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Genotype-guided tacrolimus dosing in African American kidney transplant recipients

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Abstract

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 (AA) have on average higher tacrolimus dose requirements than Caucasians; however, some have requirements similar to Caucasians. Studies in AA have primarily evaluated the *CYP3A5*3* variant; however, there are common nonfunctional variants in AA (*CYP3A5*6* and *CYP3A5*7*) which do not occur in Caucasians. These variants are associated with lower dose requirements and may explain why some AA are metabolically similar to Caucasians. We created a tacrolimus clearance model in 354 AA using a development and validation cohort. Time posttransplant, steroid and antiviral use, age, *CYP3A5*1, *3, *6* and **7* alleles were significant towards clearance. This study is the first to develop an AA specific genotype-guided tacrolimus dosing model to personalize therapy.

Keywords

tacrolimus; kidney transplant; pharmacokinetics; personalization; pharmacogenomics

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Introduction

Kidney transplantation is a common and effective treatment for end stage renal disease. African Americans (AA) represent around 34% of the candidates on the kidney transplant waiting list. (1, 2) Long-term graft survival rates are lower and all-cause mortality rates are higher in AA than in Caucasians or Asians.(3–6) There are several reasons cited for poor outcomes including greater variation in HLA, immunological differences, higher medical non-adherence, socio-economic barriers and pharmacokinetic differences of the immunosuppressive agents including tacrolimus.(7, 8)

Tacrolimus has a narrow therapeutic index (9–13) with wide interindividual variability in pharmacokinetics resulting in unpredictable blood concentrations.(14–16) This necessitates therapeutic drug monitoring to avoid subtherapeutic and supratherapeutic concentrations, which places 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 to Caucasians.(19–23) To achieve target tacrolimus trough concentrations some AA require ~1.5 to 2 times higher doses than Caucasians.(24–29) However, not all AA 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 to CYP3A4. (31) Tacrolimus is also a substrate of P-glycoprotein which 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 only CYP3A5 variants have demonstrated major clinical relevance.(23, 30, 34–44)

*CYP3A5*3* is an intronic variant which 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 L/hr/kg, *CYP3A5*1/*3* ~ 0.8 L/hr/kg vs *CYP3A5*3/*3* ~ 0.5 L/hr/kg).(14, 48, 49) Therefore, the dose requirements for *CYP3A5*1/*1* or **1/*3* carriers are about 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.(43, 52)

*CYP3A5*6* is a missense mutation that codes for a splicing defect, deleting exon 7 resulting in absence of CYP3A5 enzyme and activity.(47) *CYP3A5*7* is a frame shift mutation due to an insertion within codon 346 and termination of protein synthesis.(46, 47, 53) Few studies have evaluated the association between *CYP3A5*6* and **7* alleles and tacrolimus pharmacokinetics. (54–59) Brazilian transplant recipients carrying two CYP3A5 variant alleles (**3*, **6* or **7*) had higher tacrolimus trough concentrations compared to 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 which comprehensively includes the common AA specific CYP3A5 variants.

Methods

Subjects

The data for this analysis was 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 prior to the study. African American kidney transplant recipients (n=354) ≥18 years 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 were targeted for the first 3 months and 6–10 ng/mL for 3–6 months posttransplant. A median (range) of 18 (1–24) concentrations were obtained from each subject in the first 6 months posttransplant, 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 certified labs.

Genotypes

Genotyping was performed on recipient DNA isolated from peripheral blood. Single nucleotide polymorphisms CYP3A5*3(rs776746, g.6986A>G), CYP3A5*6 (rs10264272, g. 14690 G>A) and CYP3A5*7 (rs41303343, g.27131-27132insT) were found to be significant in our previous GWAS analysis and therefore were chosen for this analysis.(60) In addition POR*28 (rs1057868, g.1058C>T) and CYP3A4*22 (rs35599367, g.15389 C>T) were also evaluated based on data from our previous analyses in a mixed race populations suggesting their importance.(61) Genotypes were determined using a custom exome-plus Affymetrix TxArray SNP chip described elsewhere. (62) The allele frequency of *CYP3A5*3* (G allele), *CYP3A5*6* (T allele), *CYP3A5*7* (A allele), *POR*28* (T allele) and *CYP3A4*22* (A allele) were 29.0%, 12.3%, 8.8%, 19.0%, 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 build the apparent oral tacrolimus clearance (Cl/F) model and subsequent dosing equation. The validation cohort (142 subjects with 2333 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 package. Nonlinear mixed effect modeling was used to develop the Cl/F model with NONMEM (version 7.2, ICON development solutions, Maryland, USA) software on a Visual Fortran compiler (90/95). 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). R studio 3.0.3 was used for predictive performance checks. A steady-state infusion model was used to develop the pharmacokinetic base model using $PRED library in NONMEM. In absence of intravenous data for the tacrolimus, it was not possible to calculate oral bioavailability. Therefore tacrolimus apparent oral clearance (Cl/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. Cl/F was related to tacrolimus trough concentrations by the following equation:

