HHS Public Access

Author manuscript

J Clin Pharmacol. Author manuscript; available in PMC 2024 July 12.

Published in final edited form as:

J Clin Pharmacol. 2021 August ; 61(8): 1035–1044. doi:10.1002/jcph.1823.

Precision Dosing for Tacrolimus Using Genotypes and Clinical Factors in Kidney Transplant Recipients of European Ancestry

Mahmoud Al-Kofahi, DDS, PhD[1], **William S. Oetting, PhD**[1], **David P. Schladt, MS**[2], **Rory P. Remmel, PhD**[3], **Weihua Guan, PhD**[4], **Baolin Wu, PhD**[4], **Casey R. Dorr, PhD**[2,5], **Roslyn B. Mannon, MD**[6], **Arthur J. Matas, MD**[7], **Ajay K. Israni, MD**[2,5,8], **Pamala A. Jacobson, PharmD**[1]

[1]Department of Experimental and Clinical Pharmacology, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota, USA

[2]Hennepin Health Research Institute, Minneapolis, Minnesota, USA

[3]Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota, USA

[4]Department of Biostatistics, University of Minnesota, Minneapolis, Minnesota, USA

[5]Department of Medicine, Hennepin Healthcare, University of Minnesota, Minneapolis, Minnesota, USA

[6]Division of Nephrology, University of Nebraska, Omaha, Nebraska, USA

[7]Department of Surgery, University of Minnesota, Minneapolis, Minnesota, USA

[8]Department of Epidemiology & Community Health, University of Minnesota, Minneapolis, Minnesota, USA

Abstract

Genetic variation in the CYP3A4 and CYP3A5 (CYP3A4/5) genes, which encode the key enzymes in tacrolimus metabolism, is associated with tacrolimus clearance and dose requirements. Tacrolimus has a narrow therapeutic index with high intra- and intersubject variability, in part because of genetic variation. High tacrolimus clearance and low trough concentration are associated with a greater risk for rejection, whereas high troughs are associated with calcineurin-induced toxicity. The objective of this study was to develop a model of tacrolimus clearance with a dosing equation accounting for genotypes and clinical factors in adult kidney transplant recipients of European ancestry that could preemptively guide dosing. Recipients receiving immediate-release tacrolimus for maintenance immunosuppression from 2 multicenter studies were included. Participants in the GEN03 study were used for tacrolimus model development (n = 608 recipients) and was validated by prediction performance in the DeKAF Genomics study (n = 1361 recipients). Nonlinear mixed-effects modeling was used to develop the apparent oral

Corresponding Author: Pamala Jacobson, PharmD, FCCP, Department of Experimental and Clinical Pharmacology, 308 Harvard St. SE, 7–151 Weaver Densford Hall, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455 jacob117@umn.edu.

Conflicts of Interest

The authors declared no conflicts of interest.

Supplemental Information

Additional supplemental information can be found by clicking the Supplements link in the PDF toolbar or the Supplemental Information section at the end of web-based version of this article.

tacrolimus clearance (CL/F) model. CYP3A4/5 genotypes and clinical covariates were tested for their influence on CL/F. The predictive performance of the model was determined by assessing the bias (median prediction error [ME] and median percentage error [MPE]) and the precision (root median squared error [RMSE]) of the model. *CYP3A5*3, CYP3A4*22*, corticosteroids, calcium channel blocker and antiviral drug use, age, and diabetes significantly contributed to the interindividual variability of oral tacrolimus apparent clearance. The bias (ME, MPE) and precision (RMSE) of the final model was good, 0.49 ng/mL, 6.5%, and 3.09 ng/mL, respectively. Prospective testing of this equation is warranted.

Keywords

genomics; kidney transplant; pharmacogenomics; population pharmacokinetics; tacrolimus

Immunosuppressive drugs are the key to the prevention of kidney allograft rejection. Tacrolimus is a potent immunosuppressive agent that is highly effective and along with mycophenolate is the primary maintenance immunosuppressant used in transplantation. However, tacrolimus dosing is complicated by its narrow therapeutic index and wide interindividual variability in its pharmacokinetics.[1–8] These factors make defining an optimal dosing schedule for tacrolimus difficult and result in out-of-range trough concentrations and tacrolimus-related adverse effects, such as nephrotoxicity, in which subtherapeutic concentration increases the risk of de novo donor-specific antibody formation and acute rejection.[9–11]

Population pharmacokinetic analyses have been used to investigate and identify sources of the observed wide variability in the pharmacokinetics of tacrolimus.[12–17] Factors that have been identified include age, race, body weight, hematocrit, and time posttransplant.[6] Genetic variation is also a well-known influencer of tacrolimus pharmacokinetic variability. Tacrolimus is extensively metabolized in the liver and small intestine by the CYP3A4 and CYP3A5 (CYP3A4/5) isoforms of the cytochrome (CYP) P450 system, and genetic variants in these genes are important determinates of tacrolimus troughs and dose.[18,19]

The *CYP3A5*3* allele (*rs776746*) is the most common (allele frequency of ~95%) nonfunctional variant of the *CYP3A5* gene in the European-American (EA, white) population.[20,21] It is a splice-site variant and is associated with loss-of-function CYP3A5.[20,22] Individuals with the *CYP3A5*3/*3* genotype have significantly lower tacrolimus apparent clearance (CL/F) and smaller dose requirements compared with individuals with the *CYP3A5*1/*1* or **1/**3 genotype.[6,23,24] The *CYP3A5* variant alleles *CYP3A5*6* (rs10264272) and *CYP3A5*7* (rs41303343) also cause loss of enzyme function, lower tacrolimus clearance and dose requirements, but occur primarily in those with African ancestry.[25,26]

*CYP3A4*22* is a decrease-of-function allele (*rs35599367*) associated with reduced CYP3A4 protein expression.[27–30] Individuals carrying 1 or more *CYP3A4*22* alleles require significantly lower tacrolimus doses and are at risk of tacrolimus overexposure compared with those who do not carry these alleles.[27,28,31,32] The frequency of the *CYP3A4*22* allele

is significantly higher in the EA population (minor allele frequency [MAF], 0.043–0.053) relative to African Americans (MAF, 0.009) in whom it rarely occurs.[33]

Because CYP3A5 represents at least 50% of the total hepatic CYP3A content in individuals expressing CYP3A5 enzyme and is also highly expressed in intestine,[20] genetic variations in the *CYP3A5* gene explain a substantial proportion of the variability in tacrolimus clearance and dose.[34–38] In our previous work, we found that African American recipients with loss-of-function variants had a reduction in tacrolimus clearance near 50% and that 3 genetic variants (*CYP3A5*3*, **6*, and **7*) and clinical factors explained 53.9% of variability in tacrolimus troughs.[17,25] Therefore, continuing to add new genetic variants and clinical factors, as they are identified will improve the model and our ability to predict tacrolimus concentrations and dose. The aim of this study was to develop a tacrolimus clearance model and a dosing equation specific for those of European ancestry with important CYP3A4/5 variants and clinical variables. Specifically, the *CYP3A4*22* variant is included in this model, which has not been included in our previous dosing models. The long-term goal is to develop methods to personalize and guide immunosuppressive therapy.

