed the first genotype-guided dosing model for AAs.

We found that tacrolimus TVCl/F in AAs was significantly influenced by CYP3A5*1, *3, *6 and *7 alleles, days after transplant, steroid and antiviral drug coadministration and age. The TVCl/F was 54.6 l h⁻¹ and higher than reported in non-AA studies (~22–40 l h⁻¹),14,68–71 consistent with AAs being more likely to carry a *1 expressor allele than Caucasians. The CYP3A5*3, *6 and *7 alleles were each associated with a reduction in tacrolimus clearance. Approximately 50% of our subjects carried one nonfunctional allele (CYP3A5*3/*1, *6/*1 or *7/*1) that decreased tacrolimus TVCl/F by 15%. Individually, the CYP3A5*1/*3, *1/*6 and *1/*7 genotypes decreased TVCl/F by 16.2%, 8.2% and 24.1%, respectively. In addition, ∼24% of our subjects carried two nonfunctional alleles—primarily CYP3A5*3/*3, *3/*6 and *3/*7 and *6/*6. The effect of two variant alleles was large, resulting in a decrease in tacrolimus TVCl/F by 47%. We did not observe any subject with more than two *3, *6 or *7 alleles. Based on our data and haplotype analyses by others, the probability of this occurring is very low (<0.5%).72,73

The CYP3A5*6 allele is thought to encode for nonfunctional enzyme; however, there is some uncertainty about its functionality and it may express low levels of enzyme. In our study tacrolimus TVCl/F was 24% lower in CYP3A5 *1/*7 carriers but only 8.2% lower in *1/*6 carriers relative to the *1/*1 carriers, supporting that *6 may express low levels of enzyme. Others found no difference in tacrolimus concentrations between CYP3A5*1/*1 and *1/*6 genotypes groups, although the number of subjects was small.56

In another study, CYP3A5*1/*1, *1/*3 or *1/*6 carriers had lower

---

Table 3. Predictive performance of the tacrolimus clearance model

| Predictive performance measure                             | Estimate          |
|-------------------------------------------------------------|-------------------|
| Median prediction error (MPE, 95%)                          | 0.48 (0.31–0.65)  |
| Confidence interval (CI)                                    |                   |
| Median percentage prediction error (MPPE, 95% CI)           | 9.45 (6.44–12.45) |
| Median absolute prediction error (MAPE, 95% CI)             | 2.32 (2.21–2.44)  |
Personalizing tacrolimus dose in African Americans

K Sanghavi et al

The Pharmacogenomics Journal (2017), 61–68

© 2017 Macmillan Publishers Limited, part of Springer Nature.

tacrolimus troughs than CYP3A5*3/*3 carriers but no difference in area under the curve, although only one individual carried the CYP3A5*1/*6 genotype. The influence of CYP3A5*6 and CYP3A5*7 alleles has been studied toward other CYP3A substrates and the effect may be substrate specific and therefore our results may not be generalizable to other drugs.

Day after transplant was a significant covariate toward tacrolimus where TCV/F is 33% higher in the first 9 days after transplantation compared with after day 9, consistent with other studies. The higher TCV/F may be because of early physiological changes such as fluid status, hepatic and kidney function and/or decreased bioavailability from dietary changes or concomitant medications. Concomitant steroid use was associated with a 23% higher tacrolimus TCV/F most likely because steroids induce CYP3A enzymes. We also found that younger subjects (18–34 years) had a 24% higher tacrolimus TCV/F compared with older subjects. Although some studies have not observed a significant association between tacrolimus CI/F and age, we previously showed in 1967 kidney recipients that age (18–34 vs 35–64 vs 65–84 years) had a highly significant effect on tacrolimus troughs. We found that the coadministration of antivirals reduced tacrolimus TCV/F but only by 8%. The mechanism of this effect is unknown. We did not find that calcium channel blockers were associated with TCV/F. This is likely because amiodipine is the preferred agent at our centers and has a lower potential for an interaction than other calcium channel blockers. Weight was not significant toward TCV/F. Other studies have also not found weight to be significant.

