Citation: CPT Pharmacometrics Syst. Pharmacol. (2019) 8, 273–284; doi:10.1002/psp4.12392

ARTICLE

A Theoretical Physiologically-Based Pharmacokinetic Approach to Ascertain Covariates Explaining the Large Interpatient Variability in Tacrolimus Disposition

Chie Emoto[1,2], Trevor N. Johnson[3], David Hahn[1], Uwe Christians[4], Rita R. Alloway[5], Alexander A. Vinks[1,2] and Tsuyoshi Fukuda[1,2,*]

Physiologically-based pharmacokinetic (PBPK) modeling allows assessment of the covariates contributing to the large pharmacokinetic (PK) variability of tacrolimus; these include multiple physiological and biochemical differences among patients. A PBPK model of tacrolimus was developed, including a virtual population with physiological parameter distributions reflecting renal transplant patients. The ratios of predicted to observed dose-normalized maximum plasma concentration (C_{max}), 0–12-hour area under the concentration–time curve ($AUC_{0–12\ hour}$), and trough plasma concentration (C_{trough}) ranged from 0.92-fold to 1.15-fold, indicating good predictive performance. The model quantitatively indicated the impact of cytochrome P450 (CYP)3A4 abundance, hematocrit, and serum albumin levels, in addition to CYP3A5 genotype status, on tacrolimus PK and associated variability. Age-dependent change in tacrolimus trough concentration in pediatric patients was mainly attributed to the CYP3A ontogeny profile. This study demonstrates the utility of PBPK modeling as a tool for mechanistic and quantitative assessment of the impact of patient physiological differences on observed large PK variability.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Multiple physiological and biochemical differences are considered to cause large interpatient and intrapatient variability in tacrolimus pharmacokinetics (PK). Several factors have been reported to explain this variability; however, comprehensive assessment of their combined effects is still limited.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Can a physiologically-based pharmacokinetic (PBPK) model, incorporating realistic renal transplant patient physiological data and the known drug information on tacrolimus, be used to identify and quantitatively assess the effects of covariates on the observed PK variability?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Implementing realistic distributions of physiological parameters observed in the target patient population into the PBPK model enhanced tacrolimus PK predictability. The model successfully quantified contributions of cytochrome P450 (CYP)3A5 genotype and changes in CYP3A expression, hematocrit, and serum albumin related to patient status and age to the PK variability in renal transplant patients.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

☑ This study provides an example of the utility of PBPK modeling as a knowledge-integrating tool to define the physiological covariates on the PK variability within specific populations.

Physiologically-based pharmacokinetic (PBPK) models allow the mechanistic assessment of potential covariates contributing to the observed variability in drug exposure. This modeling approach can predict interpatient and intrapatient pharmacokinetic (PK) variability through interaction between system-specific parameters (human body characteristics) and drug-specific parameters (physicochemical and disposition properties). In descriptive and population PK (PopPK) analyses, the probability of identifying covariates may rely on the physiological characteristics of the target population. This often represents a study limitation in terms of the power of detection. For instance, if the range and effect of a physiological parameter observed in the target population were small, it would be challenging to identify this as an influential covariate within the study even though the parameter may be truly influential. Where multiple factors contribute to large variability, it would be more challenging to evaluate the effect of each potential covariate,

[1]Division of Clinical Pharmacology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA; [2]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA; [3]Simcyp Division, Certara UK Limited, Sheffield, UK; [4]iC42 Clinical Research and Development, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA; [5]Division of Nephrology and Hypertension, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA. *Correspondence: Tsuyoshi Fukuda (Tsuyoshi.Fukuda@cchmc.org)
Received: December 11, 2018; accepted: January 23, 2019. doi:10.1002/psp4.12392

because each covariate may work as a confounder in identifying others in the analysis.

The immunosuppressant tacrolimus was selected as a test drug in this study because of large intrapatient and interpatient variability in the PK profiles seen in patients for whom multiple factors have been proposed. Changes in covariates that affect elimination (such as genotype) are important for prediction of initial drug dosing, whereas covariates that affect whole blood binding (albumin and hematocrit) do not influence elimination but are informative only for interpreting measured concentrations in order to estimate unbound clearance.

The pharmacogenetic influence of the cytochrome P450 (CYP)3A5**1* and *CYP3A5*3* alleles, an important proven covariate of the PK variability, has previously been described by the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for tacrolimus dosing.[1] However, CYP3A5 genotype does not fully explain the PK variability of tacrolimus. CYP3A5**3* polymorphism explained around 35% and up to 50% for dose-adjusted trough blood concentrations and dose, respectively (n = 50 stable renal transplant recipients).[2]

The oral bioavailability of tacrolimus is reported to be ~ 20%, yet it can be highly variable in healthy adults.[3,4] After intravenous and oral administration of ^{14}C-labeled tacrolimus, most of the radioactivity (sum of tacrolimus and its metabolites) was found to be excreted in the feces, with < 3% of the dose excreted in the urine. Interestingly, unchanged tacrolimus concentrations accounted for < 1% of the total radioactivity excreted in both feces and urine, suggesting that tacrolimus undergoes extensive metabolism before excretion. Therefore, it seems that physiological factors related to the metabolism of tacrolimus are most likely to show the highest impact on the large PK variability.

Tacrolimus is a substrate of CYP3A4 and CYP3A5,[5] which are expressed in both small intestine[6] and liver tissue.[7] The fraction of tacrolimus escaping gut wall metabolism (F_g) was estimated to be 0.14–0.26,[8,9] which explains the low bioavailability. CYP3A abundance in human intestinal and liver microsomes is highly variable[10] and directly contributes to the variability seen in the metabolism of tacrolimus. This variability in metabolism may make it difficult to identify other significant covariates on total blood (but not unbound) elimination, such as free fraction of tacrolimus determined by protein binding and blood-to-plasma ratio.

In this study, a PBPK model of tacrolimus was developed to provide mechanistic insight into factors contributing to variability in tacrolimus PK among adult and pediatric renal transplant patients.

METHODS

Development of the tacrolimus PBPK model

A PBPK model of tacrolimus was developed using Simcyp simulator software version 17 (Certara UK Limited, Simcyp Division, Sheffield, UK). Tacrolimus drug-specific parameters, such as molecular weight, *in vitro* protein binding, blood-to-plasma ratio, *in vitro* CYP enzyme kinetic parameters, and *in vivo* renal clearance, were collected from the literature and incorporated into the compound model to describe the characteristic of tacrolimus (**Table 1**[3,8,11–15]). The simulator allows a specific clinical trial design to be generated based on age, sex, dose, number of doses, route of administration, and multiple trials of "n" individuals. The model development process is shown in **Figure 1**. Distribution parameters incorporated into a minimal PBPK model were determined using the parameter estimation function implemented in Simcyp. For CYP3A4 and CYP3A5

Table 1 Summary of physicochemical parameters, *in vitro*, and *in vivo* data of tacrolimus from the literature

Parameter	Value
Physicochemical properties	
Molecular weight (g/mol)	804.0182[a]
LogP	3.3[11]
Compound type	Neutral[11]
Blood binding properties	
Fraction unbound in serum	0.012[12]
Blood-to-plasma ratio	35[8]
Plasma binding protein	Human serum albumin
Absorption	
First-order model	
f_a	1.00[b]
k_a (hour^{-1})	3.68[b]
Lag time (hour)	0.43[b]
Distribution	
Minimal PBPK model	
k_{in} (hour^{-1})	0.68[c]
k_{out} (hour^{-1})	0.10[c]
V_{sac} (L/kg)	10.8[c]
Predicted V_{ss} (L/kg)	17.1[d]
Elimination	
CYP kinetic parameters	
CYP3A4	
K_m (μM)	0.21 (12.7, CV%)[13]
V_{max} (pmol/minute/pmol CYP)	3.8[e]
CYP3A5	
K_m (μM)	0.21 (6.4, CV%)[13]
V_{max} (pmol/minute/pmol CYP)	2.5[f]
Renal clearance (mL/minute)[g]	0.014 ± 0.008 (mean ± SD)[3]

CV%, percentage of coefficient of variation; CYP, cytochrome P450; f_a, fraction available from dosage form; k_a, first-order absorption rate constant; k_{in} and k_{out}, first-order rate constants describing the transfer of tacrolimus to a single adjusting compartment; K_m, Michaelis-Menten constant; PBPK, physiologically-based pharmacokinetic; V_{max}, maximum rate of metabolite formation; V_{sac}, volume of single adjusting compartment; V_{ss}, volume of distribution at steady state using tissue volumes for a population representative of healthy volunteers.

