# A Population Pharmacokinetic Model of Tacrolimus in Pediatric Liver Transplant Recipients.

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## INTRODUCTION

Tacrolimus, a potent immunosuppressive agent, has a narrow therapeutic index and high pharmacokinetic variability causing a wide interindividual variation of responsiveness to immunosuppressive therapy. The interpatient pharmacokinetic variability of tacrolimus is multifactorial and commonly seen in both adults and particularly pediatrics, however, limited data are available on the pharmacokinetics of tacrolimus in pediatric patients after transplantation. In addition, tacrolimus has poor and highly variable absorption (range 5–67%), is bound to erythrocytes and plasma proteins, and is extensively metabolized by the cytochrome P450 3A5 isoform (CYP3A5) in the liver and small intestine.

## OBJECTIVES

1. To quantify the population pharmacokinetics of tacrolimus in a pediatric liver transplant population using model-based methods.
2. To estimate the effects of covariates on tacrolimus pharmacokinetic parameters in this population.

## METHODS

### Patients and Data Collection

All patients were in the age range from 0.1 to 15 years old who had undergone a liver transplant surgery and were receiving routine clinical care.

### Model

Clinical observational data from routine therapeutic drug monitoring of tacrolimus in pediatric liver transplant recipients were collected and formatted for data analysis using the R software (http://www.r-project.org/). Population pharmacokinetic (PK) analyses were conducted via nonlinear mixed-effects modeling with NONMEM VII. Goodness of fit was assessed by inspection of diagnostic plots, parameter plausibility and precision, stability of the optimized minimization (e.g. through parameter gradients and consistency of minimization under different sets of initial estimates), and AIC based on minimum value of the objective function. Initial base structural models were implemented based on prior knowledge from the literature and the inclusion of allometrically scaled compartmental PK parameters. A full covariate model approach, emphasizing parameter estimation rather than stepwise hypothesis testing was implemented. Predefined covariate-parameter relationships were identified based on clinical interest, mechanistic plausibility, or prior knowledge; and then a full model was constructed, with care to avoid correlation or collinearity in predictors. Model parameters were estimated and exploratory assessment of any remaining trends was conducted by graphical inspection of all covariate effects.

### Model Evaluation

- Non-Parametric Bootstrap
  A non-parametric bootstrap procedure, which estimates the precision of the model parameters, was implemented. One thousand replicate data sets were generated by random sampling of individuals with replacement and were stratified by age to ensure adequate characterization of the pediatric population [2, 3, 4, 6]. Population parameters for each data set were subsequently estimated using NONMEM. Empirical 95% confidence intervals (CIs) were constructed by observing the 2.5th and 97.5th quantiles of the resulting parameter distributions for all bootstrap runs with successful convergence (n=266).

## RESULTS

Demographic and biologic characteristics of the patient population are presented in Table 1. Patients were between the ages of 0.1 to 15 years old. The study population consisted of 19 males and 22 females with weights ranging from 2.6 to 63.6 kg. The tacrolimus population PK dataset consisted of 643 tacrolimus concentrations with an average of 16 sparse PK samples per patient (range: 7-33). The average trough concentration of tacrolimus varied considerably over the post-transplantation period with a range of 0.42 to 61.8 ng/mL.

Available covariates included: age, sex, weight, albumin (ALB), alanine aminotransferase (ALT,) aspartate aminotransferase (AST), blood urea nitrogen (BUN), gamma glutamyl transferase (GGT), hematocrit (HCT), hemoglobin (HGB), time after transplant (POD), and CYP3A genotype. The frequency distribution of CYP3A5*1/*1, CYP3A5*1/*3 and CYP3A5*3/*3 genotypes was 2.9% (n = 1), 35.3% (n = 12) and 61.8% (n = 21), respectively, in 34 pediatric liver transplant patients. CYP3A genotype information was not available for 7 patients (14%) and all missing values were imputed with extreme cases to determine sensitivity of the covariate effect estimate to the missing data.