The POR*28 and CYP3A4*22 variants have been previously associated with tacrolimus concentrations but we were unable to find an association in our AA population. One or two POR*28 alleles were present in ~30% of subjects, whereas the CYP3A4*22 allele was infrequent (<5%). Our ability to detect an association with CYP3A4*22 was therefore limited.

A prospective trial, in a primarily Caucasian kidney transplant recipients, evaluated the effect of genotype-guided tacrolimus dosing vs traditional weight-based dosing. The study tested an initial dose of 0.3 mg kg⁻¹ per day p.o. in CYP3A5 expressors (CYP3A5*1) and 0.15 mg kg⁻¹ per day p.o. for nonexpressors (CYP3A5*3). The genotype-guided group had a higher proportion of patients with tacrolimus troughs within the target, fewer dose modifications and more rapid achievement of the target concentration. Although genotype-guided dosing did not reduce major clinical outcomes, it was an important study as it showed the value of genetic targeting in controlling systemic exposure. Data such as ours show that race-specific variants and clinical factors are necessary in future trials and may improve achievement of major clinical end points. The Clinical Pharmacogenetics Implementation Consortium recently published guidelines for initial tacrolimus dosing. The guidelines recommend increasing the starting dose by 1.5–2 times in extensive metabolizers (CYP3A5*1/*1) and intermediate metabolizers (CYP3A5*1/*3, *1/*6, *1/*7) and standard dose in poor metabolizers (CYP3A5*3/*3, *6/*6, *7/*7, *3/*6, *3/*7 and *6/*7). Our data support these recommendations where *6 and *7 allele carriers require lower doses.

One of the limitations of our study is that albumin, hematocrit and antifungal agent status was not available and not tested in our model. Our study used clinical trough concentrations that were obtained as part of clinical care and draw times were not supervised by our study personnel but instead overseen by the clinicians. Compliance was also assessed by the clinical site and not through the study protocol.

To our knowledge, this is the first study in which the effect of CYP3A5 alleles (*1, *3, *6, *7) common in AAs have been collectively studied toward tacrolimus clearance. We identified one or more nonfunctional CYP3A5 alleles (*3, *6 or *7) in 74.5% of our AA study population, whereas 90–95% of Caucasians will carry one or more CYP3A5*3 alleles. This is considerably higher than what has been previously presumed in the AA population. If the *6 or *7 alleles had not been genotyped, 27% of our subjects would have been inappropriately categorized as carrying two CYP3A5*1 alleles, and 10% categorized as carrying one CYP3A5*1 allele, thereby overestimating tacrolimus CI/F by nearly 50% in some individuals. Our data are consistent with a recent African study where only ~43% of individuals were considered CYP3A5 expressers as most carried one or more CYP3A5*3, *6 or *7 nonfunctional alleles.

This is the first study to develop and validate an AA-specific genotype-guided dosing model using variants common and relevant in the AA population. This study demonstrates the importance of race-specific genotypes to determine drug clearance. Using dosing models that account for the genotypes and clinical factors may lead to precision dosing of tacrolimus.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This project was supported by Grants (U19-AI070119 and U01-AI058103) from the National Institute of Allergy and Infectious Disease. We acknowledge the dedication of our coordinators and generous patients.

DEKA GENOMICS INVESTIGATORS

J Michael Cecka, UCLA Immunogenetics Center, Los Angeles, CA, USA. E-mail: mcecka@ucla.edu
Fernando G Cosio, Division of Nephrology, Mayo Clinic, Rochester, MN, USA. E-mail: FCosio.Fernando@mayo.edu
Robert Gaston, University of Alabama, Division of Nephrology, Birmingham, AL, USA. E-mail: rgaston@uab.edu
Sita Gourishankar, Division of Nephrology and Immunology, University of Alberta, Edmonton, Alberta, Canada. E-mail: sitag@ualberta.ca
Lawrence Hunscicker, Nephrology Division, Iowa City, IA, USA. E-mail: lawrence-hunscicker@uiowa.edu
Bertram Kasiske, Department of Medicine, Hennepin County Medical Center and the University of Minnesota, Minneapolis, MN, USA. E-mail: kasiski001@umn.edu
David Rush, Health Sciences Center, Winnipeg, MB, Canada. E-mail: drush@exchange.hsc.mb.ca