Methods

Participants

Data were obtained from 2 multicenter observational studies (GEN03 and Deterioration of Kidney Allograft Function [DeKAF]) approved by local institutional review boards. Informed consent was obtained from each participant. The studies are registered at www.clinicaltrials.gov (NCT00270712 and NCT01714440). A total of 1969 adult (≥18 years) kidney transplant recipients were eligible for this analysis. Participants were included if they were of European ancestry, as determined through principal components analysis, and received immediate-release oral tacrolimus for maintenance immunosuppression post-transplant. The GEN03 study was conducted from 2012 to 2016 and included 608 recipients from 5 centers: University of Minnesota, Hennepin County Medical Center, University of Alabama, Mayo Clinic-Rochester, and University of Iowa. The DeKAF study was conducted from 2005 to 2010 and included 1361 recipients from 7 centers: University of Minnesota, Hennepin County Medical Center, University of Alabama, Mayo Clinic-Rochester, University of Iowa, University of Manitoba, and University of Alberta. Patient demographics and dose ranges are presented in Table 1.

Participants received tacrolimus with mycophenolate along with corticosteroids for varying durations by center protocols. Induction was given per center preference but primarily contained rabbit antithymocyte globulin (rATG), basiliximab, or Campath-1H. Immunologically high-risk patients (eg, donor-specific antibody, prior pregnancies, or repeat transplants) were more likely to receive rATG. Recipient characteristics, such as serum creatinine (SCr), estimated glomerular filtration rate, and concomitant medications, were obtained from the electronic health records. Tacrolimus trough whole-blood measurements were measured clinically at each center and were analyzed in Clinical Laboratory Improvement Methods–approved laboratories; >95% were measured by liquid chromatography-mass spectrometry. Tacrolimus trough concentrations were obtained twice each week for the first 8 weeks, and 2 troughs per month in months 3, 4, 5, and 6 for a

maximum of 24 trough concentrations per patient. Tacrolimus doses and dosing intervals were adjusted based on troughs to achieve center-specific trough goals and were generally 8–12 ng/mL in months 0 to 3 and 6–10 ng/mL in months 4 to 6. Dose was also adjusted for side effects (eg, tacrolimus-associated rise in SCr) by center-specific practices. All participants received the immediate-release formulation of tacrolimus (Prograf or generic), and no participants received the extended-release formulation. A total of 34 650 trough concentrations from the 1969 recipients were analyzed.

Genotyping

Pretransplant recipient blood was collected at each center at time of transplant, and DNA was isolated at a central laboratory at the University of Minnesota. Lymphocytes were isolated by centrifugation after red blood cell lysis and the DNA isolated. Genotyping was performed on a custom exome-plus Affymetrix TxAr-ray genome-wide association study (GWAS) single-nucleotide polymorphism chip.[39] This chip contained ~782 000 markers including pharmacogenomic variants, 168 000 exonic or coding variants, and more than 16 000 putative loss-of-function variants. The *CYP3A5*3* (*rs776746*) and *CYP3A4*22* (*rs35599367*) genotypes were obtained from this chip. The variants did not diverge from Hardy-Weinberg equilibrium. Genotyping and data quality control for this chip have been previously described.[25,40] European ancestry of each individual was determined by principal components analysis (PCA) of ancestry computed from the GWAS panel and through knowledge of self-reported ancestry and was previously described.[41] There was high concordance with self-reported ancestry and PC-defined ancestry; however, when discordance was raised, PCA-defined ancestry was assigned to that individual.

Population Pharmacokinetic Modeling

Data from the GEN03 study, with 10 992 troughs from 608 recipients, was used for model development. Nonlinear mixed-effects modeling (NONMEM version 7.4 software; ICON Development Solutions, Ellicott City, Maryland) was performed to develop tacrolimus apparent oral clearance (CL/F) model and a subsequent dosing equation by first-order conditional estimation method with interaction. Exploratory analyses and diagnostic graphics were performed with R 3.6.1 (R Core Team, 2019) and RStudio 1.2.5001 (RStudio, Inc., The R Development Core Team) and Perl-speaks-NONMEM (PsN 4.9.0, Uppsala University, Uppsala, Sweden) under the Pirana interface.[42]

Our previously developed pharmacokinetic base model was used.[17] In this study, the $PRED library in NONMEM was employed and pharmacokinetic base model was developed using a steady-state infusion model. Because of the longer half-life for tacrolimus (approximately 12 hours, with a range of 3.5 to 40.5 hours), minimal peak-trough fluctuation is expected and steady-state trough concentrations were assumed to be approximately equivalent to average daily $C_{p,ss}$. Hence, C_{trough}approximately equals average daily $C_{p,ss}$. Because intravenous data for the tacrolimus were absent, it was not possible to calculate oral bioavailability. Therefore, tacrolimus CL/F, which is the ratio of total clearance (CL) to bioavailability (F), was used to regress average daily $C_{p,ss}$ to the administered dose. CL/F was related to tacrolimus trough concentrations using the following equation:

$$C_{trough} = \text{average daily } C_{p,ss}$$

$$= \text{total daily tacrolimus dose}/([CL/F] \times 24).$$

Although, the factual apparent oral clearance may differ from this approximated CL/F; this difference is negligible for drugs with longer half-lives, such as tacrolimus.

Two-level nested random effects were included in the model: interindividual variability (IIV) and intercenter variability (ICV). The interindividual variability of tacrolimus CL/F was estimated with an exponential error model and expressed as:

$$CL/F_i = TVCL/F \text{ x } \exp^{(\eta_{IIV})_i}$$

where CL/F is a function of the typical value of apparent oral clearance (TVCL/F) and the individual parameter for the ith subject, with $(\eta_{IIV})_i$ the estimate of individual deviation from TVCL/F and assumed to be normally distributed with mean 0 and variance ω^2.

In the developed model, random effects explaining variability between 1 transplant center and the next (ICV) was mapped based on the $LEVEL record in NONMEM. Each study center in the development cohort has a separate identification number in the data set. Subjects from the same center share the same random effect. NONMEM is informed such that η_{ICV} is a CL/F η that changes only with every study center and is associated by nesting with η_{IIV}, which varies with each subject, and was estimated with an exponential error model and expressed as:

$$CL/F_i = TVCL/F \text{ x } \exp^{(\eta_{IIV} + \eta_{ICV})_i}$$

where $(\eta_{ICV})_i$ is the estimate of center deviation from TVCL/F and is assumed to be normally distributed with mean 0 and variance ω^2. The reason η_{IIV} and η_{ICV} are associated together is that η in both provides random effects to the same parameter of CL/F.