[a]https://www.drugbank.ca/drugs/DB00864. [b]Estimated by the parameter estimation method using tacrolimus pharmacokinetic (PK) profile after oral administration in healthy white people. [c]Estimated by the parameter estimation method using tacrolimus PK profile after intravenous administration in healthy white people, whose weights were within 20% of their ideal body weight. The estimated values can be considered as parameters for a subject having a standard body weight of 70 kg. The rate constants are scaled within the pediatric PBPK model using body weight (BW)/70$^{-0.25}$. [d]The V_{ss} value was predicted by a minimal PBPK model based on the method by Poulin and Theil[14] with correction by Berezhkovskiy,[15] where Kp scaler was set at 13. [e]Estimated using the absorption, distribution, metabolism, and excretion simulator. [f]Estimated by sensitivity analysis. [g]The observed renal blood clearance[3] was used to estimate renal plasma clearance with the blood-to-plasma ratio,[8] as typical renal clearance in 20–30 year healthy men. This value is scaled to 0.013 ± 0.007 mL/minute for a subject having a standard body weight of 70 kg, based on the allometric scaling with exponent of 0.75.

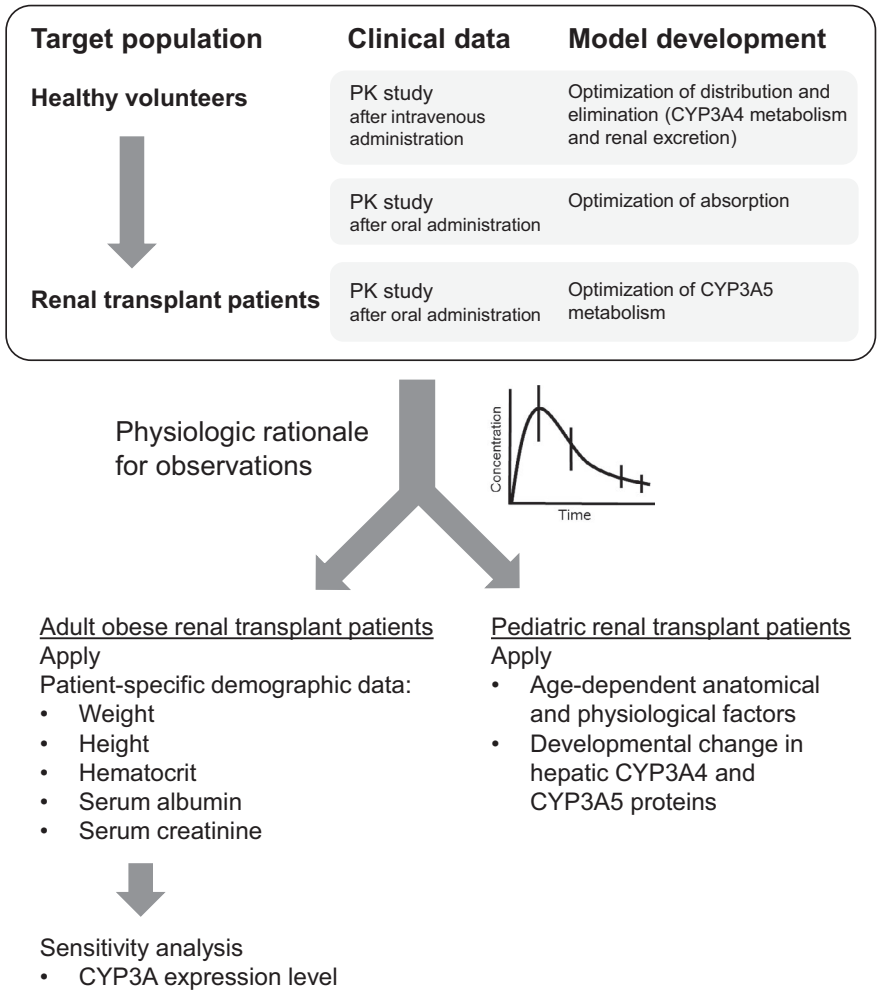

Figure 1 Schematic representation of the workflow describing physiologically-based pharmacokinetic model development of tacrolimus. CYP, cytochrome P450; IV, intravenous; PO, oral.

metabolism, Michaelis-Menten constant (K_m) values for CYP3A4 and CYP3A5 were directly obtained from previously published *in vitro* data. Maximal rate of metabolism (V_{max}) values were estimated based on clinical PK data of tacrolimus: V_{max} of CYP3A4, using back-calculation from PK parameters observed in healthy volunteers; V_{max} of CYP3A5, using sensitivity analysis based on observed mean ratio of trough concentrations of tacrolimus between CYP3A5 poor metabolizers (PMs; i.e., homozygous of CYP3A5*3) and normal metabolizers (NMs; i.e., CYP3A5*1 carrier) through the sensitivity analysis. Subsequently, the oral absorption parameters were optimized based on a first-order model using PK data obtained after oral administration in the healthy volunteers. The details on the development process for elimination pathways, including implementation of CYP3A5 metabolism, distribution, absorption, and PK evaluation, are described in the **Supplemental Methods**.

Simulation in adult renal transplant patients

Population system parameters. Patient demographic information and clinical laboratory values were obtained from a clinical study that was funded by the US National Institutes of Health and the US Food and Drug Administration (NIH/FDA U01FD004573) and was a PK study of tacrolimus in transplant patients (ClinicalTrials.gov Identifier: NCT01889758).[16] The built-in Obesity population module was manually modified based on the obtained patient data to represent a realistic renal transplant patient population. The relationships among age, body height, and body weight were used to generate virtual subjects having body mass index (BMI) similar to the target renal transplant patients. In addition, the mean and percentage of coefficient of variation (CV%) values of hematocrit, serum albumin concentrations, and serum creatinine concentrations were obtained from renal transplant patients and incorporated into the patient-specific system model. The detailed parameters are summarized in **Table S1**. Based on the developed patient-specific model, the virtual CYP3A5 PM and NM were created according to the method mentioned in **Supplemental Methods**. The protein expression levels of hepatic and intestinal CYP3A4 and CYP3A5 were also modified from 80% to 100%, where 100% was default value (see **Supplemental Methods**) for each enzyme in the built-in healthy volunteer population module. The

predicted PK parameters of tacrolimus with the modified enzyme expression level were compared with the clinically observed PK data.

PK simulations. All simulations were conducted with the generated patient-specific models for CYP3A5 PM and NM. In the simulation, tacrolimus was orally administrated twice a day for 3 weeks. Doses of tacrolimus were set at 0.02 mg/kg for CYP3A5 PM and 0.04 mg/kg for CYP3A5 NM. For the simulation, ages ranged from 20–65 years, and the proportion of women was 0.34 (34% women in the generated virtual subjects). The simulation size was 1,000 (10 individuals × 100 trials) for each CYP3A5 genotyped patient model.

Sensitivity analysis of CYP3A4 abundance and clinical laboratory values on tacrolimus PK profiles in virtual patients

Sensitivity analyses were conducted focusing on hepatic and intestinal CYP3A4 abundances, hematocrit, serum albumin concentration, and serum creatinine concentration to explore the potential impact on the tacrolimus PK profile. The sensitivity to CYP3A4 abundance was assessed within a twofold range of a default setting of hepatic and intestinal CYP3A4 abundances in healthy volunteers. The range for each clinical laboratory value was as follows: hematocrit, 30–60%; albumin, 3.0–6.0 g/dL; and serum creatinine, 0.57–2.3 mg/dL. The patient-specific system model for CYP3A5 PM was used for the sensitivity analyses. The simulation trial design included the oral administration of tacrolimus twice a day for 3 weeks (21 days) in a representative virtual subject.

