

# Dosing equation for tacrolimus using genetic variants and clinical factors

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## WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Patients with low tacrolimus troughs are at a higher risk of rejection while those with high troughs are at an increased risk for toxicity. Therefore, achieving the therapeutic range is important.
- CYP3A5 genotype and days post transplant have been previously shown individually to be associated with tacrolimus troughs.

## WHAT THIS STUDY ADDS

- This paper presents the first dosing model for tacrolimus using a combination of genetic and clinical factors in adult kidney transplant recipients. It was developed from one of the largest tacrolimus pharmacogenetic studies conducted to date (681 subjects and 11 823 trough concentrations).
- We found that CL/F was significantly influenced by days post transplant, CYP3A5 genotype, transplantation at a steroid sparing centre, recipient age and the use of a calcium channel blocker.
- Our large sample size enabled us to define the distinct differences in tacrolimus CL/F between three CYP3A5 genotype groups (\*1/\*1, \*1/\*3 and \*3/\*3).
- This study is an important step towards using pharmacogenetic information in the clinical setting.

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CYP3A5, kidney transplantation, pharmacogenetics, pharmacogenomics, pharmacokinetics, tacrolimus

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## AIM

To develop a dosing equation for tacrolimus, using genetic and clinical factors from a large cohort of kidney transplant recipients. Clinical factors and six genetic variants were screened for importance towards tacrolimus clearance (CL/F).

## METHODS

Clinical data, tacrolimus troughs and corresponding doses were collected from 681 kidney transplant recipients in a multicentre observational study in the USA and Canada for the first 6 months post transplant. The patients were genotyped for 2 724 single nucleotide polymorphisms using a customized Affymetrix SNP chip. Clinical factors and the most important SNPs (rs776746, rs12114000, rs3734354, rs4926, rs3135506 and rs2608555) were analysed for their influence on tacrolimus CL/F.

## RESULTS

The CYP3A5\*1 genotype, days post transplant, age, transplant at a steroid sparing centre and calcium channel blocker (CCB) use significantly influenced tacrolimus CL/F. The final model describing CL/F ( $\text{L h}^{-1}$ ) was:  $38.4 \times [(0.86, \text{if days } 6-10) \text{ or } (0.71, \text{if days } 11-180)] \times [(1.69, \text{if CYP3A5}^*1/*3 \text{ genotype}) \text{ or } (2.00, \text{if CYP3A5}^*1/*1 \text{ genotype})] \times (0.70, \text{if receiving a transplant at a steroid sparing centre}) \times (\text{age in years}/50)^{-0.4} \times (0.94, \text{if CCB is present})$ . The dose to achieve the desired trough is then prospectively determined using the individuals CL/F estimate.

## CONCLUSIONS

The CYP3A5\*1 genotype and four clinical factors were important for tacrolimus CL/F. An individualized dose is easily determined from the predicted CL/F. This study is important towards individualization of dosing in the clinical setting and may increase the number of patients achieving the target concentration. This equation requires validation in an independent cohort of kidney transplant recipients.

## Introduction

Tacrolimus is the most widely used calcineurin inhibitor in kidney transplantation [1]. It has a narrow therapeutic window with wide inter-individual variability in pharmacokinetics and clearance (CL) [2, 3]. Higher troughs are associated with increased risk of toxicity whereas lower troughs are associated with increased risk of rejection [4]. Two recent studies showed that with contemporary immunosuppressive regimens (tacrolimus, mycophenolate and steroids  $\pm$  antibody induction) low tacrolimus troughs in the first week post transplant were associated with a greater risk of acute rejection [5, 6]. To tailor therapy better, multiple clinical factors have been explored to determine their effects on tacrolimus pharmacokinetics. It is generally acknowledged that drug interactions, haematocrit, corticosteroid therapy, days post transplant, and race affect tacrolimus pharmacokinetics [3, 7–10]. It is also established that the cytochrome P4503A5 (CYP3A5)\*1 allele is associated with significantly higher tacrolimus CL and lower systemic exposure [11–15]. However, because tacrolimus troughs are routinely monitored and dose adjusted based on trough measurements the effect of these factors are not regarded in a consistent manner by centres and a trial and error approach to dosing is still common practice. These factors have not been used to guide dosing primarily due to the lack of robust, clinically feasible dosing models that combine important factors. Because of the growing use of steroid sparing or avoidance protocols, the importance of early immunosuppression intensity provided by the calcineurin inhibitors and/or other immunosuppressive exposure is considered important in minimizing acute rejection [16].