For the residual unexplained variability, additive and proportional error models were used and expressed as:

$$C_{ij} = C_{pred,ij} * (1 + \varepsilon_{(prop)ij}) + \varepsilon_{(add)ij}$$

where C_{ij} is the observed concentration and C_{predij} is the corresponding model-predicted concentration with the ith individual at the jth occasion, $\varepsilon_{(prop)ij}$ is proportional error and assumed to be normally distributed with mean 0 and variance σ^2, and $\varepsilon_{(add)ij}$ is additive error and is assumed to be normally distributed with mean 0 and variance σ^2.

Covariate Analysis

Demographics, clinical factors, and genetic variants were evaluated for their influence on tacrolimus TVCL/F. The demographics evaluated were recipient body weight (kg), recipient age, donor age, donor deceased status, recipient sex, donor sex, days post-transplant, smoking, ethnicity, recipient race, donor race, and glomerular filtration rate. Medications used at each trough measurement were evaluated and included steroids (prednisone, methylprednisolone), calcium channel blockers (CCBs), antiviral drugs, angiotensin-converting enzyme (ACE) inhibitors, ketoconazole, itraconazole, fluconazole, and voriconazole. Disease conditions considered were diabetes and post-transplant dialysis for delayed graft function. Genetic variants tested were *CYP3A5*3* and *CYP3A4*22* because these are the common tacrolimus variants in EAs. Recipients who did not carry any *CYP3A5*3* alleles were designated a *CYP3A5*1/*1* genotype and those who carried 1 or 2 *CYP3A5*3* allele were designated either a *CYP3A5*1/*3* or a *CYP3A5*3/*3* genotype. For *CYP3A4*, recipients were classified as *CYP3A4*1/*1, CYP3A4*1/*22* or *CYP3A4*22/*22*.

Covariate assessment was conducted by the stepwise covariate modeling in a PsN tool kit. Stepwise covariate modeling involved testing of covariate relationships in forward inclusion and backward exclusion processes. The significance of inclusion and elimination of each covariate was tested based on likelihood ratio test that follows the χ^2 distribution. A decrease in objective function value (OFV) by 3.8 or more ($P < .05$) was considered significant for forward inclusion. A full model was built that included all the covariates that showed a significant decrease in OFV following forward inclusion. Each covariate was then reevaluated through a backward elimination process. The covariates yielding an increase in OFV by 10.8 or more ($P < .001$) were considered significant and retained in the final model.

Model Evaluation and Predictive Ability of the Pharmacokinetic Parameters

The precision of the final model parameters were evaluated with sampling-importance-resampling (SIR)-based 95% confidence intervals (CIs).[43,44] The final models were evaluated using prediction-corrected visual predictive checks (pcVPC; 1000 simulations).[45] Data from the DeKAF Genomics study, with 23 658 troughs from 1361 recipients, was used to validate model prediction performance from the GEN03 data set. The final model parameters and significant covariates were fixed in NONMEM and were used to predict trough concentrations in the validation cohort subjects. Population-predicted trough concentrations (PRED) were obtained for each observed concentration (dependent variable) given their actual administered dose, the time after transplant, and the significant clinical covariates and genotypes (which were identified from the development model). The bias (median prediction error [ME], median percentage error [MPE]) and the precision (root median squared error [RMSE]) of population prediction (PRED) was used to assess the predictive performance. The following equations were used:

$$\mathrm{ME} = \mathrm{Median}\ (\mathrm{PRED} - \mathrm{DV})$$

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

$$\text{RMSE} = \sqrt{\text{Median}\ [(\text{PRED}-\text{DV})]}$$

Results

Characteristics of the participants in the development and validation cohorts are shown in Table 1. The typical value estimate of tacrolimus apparent clearance (CL/F) was 32.2 L/h. The estimates of IIV and ICV as coefficient of variation (CV%) were 41.9% and 31.3%, respectively. Steroid, calcium channel blocker, and antiviral drug use, age, diabetes, time posttransplant, and *CYP3A5*1, *3*, and *CYP3A4*22* alleles were found to significantly contribute to the interindividual variability of oral tacrolimus CL/F in EAs. The effect of genotypes and clinical covariates on tacrolimus CL/F and final parameter estimates in the model development cohort and in the SIR analysis is shown in Table 2.

The final population pharmacokinetic model from the development cohort showed that tacrolimus CL/F decreased by 13% on average if the recipient was diabetic, 5% if receiving a CCB, and 9% if receiving an antiviral drug. Tacrolimus CL/F also decreased as the recipient's age increased. Tacrolimus CL/F increased by 6% if recipient was receiving a corticosteroid at the time of tough measurement. CL/F of tacrolimus was 18% lower after day 8 posttransplant relative to day 8 or before. Other tested clinical factors were not significant ($P > .001$) on CL/F.

The effect of the genotypes was profound. In recipients with the *CYP3A5*1/*1 or CYP3A5*1/*3* genotype, the tacrolimus CL/F increased by 305%, and 181%, respectively, compared with recipients with the *CYP3A5*3/*3* genotype. For recipients with 1 or 2 *CYP3A4*22* alleles, the CL/F declined by 22% and 72%, respectively, relative to recipients with no *CYP3A4*22* alleles. The final model and dose equation are given below:

CL/F(L/h) = 32.2 (L/h) × ([1.81, if *CYP3A5*1/*3*] × [3.05, if *CYP3A5*1/*1*] × [0.78, if *1 CYP3A4*22*] × [0.28, if *2 CYP3A4*22*] × [1.06, if receiving a *steroid*] × [0.95, if receiving a calcium channel blocker] × [0.87, if diabetic] × [0.91, if receiving an antiviral drug] × [$(AGE/52)^{-0.3}$]) × (0.82, if after day 8 posttransplant)

Daily dose (mg/day) = (**CL/F** × target tacrolimus trough concentration [ng/mL] × 24 hours)/1000

Model Evaluation and Predictive Ability of Pharmacokinetic Parameters

The diagnostic scatterplots showed an acceptable overall goodness of fit of the final model (Figure 1). The final model parameter estimates were all within their SIR-based 95%CIs (Table 2). Parameter estimates for fixed and random effects obtained from the original data set fell within the prediction interval of the estimates obtained from SIR, indicating that the model is robust and reproducible.

The predictive performance of the final pharmacokinetic model, was measured through bias (ME, MPE) and precision (RMSE) in the validation cohort. The ME, MPE, and RMSE for all trough concentrations in the validation data were 0.49 ng/mL, 6.5%, and 3.09

ng/mL, respectively. This suggests that, on median, the model overpredicted the trough concentrations relative to the observed concentrations.

Discussion

We developed a tacrolimus dosing model and clinical equation that simultaneously accounts for genotypes and clinical factors in a large sample of adult kidney transplant recipients with European ancestry using a nonlinear mixed-effects modeling approach. Steroid, CCB, and antiviral drug use, age, diabetes, time post-transplant, and *CYP3A5*1, *3*, and *CYP3A4*22* alleles were identified as significant factors influencing tacrolimus clearance and were subsequently accounted for in the model. The model was then validated with data from the DeKAF Genomics data set. We previously developed and validated an African American-specific model with 3 *CYP3A5* variants along with clinical factors.[17] The data here are consistent with our previous work in which models had to combine genotypes and clinical factors to robustly explain variability in clearance.