Prediction of trough concentration in pediatric renal transplant patients via PBPK modeling and PopPK modeling

Pediatric PBPK simulation. The PBPK model of tacrolimus was incorporated within the Simcyp Pediatric simulator, where two different ontogeny profiles of hepatic CYP3A4 were tested according to that reported by Upreti and Wahlstrom[17] and one based on the data and methodology of Salem *et al.*[18,19] with modifications based on the effects of disease. The details of simulation settings for CYP3A enzyme expression and hematocrits are described in **Supplemental Methods**. For the pediatric simulations, dosing regimens were 0.05–0.2 mg/kg every 12 hours for 3 weeks. A total of 600 simulations were conducted (n = 100 for each age group) with six age groups covering the period from 1–16 years, where virtual infants were assumed to be full-term. Pediatric population models for CYP3A5 PMs and NMs were generated as mentioned above in adults.

Pediatric PK simulation based on the reported PopPK model. Trough concentrations in pediatric subjects were predicted based on the PopPK model of tacrolimus reported previously[20,21] using Berkeley Madonna version 8.3.23.0 (Berkeley Madonna, Berkeley, CA). Briefly, the PopPK model for pediatric renal transplant patients was a two-compartment model with first-order, lagged time absorption and first-order elimination, where body weight, CYP3A5 genotype, and hematocrit level were covariates for clearance of tacrolimus. The concentration–time profiles of tacrolimus were simulated with twice a day dose 0.05–0.2 mg/kg for 21 days in hypothetical pediatric subjects who have various body weights, CYP3A5 genotypes, and hematocrit levels, where a trough concentration at steady state was assumed the concentration at day 21. The model code used for the simulation is described in **Supplemental Documents**.

Statistical analysis

Statistical data of simulated and observed PK parameters, such as geometric mean, the confidence interval, and geometric SD, were calculated using GraphPad PRISM version 7.02 (GraphPad Software, La Jolla, CA). In this study, the following classification for PBPK model predictability was used: good prediction as between 0.8-fold and 1.25-fold; reasonable between 0.67-fold and 1.5-fold; and acceptable between 0.5-fold and 2-fold.

RESULTS

PK simulation of tacrolimus in adult healthy volunteers

The predicted and observed concentration–time profiles following i.v. tacrolimus administration are shown in **Figure 2a,b** and for oral administration in **Figure 2c,d**.[3,4] The observed mean concentration–time profiles were within the 5th to 95th percentile range of the PBPK simulations. In comparison with reported PK observations,[3,4] the ratio of predicted to observed parameters were in the range 0.73–1.19 (**Table S2**).

PK simulation of tacrolimus in adult renal transplant patients

The distribution of patient demographic values observed in renal transplant patients (n = 35)[16] was implemented into the PBPK model in order to generate a virtual patient population having realistic demographics (**Table S1**). The mean of body weight was 96.4 kg (**Figure S1**); most of the current renal transplant patients were categorized as obese according to the classification of the World Health Organization (WHO)[22]: 74% obese (BMI ≥ 30); 11% overweight (≥ 25, < 30); and 14% other (≥ 19, < 25). Overall hematocrit values in the patient cohort were lower than those in the reported normal range,[23] whereas serum creatinine concentrations were slightly higher.[24] Most of serum albumin concentrations were within the normal range.[25] The demographic information in generated virtual patients was comparable to observed data in the renal transplant patients.

PK parameter predictions were good after hepatic and intestinal CYP3A4 and CYP3A5 protein expression levels were changed to 90% of the default values for healthy volunteers, where only CYP3A4 expression level was modified for CYP3A5 PM (see **DISCUSSION**; **Figure S2**). The ratios of predicted to observed PK parameters, such as dose-normalized maximum plasma concentration (C_{max}), 0–12-hour area under the concentration–time curve ($AUC_{0-12\ hour}$), and trough plasma concentration (C_{trough}), ranged from 0.98-fold to 1.15-fold in CYP3A5 PMs and from 0.92-fold to 1.11-fold in CYP3A5 NMs (**Table 2**). Regarding the ratios of the PK parameters between CYP3A5 PMs and NMs, the predicted

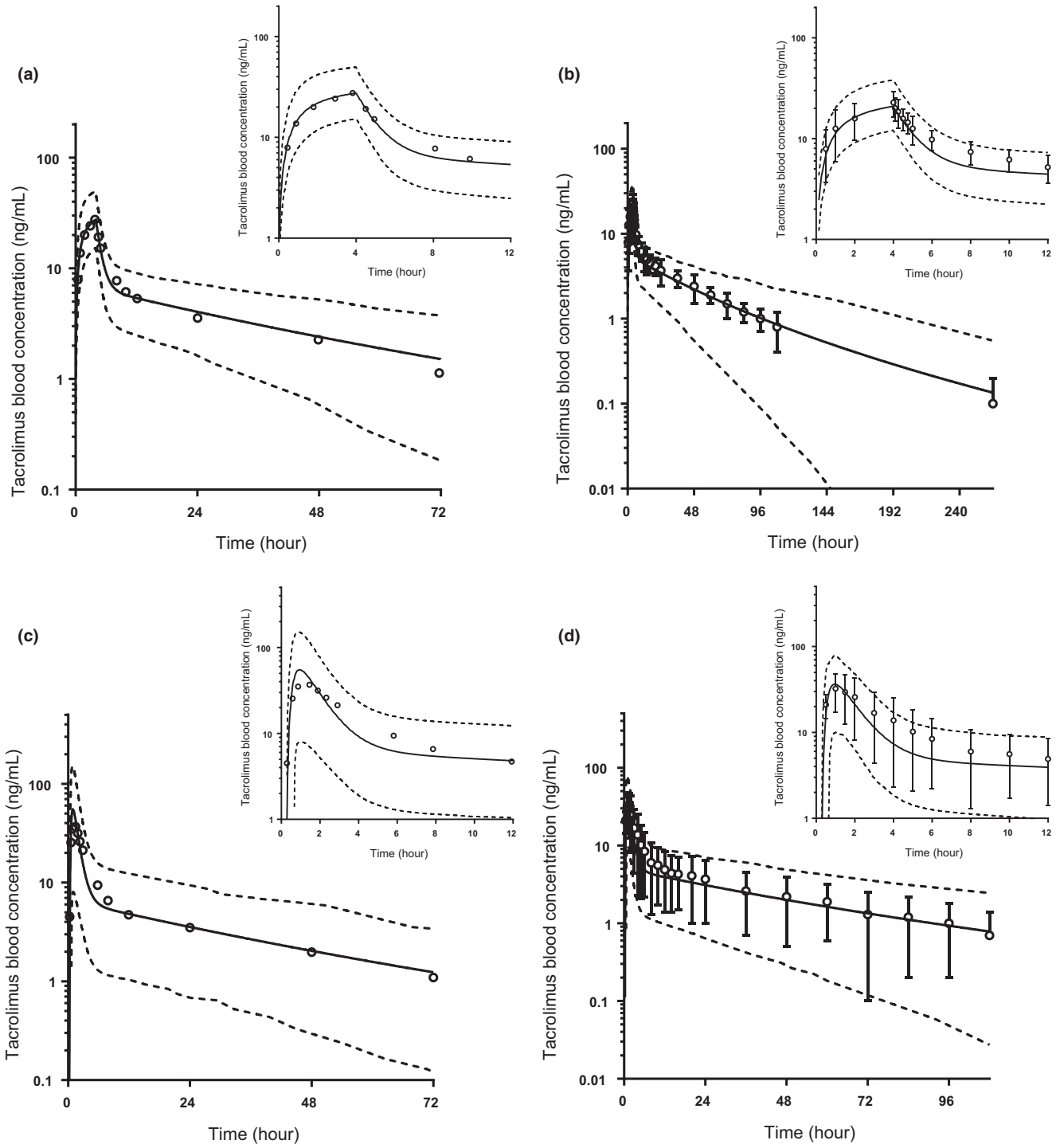

Figure 2 Observed and physiologically-based pharmacokinetic model–simulated blood concentration–time profiles of tacrolimus in healthy whites. Tacrolimus pharmacokinetic profiles in healthy whites: (**a**) and (**b**) after the intravenous administration; (**c**) and (**d**), after oral administration. Solid and dashed lines represent the mean and 5^{th}/95^{th} percentiles of the simulation results, respectively. Open circles represent the observed mean data from reported clinical studies: **a** and **c**, Mancinelli *et al.*[4]; **b** and **d**, Moller *et al.*[3] (bars represent SD). Details on parameter settings used for each simulation in this study are summarized in the Method section and **Table S3**.

ratios were 1.03-fold to 1.08-fold of the observed ratios. The observed individual PK data were mainly within the 5^{th} to 95^{th} percentile range of the PBPK simulations in both CYP3A5 PMs and NMs; the percentage of observed data outside this range were < 2% and < 3%, respectively (**Figure 3**[16]). The predicted variability CV% in each timepoint ranged from 68–85% and 65–88% in CYP3A5 PMs and NMs, whereas observed variability ranged 40–56% and 39–62%, respectively.