A recent randomized trial studied CYP3A5 genotype guided tacrolimus dosing in kidney transplant recipients [17]. In the genotype guided group, patients with one or more CYP3A5\*1 alleles received an initial tacrolimus dose of  $0.3 \text{ mg kg}^{-1} \text{ day}^{-1}$  and those without a \*1 allele received a dose of  $0.15 \text{ mg kg}^{-1} \text{ day}^{-1}$ . The non-genotype guided group was administered  $0.2 \text{ mg kg}^{-1} \text{ day}^{-1}$ . In the genotype guided group, 43.2% of subjects achieved the trough target compared with 29.1% in non-genotype guided group ( $P = 0.03$ ). Although using genotype guided dosing was significantly better, the overall proportion of patients achieving the therapeutic range may not be sufficient to justify the cost of incorporating genotyping into clinical practice. It is possible that addition of clinical factors and/or other genotypes to dosing models may further improve the initial dose estimates and the number of patients achieving the therapeutic target. However, defining these factors and the development of a robust dosing model for clinical use requires a large study population. Therefore, we studied the effect of clinical and genetic factors on tacrolimus apparent clearance (CL/F) in a large kidney transplant population through a multicentre study. Our objective was to define the important clinical and

genetic factors pertinent towards tacrolimus CL/F and develop a dosing model which would be suitable for the clinical setting.

## Methods

Subjects for this analysis were obtained from the first 1000 patients with end-stage renal dysfunction undergoing kidney or kidney–pancreas transplant enrolled in the Deterioration of Kidney Allograft Function (DeKAF) Genomics study between 2006 and 2008. This was a multicentre observational trial to define genetic and clinical determinants associated with clinical outcomes after kidney transplant. Details of the trial have been published elsewhere and are registered at <http://www.clinicaltrials.gov> (NCT00270712). A total of 681 patients,  $\geq 18$  years of age, who received tacrolimus at any time in the first 6 months post transplant were selected for this analysis. The remaining subjects received ciclosporin and were not studied. Institutional Review Board Human Subjects Committee approval was obtained at each participating centre. All recipients provided written informed consent.

Tacrolimus trough concentrations ( $n = 11\,823$ ) in 681 patients were obtained during oral administration in the first 6 months post transplant. All patients were administered tacrolimus (Prograf) either once or twice daily. Troughs were obtained as part of clinical care at the treating centre. The initial tacrolimus dosing was based on an individual's body weight and subsequent doses were adjusted based on trough concentrations using institution-specific targets. In general, troughs of  $8\text{--}12 \text{ ng ml}^{-1}$  were targeted in the first 3 months post transplant and of  $6\text{--}10 \text{ ng ml}^{-1}$  were desired in the months 3–6 post transplant. Trough concentrations, if available, were obtained twice in each of weeks 1–8 post transplant and twice in each of months 3, 4, 5 and 6 post transplant. There were a mean of 17 trough concentrations per patient (range 1–24). To ensure that tacrolimus was at or near steady-state, only trough concentrations measured after day 2 post transplant were used in this analysis. Trough concentrations were measured in the clinical laboratories of the participating centres. The majority (97.1%) of tacrolimus whole blood concentrations were obtained from centres using liquid chromatography-mass spectroscopy to measure trough concentrations. All troughs were measured in centres using CLIA certified assays or CLIA quality assays.

Recipient and donor demographics and clinical characteristics were obtained from medical records and are listed in Table 1. Baseline clinical factors collected were recipient age, weight, gender and race (African American (AA) or non-AA), donor type (living or deceased), donor gender, pre-emptive transplant, number of prior transplants, immunosuppressive regimen and enrolling centre. The

**Table 1**

Characteristics in all subjects, non-African Americans (AA) and AA

Clinical characteristics	All subjects (Non-AA and AA)	Non-AA	AA
Number of subjects	681	540 (79.3%)	141 (20.7%)
Age (years) of recipient*	50.2 ± 12.2	50.1 ± 12.2	46.9 ± 11.5
Baseline weight (kg) of recipient*	81.3 ± 18.7	81.1 ± 18.8	81.9 ± 17.9
Gender of recipient (Male/Female)	429 (63%)/252 (37%)	338 (63%)/202 (37%)	91 (65%)/50 (35%)
Number of transplants			
1	550 (81%)	417 (77%)	133 (94%)
≥2	130 (19%)	122 (23%)	8 (6%)
Number transplanted at a steroid sparing centre	205 (30%)	197 (36.5%)	8 (5.5%)
Living donor	398 (59%)	355 (66%)	43 (31%)
Number of troughs	11 823	9523	2300
Mean tacrolimus daily dose in mg kg <sup>-1</sup> †	0.08 ± 0.05	0.07 ± 0.05	0.09 ± 0.04
Mean tacrolimus trough in ng ml <sup>-1</sup> †	8.31 ± 3.48	8.66 ± 3.37	6.82 ± 3.51
Number of patients with troughs <8 ng ml <sup>-1</sup>			
Week 1	413 (75%)	307 (69%)	106 (96%)
Week 2	331 (55%)	228 (48%)	103 (82%)
Calcium channel blocker use‡	5082 (43%)	3915 (41%)	1167 (51%)
CYP3A5 genotype			
*1/*1	72 (11%)	9 (2%)	63 (45%)
*1/*3	129 (19%)	70 (13%)	59 (42%)
*3/*3	476 (70%)	457 (85%)	19 (13%)

\*Reported age and baseline weight are those measured at the time of transplant and are mean ± SD, †doses and troughs are over the 6-month study period and are mean ± SD, ‡calcium channel blocker use at the time of trough collection.