Our analysis identified a significant effect of several medications on tacrolimus CL/F. Concomitant use of steroids was reported with 64% of the trough concentrations and was associated with an increase in tacrolimus CL/F by 6%. Literature reports have also shown a significant association between relative tacrolimus clearance and steroid use.[46] Corticosteroids are inducers of CYP3A enzymes[47–50] and are well known for their drug interactions with CYP3A substrates. CCB and antiviral drugs were concomitantly present in 40% and 52%, respectively, of the troughs. For subjects receiving a CCB in the development cohort, 57% of them received amlodipine, felodipine, nimodipine, or nisoldipine; 6.5% received isradipine, nicardipine, or nifedipine; and 6.5% received diltiazem or verapamil for up to 6 months. Both CCB and antiviral drug use decreased tacrolimus CL/F by 5% and 9%, respectively. Drug interactions between CCBs and tacrolimus have been previously described, especially with diltiazem and verapamil, which are mechanism-based inhibitors of P450.[51,52]

We explored the potential effect of ACE inhibitors and antifungal drugs on tacrolimus CL/F, but no significant effects were detected. Tacrolimus is not known to interact with ACE inhibitors clinically, and our data are consistent with this knowledge. Antifungal drugs can inhibit CY3A4/5 and tacrolimus metabolism.[53,54] Only 65 recipients, mostly on fluconazole, an antifungal drug, were in the GEN03 cohort, and the absence of an effect may be because of the small sample size and the relatively weak inhibition of CYP3A4/5 of fluconazole relative to itraconazole.

We found that tacrolimus CL/F decreased by 18% after day 8 posttransplant up to 6 months posttransplant relative to day 8 or before. We and others have previously described that tacrolimus clearance declines with increasing time posttransplantation and may be because of a reduction in CYP3A activity, a rising hematocrit, physiological changes occurring in hepatic and kidney function, and/or gastrointestinal motility.[13,15–17,55] We also found that tacrolimus CL/F decreased as recipient age increased; other studies have shown no significant relationship between age and tacrolimus.[12,14,56–59] Age is a factor known to influence drug metabolism of many other drugs.[60,61] About 30% of our enrolled participants

were diabetic at the time of transplant, which was associated with a 13% decrease in tacrolimus CL/F relative to nondiabetic subjects. Hematocrit is also a significant factor for whole-blood tacrolimus concentrations because of high accumulation in erythrocytes. One of the limitations of our study is that hematocrit data were not available in our study and not tested in our model. Low hematocrit levels are associated with lower whole-blood tacrolimus concentrations and can be incorrectly interpreted as an increase in CL/F when concentrations and clearance are actually unchanged.[62–64] Hematocrit levels are generally low in the early posttransplant period and improve in the first 1–3 months posttransplant.

The distribution of *CYP3A5* alleles varies significantly by ancestry. The *CYP3A5*3* allele has a high frequency in EAs.[2] Our analysis was conducted only in those of European ancestry, and most participants (about 88%) carried 2 nonfunctional *CYP3A5*3* alleles (*CYP3A5*3/*3*). About 10.7% of our participants carried 1 nonfunctional *CYP3A5*3* allele (*CYP3A5*1/*3*), which increased tacrolimus CL/F by 81% relative to those with *CYP3A5*3/*3*. As expected, only 0.5% of those enrolled in our study population carried the *CYP3A5*1/*1* genotype and were associated with an increase in tacrolimus CL/F by 205% relative to *CYP3A5*3/*3. CYP3A5*6* and *CYP3A5*7* alleles are usually not present in EAs.[2,26] Only 1 participant carried *CYP3A5*6*.

The *CYP3A4*22* allele was associated with a reduction in tacrolimus clearance and has a frequency that is higher than other ancestry groups.[30,33,41] About 8.7% of our enrolled subjects carried 1 nonfunctional *CYP3A4*22* allele (*CYP3A5*22/*1*), which decreased tacrolimus CL/F by 22% relative to subjects carrying *CYP3A4*1/*1*. Only 0.5% of our enrolled subjects carried 2 nonfunctional *CYP3A4*22* alleles (*CYP3A5*22/*22*) that decreased tacrolimus CL/F by 72% relative to subjects carrying *CYP3A4*1/*1*. We previously evaluated the *CYP3A4*22* allele in African American recipients in whom the allele frequency was lower (~4%), and we were unable to detect an association with tacrolimus CL/F, possibly because of the small sample size.[17]

The Clinical Pharmacogenetics Implementation Consortium (CPIC) gives CYP3A5 and tacrolimus an A level of evidence and categorizes individuals as extensive (*CYP3A5*1/*1*), intermediate (*CYP3A5*1/*3*), or poor (*CYP3A5*3/*3*) metabolizers.[65] CPIC recommends that CYP3A5 poor metabolizers receive the standard recommended starting dose, whereas extensive or intermediate metabolizers receive 1.5–2 times the standard dose without exceeding 0.3 mg/kg/day[65] but does not account for clinical factors. Our results in general support these recommendations, but our model includes clinical factors and drugdrug interactions that are well known to change the pharmacokinetics of tacrolimus, thereby creating a more precise starting dose. CPIC does not provide guidance for *CYP3A4*22*, but our results show that it has a significant effect on tacrolimus clearance and should be considered in dosing decisions. Although rare, carriers of 2 *CYP3A4*22* alleles along with 2 nonfunctional *CYP3A5*3* alleles have profoundly reduced clearance.[31]

The model evaluation techniques including SIR and prediction-corrected visual predictive check (on a log scale; Figure 2) showed that the model is able to adequately predict the observed data. The predictability of the model was further tested by external validation, which showed acceptable bias (ME, 0.49 ng/mL; MPE, 6.5%) and imprecision

(population RMSE, 3.09 ng/mL; individual RMSE, 1.73 ng/mL) values for tacrolimus trough concentrations. Although the model slightly overpredicted trough concentrations relative to the observed concentrations (median tacrolimus trough in validation cohort, 8.4 ng/mL), the current analysis reported a population median error of 36.8% and an individual median error of 20.5% with reasonable forecasting of future predictions. Use of the model should be coupled with clinical judgment to account for clinical factors potentially affecting clearance not included in the model.

A limitation to the study is the observational design. The exact times of the tacrolimus troughs were not confirmed with patients. If adherence concerns were noted in the electronic medical record, the trough was not collected or used in the analysis. For all other troughs, we assumed adherence was high. There may also be effects from varying hematocrit levels, rare variants and/or concomitant medications (eg, over-the-counter, neutraceuticals) that affect the clearance of tacrolimus that we have not accounted for.

Conclusion

Tacrolimus exhibits considerable interindividual variability in its pharmacokinetics in kidney transplant recipients of European ancestry. This study complements our previously published study that demonstrated the importance of population-specific genotypes to better understanding tacrolimus pharmacokinetics.[17] The current analysis of recipients of genetically confirmed European ancestry, which accounts for variants and clinical factors, provides a tool to preemptively individualize the dose of tacrolimus and ultimately improve clinical immunosuppressant outcomes in kidney transplant recipients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding

Support was received by the National Institutes of Health NIAID Genomics of Transplantation (5U19-AI070119) and ARRA supplement (5U19-AI070119).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author on reasonable request.