Table 2 Comparison between predicted and observed pharmacokinetic parameters of tacrolimus in renal transplant patients

Parameters	C_{max}/dose (ng/mL)/(mg/kg)			$AUC_{0-12\ hour}$/dose (ng·hour/mL)/(mg/kg)			C_{trough}/dose (ng/mL)/(mg/kg)		
CYP3A5	**PM**	**NM**	**Ratio**	**PM**	**NM**	**Ratio**	**PM**	**NM**	**Ratio**
Predicted	605 (582–630)	403 (388–419)	1.50	4,145 (3,958–4,340)	2,258 (2,162–2,358)	1.84	277 (263–291)	136 (129–143)	2.04
Observed[a]	616 (551–690)	421 (331–534)	1.46	4,154 (3,638–4,744)	2,442 (2,024–2,946)	1.70	240 (206–279)	123 (102–148)	1.95
P/O ratio[b]	0.98	0.96	1.03	1.00	0.92	1.08	1.15	1.11	1.04

Data represent geometric mean (95% confidence interval).
$AUC_{0-12\ hour}$, 0–12-hour area under the concentration–time curve; C_{max}, maximum plasma concentration; C_{trough}, trough plasma concentration; CYP, cytochrome P450; NM, normal metabolizer; PM, poor metabolizer; P/O, predicted to observed data..
[a]Forty-six and 24 data points were available from 23 CYP3A5 PMs and 12 CYP3A5 NMs, respectively. [b]Ratio of predicted to observed data.

The impact of CYP3A4 abundances, hematocrit, serum albumin, and creatinine levels on tacrolimus PKs in virtual CYP3A5 PMs

In addition to the effect of CYP3A5 expression, the impact of CYP3A4 abundances, hematocrit, serum albumin, and creatinine levels on tacrolimus PK were assessed in virtual CYP3A5 PMs using sensitivity analysis. The predicted tacrolimus whole blood concentration–time profiles were sensitive to changes in hepatic and intestinal CYP3A4 abundance, hematocrit, and serum albumin but not to serum creatinine (**Figure 4**). The simulated trough concentration of tacrolimus was more sensitive to hepatic and intestinal CYP3A4 abundances compared with hematocrit and serum albumin concentration within the tested ranges for each factor.

Age-dependent trough concentration in CYP3A5-genotyped pediatric kidney transplant patients

The age-dependent effects on total tacrolimus trough concentration were examined with special attention to CYP3A5 genotype and hematocrit level using the pediatric PBPK model of tacrolimus, where two hepatic CYP3A4 ontogeny profiles were assessed. Predicted tacrolimus trough concentrations were displayed with body weight, which were similar to the findings based on the PopPK model.[20,21] This demonstrates an age-dependent trend due to colinearity between age and body weight. The trough concentrations predicted by the PopPK model mostly fell into the twofold range of the geometric mean of trough concentrations predicted by the PBPK model (**Figure 5**[17,20,21]). Of note, the PBPK model simulated trough concentrations in virtual pediatric subjects with a body weight of 10 kg trended to be higher compared with the reported PopPK model, especially in subjects having lower hematocrit levels, when the CYP3A4 ontogeny profile reported by Upreti and Wahlstrom[17] was used. This overestimation was improved when a hepatic CYP3A4 ontogeny profile was derived using the methods described by Salem *et al*.[18,19] and accounting for the effects of disease was used (see **Supplemental Methods**).

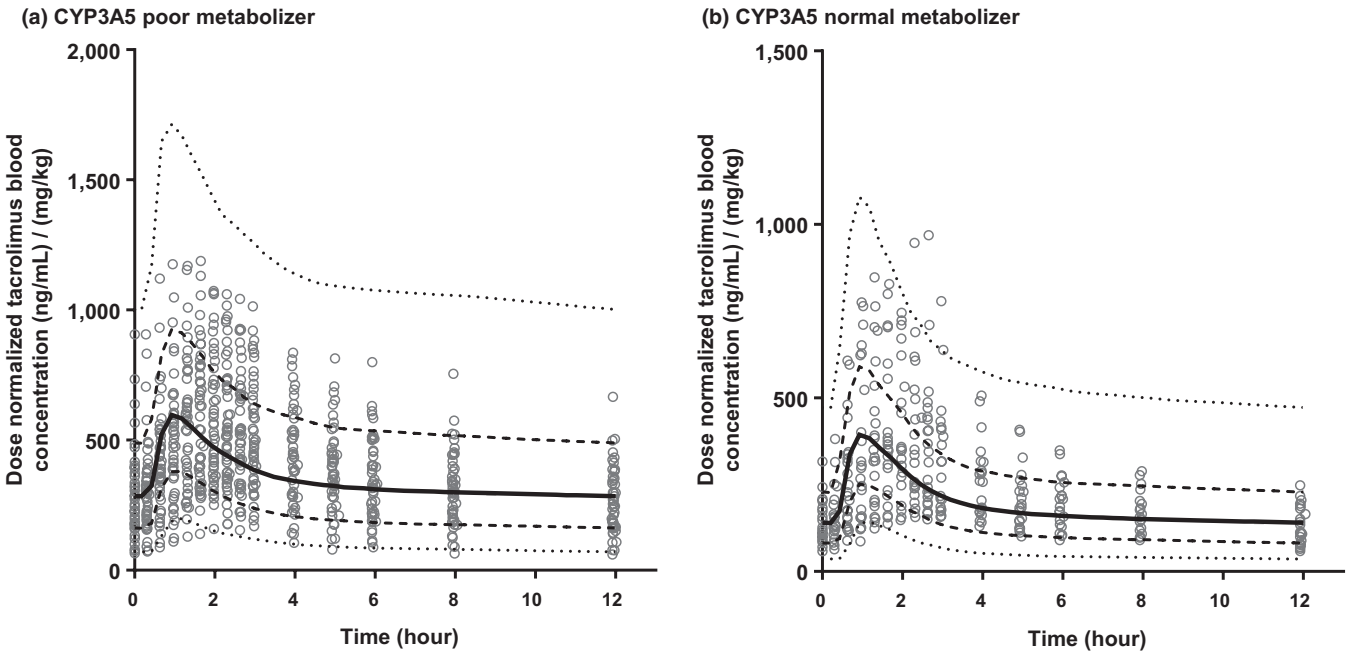

Figure 3 Predicted and observed dose-normalized blood concentration–time profiles of tacrolimus in renal transplant patients. Pharmacokinetic (PK) profiles of tacrolimus were simulated with (**a**) virtual cytochrome P450 (CYP)3A5 poor metabolizers and (**b**) CYP3A5 normal metabolizers. Solid, dashed, and dotted lines represent the median, 25th/75th, 5th/95th percentiles of the simulation results, respectively. Open circles represent observed data from renal transplant patients, where each individual patient has duplicate PK profiles from two separate visits.[16]