REFERENCES

1. McCullough KP, Keith DS, Meyer KH, Stock PG, Brayman KL, Leichtman AB. Kidney and pancreas transplantation in the United States, 1998-2007: access for patients with diabetes and end-stage renal disease. Am J Transplant 2009; 9: 894–906.
2. Matas AJ, Smith JM, Skeens MA, Thompson B, Gustafson SK, Schnitzler MA et al. OPTN/SRTR 2012 Annual Data Report: kidney. Am J Transplant 2014; 14 (Suppl 1): 11–44.
3. Fan PY, Ashby VB, Fuller DS, Boulware LE, Kao A, Norman SP et al. Access and outcomes among minority transplant patients, 1999-2008, with a focus on determinants of kidney graft survival. Am J Transplant 2010; 10: 1090–1107.
4. Gondos A, Dahlér B, Brenner H, Opelz G. Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. Transplantation 2013; 95: 267–274.
5. Press R, Carrasquillo O, Nickolas T, Radhakrishnan J, Shea S, Barr RG. Race/ethnicity, poverty status, and renal transplant outcomes. Transplantation 2005; 80: 917–924.
6. Young CJ, Gaston RS. Renal transplantation in black Americans. N Engl J Med 2000; 343: 1545–1552.
7. Eckhoff DE, Young CJ, Gaston RS, Fineman SW, Deierhoi MH, Foushee HT et al. Racial disparities in renal allograft survival: a public health issue? J Am Coll Surg 2007; 204: 894–902.
8. Martins D, Tareen N, Norris KC. The epidemiology of end-stage renal disease among African Americans. Am J Med Sci 2002; 323: 65–71.
9. deJonge H, Naesens M, Kuypers DR. New insights into the pharmacokinetics and pharmacodynamics of the calcineurin inhibitors and mycophenolic acid: possible
Personalizing tacrolimus dose in African Americans
K Sanghavi et al

consequences for therapeutic drug monitoring in solid organ transplantation. Ther Drug Monit 2009; 31: 416–435.

Kahan BD, Keown P, Levy GA, Johnston A. Therapeutic drug monitoring of immunosuppressant drugs in clinical practice. Clin Ther 2002; 24: 330–350.

Kershner RP, Fitzsimmons WE. Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. Transplantation 1996; 62: 920–926.

McMaster P, Mirza DF, Ismail T, Vennarecci G, Patapas P, Mayer AD. Therapeutic drug monitoring of tacrolimus in clinical transplantation. Ther Drug Monit 1995; 17: 602–605.

Monchaud C, Marquet P. Pharmacokinetic optimization of immunosuppressive therapy in thoracic transplantation: part I. Clin Pharmacokinet 2009; 48: 419–462.

Staats CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. Clin Pharmacokinet 2004; 43: 623–653.

Shaw LM, Holt DW, Keown P, Venkataramanan R, Yatscoff RW. Current opinions on therapeutic drug monitoring of immunosuppressive drugs. Clin Ther 1999; 21: 1632–1652.

Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet 1995; 29: 404–430.

Staats C, Taylor P. Low tacrolimus concentrations and increased risk of early acute rejection in adult renal transplantation. Nephrol Dial Transplant 2001; 16: 1905–1909.

Undre NA, van Hooff J, Christiansia M, Vanrenterghem Y, Donck J, Heeman U et al. Low systemic exposure to tacrolimus correlates with acute rejection. Transplant Proc 1999; 31: 296–298.

Dirks NL, Huth B, Yates CR, Mebohm B. Pharmacokinetics of immunosuppressants: a perspective on ethnic differences. Int J Clin Pharmacol Ther 2004; 42: 701–718.

Fitzsimmons WE, Behersky I, Dressler D, Raye K, Hodosh E, Melki Q. Demographic considerations in tacrolimus pharmacokinetics. Transplant Proc 1998; 30: 1359–1364.