References

1. de Jonge H, Naesens M, Kuypers DR. New insights into the pharmacokinetics and pharmacodynamics of the calcineurin inhibitors and mycophenolic acid: possible consequences for therapeutic drug monitoring in solid organ transplantation. Ther Drug Monit. 2009;31(4):416–435. [PubMed: 19536049]
2. Kahan BD, Keown P, Levy GA, Johnston A. Therapeutic drug monitoring of immunosuppressant drugs in clinical practice. Clin Ther. 2002;24(3):330–350; discussion 329. [PubMed: 11952020]

3. Kershner RP, Fitzsimmons WE. Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. Transplantation. 1996;62(7):920–926. [PubMed: 8878385]
4. McMaster P, Mirza DF, Ismail T, Vennarecci G, Patapis P, Mayer AD. Therapeutic drug monitoring of tacrolimus in clinical transplantation. Ther Drug Monit. 1995;17(6):602–605. [PubMed: 8588228]
5. Monchaud C, Marquet P. Pharmacokinetic optimization of immunosuppressive therapy in thoracic transplantation: part I. Clin Pharmacokinet. 2009;48(7):419–462. [PubMed: 19691367]
6. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. Clin Pharmacokinet. 2004;43(10):623–653. [PubMed: 15244495]
7. Shaw LM, Holt DW, Keown P, Venkataramanan R, Yatscoff RW. Current opinions on therapeutic drug monitoring of immunosuppressive drugs. Clin Ther. 1999;21(10):1632–1652; discussion 1631. [PubMed: 10566561]
8. Venkataramanan R, Swaminathan A, Prasad T, et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet. 1995;29(6):404–430. [PubMed: 8787947]
9. Staatz C, Taylor P, Tett S. Low tacrolimus concentrations and increased risk of early acute rejection in adult renal transplantation. Nephrol Dial Transplant. 2001;16(9):1905–1909. [PubMed: 11522877]
10. Undre NA, van Hooff J, Christiaans M, et al. Low systemic exposure to tacrolimus correlates with acute rejection. Transplant Proc. 1999;31(1–2):296–298. [PubMed: 10083114]
11. Wiebe C, Rush DN, Nevins TE, et al. Class II Eplet Mismatch modulates tacrolimus trough levels required to prevent donor-specific antibody development. J Am Soc Nephrol. 2017;28(11):3353–3362. [PubMed: 28729289]
12. Staatz CE, Willis C, Taylor PJ, Tett SE. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. Clin Pharmacol Ther. 2002;72(6):660–669. [PubMed: 12496747]
13. Staatz CE, Willis C, Taylor PJ, Lynch SV, Tett SE. Toward better outcomes with tacrolimus therapy: population pharmacokinetics and individualized dosage prediction in adult liver transplantation. Liver Transpl. 2003;9(2):130–137. [PubMed: 12548506]
14. Han N, Ha S, Yun HY, et al. Population pharmacokinetic-pharmacogenetic model of tacrolimus in the early period after kidney transplantation. Basic Clin Pharmacol Toxicol. 2014;114(5):400–406. [PubMed: 24238261]
15. Bergmann TK, Hennig S, Barraclough KA, Isbel NM, Staatz CE. Population pharmacokinetics of tacrolimus in adult kidney transplant patients: impact of CYP3A5 genotype on starting dose. Ther Drug Monit. 2014;36(1):62–70. [PubMed: 24089074]
16. Antignac M, Hulot JS, Boleslawski E, et al. Population pharmacokinetics of tacrolimus in full liver transplant patients: modelling of the post-operative clearance. Eur J Clin Pharmacol. 2005;61(5–6):409–416. [PubMed: 15991041]
17. Sanghavi K, Brundage RC, Miller MB, et al. Genotype-guided tacrolimus dosing in African-American kidney transplant recipients. Pharmacogenomics J. 2017;17(1):61–68. [PubMed: 26667830]
18. Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part I. Clin Pharmacokinet. 2010;49(3):141–175. [PubMed: 20170205]
19. Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part II. Clin Pharmacokinet. 2010;49(4):207–221. [PubMed: 20214406]
20. Kuehl P, Zhang J, Lin Y, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet. 2001;27(4):383–391. [PubMed: 11279519]
21. Lamba J, Hebert JM, Schuetz EG, Klein TE, Altman RB. PharmGKB summary: very important pharmacogene information for CYP3A5. Pharmacogenet Genomics. 2012;22(7): 555–558. [PubMed: 22407409]

22. Rekers NV, Flaig TM, Mallat MJK, et al. Donor genotype and intragraft expression of CYP3A5 reflect the response to steroid treatment during acute renal allograft rejection. Transplantation. 2017;101(9):2017–2025. [PubMed: 27926596]

23. de Jonge H, de Loor H, 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(12):1467–1480. [PubMed: 24024898]

24. Passey C, Birnbaum AK, Brundage RC, Oetting WS, Israni AK, Jacobson PA. Dosing equation for tacrolimus using genetic variants and clinical factors. Br J Clin Pharmacol. 2011;72(6):948–957. [PubMed: 21671989]

25. Oetting WS, Schladt DP, Guan W, et al. Genomewide Association study of tacrolimus concentrations in African American kidney transplant recipients identifies multiple CYP3A5 alleles. Am J Transplant. 2016;16(2):574–582. [PubMed: 26485092]

26. Lee SJ, Usmani KA, Chanas B, et al. Genetic findings and functional studies of human CYP3A5 single nucleotide polymorphisms in different ethnic groups. Pharmacogenetics. 2003;13(8):461–472. [PubMed: 12893984]

27. Elens L, Capron A, van Schaik RH, et al. Impact of CYP3A4*22 allele on tacrolimus pharmacokinetics in early period after renal transplantation: toward updated genotype-based dosage guidelines. Ther Drug Monit. 2013;35(5):608–616. [PubMed: 24052064]

28. Abdel-Kahaar E, Winter S, Tremmel R, et al. The impact of CYP3A4*22 on tacrolimus pharmacokinetics and outcome in clinical practice at a single kidney transplant center. Front Genet. 2019;10:871. 10.3389/fgene.2019.00871 [PubMed: 31616470]

29. Wang D, Guo Y, Wrighton SA, Cooke GE, Sadee W. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. Pharmacogenomics J. 2011;11(4):274–286. [PubMed: 20386561]

30. Okubo M, Murayama N, Shimizu M, Shimada T, Guengerich FP, Yamazaki H. CYP3A4 intron 6 C>T polymorphism (CYP3A4*22) is associated with reduced CYP3A4 protein level and function in human liver microsomes. J Toxicol Sci. 2013;38(3):349–354. [PubMed: 23665933]

31. Scheibner A, Remmel R, Schladt D, et al. Tacrolimus elimination in four patients with a CYP3A5*3/*3 CYP3A4*22/*22 genotype combination. Pharmacotherapy. 2018.