**Table 2**

Analysed variants and their allele frequencies in all subjects, non-African Americans (AA) and AA

Reference sequence (rs) number	Gene name	Allele	Overall allele frequency* (%)	Non-AA allele frequency† (%)	AA allele frequency‡ (%)
rs776746	CYP3A5	A	20.16	8.21	65.60
rs12114000	CYP3A4	A	3.82	0.09	18.09
rs3734354	SIM1	A	11.40	13.45	3.55
rs4926	SERPING1	A	23.45	26.63	11.35
rs3135506	APOA5	C	6.12	6.33	5.32
rs2608555	GAN	T	16.45	15.93	18.44

\*Allele frequency in the whole population, †allele frequency in the non-African American, ‡allele frequency in the African America.

concomitant use of a calcium channel blocker (CCB) and days post transplant at the time of each trough measurement was also obtained.

### Genotyping and selection of variants for analysis

Recipient pre-transplant DNA was obtained from the lymphocytes isolated from peripheral blood. DNA was genotyped for 2724 variants primarily using a customized Affymetrix GeneChip (Affymetrix, Santa Clara, CA, USA) [18, 19]. Additional variants were genotyped using the SNPlex (Applied Biosystems, Foster City, CA, USA) and Sequenom (Sequenom, San Diego, CA, USA) systems. In a prior analysis, these variants were evaluated for their association with dose-normalized tacrolimus concentrations using standard regression analysis [20]. As it was not feasible to evalu-

ate 2724 variants and their combinations in a population pharmacokinetic approach to estimate CL/F, the SNPs with a *P* value < 0.01 from the AA and non-AA populations from the regression analysis, were selected for this analysis. The variants evaluated towards tacrolimus were rs776746, rs12114000, rs3734354, rs4926, rs3135506 and rs2608555. The variants and their allele frequencies are given in Table 2.

### Population modelling of troughs

The data were analysed by a non-linear mixed-effects approach using the NONMEM software (Version 7.1, NONMEM Project Group, GloboMax LLC, MD, USA) with a Visual Fortran compiler (Professional Edition for Windows 11.1, Intel®) and PdxPop (Version 4.0, ICON Development Solutions). R 2.4.1 was used for the generation of

diagnostic plots. Given the long half-life of tacrolimus (12 h) [21, 22] relative to the dosing intervals, and the absence of pharmacokinetic sampling at times other than the troughs, the concentrations were analysed according to a steady-state infusion model. It was assumed that the trough concentrations ( $C_{\min}$ ) were well approximated by the average steady-state plasma concentration ( $C_{ss, av}$ ) of tacrolimus due to its long elimination half-life and were related to the tacrolimus dose through  $(CL/F)^*$ :

$$C_{\text{obs}} = \text{Dosing rate} / (CL/F)^*$$

where  $C_{\text{obs}}$  is the observed tacrolimus trough concentration and dosing rate is the total daily dose of tacrolimus (in mg) divided by 24 h. The  $(CL/F)^*$  is a regression parameter that predicts the trough concentrations. It approximates  $CL/F$  when  $C_{\min} \approx C_{ss, av}$ , as in the case of drugs that have a long half-life such as tacrolimus. Using  $(CL/F)^*$  as an approximation to  $CL/F$  has the advantage of giving the regression parameter clinically relevant meaning in terms of magnitude, units and interpretation. However, we caution the reader that the apparent clearance we describe in the manuscript is only an approximation of the actual apparent tacrolimus clearance.

The first order conditional approximation estimation with interaction (FOCE-I) was utilized. The pharmacokinetic parameter obtained was the  $CL/F$  for tacrolimus. The inter-individual variability (IIV) in  $CL/F$  was modeled using an exponential error model as shown in the following equation:

$$CL/F = TVCL \times e^{\eta(1)}$$

where  $TVCL$  represents the typical value of  $CL/F$  in the population and  $\eta(1)$  represents the IIV in tacrolimus  $CL/F$ ,  $\eta \sim N(0, \omega^2)$ . An additive error model was utilized to characterize the residual unexplained variability (RUV) as shown in the following equation:

$$C_{ij} = C_{\text{pred},ij} + \varepsilon_{ij}$$

where  $C_{ij}$  is the  $j^{\text{th}}$  observed concentration in the  $i^{\text{th}}$  individual,  $C_{\text{pred},ij}$  is the  $j^{\text{th}}$  predicted concentration in the  $i^{\text{th}}$  individual and  $\varepsilon_{ij}$  is the RUV in tacrolimus  $CL/F$ ,  $\varepsilon \sim N(0, \sigma^2)$ .

