

# Impact of CYP3A5 phenotype on tacrolimus time in therapeutic range and clinical outcomes in pediatric renal and heart transplant recipients

Abbie D. Leino  | Jeong M. Park  | Amy L. Pasternak 

Department of Clinical Pharmacy, College of Pharmacy, University of Michigan, Ann Arbor, Michigan, USA

## Correspondence

Amy L. Pasternak, College of Pharmacy, University of Michigan, 428 Church St, Ann Arbor, MI 48109, USA.  
Email: amyip@med.umich.edu

## Funding information

National Center for Advancing Translational Sciences, Grant/Award Number: UL1TR002240

## Abstract

**Study Objective:** This study investigated the effect of CYP3A5 phenotype on time in therapeutic range (TTR) of tacrolimus post-transplant in pediatric patients.

**Design and Data Source:** This retrospective study assessed medical records of pediatric kidney and heart recipients with available CYP3A5 genotype for tacrolimus dosing, troughs, and the clinical events (biopsy-proven acute rejection [BPAR] and de novo donor-specific antibodies [dnDSA]).

**Measurements and Main Results:** The primary outcome, mean TTR in the first 90 days post-transplant, was 9.0% (95% CI: -16.1, -1.9) lower in CYP3A5 expressers ( $p = 0.014$ ) when adjusting for time to therapeutic concentration and organ type. There was no difference between CYP3A5 phenotypes in time to the first clinical event using TTR during the first 90 days. When applying TTR over the first year, there was a significant difference in event-free survival (EFS) which was 50.0% for CYP3A5 expressers/TTR < 35%, 45.5% for expressers/TTR  $\geq$  35%, 38.1% for nonexpressers/TTR < 35%, and 72.9% for nonexpressers/TTR  $\geq$  35% (log-rank  $p = 0.03$ ). A post hoc analysis of EFS identified CYP3A5 expressers had lower EFS compared to nonexpressers in patients with TTR  $\geq$  35% ( $p = 0.04$ ) but no difference among patients with TTR < 35% ( $p = 0.6$ ).

**Conclusions:** The relationship between TTR and CYP3A5 phenotype suggests that achieving a TTR  $\geq$  35% during the first year may be a modifiable factor to attenuate the risk of BPAR and dnDSA.

## KEYWORDS

cytochrome P-450 CYP3A5, pediatric transplant, tacrolimus, time in therapeutic range

## 1 | INTRODUCTION

Tacrolimus is the primary immunosuppressant used following pediatric solid organ transplantation.<sup>1,2</sup> Tacrolimus has a narrow therapeutic index; low exposure, defined by trough concentrations, contributes to therapeutic failure (e.g., acute rejection, de novo donor-specific antibody (dnDSA), and graft loss) and high exposure increases the rate of adverse events. Moving beyond the assessment

of single trough concentrations, tacrolimus time in therapeutic range (TTR) has emerged as an additional risk factor for poor long-term transplant outcomes, including acute rejection, dnDSA formation, and graft loss in adult transplant recipients. TTR calculates the proportion of time a patient is within the target range for tacrolimus trough concentration. This method is the standard of care for the classic narrow therapeutic index drug, warfarin, and is predictive of warfarin effectiveness and adverse events.<sup>3-5</sup> The hypothesized

benefit of TTR is that it provides information about tacrolimus stability within the target concentration over time and may better explain the risk of poor outcomes.

Understanding the causes of low TTR is vital to address the associated risk through effective interventions. Drug-drug interactions, food intake, gastrointestinal disorders, medication adherence, chronobiology, and pharmacogenetics, among others, are hypothesized to contribute to TTR but have not been thoroughly evaluated. Pharmacogenetics has been reported to be one of the least well-understood potential causes.<sup>6</sup> Tacrolimus is extensively metabolized by cytochrome P450 enzymes (CYP) 3A4 and 3A5, regulating the bioavailability and hepatic clearance. A theorized mechanism for effect on TTR is that CYP3A5 nonexpressers rely on CYP3A4 for tacrolimus metabolism, which is more sensitive to induction and inhibition.<sup>7,8</sup> The corresponding changes in tacrolimus trough concentrations would then increase the time spent outside the therapeutic range. As CYP3A5 phenotype may be expected to have a more significant impact in the early post-transplant period given its relationship to initial trough concentrations, the primary purpose of this study was to investigate the effect of CYP3A5 phenotype on tacrolimus TTR early post-transplant in pediatric transplant recipients.<sup>9-12</sup>