Mancinelli LM, Frassetto L, Floren LC, Dressler D, Carrier S, Bekersky I et al. The pharmacokinetics and metabolic disposition of tacrolimus: a comparison across ethnic groups. Clin Pharmacol Ther 2001; 69: 24–31.

Hicklin DE, Anton HA, Krauss TC, Rodriguez V, Seaman D, Siegel C et al. Outcomes of African American kidney transplant recipients treated with sirolimus, tacrolimus, and corticosteroids. Transplantation 2002; 74: 189–193.

Jackson PS, Oetting WS, Brearley AM, Leduc R, Guan W, Schlacht D et al. Novel polymorphisms associated with tacrolimus trough concentrations: results from a multicenter kidney transplant consortium. Transplantation 2011; 91: 300–308.

Andrews PA, Sen N, Chang RW. Racial variation in dosage requirements of calcineurin inhibitors. J Clin Pharmacol 2005; 45: 3498–3501.

Hayworth S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci USA 2000; 97: 3473–3478.

Kuppers DR, de Loo R, Naesens M, Coopmans T, Hendriks H et al. Combined effects of CYP3A5*1, POR*28, and CYP3A4*22 single nucleotide polymorphisms on early concentration-controlled tacrolimus exposure in de-novo renal recipients. Pharmacogenet Genomics 2014; 24: 597–606.

Magee IA, Fredericks S, Mabile MM, Boll M, Moreton M, Carter ND, Johnston A et al. Tacrolimus pharmacogenetics: the CYP3A5*1 allele predicts low-dose-normalized tacrolimus blood concentrations in whites and South Asians. Transplantation 2005; 79: 499–502.

Pallet N, Jennos AT, El Bahri M, Etienne L, Buchler M, de Ligny BH et al. Kidney transplant recipients carrying the CYP3A4*22 allelic variant have reduced tacrolimus clearance and often reach supratherapeutic tacrolimus concentrations. Am J Transplant 2013; 13: 600–612.

Busi F, Crestell T. CYP3A5 mRNA degradation by nonsense-mediated mRNA decay. Mol Pharmacol 2005; 68: 808–815.

Hustert E, Haberl M, Turk O, Wolbold R, He YQ, Klein K et al. The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics 2001; 11: 773–779.

Kuehl P, Zhang J, Lin Y, Lamba J, Assm M, Schuetz J et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A expression. Nat Genet 2001; 27: 383–391.

de Jonge H, de Loo R, Verbeke K, Vanrenterghem Y, Kuypers DR. Impact of CYP3A5 genotype on tacrolimus versus midazolam clearance in renal transplant recipients: new insights in CYP3A5-mediated drug metabolism. Pharmacogenomics 2013; 14: 1467–1480.

Passey C, Binbaum AK, Brundage RC, Oetting WS, Israni AK, Jacobson PA. Dosing equation for tacrolimus in CYP3A5*3 null recipients. Clin Pharmacol Ther 2001; 69: 948–957.

Rojas L, Neumann I, Herrero MJ, Boso V, Reij J, Poveda JL et al. Effect of CYP3*5 on kidney transplant recipients treated with tacrolimus: a systematic review and meta-analysis of observational studies. Pharmacogenomics J 2014; 15: 38–48.

Vamprasaht S, Reungjui S, Supanya D, Srivongs D, Pongsukul C, Avringsanon Y et al. Personalized tacrolimus dosing determined by CYP3A5 genotype for induction and maintenance phases of kidney transplantation. Clin Ther 2013; 35: 1762–1769.

Roy BN, Barama A, Poirier C, Vinet B, Roger M. Cyp3a4, Cyp3A5, and MDR-1 genetic influences on tacrolimus pharmacokinetics in renal transplant recipients. Pharmacogenomics Genomics 2006; 16: 659–665.

Lee SJ, Usmani KA, Chanas B, Ghahreyam B, Xi T, Hodgson E et al. Genetic findings and functional studies of human CYP3A5 single nucleotide polymorphisms in different ethnic groups. Pharmacogenomics 2003; 13: 461–472.