The clinical factors and the six variants described in Tables 1 and 2 were tested for their influence on tacrolimus  $CL/F$  in the model building phase. Forward inclusion and backward elimination were used to analyse the covariates. A decrease in objective function value (OFV, a goodness-of-fit statistic) of 6.63 or more ( $P < 0.01$ ) was considered significant ( $\chi^2$ : 1 degree of freedom, d.f.) in the forward inclusion step. Backward elimination was performed using a more stringent increase in OFV of 10.83 or more ( $P < 0.001$ , 1 d.f.). Clinical factors that were tested included categorical covariates (days post transplant of trough, recipient gender, race, donor type, donor gender, pre-emptive transplant, number of prior transplants and enrolling centre) and continuous covariates (age and weight). Race

(Caucasian, AA, Asian, Native American/Aleut/Inuit and Hawaiian/Pacific Islander) was categorized as AA or non-AA. Centres were designated as using a steroid sparing immunosuppressive regimen if they administered steroids for  $\leq 7$  days post transplant. Age was tested by standardizing it by the median age (50 years) in the population. Days post transplant were converted to an ordered categorical covariate and classified as: immediate post transplant (days 3–5), early post transplant (days 6–10) and late post transplant (days 11–180). Categorization was done as the model failed to converge when days post transplant was modelled as a continuous function (such as a simple continuous function, Bateman function,  $E_{\text{max}}$  model, etc.). The concomitant use of a CCB at the time of the trough measurement was also analysed. Variants were initially classified as homozygous for the major allele, heterozygous for the minor allele or homozygous for the minor allele. However, the frequency of homozygous variant carriers in five of the six variants was  $\leq 5\%$ . Therefore, for these five variants, the genotypes were categorized as two variables (carriers or non-carriers of the minor allele). The minor allele frequency (MAF) of CYP3A5\*1 was high and, therefore, we were able to categorize the genotypes as CYP3A5\*3/\*3, CYP3A5\*1/\*3, or CYP3A5\*1/\*1.

### Model building

The covariates were tested in a step-wise manner. In the first step, the OFV for the base model (pharmacokinetic model with no covariates) was observed. The covariates were analysed next by forward inclusion by the following procedure: (a) univariate analysis was performed, (b) the insignificant covariates were removed from further consideration, (c) the significant covariates (OFV  $> 6.63$  for 1 d.f.) were ranked in terms of their significance towards tacrolimus  $CL/F$ , as assessed by the magnitude of drop in the OFV, (d) the covariate with the highest rank (i.e. most significant), was included into an updated model and (e) the remaining significant covariates were tested for significance by adding them univariately to the updated model. Steps (b) through (e) were repeated until all the significant covariates were included into the forward inclusion model and all insignificant covariates were discarded. Backward elimination was then performed by removing each group within a covariate at a time (e.g. days 6–10 and 11–180 separately). An increase in OFV ( $> 10.83$  for 1 d.f.) was used to obtain the final model.

### Model evaluation

For the purpose of model evaluation, a non-parametric bootstrap approach was employed. A single bootstrap run generated a new dataset, by random sampling with replacement from the original dataset. Bootstrap runs ( $n = 1\ 000$ ) were performed and non-parametric statistics, median and 2.5th and 97.5th percentile were obtained from successful bootstrap runs (successful was defined as achieving both convergence and covariance step). A close



**Table 3**

Final parameter estimates and effect of genetic and clinical factors on tacrolimus CL/F

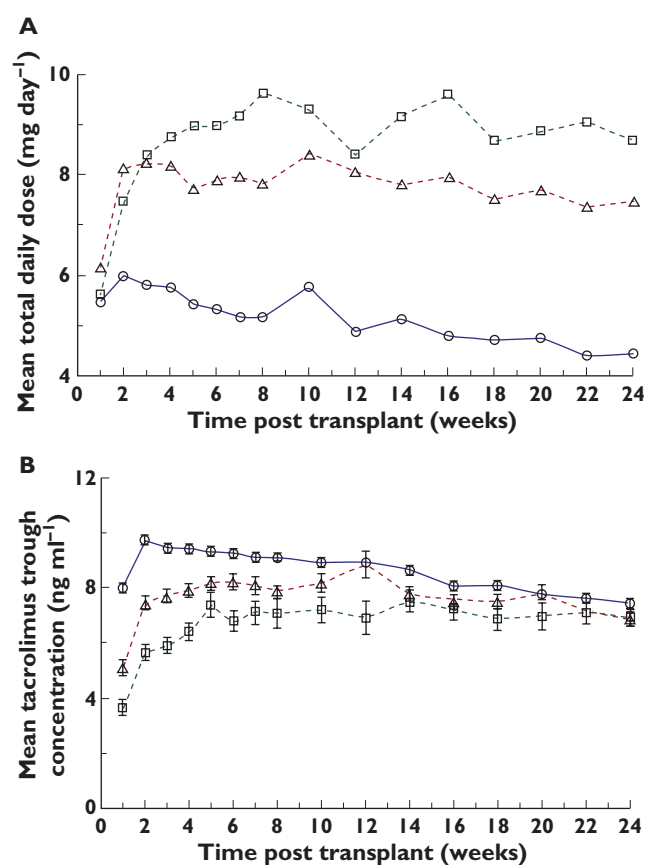
Parameter	Study population <sup>1</sup> Estimate (%RSE) <sup>3</sup>	95% CI <sup>4</sup>	Bootstrap analysis <sup>2</sup> Median	2.5th–97.5th percentiles
Tacrolimus CL/F <sup>5</sup>	38.4 (4.14)	35.3, 41.5	38.3	35.5, 41.7
<b>Factors affecting CL/F</b>				
6–10 DPT <sup>6</sup>	0.86 (4.13)	0.80, 0.93	0.86	0.80, 0.93
11–180 DPT <sup>6</sup>	0.71 (4.14)	0.66, 0.77	0.72	0.66, 0.77
CYP3A5*1/*3	1.70 (3.99)	1.56, 1.82	1.69	1.56, 1.82
CYP3A5*1/*1	2.00 (5.90)	1.77, 2.23	1.99	1.77, 2.23
Steroid sparing centre	0.70 (3.50)	0.65, 0.75	0.70	0.65, 0.75
(Age/50) <sup>8</sup>	-0.40 (13.5)	-0.50, -0.30	-0.39	-0.50, -0.29
CCB use <sup>7</sup>	0.94 (2.43)	0.89, 0.98	0.94	0.90, 0.99
ILV <sup>9</sup> for CL/F, %CV	40.1	37.4, 43.6	40.0	37.4, 43.6
RUV <sup>9</sup> , additive, SD	3.19	3.07, 3.32	3.19	3.08, 3.32