## 2 | PATIENTS AND METHODS

### 2.1 | Patient population

This single-center retrospective analysis included all pediatric patients who underwent renal or heart transplant from 6/1/14 to 12/31/18 and were initiated on tacrolimus with whole blood or buffy coat samples available for genotyping.<sup>13</sup> Clinical patient data were collected from the medical record or linked data collection systems from the date of transplant through the first year after transplant.<sup>14</sup> The study was approved by the local institutional review board.

Most patients received tacrolimus orally twice daily except for kidney recipients <40 kg, who could be initiated on dosing every 8 h at the discretion of the provider. The protocolized tacrolimus starting dose was 0.1 mg/kg/dose in kidney recipients and 0.05 mg/kg/dose in heart recipients. Target tacrolimus trough concentrations ( $C_0$ ) were 10–12 ng/ml for months 1–2, 8–10 ng/ml for months 3–6, 6–8 ng/ml for months 7–9 and 4–6 ng/ml for months 10–12 in kidney recipients, and 10–15 ng/ml for the 1st year in heart recipients. The medical record was also reviewed for individualized target ranges. Outpatient tacrolimus trough concentrations were obtained per protocol and at clinician discretion. In kidney recipients, per protocol, troughs were obtained weekly for the first three months, every two weeks for the next three months, then monthly. The protocol in heart recipients dictated tacrolimus concentrations at 7–10 days, one month, two months, then every six weeks until month 9, then again at one year.

A triple immunosuppression regimen with mycophenolate mofetil (kidney: 300 mg/m<sup>2</sup> every 12 h; heart: 600 mg/m<sup>2</sup> every 12 h) and prednisone was used in most patients. Details of the

steroid protocols are provided in the supplemental materials. For kidney recipients receiving a steroid avoidance protocol, mycophenolate mofetil was dosed at 450 mg/m<sup>2</sup> every twelve hours. Induction therapy with basiliximab or rabbit antithymocyte globulin was at the provider's discretion based on immunologic risk. Biopsy in kidney recipients was based on the selected immunosuppression protocol. For patients receiving steroid-avoidant maintenance immunosuppression, protocol biopsy was performed at 6 weeks and between 8 to 12 months post-transplant. The remaining kidney recipients only underwent biopsy for clinical indications. The standard of care protocol for DSA testing in kidney recipients was based on rejection risk or need for indication biopsy. Kidney recipients at high risk for antibody-mediated rejection (positive crossmatch, history of DSA, desensitization, or peak PRA > 50%) had DSA drawn at approximately 7 days, monthly until 6 months, then at 1 year. Kidney recipients considered to be at a high of T-cell-mediated rejection (peak PRA 21%–50%, re-transplant, African American, or history of Banff 2A or greater rejection) had DSA drawn at 3, 6, and 12 months. In the remaining kidney recipients, DSA was obtained at one year. Heart recipients underwent protocol biopsy at approximately day 7, 30, 60, 90, 180, 270, and 365. DSA was obtained at the time of all biopsies in all heart recipients.

### 2.2 | Study outcomes

The primary outcome was tacrolimus TTR within the first 90 days after transplantation. All trough levels available for the patient were included in the estimation of TTR. The Rosendaal linear interpolation method was used to calculate time in the therapeutic range.<sup>15</sup> Assuming a linear relationship, a daily concentration is assigned for each day between two measured concentrations. The daily concentration is estimated to increment equally up or down, as appropriate, from the previous level until reaching the value of the next level. The TTR is the sum of days during which the tacrolimus trough concentration was assumed to be within the defined target range. To account for intentional deviations from the protocolized therapeutic range due to circumstances such as adverse effects, infection, or rejection, TTR was calculated for each patient using the individualized therapeutic range documented by the treating provider in the medical record.