32. Pallet N, Jannot AS, El Bahri M, et al. Kidney transplant recipients carrying the CYP3A4*22 allelic variant have reduced tacrolimus clearance and often reach supratherapeutic tacrolimus concentrations. Am J Transplant. 2015;15(3):800–805. [PubMed: 25588704]

33. Kitzmiller JP, Luzum JA, Baldassarre D, Krauss RM, Medina MW. CYP3A4*22 and CYP3A5*3 are associated with increased levels of plasma simvastatin concentrations in the cholesterol and pharmacogenetics study cohort. Pharmacogenet Genomics. 2014;24(10):486–491. [PubMed: 25051018]

34. Press RR, Ploeger BA, den Hartigh J, et al. Explaining variability in tacrolimus pharmacokinetics to optimize early exposure in adult kidney transplant recipients. Ther Drug Monit. 2009;31(2):187–197. [PubMed: 19258929]

35. Birdwell KA, Grady B, Choi L, et al. The use of a DNA biobank linked to electronic medical records to characterize pharmacogenomic predictors of tacrolimus dose requirement in kidney transplant recipients. Pharmacogenet Genomics. 2012;22(1): 32–42. [PubMed: 22108237]

36. Haufroid V, Mourad M, Van Kerckhove V, et al. The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. Pharmacogenetics. 2004;14(3):147–154. [PubMed: 15167702]

37. Jacobson PA, Oetting WS, Brearley AM, et al. Novel polymorphisms associated with tacrolimus trough concentrations: results from a multicenter kidney transplant consortium. Transplantation. 2011;91(3):300–308. [PubMed: 21206424]

38. Tang JT, Andrews LM, van Gelder T, et al. Pharmacogenetic aspects of the use of tacrolimus in renal transplantation: recent developments and ethnic considerations. Expert Opin Drug Metab Toxicol. 2016;12(5):555–565. [PubMed: 27010623]

39. Li YR, van Setten J, Verma SS, et al. Concept and design of a genome-wide association genotyping array tailored for transplantation-specific studies. Genome Med. 2015;7:90. [PubMed: 26423053]

40. Oetting WS, Wu B, Schladt DP, et al. Genome-wide association study identifies the common variants in CYP3A4 and CYP3A5 responsible for variation in tacrolimus trough concentration in Caucasian kidney transplant recipients. Pharmacogenomics J. 2018;18(3):501–505. [PubMed: 29160300]
41. Mohamed ME, Schladt DP, Guan W, et al. Tacrolimus troughs and genetic determinants of metabolism in kidney transplant recipients: A comparison of four ancestry groups. Am J Transplant. 2019;19(10):2795–2804. [PubMed: 30953600]
42. Keizer RJ, van Benten M, Beijnen JH, Schellens JH, Huitema AD. Pirana and PCluster: a modeling environment and cluster infrastructure for NONMEM. Comput Methods Programs Biomed. 2011;101(1):72–79. [PubMed: 20627442]
43. Dosne AG, Bergstrand M, Karlsson MO. An automated sampling importance resampling procedure for estimating parameter uncertainty. J Pharmacokinet Pharmacodyn. 2017;44(6):509–520. [PubMed: 28887735]
44. Dosne AG, Bergstrand M, Harling K, Karlsson MO. Improving the estimation of parameter uncertainty distributions in nonlinear mixed effects models using sampling importance resampling. J Pharmacokinet Pharmacodyn. 2016;43(6):583–596. [PubMed: 27730482]
45. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. AAPS J. 2011;13(2): 143–151. [PubMed: 21302010]
46. Undre NA, Schafer A. Factors affecting the pharmacokinetics of tacrolimus in the first year after renal transplantation. European Tacrolimus Multicentre Renal Study Group. Transplant Proc. 1998;30(4):1261–1263. [PubMed: 9636512]
47. Eeckhoudt SL, Horsmans Y, Verbeeck RK. Differential induction of midazolam metabolism in the small intestine and liver by oral and intravenous dexamethasone pretreatment in rat. Xenobiotica. 2002;32(11):975–984. [PubMed: 12487727]
48. Hukkanen J, Vaisanen T, Lassila A, et al. Regulation of CYP3A5 by glucocorticoids and cigarette smoke in human lung-derived cells. J Pharmacol Exp Ther. 2003;304(2): 745–752. [PubMed: 12538830]
49. Ogg MS, Williams JM, Tarbit M, Goldfarb PS, Gray TJ, Gibson GG. A reporter gene assay to assess the molecular mechanisms of xenobiotic-dependent induction of the human CYP3A4 gene in vitro. Xenobiotica. 1999;29(3):269–279. [PubMed: 10219967]
50. Schuetz EG, Wrighton SA, Barwick JL, Guzelian PS. Induction of cytochrome P-450 by glucocorticoids in rat liver. I. Evidence that glucocorticoids and pregnenolone 16 alpha-carbonitrile regulate de novo synthesis of a common form of cytochrome P-450 in cultures of adult rat hepatocytes and in the liver in vivo. J Biol Chem. 1984;259(3):1999–2006. [PubMed: 6141167]
51. Li JL, Wang XD, Chen SY, et al. Effects of diltiazem on pharmacokinetics of tacrolimus in relation to CYP3A5 genotype status in renal recipients: from retrospective to prospective. Pharmacogenomics J. 2011;11(4):300–306. [PubMed: 20514078]
52. Zhu Y, Wang F, Li Q, et al. Amlodipine metabolism in human liver microsomes and roles of CYP3A4/5 in the dihydropyridine dehydrogenation. Drug Metab Dispos. 2014;42(2):245–249. [PubMed: 24301608]
53. Groll AH, Townsend R, Desai A, et al. Drug-drug interactions between triazole antifungal agents used to treat invasive aspergillosis and immunosuppressants metabolized by cytochrome P450 3A4. Transpl Infect Dis. 2017;19(5).
54. Vanhove T, Bouwsma H, Hilbrands L, et al. Determinants of the magnitude of interaction between tacrolimus and voriconazole/posaconazole in solid organ recipients. Am J Transplant. 2017;17(9):2372–2380. [PubMed: 28224698]
55. de Jonge H, Vanhove T, de Loor H, Verbeke K, Kuypers DR. Progressive decline in tacrolimus clearance after renal transplantation is partially explained by decreasing CYP3A4 activity and increasing haematocrit. Br J Clin Pharmacol. 2015;80(3):548–559. [PubMed: 26114223]
56. Jacobson PA, Schladt D, Oetting WS, et al. Lower calcineurin inhibitor doses in older compared to younger kidney transplant recipients yield similar troughs. Am J Transplant. 2012;12(12):3326–3336. [PubMed: 22947444]