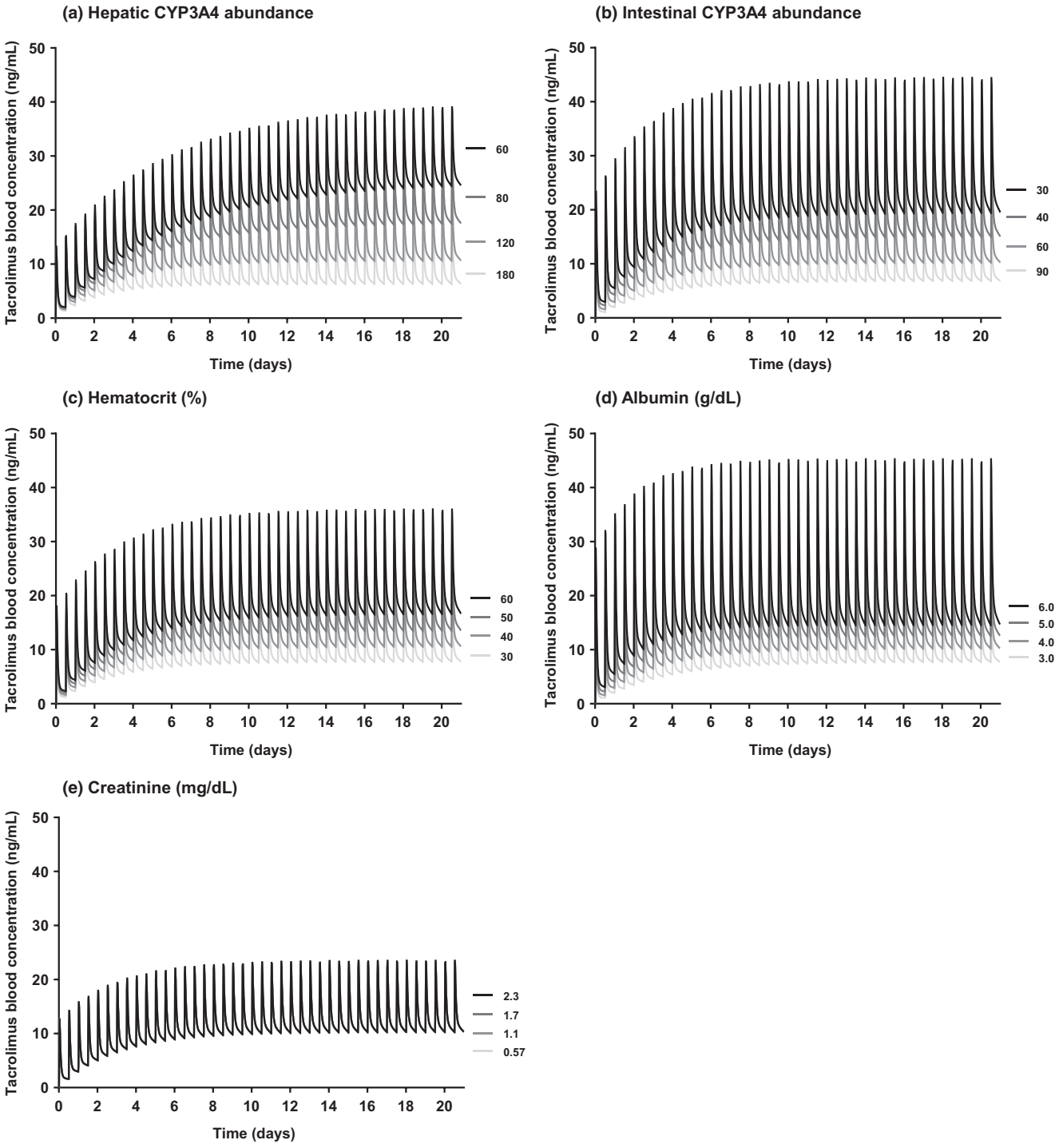

Figure 4 Sensitivity analyses results showing the effect of changes in clinical laboratory values on tacrolimus pharmacokinetic profiles in virtual renal transplant patients assuming poor metabolizer status cytochrome P450 (CYP)3A5. Sensitivity analyses were conducted for: (**a**) hepatic CYP3A4 abundance, (**b**) intestinal CYP3A4 abundance, (**c**) hematocrit, (**d**) serum albumin concentration, and (**e**) serum creatinine concentration. The range of change for each factor was as follows: hepatic CYP3A4 abundance, 60–180 pmol/mg microsomal protein; intestinal CYP3A4 abundance, 30–90 nmol/small intestine; hematocrit, 30–60%; albumin, 3.0–6.0 g/dL; and serum creatinine, 0.57–2.3 (mg/dL).

DISCUSSION

Multiple factors have been reported to explain the large PK variability of tacrolimus; however, a comprehensive assessment of these factors is limited. The general covariate analyses are assumed challenging due to simultaneous changes in multiple covariates and/or small sample sizes of clinical studies, especially in the pediatric field.

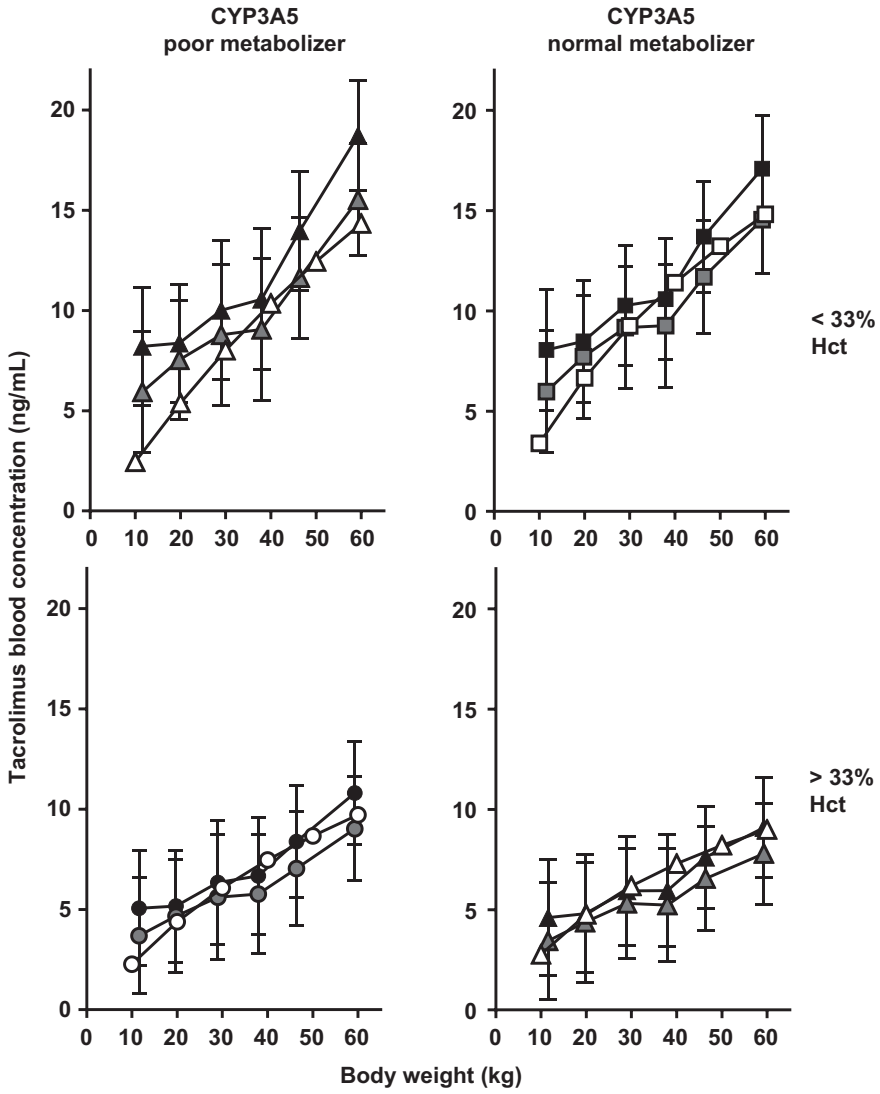

Figure 5 Comparison between physiologically-based pharmacokinetic (PBPK) model–based and population pharmacokinetic (PopPK) model–based simulated tacrolimus steady-state trough concentrations in pediatric patients. Tacrolimus steady-state trough concentrations simulated by the PBPK model (black symbols, Upreti and Wahlstrom model[17]; gray symbols, Salem *et al*.[18,19] model) and the PopPK model (open symbols) by Zhao *et al.*[20] and Lancia *et al.*[21] Individual graphs are separated into genotypes, with lower and normal hematocrit (Hct) levels, with body weights of 10–60 kg, and receiving body weight–based dosage regimens of 0.05 (circles), 0.1 (triangles), and 0.2 (squares) mg/kg twice daily for 3 weeks. For the PBPK model–based simulations, each symbol with bars represents the geometric mean with SD of tacrolimus trough concentrations.