Secondary outcomes included the time to first therapeutic trough and time to stable therapeutic trough defined as two consecutive  $C_0$  values within  $\pm 1$  ng/ml of the target range, TTR during the 1st year post-transplant, and coefficient of variation (CV) of tacrolimus trough concentrations.<sup>16</sup>

Exploratory outcomes included event-free survival (EFS), defined as no biopsy-proven acute rejection (BPAR), and dnDSA formation during the 1st year post-transplant. BPAR was diagnosed by allograft biopsy according to the most updated consensus criteria at the time of biopsy (Banff classification or ISHLT grading system).<sup>17,18</sup> Detection of dnDSA was performed using a solid-phase assay and considered positive if DSA was newly detected post-transplant.

## 2.3 | Genotyping

DNA was extracted from the buffy coat or whole blood sample via the Qiamp DNA Blood and Tissue Kit. Commercial Taqman assays were used as described previously.<sup>13</sup> Genotypes were dichotomized into phenotype based on diplotype for analysis as CYP3A5 expressers (CYP3A5 \*1/\*1, \*1/\*3, \*1/\*6, \*1/\*7) or CYP3A5 nonexpressers (CYP3A5 \*3/\*3, \*6/\*6, \*7/\*7, \*3/\*6, \*3/\*7, \*6/\*7).

## 2.4 | Statistical analysis

Descriptive statistics were used to describe the baseline and clinical characteristics of the cohort by CYP3A5 phenotype. Kidney and heart recipients were analyzed together for the primary analysis unless otherwise noted. The primary endpoint compared 90-day TTR between CYP3A5 expressers and CYP3A5 nonexpressers using simple and multivariable linear regression. A multivariable analysis was planned to follow the univariate screening of the following variables: organ type, time to therapeutic concentrations, and age greater than 8 years based on previous literature.<sup>19–27</sup> Variables with *p* value <0.1 were included in the multivariable model. Starting dose was considered for inclusion but was closely related to organ type, which was determined to be more important for inclusion to adjust for unspecified differences in management between organ groups. For the secondary endpoint, time to stable therapeutic trough concentration was described using Kaplan–Meier survival analysis with the log-rank test to evaluate stratum differences. The exploratory time-to-event analysis was conducted for immune-mediated clinical events defined as the composite of first BPAR episode or development of dnDSA using Kaplan–Meier and log-rank methods. Data were right-censored for death or graft loss unrelated to rejection. For this analysis, TTR was dichotomized at the mean value of this study cohort (35%) to form four groups based on the CYP3A5 phenotype. For consistency, the TTR value included in the EFS analysis was the same used for the primary (all levels in the first 90 days) and secondary (all levels in the first 365 days) analyses. Post hoc sensitivity analyses were undertaken, (1) dividing the groups using the TTR threshold of 30% presented in the adult literature and (2) censoring the tacrolimus concentrations included in the TTR calculation at the time of event.<sup>28</sup> Cox proportional hazards analysis was also performed adjusting for organ type and induction therapy (selected based on the established impact on the outcome). Statistical tests were two-tailed with an alpha of 0.05 and completed using RStudio version 3.6.1.

## 3 | RESULTS

A total of 85 pediatric transplant patients were analyzed: 37 heart recipients and 48 kidney recipients. Nineteen patients (22.4%) were CYP3A5 expressers, and 66 (77.6%) were CYP3A5 nonexpressers.

**TABLE 1** Demographic and clinical characteristics stratified by CYP3A5 phenotype

	Nonexpresser <sup>c</sup> (n = 66)	Expresser <sup>b</sup> (n = 19)	<i>p</i> Value
Age in years, median (range)	11.5 (0.13–17.9)	8.7 (0.27–17.4)	0.457
Male, n (%)	41 (62.1)	11 (57.9)	0.947
Organ Type, n (%)			
Heart	31 (47.0)	6 (31.6)	0.352
Kidney	35 (53.0)	13 (68.4)	
Deceased donor kidney, n (%)	16 (45.7)	10 (76.9)	0.109
Race, n (%)			
White	56 (84.8)	9 (47.4)	<0.001
Black	1 (1.5)	9 (47.4)	
Asian	0 (0)	1 (5.3)	
Other/Unknown	9 (13.6)	0 (0)	
Induction, n (%)			
Rabbit antithymocyte globulin	34 (51.5)	9 (47.4)	0.519
Basiliximab	13 (19.7)	6 (31.6)	
None	19 (28.8)	4 (21.0)	
Antiproliferative, n (%)			
Mycophenolate	65	17	0.124
mTOR inhibitor	1	1	
Azathioprine	0	1	
Steroid avoidant protocol, n (%) <sup>a</sup>	19 (28.8)	7 (36.8)	0.999
Azole antifungal use, n (%)	9 (13.6)	7 (36.8)	0.041
Other CYP3A interactions, n (%)	11 (16.7)	3 (15.8)	0.999

Abbreviation: mTOR, mammalian target of rapamycin.