$$\mathrm{Css}=\mathrm{Total\ daily\ dose}/[(\mathrm{Cl/F})*24] \quad (1)$$

Due to the longer half-life of tacrolimus, steady-state trough concentrations were assumed to be approximately equivalent to average steady-state concentrations (Css). Actual apparent oral clearance may vary from this approximated Cl/F; however, this difference is negligible for drugs with longer half-lives, such as tacrolimus.

An exponential error model was used to explain the inter-individual variability in Cl/F as shown in the following equation:

$$\mathrm{Cl/F}=\mathrm{Typical\ value\ of\ Cl/F\ (TVCl/F)}\times \exp^{\eta_{(1)}} \quad (2)$$

where, $\eta_{(1)}$ is the estimate of deviation of individual Cl/F from TVCl/F. $\eta_{(1)}$ is assumed to be normally distributed mean of zero and variance ω^2.

An additive error model adequately explained the residual unexplained variability.

$$C_{ij}=C_{\mathrm{pred,ij}}+\varepsilon_{ij} \quad (3)$$

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

Covariate analysis

Clinical factors and genotypes were tested for their influence on tacrolimus TVCl/F. Covariates tested were recipient and donor age, gender, days posttransplant, steroid use (prednisone, methylprednisolone) at each trough measurement, calcium channel blocker use at each trough measurement, ACE-inhibitor use at each trough measurement, CMV sero-status at time of transplant (antibody positive or negative), anti CMV viral drug (as prophylaxis) use at each trough measurement, diabetes diagnosis at 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 time of trough measurement as a time varying covariate. Alleles tested were *CYP3A5*3, CYP3A5*6, CYP3A5*7, POR*28*, and *CYP3A4*22*. Recipients who did not carry any *CYP3A5*3, *6* or **7* alleles were designated as *CYP3A5*1/*1* genotype and those who carried one *CYP3A5*3, *6* or **7* allele were designated *CYP3A5*1/*3, *1/*6* or **1/*7* genotype, respectively. Recipients were classified into one of nine *CYP3A5* genotypes (*CYP3A5 *3/*3, *3/*6, *3/*7, *6/*7, *6/*6, *1*3, *1*6*, and **1*7* and **1/*1*). 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 posttransplant 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 (OFV); 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 a chi square distribution. A lower OFV is considered to be a better fit and a decrease in the OFV by 3.8 ($p<0.05$) or more was considered significant for forward inclusion and an increase in OFV by 6.6 ($p < 0.01$) was chosen for backward elimination.

Model evaluation

To evaluate the precision of the parameter estimates, a non-parametric bootstrap approach was performed using the development cohort. The method used random sampling with replacement to generate 1000 bootstrapped datasets using PsN toolkit. The final model developed with NONMEM was fit to each of the bootstrapped datasets 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:

$$\mathrm{MPE}=\mathrm{Median}\,(\mathrm{PRED}-\mathrm{DV})$$
$$\mathrm{MPPE}=\mathrm{Median}\,[(\mathrm{PRED}-\mathrm{DV})/\mathrm{DV}\times 100]$$
$$\mathrm{MAPE}=\mathrm{Median}\,[|(\mathrm{PRED}-\mathrm{DV})|]$$

Results

Characteristics of the subjects in the development and validation cohorts are shown in Table 1. The median (range) daily dose and trough concentrations did not differ between the cohorts. The median tacrolimus concentrations were low during the first week post