In one of the larger PopPK studies by Størset *et al*.,[26] CYP3A5 phenotype and hematocrit were identified as significant covariates in whole blood tacrolimus PK in adult subjects; the model was used to simulate initial and Bayesian revised tacrolimus doses to achieve target concentrations. To complement the PopPK approach, PBPK modeling can provide possible PK simulations even when actual PK data are very limited (e.g., in pediatrics) and allows the user to theoretically ascertain the contribution of covariates to the PK variability of drugs in a virtual target population. This can be achieved as these virtual populations within PBPK models are not limited by size and, thus, the power to detect significant covariates, nor patient characteristics, the latter allowing the flexibility to run "what if" scenarios to improve our mechanistic understanding of important factors behind PK variability (e.g., changes in hepatic CYP3A in the renal transplant population). These approaches would be supported by our continuous evaluation and accumulation of knowledge regarding the influence of patient physiological and genetic factors. In this study, the factors contributing to the large PK variability of tacrolimus were assessed using a PBPK model incorporating patient-specific demographic and CYP3A5 genotype data. Sensitivity analysis was performed on key covariates.

The influential covariates are likely to be related to elimination pathways that will influence both total and unbound blood concentrations. The clearance of tacrolimus after i.v. administration was estimated to be 37.5 mL/minute

(= 2.25 L/hour), which was reported to be 3% of liver blood flow indicating a low hepatic extraction ratio.[3] Therefore, the total hepatic clearance of tacrolimus should be determined by the intrinsic clearance and the free fraction of tacrolimus in blood, according to the Well-Stirred model.[27] Intestinal metabolism also plays an important role on tacrolimus PK. The fraction of tacrolimus escaping gut wall metabolism (F_g) was reported to be low (0.14), which could be explained by the interplay between intestinal metabolism and permeability based on the Q_{gut} model.[28] Regarding renal excretion, urinary excretion of tacrolimus was < 3% and so, unsurprisingly, was not a significant covariate for tacrolimus total elimination.

Based on the observations on total tacrolimus clearance, CYP3A5 genotype, body weight, hematocrit, serum albumin, and creatinine levels were selected to define patient-specific characteristics and described in the model based on observed distributions. The PBPK model adequately predicted the PK profiles observed in CYP3A5-genotyped renal transplant patients, whereas the base PBPK model with the predefined distributions in a healthy population demonstrated the ratio of predicted to observed PK parameters of 0.85–1.28 for CYP3A5 PMs and 0.73–1.38 for CYP3A5 NMs (**Figure S3**). This indicates that the matching of key factors between the virtual generated population and the actual patients contributes to PBPK model predictive performance.

CYP3A abundance was identified to be the most influential factor contributing to individual variability in tacrolimus PK through the sensitivity analysis. Barter *et al.*[29] also reported the impact of changes in hepatic CYP3A contents on oral clearance of tacrolimus, where correlated hepatic CYP3A4 and CYP3A5 expression was incorporated within the virtual population. However, it is difficult to directly determine CYP3A levels in individual patients in the clinical settings, although there has been research on the genotyping of CYP3A enzymes. The pharmacogenetic influence of alleles of *CYP3A5*1* and *CYP3A5*3* has been described as an important covariate for tacrolimus dosing in the CPIC guideline.[1] There is still lack of concrete understanding of the effects of CYP3A4 genotypes on the large PK variability of tacrolimus, despite continued pharmacogenetic efforts to quantify the effects of specific genotypes (e.g., *CYP3A4*1B* and *CYP3A4*22*) and others factors related to CYP3A expression and activity (e.g., Pregnane X receptor; P450 oxidoreductase).[30] Regarding the *CYP3A4*22* allele, Elens *et al.*[31] discussed a potential tissue-specific regulation affected by the *CYP3A4*22* allele according to the findings that the association between reduced mRNA expression and *CYP3A4*22* (i.e., T-variant allele) was not observed in 106 small intestine samples, although the effect of the *CYP3A4*22* allele on hepatic expression was confirmed by measuring total CYP3A4 mRNA levels in 93 liver samples. In the current study, sensitivity analyses demonstrated 30–50% decreases in hepatic CYP3A4 resulted in 1.6-fold to 2.3-fold increases in trough concentration in a virtual CYP3A5 PM, where intestinal CYP3A4 expression level was not changed. In fact, Scheibner *et al.*[32] recently reported that the median dose-normalized tacrolimus trough concentration in *CYP3A5 *3/*3* and *CYP3A4 *22/*22* (3.05 ng/mL/mg of total daily dose, $n = 4$) was 1.9-fold higher compared with *CYP3A5 *3/*3* and *CYP3A4 *1/*1* (1.60 ng/mL/mg of total daily dose; $n = 1{,}048$) in the first 6 months post transplant. This theoretical approach may provide an additional quantitative insight on the effect of CYP3A4*22 allele on hepatic CYP3A4 activity as observed increased tacrolimus trough concentration. In addition to these findings, we need to seriously consider a relation in CYP3A4 expression between genotype-related and disease-specific physiology, including recovery post transplant, with clinical confirmation. Further investigations are needed to explain the variability in CYP3A4 abundance or activity due to pharmacogenetic effects. Although genetic testing for CYP3A is becoming more accessible, it should be noted that dosing recommendations based on genetic information alone may not be sufficient. Age-dependent changes in the trough tacrolimus concentrations predicted by a PBPK model implementing CYP3A ontogeny profiles[17–19] were also observed. In pediatric patients, the genotype–phenotype relationship may be influenced by the developmental changes that occur between birth and adolescence.

Another approach to studying this further is the use of probe substrates. The study by de Jonge *et al.*[33] assessed *in vivo* CYP3A4 activity using midazolam, which is metabolized by CYP3A4 in liver and intestine. *In vivo* CYP3A4 activity was significantly correlated with weight-corrected tacrolimus daily dose requirements and dose-corrected tacrolimus PK parameters, and weight-corrected tacrolimus steady-state clearance. In addition to the genetic effects, CYP3A4 abundance or activity in individual patients can be affected by drug–drug interactions. For example, glucocorticoids (e.g., methylprednisolone and prednisone[34]), which are used after renal transplantation as part of the immunosuppressive regimen, are known to influence CYP3A4 activity in renal transplant patients. Decreasing CYP3A4 activity over time during the first month post graft contributes significantly to the decline in tacrolimus clearance, which could be explained in part by steroid tapering in adult kidney transplant patients.[35]

Hematocrit and albumin plasma concentrations influence the free fraction but not unbound concentration of tacrolimus in blood as unbound clearance is unchanged as is the dose required to maintain the target unbound concentration. Likewise, doses predicted from genotypes affecting tacrolimus metabolism are not influenced by concomitant differences in albumin and hematocrit on the tacrolimus therapy targeting unbound concentration. The decreases in hematocrit and albumin levels increase the free fraction of tacrolimus in blood, thus there is an increased whole blood clearance with a parallel increase in volume of distribution and an associated fall in total blood concentration. These changes are, however, important for the correct interpretation of therapeutic drug monitoring results. The difference in the effect of changes in albumin and hematocrit between total and unbound concentrations of tacrolimus in blood was simulated using the developed PBPK model with two virtual healthy volunteer populations: one with hematocrit = 45% and albumin = 45 g/L as typical settings and the other with hematocrit = 35% and albumin = 25 g/L. A 2.5-fold decrease in average trough total concentration in blood was predicted due to the decreases in hematocrit and albumin levels in

the virtual populations after oral administration of 0.03 mg/kg twice a day for 14 days. However, trough unbound/free blood concentrations were almost unchanged. In CYP3A5 PMs, a significant correlation was found between hematocrit and total tacrolimus clearance (**Figure S4a**). Gérard *et al.*[36] analyzed hematocrit and plasma free fraction as influential factors on tacrolimus trough concentrations in liver transplant patients through a PBPK modeling approach. These findings were also supported by other clinical data showing the contribution of hematocrit and serum albumin levels on total tacrolimus PK in renal transplant patients,[33,37] although the influence of serum albumin levels was not statistically detected in our clinical study. In actual clinical settings, large variability in CYP3A activity mentioned above might make it difficult to identify hematocrit and albumin contributions to the PK variability of tacrolimus.