57. Miura M, Satoh S, Kagaya H, et al. No impact of age on dose-adjusted pharmacokinetics of tacrolimus, mycophenolic acid and prednisolone 1 month after renal transplantation. Eur J Clin Pharmacol. 2009;65(10):1047–1053. [PubMed: 19730841]
58. Staatz CE, Tett SE. Pharmacokinetic considerations relating to tacrolimus dosing in the elderly. Drugs Aging. 2005;22(7):541–557. [PubMed: 16038570]
59. Stratta P, Quaglia M, Cena T, et al. The interactions of age, sex, body mass index, genetics, and steroid weight-based doses on tacrolimus dosing requirement after adult kidney transplantation. Eur J Clin Pharmacol. 2012;68(5):671–680. [PubMed: 22101623]
60. Waring RH, Harris RM, Mitchell SC. Drug metabolism in the elderly: A multifactorial problem? Maturitas. 2017;100:27–32. 10.1016/j.maturitas.2017.03.004. [PubMed: 28539174]
61. Kinirons MT, O'Mahony MS. Drug metabolism and ageing. Br J Clin Pharmacol. 2004;57(5):540–544. [PubMed: 15089805]
62. Schijvens AM, van Hesteren FHS, Cornelissen EAM, et al. The potential impact of hematocrit correction on evaluation of tacrolimus target exposure in pediatric kidney transplant patients. Pediatr Nephrol. 2019;34(3):507–515. [PubMed: 30374607]
63. Limsrichamrern S, Chanapul C, Mahawithitwong P, et al. Correlation of hematocrit and tacrolimus level in liver transplant recipients. Transplant Proc. 2016;48(4):1176–1178. [PubMed: 27320581]
64. Brown NW, Gonde CE, Adams JE, Tredger JM. Low hematocrit and serum albumin concentrations underlie the overestimation of tacrolimus concentrations by microparticle enzyme immunoassay versus liquid chromatography-tandem mass spectrometry. Clin Chem. 2005;51(3):586–592. [PubMed: 15650031]
65. Birdwell KA, Decker B, Barbarino JM, et al. Clinical pharmacogenetics implementation consortium (CPIC) guidelines for CYP3A5 genotype and tacrolimus dosing. Clin Pharmacol Ther. 2015;98(1):19–24. [PubMed: 25801146]

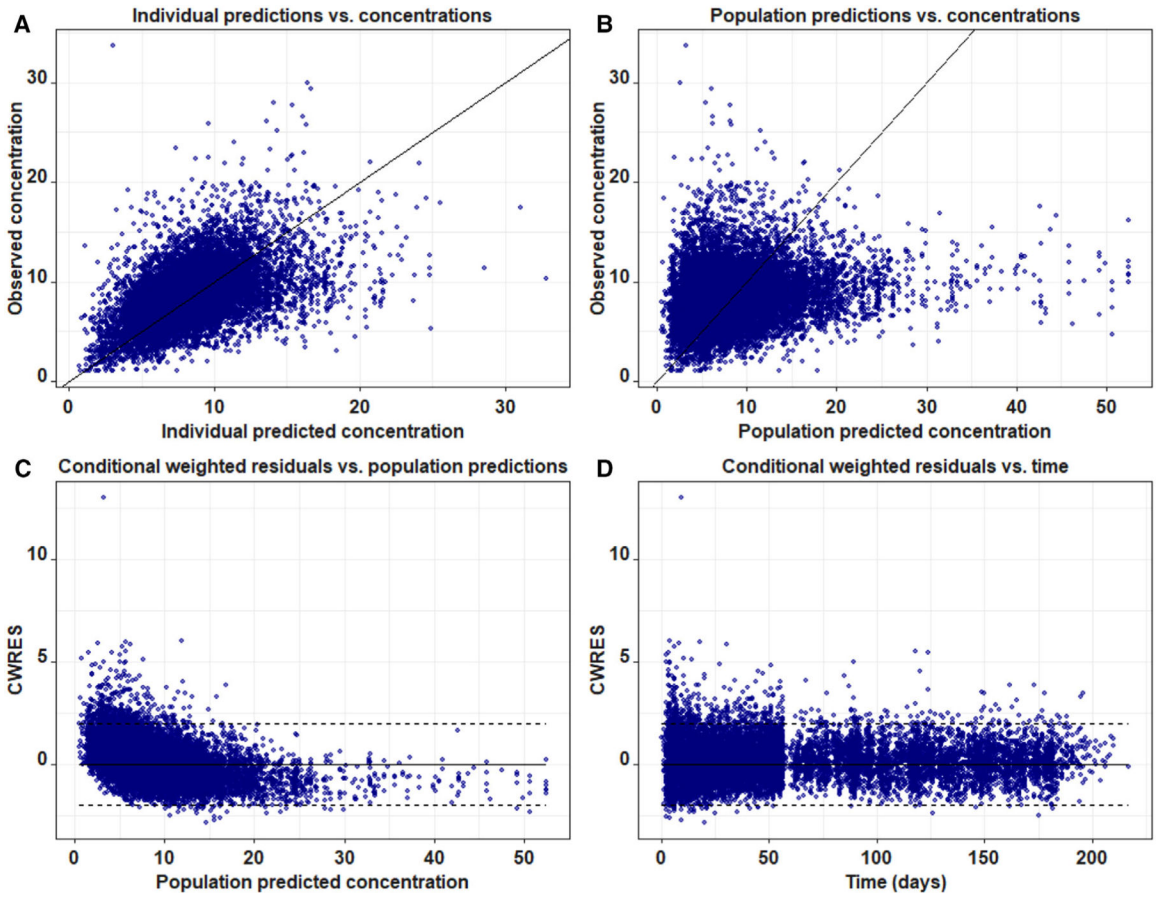

Figure 1.

Goodness-of-fit plots for the final tacrolimus model. (A) Observed concentrations (ng/mL) versus individual-predicted concentrations (ng/mL). (B) Observed concentrations (ng/mL) versus population-predicted concentrations (ng/mL). Dots represent the observed tacrolimus trough concentrations. Solid line represents the line of unity.(C) Conditional weighted residuals (CWRES) versus population-predicted concentrations (ng/mL). (D) CWRES versus time (days). Dots represent the observed tacrolimus trough concentrations. Solid line is the line at $y = 0$.