<sup>1</sup>681 patients analysed in this study, <sup>2</sup>results from 989 bootstrap runs with successful convergence and successful covariance step, <sup>3</sup>per cent relative standard error, <sup>4</sup>confidence interval: estimate ± (1.96\*standard error of estimate), <sup>5</sup>typical value of CL/F in l h<sup>-1</sup>, <sup>6</sup>days post transplant, <sup>7</sup>calcium channel blocker use at the time of trough measurement, <sup>8</sup>inter-individual variability, <sup>9</sup>random unexplained variability.

agreement between the non-parametric statistics and the estimates from the original dataset (≤10%) was indicative of the stability and performance of the population model.

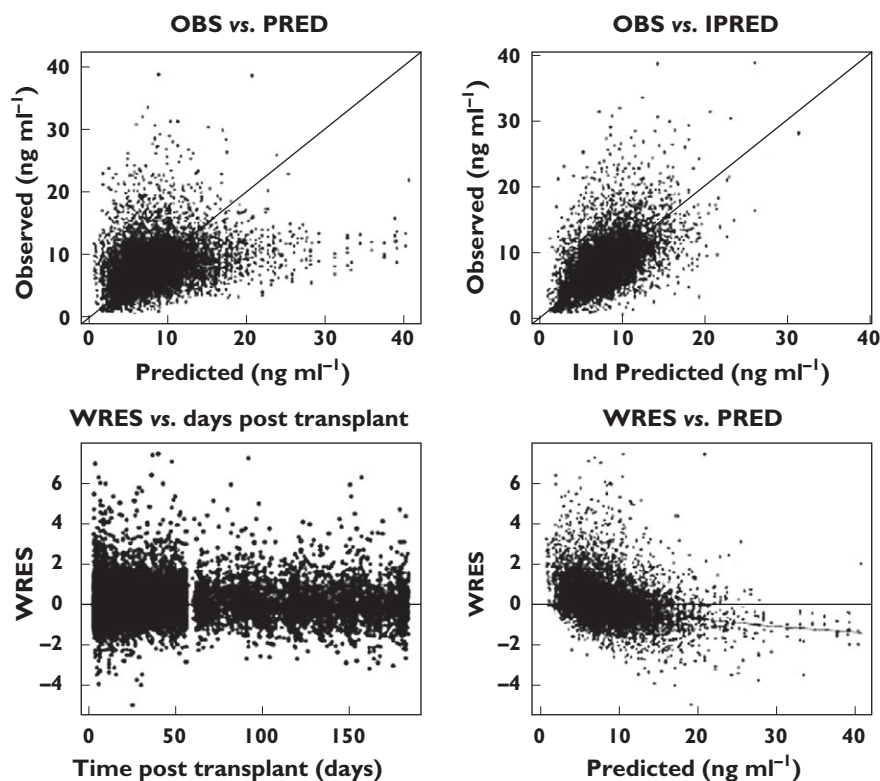
## Results

Tacrolimus troughs ( $n = 11\,823$ ) from 681 adult kidney transplant recipients obtained in the first 6 months post transplant were studied. Patient characteristics, tacrolimus doses and troughs are given in Table 1. The mean ± SD daily doses over the 6 months period for the non-AA and AA were  $0.07 \pm 0.05$  and  $0.09 \pm 0.04$  mg kg<sup>-1</sup>, respectively. The mean ± SD troughs for the non-AA and AA patients were  $8.66 \pm 3.37$  and  $6.82 \pm 3.51$  ng ml<sup>-1</sup>, respectively. A majority (59%) of the subjects received a living donor transplant and 21% of the population was AA. In our study, in the first week post transplant, 75% ( $n = 413$ ) of all transplant recipients, 69% ( $n = 307$ ) of non-AA and 96% ( $n = 106$ ) of AA, had a trough concentration <8 ng ml<sup>-1</sup>. In the second week post transplant, 55% ( $n = 331$ ) of all transplant recipients, 48% ( $n = 228$ ) of non-AA and 82% ( $n = 103$ ) of AA, had a trough <8 ng ml<sup>-1</sup>. The six genetic factors and their allele frequencies are shown in Table 2. Factors affecting tacrolimus CL/F in order of their importance were days post transplant, presence of CYP3A5\*1 allele (rs776746), transplantation at a steroid sparing centre, recipient age and CCB use (Table 3). The typical value of CL/F was 38.4 l h<sup>-1</sup> and decreased by 14% (0.86) in days 6–10 post transplant and by 29% (0.71) in days 11–180 post transplant, relative to the days 3–5 post transplant. Patients who carried one or more CYP3A5\*1 alleles had lower troughs than those with the CYP3A5\*3 allele. Troughs and dose requirements by genotype are shown in Figure 1 (panels A and B). CL/F was increased by 69% (1.69) in subjects with the CYP3A5\*1/\*3 genotype and by 100%