**Table 1:** *Demographic and Biologic Data from 41 Patients*

| Variables | Mean | Median | Range |
|---|---|---|---|
| **Patient Data** | | | |
| Age (year) | 4.4 ± 4.6 | 2 | 0.1-15 |
| Weight (kg) | 18.8 ± 16.2 | 10.6 | 2.6-63.6 |
| ALT (u/L) | 546.8 ± 637.5 | 362.7 | 20-3780 |
| AST (u/L) | 777.5 ± 753.8 | 510.5 | 42-3625 |
| Albumin (g/L) | 29.1 ± 6.0 | 28 | 19.4-42.5 |
| HCT (g/dL) | 0.319 ± 0.043 | 0.32 | 0.250-0.440 |
| GGT (u/L) | 115.1 ± 152.4 | 51.5 | 16-724 |
| POD (day) | | 7 | 0-15 |
| **Pharmacokinetic Data** | | | |
| Total Samples | 643 | | |
| Samples Per Patient | 15.7 ± 7.6 | 13 | 7-33 |
| Tacrolimus Concentration (ng/mL) | | | 0.42-61.8 |
| **Patient Genotype Data** | | | |
| | **N** | **Frequency** | |
| CYP3A *1/*1 | 1 | 2.9 | |
| CYP3A *1/*3 | 12 | 35.3 | |
| CYP3A *3/*3 | 21 | 61.8 | |
| Unknown | 7 | | |

ALT: alanine aminotransferase, AST: aspartate aminotransferase, HCT: hematocrit, GGT: gamma glutamyl transferase, POD: post-operative days

The PK of tacrolimus in this population was adequately described by a one-compartment model with first-order elimination, parameterized in terms of apparent oral clearance (CL/F) and apparent volume of distribution (V/F) due to the lack of reference intravenous data. The absorption rate constant could not be determined since only a minimal number of PK samples were taken during the absorption phase, and was fixed to 4.48 $hr^{-1}$ [5]. CL/F and V/F were allometrically scaled by weight (CL/F: exponent fixed at 0.75, V/F: exponent fixed at 1). Sub-models for PK parameter typical values were estimated in a log-transformed parameterization, but transformed back to the linear scale for presentation of results. For the PK observations in this analysis, the residual error model was described by a combined additive and proportional error model. Inter-individual random variation on CL/F and V/F was modeled exponentially with an estimated covariance of these random effects. Covariates included in the full model were: weight, age, ALB, AST, CYP3A genotype, HCT, and POD. The model for CL/F (linear scale) was:

$$\theta 1 * (WT/70)^{0.75} * (POD/7)^{\theta 4} * \theta 5^{CYP3A} * (AST/510.5)^{\theta 6} * (ALB/28)^{\theta 7} * \theta 8^{HCT} * (AGE/2)^{\theta 9}$$

CYP3A: genotype *3/*3=0, *1/*1 or *1/*3=1, HCT: 0 if hematocrit is less than 35%, otherwise 1.

There were no covariate effects on V/F other than the fixed allometric weight. The population estimates of tacrolimus CL/F and V/F were 25.8 L/h and 2490 L, respectively, for a reference individual of 70 kg. Goodness-of-fit criteria indicated the final model did not demonstrate any systematic bias, except for a trend toward under-prediction of peak concentrations (Figure 1).