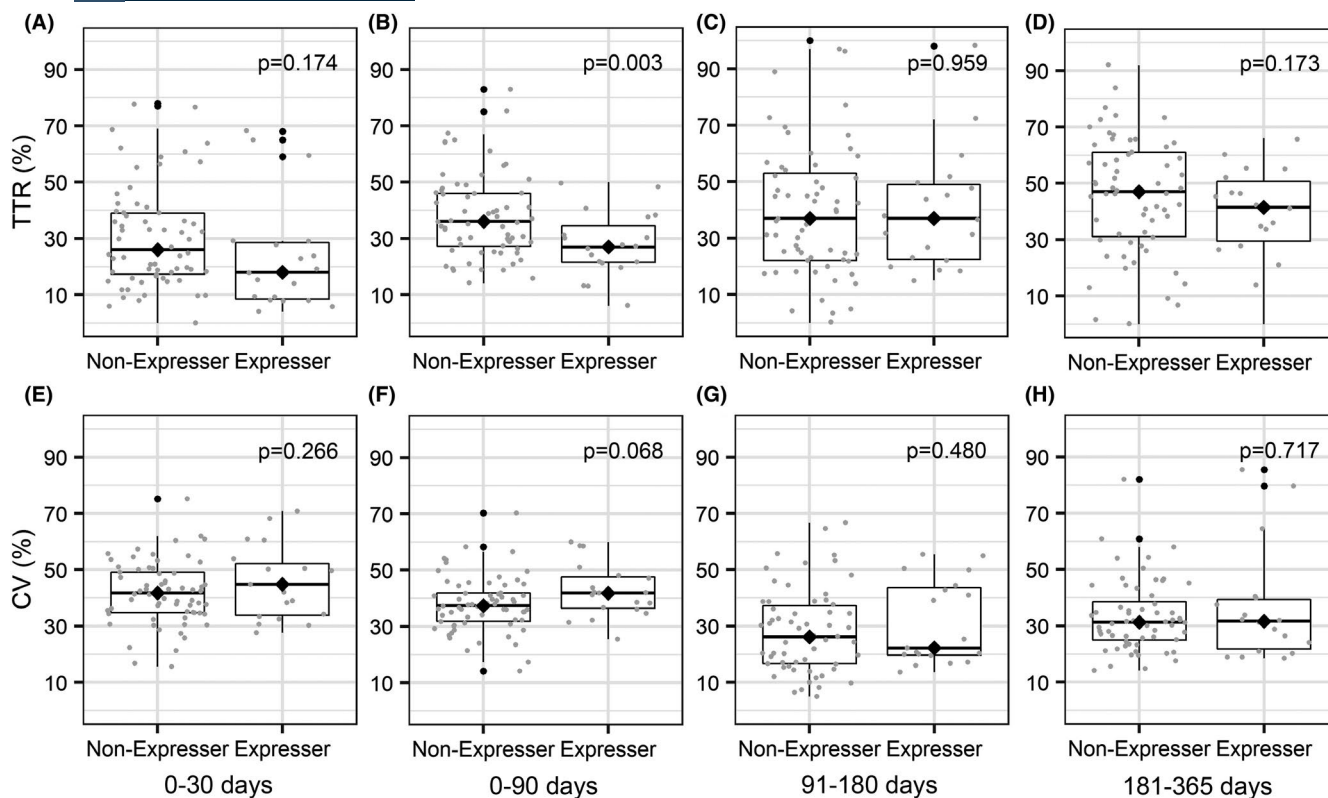
<sup>a</sup>Kidney recipients only (all heart recipients remained on steroid therapy).

<sup>b</sup>\*1/\*1, \*1/\*3, \*1/\*6, or \*1/\*7 diplotype.