**Figure 1**

Tacrolimus doses and troughs by CYP3A5 genotype (○ CYP3A5\*3/\*3, △ CYP3A5\*1/\*3, □ CYP3A5\*1/\*1) over the first 6 months post transplant. (A) Total daily doses by CYP3A5 genotype. (B) Trough concentrations by CYP3A5 genotype (mean ± SE)



**Figure 2**

Goodness-of-fit plots for the final tacrolimus model: IPRED, individual predicted concentrations (ng ml<sup>-1</sup>); WRES, weighted residuals

(2.00) in subjects with the CYP3A5\*1/\*1 genotype. CL/F was reduced by 30% (0.70) in patients undergoing transplantation at a centre using a steroid sparing immunosuppressive regimen and by 6% (0.94) when a CCB was co-administered compared with no CCB. CL/F increased until the median age of 50 years and then decreased thereafter. Recipient weight, gender, race, donor type and gender, pre-emptive transplant, number of prior transplants, and the variants rs12114000, rs3734354, rs4926, rs3135506 and rs2608555 were not significant. The following equation describes the final model for estimation of tacrolimus CL/F in the first 6 months post transplant.

$$\begin{aligned} \text{CL/F (l h}^{-1}\text{)} = & 38.4 \times [(0.86, \text{ if days 6–10}) \text{ or } (0.71, \text{ if days 11–180})] \\ & \times [(1.69, \text{ if CYP3A5 } *1/*3 \text{ genotype}) \text{ or } (2.00, \\ & \text{ if CYP3A5 } *1/*1 \text{ genotype})] \times (0.70, \text{ if receiving a} \\ & \text{ transplant at a steroid sparing centre}) \\ & \times [(\text{age in years}/50)^{-0.4}] \times (0.94, \text{ if CCB is present}) \end{aligned}$$

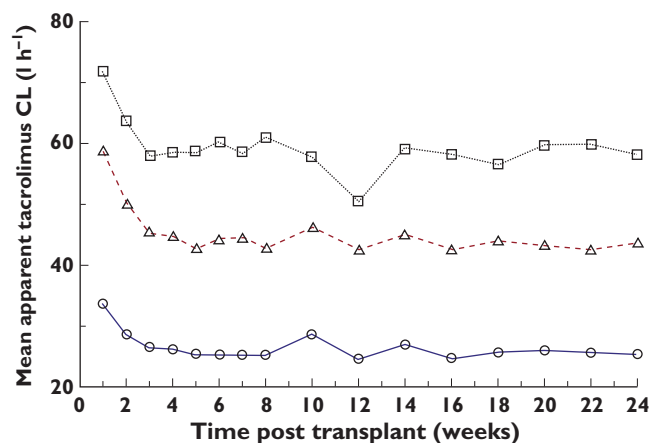
The total daily dose (TDD) requirement is then calculated from the estimated tacrolimus CL/F above and the desired goal trough concentration.

$$\begin{aligned} \text{TDD (mg)} = & [\text{CL/F (l h}^{-1}\text{)} \times \text{tacrolimus trough goal (ng ml}^{-1}\text{)} \times 24 \text{ h}] / 1000 \end{aligned}$$

The base model had an IIV of 52.3% in CL/F. The final model had an IIV of 40.1% in CL/F. Thus, the final model was able to explain 23.2% of the relative IIV in CL/F. Plots of the observed (OBS) concentrations vs. the model predicted (PRED) concentrations and OBS vs. the individual predicted (IPRED) concentrations are shown in Figure 2. A plot of the weighted residuals (WRES) vs. time shows that most of the data lies within 3 units from the zero-ordinate (Figure 2). In addition, the mean parameter estimates from the study population and the 989 successful bootstrap runs are highly comparable, thereby confirming the accuracy and precision of the estimates as well as stability of the final model (Table 3). The *post hoc* estimates of CL/F from the final model by the three different genotypes are shown in Figure 3.