**Figure 1:** *Diagnostic Plots of the Population Pharmacokinetic Model*

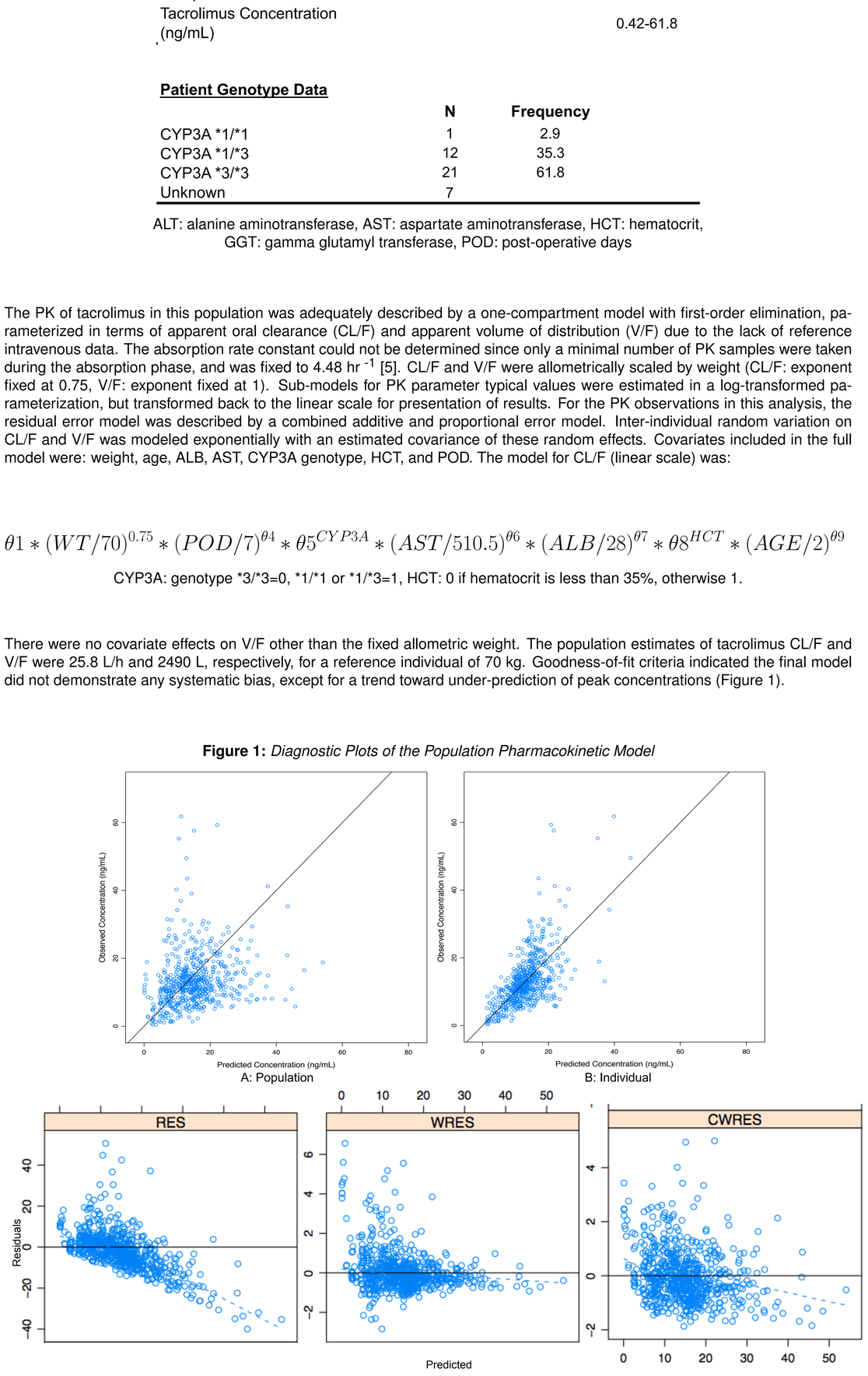


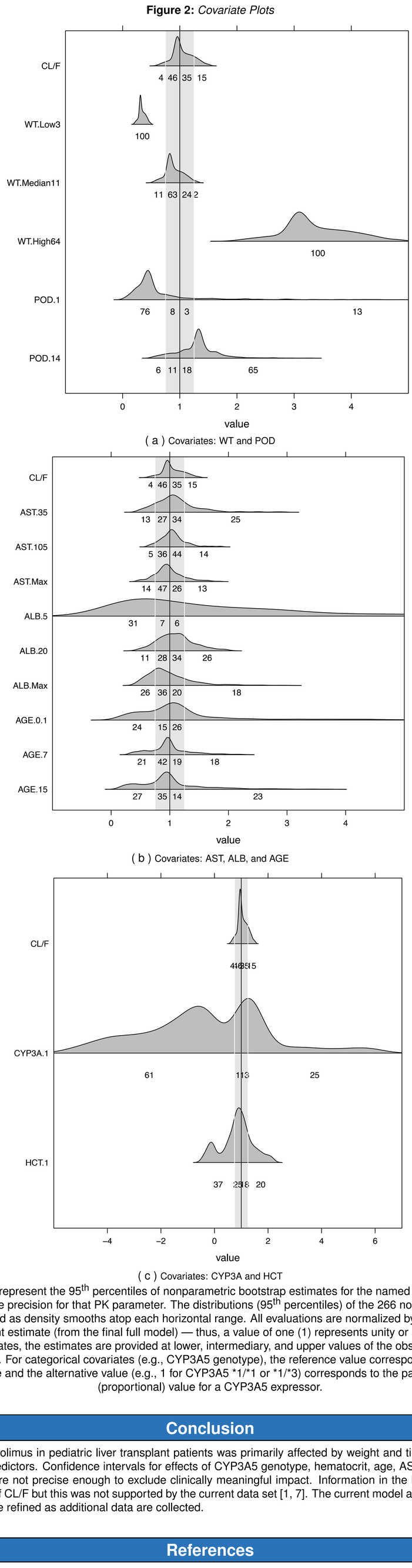

**Table 2:** *Parameter Estimates from the Population Pharmacokinetic Model**

| | | | | NONMEM SE | | Bootstrap CI | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **Model** | **Estimate** | **PRSE** | **Lower CI** | **Upper CI** | **Lower CI** | **Upper CI** |
| CL/F (θ1) | $\theta 1 \cdot (WT/70)^{0.75}$ | 25.8 L/h | 4.45 | 23.5 | 28.1 | 22.9 | 30.0 |
| V/F (θ2) | $\theta 2 \cdot (WT/70)^{1}$ | 2490 L | 0.0467 | 2488 | 2492 | 2186 | 3165 |
| KA (θ3) | | 4.48 $hr^{-1}$ | | | | | |
| POD (θ4) | $(POD/7)^{\theta 4}$ | 0.409 | 21.7 | 0.235 | 0.583 | 0.365 | 0.498 |
| CYP3A (θ5) | $\theta 5^{CYP3A}$ | 1.24 | 17.4 | 0.817 | 1.66 | 1.05 | 1.52 |
| AST (θ6) | $(AST/510.5)^{\theta 6}$ | -0.0364 | 210.7 | -0.187 | 0.114 | -0.0818 | 0.0452 |
| ALB (θ7) | $(ALB/28)^{\theta 7}$ | -0.357 | 120.7 | -1.20 | 0.488 | -0.642 | 0.551 |
| HCT (θ8) | $\theta 8^{HCT}$ | 0.993 | 21.7 | 0.572 | 1.41 | 0.730 | 1.27 |
| AGE (θ9) | $(AGE/2)^{\theta 9}$ | -0.0310 | 188.4 | -0.145 | 0.083 | -0.0778 | 0.0212 |
| $IIV_{CL/F}$ (Ω1.1) | | 0.554 | 71.7 | -0.224 | 1.33 | 0.101 | 0.930 |
| $IIV_{V/F}$ (Ω2.2) | | 0.803 | 42.5 | 0.135 | 1.47 | 0.376 | 1.11 |
| prop. err. (σ1.1) | | 0.135 | 16.1 | 0.0925 | 0.178 | 0.0891 | 0.151 |
| add. err. (σ2.2) | | 6.29 | 33.5 | 2.15 | 10.4 | 4.38 | 18.2 |

PRSE: percent relative standard error, CL: clearance, F: bioavailability, Ka: absorption constant, POD: post-operative days, AST: aspartate aminotransferase, ALB: albumin, HCT: hematocrit, Ω: interindividual variance, σ: residual variance (proportional or additive), CI: confidence interval (95%)
* Parameters presented represent the case where missing genotype data was imputed as non-expressors, due to the fact that this is the predominant category