In our clinical study[16] involving renal transplant patients, hematocrit and serum albumin levels were lower than reported normal ranges. At least 70% of kidney transplant recipients had some degree of anemia in the first 6 months after transplantation, and up to one-third were anemic between 1 and 5 years post transplantation.[38] The decreasing trend of serum albumin level was also consistent with a previous observation that albumin concentration was significantly lower in kidney transplant patients than in healthy subjects.[39] There are also reports that hematocrit and albumin levels were different depending on the time period after transplantation;[38,40] therefore, their real-time values post transplant would be important when simulating tacrolimus PK. This could be incorporated into a PBPK model as time-dependent physiological changes.[41]

Serum creatinine is routinely monitored in renal transplant patients treated with tacrolimus. Our theoretical PBPK modeling analysis showed that the disposition of tacrolimus was not sensitive to differences in serum creatinine values among virtual subjects. This indicates that the glomerular filtration rate itself is unlikely to be associated with the disposition of tacrolimus. This is unsurprising as renal clearance accounts for < 1% of the total body clearance after intravenous infusion in healthy, male, white nonsmokers.[3] However, monitoring renal function remains important for the detection of renal toxicity due to tacrolimus, because renal failure might alter nonrenal drug disposition as well as the renal excretion.[42] In a rat study, the bioavailability of tacrolimus was increased by ~ 35% in rats with impaired renal function compared with normal control rats.[43] Although the mechanism is still unclear, the downregulation of hepatic and intestinal CYP3A was observed in rats with chronic renal failure[44,45] in addition to very limited clinical findings on increased bioavailability of CYP3A substrates drugs.[46,47] There is good evidence that CYP3A is also downregulated in humans with renal impairment.[48]

Most of the adult renal transplant patients participating in this study had relatively high BMI values and were considered obese according to the WHO classification.[22] In terms of the development of a target patient-specific system model, a 10% decrease in protein expression for hepatic and intestinal CYP3A4 and CYP3A5 compared with healthy volunteers (default expression levels implemented in the Simcyp software) resulted in reasonable predictability of tacrolimus PK. Ghobadi *et al.*[49] analyzed the difference in *in vivo* clearance between obese and nonobese subjects. Slightly decreasing trends of clearance in obese subjects were observed for triazolam, alprazolam, midazolam (intravenous and oral), and cyclosporine, mainly metabolized by CYP3A4; however, there was no significant difference for each tested drug, which seems to be comparable to a possible slight decrease in CYP3A4 protein expression as observed in the current PBPK simulations. Further investigations on physiological changes with clinical PK confirmation are needed. In addition, for clinical PK analyses in an obese population, the best performing body descriptor (e.g., fat-free mass or lean body mass, etc.) should be considered to characterize physiological changes appropriately.

One limitation of the current study is that we have not considered the contribution of P-glycoprotein (P-gp) to tacrolimus PK profiles. Although tacrolimus is a substrate of P-gp, the pharmacogenetic effect on tacrolimus PK reported in the literature has been inconsistent.[5,50] Regarding the gut absorption, tacrolimus shows relatively high permeability in the Caco-2 monolayer cell system, where P-gp is expressed.[8] Therefore, both passive diffusion and active P-gp efflux of tacrolimus could contribute to prolonged exposure to intestinal CYP3A enzymes.[51] Further investigation is needed to define the contribution of P-gp to tacrolimus disposition, separately from intestinal CYP3A, especially considering regional differences in intestinal absorption.

This study clearly demonstrates that PBPK modeling allows evaluation of factors contributing to the large variability in the PK of tacrolimus in a quantitative manner. In addition, an inclusion of patient characteristics can fill in the gap in PK between virtual and actual subjects, thereby improving the predictability of a tacrolimus PBPK model. One of the advantages of PBPK modeling is the ability to apply the developed patient-specific physiological characteristics for other drugs administered to the same patient population. Although further studies will be needed to validate the integration of physiological characteristics demonstrated in this study, such efforts would accelerate the usage of PBPK modeling and simulation for personalized medicine.

Supporting Information. Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website (www.psp-journal.com).

Figure S1. Comparison between simulated and observed physiological parameters in renal transplant patients: (**a**) body weight; (**b**) body height; (**c**) hematocrit levels for male patients; (**d**) hematocrit levels for female patients; (**e**) serum creatinine concentration for male patients; (**f**) serum creatinine concentration for female patients; and (**g**) serum albumin concentrations.

Figure S2. Sensitivity analysis results focusing on CYP3A4 and CYP3A5 protein expression levels for dose-normalized C_{max}, $AUC_{0-12\,hour}$, and trough concentrations of tacrolimus between CYP3A5 PMs and NMs.

Figure S3. Predicted and observed dose-normalized blood concentration–time profiles of tacrolimus.

Figure S4. Relationship of steady-state clearance of tacrolimus after oral administration (CLss/F) in virtual and actual CYP3A5 PM with hematocrit (**a**), serum albumin (**b**), and serum creatinine (**c**).

Table S1. Demographic and clinical laboratory values observed in renal transplant patients.
Table S2. Comparison of predicted and observed pharmacokinetics parameters in healthy white people.
Table S3. Overview of the simulation settings for clinical studies in healthy white people.
Supplementary Material S1. Supplemental Methods.

Acknowledgments. We thank Farzaneh Salem, David Turner, and Brooks McPhail for their scientific input.

Funding. The pharmacokinetic and patient data obtained from the study funded by United States NIH/FDA grant U01FD004573 were partly used as a reference dataset in this study for a secondary analysis.

Conflicts of Interest. T.N.J. is an employee of Certara UK Limited, Simcyp Division. The other authors declared no conflicts of interest.

Author Contribution. C.E., T.N.J., D.H., U.C., R.R.A., A.A.V., and T.F. wrote the manuscript. C.E., T.N.J., U.C., R.R.A., A.A.V., and T.F. designed the research. C.E., D.H., U.C., R.R.A., A.A.V., and T.F. performed the research. C.E., T.N.J., D.H., and T.F. analyzed the data. C.E. and T.N.J. contributed new reagents/analytical tools.