transplant and slowly increased over time until month 2 (2.8, 5.3, 6, 6.3, 6.9, 6.9, 7, 7.1, ng/mL in weeks 1–8 and 7.4, 7.2, 6.9 and 7 ng/mL in months 3–6, respectively). Tacrolimus TVCl/F was 54.6 L/hr and was significantly influenced by recipient age, steroid and antiviral coadministration, days posttransplant 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 inter-individual variability in TVCl/F after inclusion of covariates was 48.6%. Days posttransplant was the most important covariate where TVCl/F was 33% higher in the first 9 days posttransplant compared to after 9 days. Days post-transplant was first tested as continuous covariate however the model failed to converge and hence modeled as a categorical covariate. The plot of dose normalized trough concentrations over time showed a general increase in concentrations early posttransplant (up to day 9) and stabilized later. Several cut points were tested to understand the effect of time. There was also a break point in Cl/F at day 9 similar to that observed for concentrations. Addition of a third ordered category for days post transplant was not significant, hence only categorized as a bivariate. Tacrolimus TVCl/F increased by 23% with concomitant steroid use and reduced by 8% with concomitant antiviral use. Tacrolimus TVCl/F was 24% greater in subjects under the age of 34 years vs older subjects. Similar to days post-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 (18–34 years), middle age (35–64 years) and older age (>64 years). In the current study, only 6% of AA patients were older than 64 years, 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 TVCl/F decreased by 16.2%, 8.2%, and 24.1%, respectively, compared to *CYP3A5*1/*1* genotype. *For CYP3A5*3/*3, *3/*6, *3/*7* or **6/*7* the TVCl/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 evaluable 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 TVCl/F and re-ran the model. The tacrolimus TVCl/F decreased by 47% in subjects carrying two loss of function alleles (*CYP3A5*3/*3* or **3/*6* or **3/*7* or **6/*7, or *6/*6*) and by 15% in subjects carrying one loss of function allele (*CYP3A5*1/*3, *1/*6* or **1/*7*) compared to the *CYP3A5*1/*1.* The *POR*28* and *CYP3A4*22* genotypes did not influence TVCl/F.

To examine the goodness of fit, diagnostic plots were assessed during model development. Histograms of $\eta_{(1)}$s and Cl/F satisfied conditions of normal and log-normal distribution, respectively. Figures 1A and 1B shows the plots of observed concentration vs population predicted concentration, observed concentrations vs individual predicted concentrations. Figures 1C and 1D show the conditional weighted residuals (CWRES) vs independent variables, population predicted concentration and time. Although the model under-predicted slightly at higher concentrations, most of the data are evenly distributed across the line of unity. Also the CWRES do not show any specific trends of model misspecification. Thus the

model adequately explains the observed data. The final tacrolimus TVCl/F model with clinical factors and genotypes is as follows:

> Tacrolimus TVCl/F (L/hr)=54.6 L/hr x (1.33, if days less than 9 posttransplant) x [(0.53, if *CYP3A5*3/*3* or *CYP3A5*3/*7* or *CYP3A5*3/*6* or *CYP3A5*6/*7or CYP3A5*6/*6*)] x (0.85, if *CYP3A5*1/*3* or *CYP3A5*1/*6* or *CYP3A5*1/*7*) x (1.23, if receiving a steroid) x (0.92, if receiving an anti CMV viral drug) x (1.24, if recipient age 18–34 years)

Using the TVCl/F calculated using the model above and a desired target tacrolimus trough concentration; the daily tacrolimus dose can be calculated by: Daily dose (mg/day) = [TVCl/F x target tacrolimus trough concentration (ng/ml) x 24hrs]/1000

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 dataset fell within the prediction interval of the estimates obtained from bootstrap therefore indicating that the model is robust and reproducible.

Model evaluation using the validation cohort

Table 3 shows the prediction performance of the tacrolimus TVCl/F model. The median prediction error with 95% CI was 0.48 (0.31–0.65) ng/mL and median percentage prediction error was 9.45% (6.44–12.45). Therefore, the model over-predicted the trough concentrations relative to the observed concentrations. Median absolute prediction error was 2.32 (2.21–2.44) ng/ml.

Discussion

African Americans 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.(3, 28) On average AA 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 which lead to temporary cessation of therapy and/or dose reductions. Whereas others may require even higher doses of tacrolimus to avoid insufficient blood concentrations. Most tacrolimus pharmacogenomic studies in AAs and Caucasians have classified CYP3A5 metabolism based on the presence or absence of the nonfunctional *CYP3A5*3* allele. The *CYP3A5*3* allele frequency has a minor allele frequency of 18–35% in AA and 88–95% in Caucasians.(34, 47, 53, 65–67) However, AAs also carry *CYP3A5*6* and/or *7* alleles which also encode for low activity or nonfunctional enzyme which 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, 65, 66, 68, 69) 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 posttransplant, steroid and antiviral drug coadministration and age. The TVCl/F was 54.6 L/hr and higher than reported in non-AA studies (~22–40 L/hr) (14, 70–73) which is consistent with AAs being more likely to carry a *1* expresser allele than Caucasians. The *CYP3A5*3, *6* and *7* alleles were each associated with a reduction in tacrolimus clearance. About 50% of our subjects carried one nonfunctional allele (*CYP3A5*3/*1, *6/*1* or *7/*1*) which 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, about 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%).(74, 75)