## Discussion

We report here the first dosing model for tacrolimus using a combination of genetic and clinical factors in adult kidney transplant recipients that was developed from one of the largest tacrolimus pharmacogenetics studies ( $n = 681$ ) conducted to date. We found that CL/F was significantly influenced by days post transplant, CYP3A5 genotype, transplantation at a steroid sparing centre,



**Figure 3**

Post hoc estimates of tacrolimus CL/F from the final model over the first 6 months post transplant by CYP3A5 genotype (○ CYP3A5\*3/\*3, △ CYP3A5\*1/\*3, □ CYP3A5\*1/\*1)

recipient age and the use of a CCB. The population CL/F was  $38.4 \text{ l h}^{-1}$  and is close to estimates from other pharmacokinetic studies (ranging from  $21$  to  $35 \text{ l h}^{-1}$ ) [10, 23, 24]. Our estimate of CL/F is slightly larger than previously reported studies. This is expected because, as stated earlier, we modelled  $(\text{CL}/F)^*$ , an approximation to the tacrolimus apparent clearance. In addition, differences in patient populations between the studies cannot be ignored, nor can the influence of covariates that have been included in our model.

In our study, 75% ( $n = 413$ ) and 55% ( $n = 331$ ) of the transplant recipients had tacrolimus trough concentrations  $< 8 \text{ ng ml}^{-1}$  in the first week and second week post transplant, respectively. These troughs are below the usual trough goal in centres participating in this study ( $8\text{--}12 \text{ ng ml}^{-1}$  in the first 3 months). These troughs were achieved through the use of the typical  $\text{mg kg}^{-1}$  dosing and it is possible that a higher number of patients would achieve troughs  $> 8 \text{ ng ml}^{-1}$  if dose individualization using clinical and genetic factors had been used.

Days post transplant was the most significant clinical factor affecting tacrolimus CL/F and has been previously shown to be associated with CL/F [9, 10, 25–27]. Tacrolimus CL/F decreased by 14% in days 6–10 post transplant and by 29% in days 11–180 post transplant, relative to the immediate post-transplant period (days 3–5). This decrease in CL/F is consistent with previous findings [10, 26]. A decrease in CL/F may be attributable to an increase in tacrolimus bioavailability over time as the patients clinical status improves. CL/F may also decrease due to an increase in haematocrit and albumin concentrations with time as kidney function is restored.

The CYP3A5 genotype was also highly associated with CL/F. Individuals who carry one or more CYP3A5\*1 alleles express the CYP3A5 enzyme [28, 29]. Tacrolimus is a substrate for CYP3A5 and it is well established that individuals

carrying one or more \*1 alleles have a higher tacrolimus CL and lower trough concentrations [11–14, 17]. Several previous studies have highlighted the differences in CL/F and dose requirements for the CYP3A5\*1 carriers compared with individuals with the CYP3A5\*3/\*3 genotype [24, 30–34]. In a study involving paediatric kidney transplant recipients, tacrolimus CL/F was about 50% higher in children with the CYP3A5\*1/\*1 or \*1/\*3 genotype as compared with the CYP3A5\*3/\*3 genotype [8]. A recent pharmacokinetic study found tacrolimus CL/F to be about twofold higher in CYP3A5 expressors (\*1/\*1 or \*1/\*3 genotype) as compared with the CYP3A5 non-expressors (\*1/\*1 genotype) [31]. In an earlier study on the pharmacokinetics of tacrolimus in healthy Japanese subjects, CL/F was about 1.5 times higher in the CYP3A5\*1 carriers (\*1/\*1 or \*3/\*3 genotype) as compared with the CYP3A5\*3/\*3 genotype [35]. Most studies have combined genotype groups (\*1/\*1 with \*1/\*3) due to sample size limitations [13–15, 36]. Given our large sample size, we were able to define the differences in tacrolimus CL/F between three CYP3A5 genotype groups. In our study, subjects with the CYP3A5\*1/\*3 and \*1/\*1 genotypes had a CL/F that was 70% and 100% higher, respectively, than those with the CYP3A5\*3/\*3 genotype. These data demonstrate for the first time that the three CYP3A5 genotypes have distinctive CL/F estimates and dose requirements. The majority (85%) of our non-AA population had the CYP3A5\*3/\*3 genotype, whereas the majority (87%) of the AAs had the CYP3A5\*1/\*1 or CYP3A5\*1/\*3 genotype. Despite this, race was insignificant in our final dosing model most likely due to collinearity between CYP3A5 genotype and race. Although the CYP3A5 genotype is associated with tacrolimus CL/F, it is not associated with acute rejection [37–39]. The lack of association is not surprising given that multiple factors increase the risk of rejection (i.e. donor type, recipient age, HLA compatibility, low calcineurin inhibitor concentrations). Importantly though, data show that patients with the CYP3A5\*1/\*3 or \*1/\*1 genotype have earlier acute rejection and are slower to achieve target concentrations [14].