Hafrud V, Wallmacc P, Vanerkerv vh, Elens L, de Meyer M, Eddour DC et al. CYP3A4 and ABCB1 polymorphisms and tacrolimus pharmacokinetics in renal transplant candidates: guidelines from an experimental study. Am J Transplant 2006; 6: 2706–2713.

van den Eijken DA, van Schaik RH, van der Heiden IP, van der Werf M, Gregoor PJ, Lindemans J et al. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. Clin Pharmacol Ther 2003; 74: 245–254.

Lopez-Montenegro Soria MA, Kanter Berga J, Beltran Catalan S, Millar P, Pallardo Mateu LM, Jimenez Torres NV. Genetic polymorphisms and individualized tacrolimus dosing. Transplant Proc 2010; 42: 3031–3033.
Personalizing tacrolimus dose in African Americans
K Sanghavi et al

57 Santoro A, Felipe CR, Tedesco-Silva H, Medina-Pestana JO, Struchiner CJ, Ojopi EB et al. Pharmacogenetics of calcineurin inhibitors in Brazilian renal transplant patients. Pharmacogenomics 2011; 12: 1293–1303.

58 Santoro AB, Struchiner CJ, Felipe CR, Tedesco-Silva H, Medina-Pestana JO, Suarez-Kurtz G. CYP3A5 genotype, but not CYP3A4*1b, CYP3A4*22, or hematocrit, predicts tacrolimus dose requirements in Brazilian renal transplant patients. Clin Pharmacol Ther 2013; 94: 201–202.

59 Zheng S, Tarsif Y, Hebert MF, Davis CL, Shitara Y, Calamia JC et al. Measurement and compartmental modeling of the effect of CYP3A5 gene variation on systemic and intrarenal tacrolimus disposition. Clin Pharmacol Ther 2012; 92: 737–745.

60 Oetting W, Schaldt D, Guan W, Israni A, Remmel R, Dorr C et al. Identification of genetic variants associated with variation of tacrolimus levels in African Americans using GWAS. Am J Transplant 2015; doi: 10.1111/ajt.13495; e-pub ahead of print.

61 Pulk R, Schaldt D, Guan W, Oetting W, Israni A, Matas A et al. Multi-genie pharmacogenomics of tacrolimus troughs in kidney transplant recipients. Pharmacogenomics 2014; 16: 841–854.

62 Li YR, van Setten J, Verma SS, Lu Y, Holmes MV, Gao H et al. CYP3A variation and the evolution of salt-sensitivity variants. Am J Hum Genet 2004; 75: 1059–1069.

63 Zhang J, Zhang X, Liu L, Tong W. Value of CYP3A5 genotyping on determining initial dosages of tacrolimus for Chinese renal transplant recipients. Transplant Proc 2010; 42: 3459–3464.

64 Roy JN, Lajoie J, Zijenah LS, Barama A, Poirier C, Ward BJ et al. Explaining variability in tacrolimus pharmacokinetics to optimize early exposure and the influence of new genetic variants on tacrolimus pharmacokinetics in Chinese adult renal transplant recipients. Transplantation 2010; 89: 144–146.

65 Zhang J, Zhang X, Liu L, Tong W. Value of CYP3A5 genotyping on determining initial dosages of tacrolimus for Chinese renal transplant recipients. Transplant Proc 2010; 42: 3459–3464.

66 Li YR, van Setten J, Verma SS, Lu Y, Holmes MV, Gao H et al. Concept and design of a genome-wide association genotyping array tailored for transplantation-specific studies. Genome Med 2015; 7: 90.

67 Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE et al. The integrated map of genetic variation from 1,092 human genomes. Nature 2012; 491: 56–65.

68 Thompson EE, Kuttab-Boulos H, Poirier C, Ward BJ et al. The Pharmacogenomics Journal (2017), 61 ahead of print.

69 Li YR, van Setten J, Verma SS, Lu Y, Holmes MV, Gao H et al. Concept and design of a genome-wide association genotyping array tailored for transplantation-specific studies. Genome Med 2015; 7: 90.

70 Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE et al. The integrated map of genetic variation from 1,092 human genomes. Nature 2012; 491: 56–65.