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 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.(54) The influence of *CYP3A5*6* and *CYP3A5*7* alleles has been studied towards other CYP3A5 substrates and the effect may be substrate specific therefore our results may not be generalizable to other drugs. (75–81)

Day posttransplant was a significant covariate towards tacrolimus where TVCl/F is 33% higher in the first nine days posttransplant compared to after day 9 which is consistent with other studies.(14, 23, 70, 71, 82, 83) The higher TVCl/F may be due to 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 TVCl/F most likely because steroids induce CYP3A enzymes. (84–87) We also found that younger subjects (18–34 years) had a 24% higher tacrolimus TVCl/F compared to older subjects. While some studies have not observed a significant association between tacrolimus Cl/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.(14, 26, 70, 73, 88–90) We found that the co-administration of antivirals reduced tacrolimus TVCl/F but only by 8%. The mechanism of this effect is unknown. We did not find that calcium channel blockers were associated with TVCl/F. This is likely

because amlodipine is the preferred agent at our centers and has a lower potential for an interaction than other calcium channel blockers.(91–93) Weight was not significant towards TVCl/F. Other studies have also not found weight to be significant.(94, 95)

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.(35–38, 42, 58, 96) 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.(97) The study tested an initial dose of 0.3 mg/kg/day PO in CYP3A5 expressors (CYP3A5*1) and 0.15 mg/kg/day PO for non-expressors (*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 shows that race specific variants and clinical factors is necessary in future trials and may improve achievement of major clinical endpoints. 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*).(98) Our data supports these recommendations where *6 and *7 allele carriers require lower doses.

One of the limitations of our study is that albumin, hematocrit and antifungal agents status was not available and not tested in our model.(14) 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 towards 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. (53) 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 Cl/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 since most carried one or more *CYP3A5*3, *6* or **7* nonfunctional alleles.(74)

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 which account for the genotypes and clinical factors may lead to precision dosing of tacrolimus.

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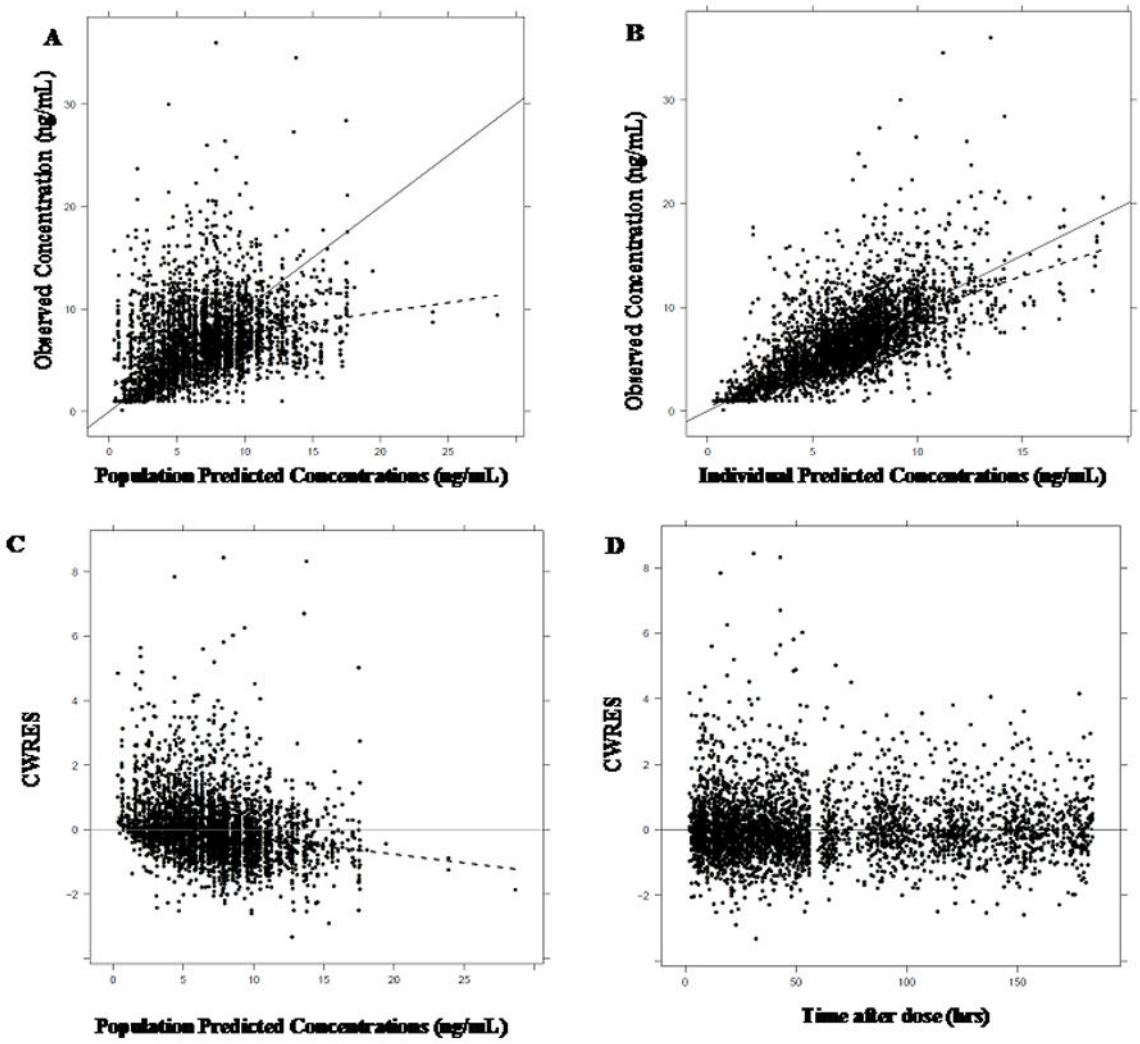