The final full model parameter estimates are shown in Table 2 and the covariate effect plots are shown in Figure 2. On average, CL/F ranged from a value that was 55% lower (POD = 1 day) to 36% higher (POD = 15 days) than the reference CL/F at POD=7 days. Similar to prior reports, tacrolimus metabolism was affected by CYP3A5 polymorphisms. CL/F generally was greater in patients with CYP3A5 *1/*1 or CYP3A5 *1/*3 (expressors) compared to those with CYP3A5 *3/*3 (non-expressors) genotype. Sensitivity to the missing genotype data was minimal, with relative fractional effect estimates of 1.24, (17.4% RSE) and 1.14, (16.4% RSE) for cases with all missing data imputed as non-expressors or expressors, respectively. Other model parameters were unaffected by the missing covariate data.

**Figure 2:** *Covariate Plots*




The horizontal lines represent the 95th percentiles of nonparametric bootstrap estimates for the named PK parameter, and so represent the relative precision for that PK parameter. The distributions (95th percentiles) of the 266 nonparametric bootstrap estimates are provided as density smooths atop each horizontal range. All evaluations are normalized by dividing by the named PK parameter's point estimate (from the final full model) — thus, a value of one (1) represents unity or a null covariate effect. For continuous covariates, the estimates are provided at lower, intermediary, and upper values of the observed covariate's range for each parameter. For categorical covariates (e.g., CYP3A5 genotype), the reference value corresponds to the typical PK parameter value and the alternative value (e.g., 1 for CYP3A5 *1/*1 or *1/*3) corresponds to the parameter's relative (proportional) value for a CYP3A5 expressor.

## Conclusion

Systemic CL/F of tacrolimus in pediatric liver transplant patients was primarily affected by weight and time since transplant, increasing with both predictors. Confidence intervals for effects of CYP3A5 genotype, hematocrit, age, AST, and ALB all included the null value, but were not precise enough to exclude clinically meaningful impact. Information in the literature identified AST levels as a predictor of CL/F but this was not supported by the current data set [1, 7]. The current model and associated covariate effect estimates will be refined as additional data are collected.

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