1. Birdwell, K.A. *et al.* Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for CYP3A5 genotype and tacrolimus dosing. *Clin. Pharmacol. Ther.* **98**, 19–24 (2015).
2. Haufroid, 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* **14**, 147–154 (2004).
3. Moller, A. *et al.* The disposition of 14C-labeled tacrolimus after intravenous and oral administration in healthy human subjects. *Drug Metab. Dispos.* **27**, 633–636 (1999).
4. Mancinelli, L.M. *et al.* The pharmacokinetics and metabolic disposition of tacrolimus: a comparison across ethnic groups. *Clin. Pharmacol. Ther.* **69**, 24–31 (2001).
5. Iwasaki, K. Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. *Drug Metab. Pharmacokinet.* **22**, 328–335 (2007).
6. Paine, M.F. *et al.* The human intestinal cytochrome P450 "pie". *Drug Metab. Dispos.* **34**, 880–886 (2006).
7. Shimada, T., Yamazaki, H., Mimura, M., Inui, Y. & Guengerich, F.P. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J. Pharmacol. Exp. Ther.* **270**, 414–423 (1994).
8. Gertz, M., Harrison, A., Houston, J.B. & Galetin, A. Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. *Drug Metab. Dispos.* **38**, 1147–1158 (2010).
9. Oda, K. & Yamano, K. Effect of telaprevir on the metabolism and hepatic uptake of tacrolimus (FK506). *Biopharm. Drug Dispos.* **35**, 501–512 (2014).
10. Lin, Y.S. *et al.* Co-regulation of CYP3A4 and CYP3A5 and contribution to hepatic and intestinal midazolam metabolism. *Mol. Pharmacol.* **62**, 162–172 (2002).
11. Gertz, M., Houston, J.B. & Galetin, A. Physiologically based pharmacokinetic modeling of intestinal first-pass metabolism of CYP3A substrates with high intestinal extraction. *Drug Metab. Dispos.* **39**, 1633–1642 (2011).
12. Zahir, H., Nand, R.A., Brown, K.F., Tattam, B.N. & McLachlan, A.J. Validation of methods to study the distribution and protein binding of tacrolimus in human blood. *J. Pharmacol. Toxicol. Methods* **46**, 27–35 (2001).
13. Dai, Y. *et al.* Effect of CYP3A5 polymorphism on tacrolimus metabolic clearance in vitro. *Drug Metab. Dispos.* **34**, 836–847 (2006).
14. Poulin, P. & Theil, F.P. Prediction of pharmacokinetics prior to in vivo studies. 1. Mechanism-based prediction of volume of distribution. *J. Pharm. Sci.* **91**(12), 9–156 (2002).
15. Berezhkovskiy, L.M. Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination. *J. Pharm. Sci.* **93**, 1628–1640 (2004).
16. Alloway, R.R. *et al.* Bioequivalence between innovator and generic tacrolimus in liver and kidney transplant recipients: a randomized, crossover clinical trial. *PLoS Med.* **14**, e1002428 (2017).
17. Upreti, V.V. & Wahlstrom, J.L. Meta-analysis of hepatic cytochrome P450 ontogeny to underwrite the prediction of pediatric pharmacokinetics using physiologically based pharmacokinetic modeling. *J. Clin. Pharmacol.* **56**, 266–283 (2016).
18. Salem, F., Johnson, T.N., Abduljalil, K., Tucker, G.T. & Rostami-Hodjegan, A. A re-evaluation and validation of ontogeny functions for cytochrome P450 1A2 and 3A4 based on in vivo data. *Clin. Pharmacokinet.* **53**, 625–636 (2014).
19. Salem, F., Johnson, T.N., Abduljalil, K., Tucker, G.T. & Rostami-Hodjegan, A. Erratum to: A re-evaluation and validation of ontogeny functions for cytochrome P450 1A2 and 3A4 based on in vivo data. *Clin. Pharmacokinet.* **54**, 671 (2015).
20. Zhao, W. *et al.* Population pharmacokinetics and pharmacogenetics of tacrolimus in de novo pediatric kidney transplant recipients. *Clin. Pharmacol. Ther.* **86**, 609–618 (2009).
21. Lancia, P., Jacqz-Aigrain, E. & Zhao, W. Choosing the right dose of tacrolimus. *Arch. Dis. Child.* **100**, 406–413 (2015).
22. World Health Organization (WHO). Obesity and overweight <http://www.who.int/mediacentre/factsheets/fs311/en/>. Accessed September 11, 2018.
23. Medscape. Hematocrit <http://emedicine.medscape.com/article/2054320-overview>. Accessed September 11, 2018.
24. Medscape. Serum creatinine <http://emedicine.medscape.com/article/2054342-overview>. Accessed September 11, 2018.
25. Medscape. Serum albumin <http://emedicine.medscape.com/article/2054430-overview>. Accessed September 11, 2018.
26. Størset, E. *et al.* Improved prediction of tacrolimus concentrations early after kidney transplantation using theory-based pharmacokinetic modelling. *Br. J. Clin. Pharmacol.* **78**, 509–523 (2014).
27. Benet, L.Z. & Ziaamirhosseini, P. Basic principles of pharmacokinetics. *Toxicol. Pathol.* **23**, 115–123 (1995).
28. Yang, J.S., Jamei, M., Yeo, K.R., Tucker, G.T. & Rostami-Hodjegan, A. Prediction of intestinal first-pass drug metabolism. *Curr. Drug Metab.* **8**, 676–684 (2007).
29. Barter, Z.E. *et al.* Determination of a quantitative relationship between hepatic CYP3A5*1/*3 and CYP3A4 expression for use in the prediction of metabolic clearance in virtual populations. *Biopharm. Drug Dispos.* **31**, 516–532 (2010).
30. Tang, J.T. *et al.* Pharmacogenetic aspects of the use of tacrolimus in renal transplantation: recent developments and ethnic considerations. *Expert Opin. Drug Metab. Toxicol.* **12**, 555–565 (2016).
31. Elens, L., van Gelder, T., Hesselink, D.A., Haufroid, V. & van Schaik, R.H. CYP3A4*22: promising newly identified CYP3A4 variant allele for personalizing pharmacotherapy. *Pharmacogenomics* **14**, 47–62 (2013).
32. Scheibner, A. *et al.* Tacrolimus elimination in four patients with a CYP3A5*3/*3 CYP3A4*22/*22 genotype combination. *Pharmacotherapy* (2018) May 26. https://doi.org/10.1002/phar.2131. [Epub ahead of print]
33. de Jonge, H., de Loor, H., Verbeke, K., Vanrenterghem, Y. & Kuypers, D.R. In vivo CYP3A4 activity, CYP3A5 genotype, and hematocrit predict tacrolimus dose requirements and clearance in renal transplant patients. *Clin. Pharmacol. Ther.* **92**, 366–375 (2012).
34. Steiner, R.W. & Awdishu, L. Steroids in kidney transplant patients. *Semin. Immunopathol.* **33**, 157–167 (2011).
35. de Jonge, H., Vanhove, T., de Loor, H., Verbeke, K. & Kuypers, D.R. Progressive decline in tacrolimus clearance after renal transplantation is partially explained by decreasing CYP3A4 activity and increasing haematocrit. *Br. J. Clin. Pharmacol.* **80**, 548–559 (2015).
36. Gérard, C. *et al.* Determination of the most influential sources of variability in tacrolimus trough blood concentrations in adult liver transplant recipients: a bottom-up approach. *AAPS J.* **16**, 379–391 (2014).
37. Benkali, K. *et al.* Tacrolimus population pharmacokinetic-pharmacogenetic analysis and Bayesian estimation in renal transplant recipients. *Clin. Pharmacokinet.* **48**, 805–816 (2009).
38. Mix, T.C. *et al.* Anemia: a continuing problem following kidney transplantation. *Am. J. Transplant.* **3**, 1426–1433 (2003).
39. Grossman, S.H., Davis, D., Kitchell, B.B., Shand, D.G. & Routledge, P.A. Diazepam and lidocaine plasma protein binding in renal disease. *Clin. Pharmacol. Ther.* **31**, 350–357 (1982).
40. Undre, N.A. & Schafer, A. Factors affecting the pharmacokinetics of tacrolimus in the first year after renal transplantation. European Tacrolimus Multicentre Renal Study Group. *Transplant Proc.* **30**, 1–1263 (1998).
41. Abduljalil, K., Jamei, M., Rostami-Hodjegan, A. & Johnson, T.N. Changes in individual drug-independent system parameters during virtual paediatric pharmacokinetic trials: introducing time-varying physiology into a paediatric PBPK model. *AAPS J.* **16**, 568–576 (2014).
42. Sun, H., Frassetto, L. & Benet, L.Z. Effects of renal failure on drug transport and metabolism. *Pharmacol. Ther.* **109**, 1–11 (2006).
43. Okabe, H., Hashimoto, Y. & Inui, K.I. Pharmacokinetics and bioavailability of tacrolimus in rats with experimental renal dysfunction. *J. Pharm. Pharmacol.* **52**, 1467–1472 (2000).
44. Dreisbach, A.W. & Lertora, J.J. The effect of chronic renal failure on drug metabolism and transport. *Expert Opin. Drug Metab. Toxicol.* **4**, 1065–1074 (2008).
45. Leblond, F. *et al.* Downregulation of hepatic cytochrome P450 in chronic renal failure. *J. Am. Soc. Nephrol.* **12**, 326–332 (2001).
46. Dreisbach, A.W. The influence of chronic renal failure on drug metabolism and transport. *Clin. Pharmacol. Ther.* **86**, 553–556 (2009).

47. Dowling, T.C. *et al.* Characterization of hepatic cytochrome p4503A activity in patients with end-stage renal disease. *Clin. Pharmacol. Ther.* **73**, 427–434 (2003).
48. Rowland Yeo, K., Aarabi, M., Jamei, M. & Rostami-Hodjegan, A. Modeling and predicting drug pharmacokinetics in patients with renal impairment. *Expert Rev. Clin. Pharmacol.* **4**, 261–274 (2011).
49. Ghobadi, C. *et al.* Application of a systems approach to the bottom-up assessment of pharmacokinetics in obese patients: expected variations in clearance. *Clin. Pharmacokinet.* **50**, 809–822 (2011).
50. Hashida, T. *et al.* Pharmacokinetic and prognostic significance of intestinal MDR1 expression in recipients of living-donor liver transplantation. *Clin. Pharmacol. Ther.* **69**, 308–316 (2001).
51. Tamura, S., Ohike, A., Ibuki, R., Amidon, G.L. & Yamashita, S. Tacrolimus is a class II low-solubility high-permeability drug: the effect of P-glycoprotein efflux on regional permeability of tacrolimus in rats. *J. Pharm. Sci.* **91**, 719–729 (2002).