Figure 1. Goodness of fit plots for the final tacrolimus model

(A) observed concentrations (ng/mL) vs population predicted concentrations (ng/mL) and (B) observed conc. (ng/mL) vs individual predicted concentrations (ng/mL). The black dots represent the observed tacrolimus trough concentrations, the solid line represents the line of unity and the dashed line represents the loess smooth.

(C) conditional weighted residuals (CWRES) vs population predicted concentrations (ng/mL) and (D) CWRES vs time after dose (hrs). The dots represent the observed tacrolimus trough concentrations, the solid line is the line at y=0 and the dashed line represents the loess smooth.

Table 1

Patient demographics

	All subjects	Development Cohort subjects	Validation Cohort subjects	P-value[a]
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	6037	3704	2333	0.09
Tacrolimus trough (ng/mL)[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/1.73m^2 [b,d]	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

	All subjects	Development Cohort subjects	Validation Cohort subjects	P-value[a]
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	
CYP3A5*1/*6	47 (13)	30 (14)	17 (12)	0.55
CYP3A5*6/*6	4 (1)	1 (0.5)	3 (2)	0.15
CYP3A5*3/*6	21()	15 (7)	6 (4)	0.26
CYP3A5*3/*7	15 (4)	8 (4)	7 (5)	0.59
CYP3A5*6/*7	11 (3)	5 (2)	6 (4)	0.32
CYP3A5*1*1	80 (23)	49 (23)	31 (21)	0.77
CYP Not determined[c]	10	5	5	
POR*1/*1	151 (43)	91 (43)	60 (42)	0.90
POR*1/*28	86 (25)	55 (26)	31 (22)	0.37
POR*28/*28	25 (7)	15 (7)	10 (7)	0.99
CYP3A4*1/*1	229 (65)	140 (66)	89 (63)	0.52
CYP3A4*1/*22	17 (4)	12 (6)	5 (4)	0.35
CYP3A4*22/*22	0	0	0	

[a] p-value is the comparison of model development and validation cohorts

[b] data are median (range)

[c] These individuals did not have one or more of the CYP3A5 genotypes available and were excluded from the all analyses

[d] GFR is glomerular filtration rate calculated by Modification of Diet in Renal Disease (MDRD) equation

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[a]) of the effect on TVCl/F	Bootstrap analysis. Median (95% confidence interval)
Typical Value of Cl/F (TVCl/F) in L/hr	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 alleles (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 posttransplant	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 yrs)	1.24 (7.8%)	1.24 (1.07–1.47)
Between subject variability[b]	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)	2.76 (7.5%)	2.75 (2.55–2.96) ng/mL

[a] RSE is relative standard error

[b] 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 {[exp (variance)]−1}

Table 3

Predictive performance of the tacrolimus clearance model

Predictive performance measure	Estimate
Median prediction error (MPE, 95% CI)	0.48(0.31–0.65)
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)