Our analysis found an important centre effect. Centres were categorized by the use of a steroid sparing immunosuppressive regimen or not. A steroid sparing immunosuppressive protocol administers corticosteroids for a short period of time (typically 7–14 days) in the early post-transplant period whereas non-sparing centres maintain steroids throughout the first 6 months or longer [40, 41]. We found that CL/F was decreased by 30% in patients transplanted at a steroid sparing centre. We hypothesize that the centre effect is related to steroid use and that continuous therapy leads to an induction of CYP enzymes and higher tacrolimus CL/F. Various studies have demonstrated an induction of CYP3A enzymes by corticosteroid therapy [42–44]. Although controversial, studies have identified corticosteroid therapy as a significant factor towards tacrolimus CL/F [9, 27, 45–47]. Clinical practice varies substantially between centres. Therefore, the attribution of the

effect to steroids cannot be confirmed in our study and must be directly tested in future analyses.

Age and CCB use were also identified as significant covariates towards CL/F. CL/F increased progressively up to age 50 years and then decreased thereafter. The concomitant use of a CCB was associated with a small (6%) decrease in tacrolimus CL/F. Multiple CCBs were used in our study and we did not collect the specific CCB prescribed. The CCB, diltiazem, is a well-known potent inhibitor of the CYP3A enzyme and decreases tacrolimus CL/F [48, 49]. Other CCBs (such as amlodipine) are not potent inhibitors of CYP3A [50]. Therefore, the inhibitory effects of some CCBs may be overestimated whereas the effect of diltiazem is likely underestimated.

As this study is based on a large population, we expect the estimates from this study to be fairly reliable. However, the data for this analysis came from an observational study where supervision of trough measurements was conducted by the clinical teams. Haematocrit and aspartate aminotransferase (AST) have been previously shown to be important clinical factors [10] and neither was collected in our study. Future studies should also consider concomitant administration of anti-fungals given the potential for drug interactions [51, 52].

The dosing equation which we developed is simple and uses common clinical factors and one genotype. The availability of this equation now allows for the testing of clinical and genetic factor guided vs. traditional weight-based initial dosing. As the achievement of therapeutic concentrations early post transplant is of importance, we would expect the use of individualized dosing to result in a higher proportion of patients within the target range and thereby lower the risk for acute rejection. This model was developed from data in adult individuals within the first 6 months post transplant. Whether it can be extrapolated beyond this time frame is not known. An advantage with our equation is that any tacrolimus trough may be targeted including patients where it is desirable to provide a higher level of immunosuppression as well as those who may be candidates for calcineurin sparing.

An example of how this model is applied clinically is as follows. Suppose we wish to determine prospectively the oral tacrolimus dose in a 50-year-old, 85 kg kidney transplant recipient on day 3 post transplant with a goal tacrolimus trough of 10 ng ml<sup>-1</sup> and a genotype of CYP3A5\*1/\*1, in a steroid using centre, receiving a CCB.

$$\begin{aligned} \text{CL/F (l h}^{-1}\text{)} &= 38.4 \times (2.00 \text{ for CYP3A5}^*1/*1 \text{ genotype}) \\ &\quad \times [(50/50)^{-0.4}] \times (0.94 \text{ for CCB use}) \\ &= 72.2 \text{ l h}^{-1} \end{aligned}$$

The total daily dose (TDD) requirement is:

$$\text{TDD (mg)} = [72.2 \text{ l h}^{-1} \times 10 \text{ ng ml}^{-1} \times 24 \text{ h}] / 1000 = 17.5 \text{ mg}$$

The daily dose is 17.5 mg or 8.5–9 mg twice daily. Should this patient have received a typical weight-based dosing

(0.1 mg kg<sup>-1</sup> day<sup>-1</sup>) the estimated dose would be 8.5 mg day<sup>-1</sup> or 4–4.5 mg twice daily which would have likely under-dosed this patient.

As another example, let us suppose that we wish to determine the oral tacrolimus dose in a 50-year-old, 85 kg kidney transplant recipient on day 100 post transplant with a goal tacrolimus trough of 8 ng ml<sup>-1</sup> and a genotype of CYP3A5\*3/\*3, who underwent transplantation at a steroid sparing centre, and is not receiving a CCB.

$$\begin{aligned} \text{CL/F (l h}^{-1}\text{)} &= 38.4 \times (0.71 \text{ for days 11–180}) \\ &\quad \times (0.70 \text{ for transplant at a steroid} \\ &\quad \text{sparing centre}) \times [(50/50)^{-0.4}] \\ &= 19.1 \text{ l h}^{-1} \end{aligned}$$

The total daily dose (TDD) requirement is:

$$\text{TDD (mg)} = [19.1 \text{ l h}^{-1} \times 8 \text{ ng ml}^{-1} \times 24 \text{ h}] / 1000 = 3.6 \text{ mg}$$

The daily dose is 3.6 mg or 1.5–2 mg twice daily. Should this patient have received our typical weight-based dosing (0.1 mg kg<sup>-1</sup> day<sup>-1</sup>) the estimated dose would be 8.5 mg day<sup>-1</sup> or 4–4.5 mg twice daily which would have likely overdosed this patient.

This model now requires confirmation in an independent population and prospective testing.

## Competing Interests

Chaitali Passey, Angela K. Birnbaum, William S. Oetting and Pamala A. Jacobson have no conflict of interest. Richard C. Brundage owns stocks in Glaxo Smith Kline.

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