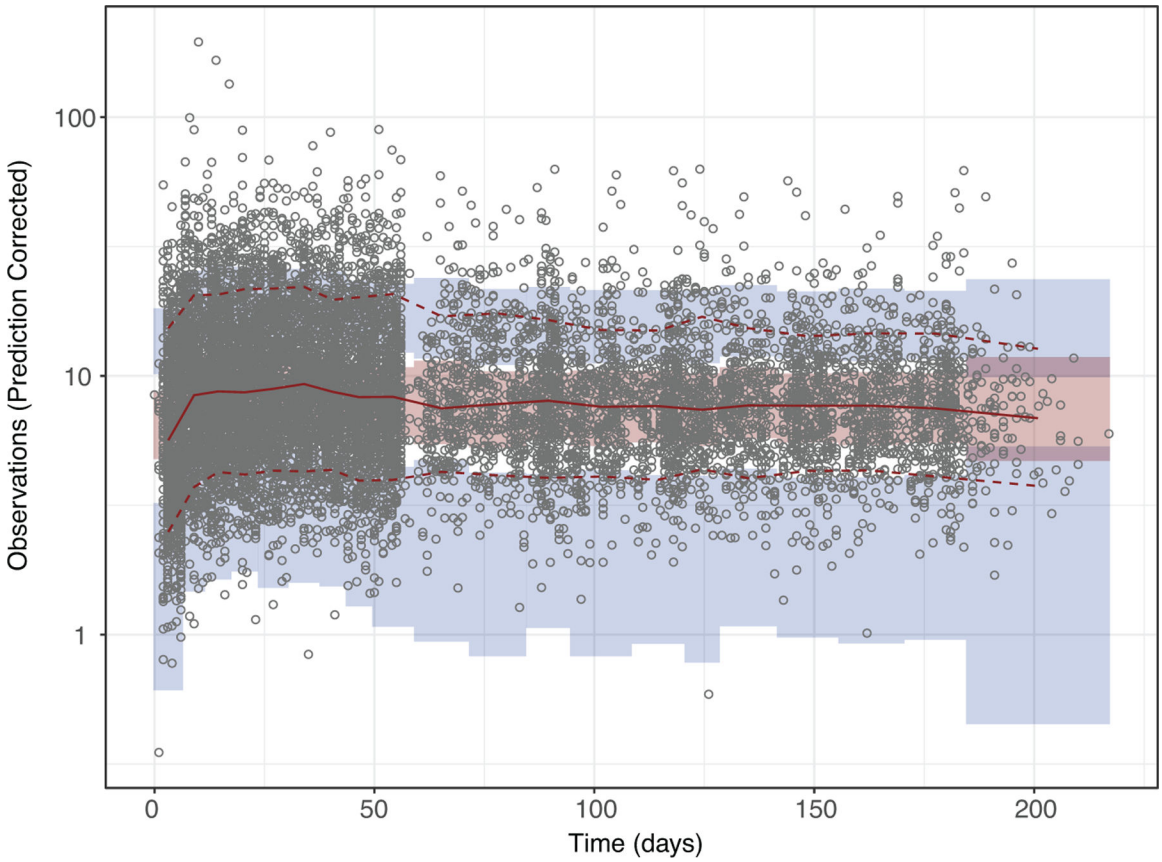

Figure 2.

Prediction-corrected visual predictive check (pcVPC) on a log scale for the final tacrolimus model. The solid red line represents the median observed trough concentrations (ng/mL; prediction-corrected trough concentration), and the semitransparent red field represents a simulation-based 95% confidence interval for the median. The observed 10th and 90th percentiles are presented with dashed red lines, and the 95% confidence intervals for the corresponding model-predicted percentiles are shown as semitransparent blue fields. The observed trough concentrations (prediction corrected) are represented by open circles. All tacrolimus observed concentrations are trough concentrations.

Table 1.

Study Population Characteristics

Characteristics					
Cohort		Development Cohort (GEN03)		Validation Cohort (DeKAF Genomics)	
Participants, n		608		1361	
Troughs, n		10 992		23 658	
		Median (range)			
Daily dose (mg)		5 (0.3–35)		5.5 (0.1–36)	
Tacrolimus trough (ng/mL)		8.3 (1–33.7)		8.4 (0.3–75.9)	
Age (years)		52 (18–81)		52 (18.4–83.4)	
Weight (kg)		79.8 (37.8–161)		82.3 (38.6–158)	
		n (%)			
Sex		Male	372 (61.2)	Male	861 (63.3)
		Female	236 (38.8)	Female	500 (36.7)
Living donor		437 (71.9)		903 (66.3)	
Diabetes at transplant		179 (29.4)		525 (38.6)	
Troughs with CCB[a]		4394 (40)		8610 (36.4)	
Troughs with antiviral drug[a]		5755 (52.4)		13 557 (57.3)	
Troughs with steroid[a]		7036 (64)		15 279 (64.6)	
Genotype	*CYP3A5*	**3/*3*	537 (88.3)	**3/*3*	1179 (86.6)
		**1/*3*	65 (10.7)	**1/*3*	175 (12.9)
		**1/*1*	3 (0.5)	**1/*1*	7 (0.5)
		indeterminate	3 (0.5)	indeterminate	0 (0)
	CYP3A4	**1/*1*	553 (91)	**1/*1*	1210 (88.9)
		**1/*22*	53 (8.7)	**1/*22*	147 (10.8)
		**22/*22*	2 (0.3)	**22/*22*	4 (0.3)

CCB, calcium channel blocker; CYP, cytochrome.

[a]Number of tacrolimus troughs that were obtained while recipient was concomitantly receiving this drug.

Table 2.

Final Tacrolimus Pharmacokinetic Parameter Estimates, Precision, and Shrinkage in Development Cohort

Parameter	Estimate (RSE %)	SIR Median (95%CI)	Shrinkage[a] (%)
Oral tacrolimus PK parameters			
CL/F (L/h)	32.2 (4.2)	32.18 (30.1–34.5)	
Effect of covariates on tacrolimus CL/F			
Age	$(age/52) \times 10^{-0.3}$ (17.2)	−0.29 (−0.39 to −0.19)	
if receiving an antiviral drug	0.91 (1.4)	0.91 (0.89–0.93)	
if diabetic	0.87 (3.9)	0.87 (0.81–0.94)	
if receiving a CCB	0.95 (2.1)	0.95 (0.93–0.98)	
if receiving a steroid	1.06 (3.8)	1.06 (1.01–1.11)	
if tacrolimus is after day 8 posttreatment	0.82 (2.3)	0.81 (0.79–0.84)	
Effect of CYP3A5 variant on tacrolimus CL/F compared with *CYP3A5*3/*3*			
if *CYP3A5*1/*3*	1.81 (4.9)	1.81 (1.62–2)	
if *CYP3A5*1/*1*	3.05 (12.5)	3.03 (2.17–3.93)	
Effect of CYP3A4 variant on tacrolimus CL/F compared with *CYP3A4*1/*1*			
if 1 *CYP3A4*22*	0.78 (4.9)	0.78 (0.7–0.86)	
if 2 *CYP3A4*22*	0.28 (11.8)	0.28 (0.2–0.35)	
Intercenter variability, CV%[b]			
CL/F	31.3 (10.8)	31.3 (27.2–35)	0.1
InterIndividual Variability, CV%[b]			
CL/F	41.9 (8.6)	41.9 (38.9–45.7)	2.1
Residual unexplained variability[c]			
Proportional, CCV%	21.7 (13.8)	21.7 (20.4–23)	2.7
Additive, SD	2.3 (11.4)	2.3 (2.2–2.4)	2.7

CCB, calcium channel blockers; CL/F, apparent oral clearance; CCV, constant coefficient of variation; CV, coefficient of variation; SIR, sampling-importance resampling.

[a] Shrinkage was calculated,for both η and ε.

[b] CV% was calculated as $\sqrt{e^{\omega^2} - 1}$

[c] CCV% and SD were calculated as $\sqrt{\sigma^2}$.