<sup>c</sup>\*3/\*3, \*6/\*6, \*7/\*7, \*3/\*6, \*3/\*7, or \*6/\*7 diplotype.

There were no significant differences between CYP3A5 phenotypes in age, gender, or organ type. As expected, there was a significant difference between groups in the self-reported race with CYP3A5 expression more common among patients reporting Black or Asian race. Expressers were also more likely to receive azole antifungal therapy though the indication was generally to leverage the interaction to boost tacrolimus exposure. The characteristics of participants by the CYP3A5 phenotype are described in Table 1.

The primary outcome, mean TTR in the first 90 days post-transplant, was 10.2% (95% CI: –17.6, –2.8) lower in CYP3A5 expressers (*p* = 0.003). A similar difference was noted for the first 60 days post-transplant (mean difference: –10.5%; 95% CI: –19.0, –1.9). However, in the first 30 days and after 90 days, there was no difference in TTR by CYP3A5 phenotype. The CV was not different



**FIGURE 1** Association of tacrolimus time in therapeutic range (A–D) and coefficient of variation (E–H) with CYP3A5 phenotype by time post-transplant. Abbreviations: CV, coefficient of variation; TTR, time in therapeutic range

between CYP3A5 groups at any time point. Figure 1 presents box-plots of TTR and CV at various times post-transplant by CYP3A5 status. In the multivariable model controlling for organ type and time to stable therapeutic concentration, TTR in the first 90 days was 9.0% (95% CI: -16.1, -1.9) lower in CYP3A5 expressers compared to nonexpressers ( $p = 0.014$ ).

The CYP3A5 expresser phenotype resulted in a higher dose to achieve similar trough concentrations at all evaluated time points (Table 2). However, there was no difference in the time to first therapeutic concentration or stable therapeutic concentrations. The median time to the first therapeutic concentration was 6 days (IQR 4–9 days) for CYP3A5 nonexpressers and 7 days (IQR 6–9 days) for CYP3A5 expressers ( $p = 0.3$ ). The median time to achieve stable therapeutic concentrations was 11 days (IQR 7–20 days) for CYP3A5 nonexpressers compared to 12 days (IQR 7–31 days) for CYP3A5 expressers (Figure 2;  $p = 0.5$ ). Table 2 also contains details on the frequency of dose changes and the number of levels included in the TTR calculation over the first year.

In the first year, 24 patients (28.2% overall, 10 kidney and 14 heart recipients) experienced at least one BPAR event and 19 patients (22.4% overall, 4 kidney and 15 heart recipients) developed dnDSA with nine subjects experiencing only dnDSA. One patient expired prior to any events and was right-censored on day 204. The mean TTR over the first 90 days for the entire cohort was 35%, and the results of the immune-mediated clinical events analysis are shown in Figure 3. EFS for each organ group is presented in the supplemental

materials (Figure S1). There was no difference among the CYP3A5 phenotype/TTR groups in the time to first clinical event for the 90-day TTR, although there was a significant difference for TTR over the first year (log-rank  $p = 0.03$ ). One year EFS was 50.0% (95% CI: 25.0%–100%) for patients with CYP3A5 expression and TTR < 35%, 45.5% (95% CI: 23.8%–86.8%) for patients with CYP3A5 expression and TTR  $\geq 35\%$ , 38.1% (95% CI: 20.9%–69.3%) for CYP3A5 nonexpressers with TTR < 35%, and 72.9% (95% CI: 61.4–86.6%) for CYP3A5 nonexpressers with TTR  $\geq 35\%$ . Pairwise comparison using the log-rank test identified the only significant difference was between CYP3A5 nonexpressers with TTR < 35% and  $\geq 35\%$  ( $p = 0.027$ ). A post hoc analysis of EFS identified CYP3A5 expressers had lower EFS than nonexpressers over the first year in patients with TTR  $\geq 35\%$  (Figure S2;  $p = 0.04$ ).

The sensitivity analysis using a 30% threshold for TTR over the first year resulted in better visual separation of the curves with 1 year event-free survival of 25% (95% CI: 4.6%–100%) for CYP3A5 expressers with TTR < 30%, 53.3% (95% CI: 33.2%–85.6%) for CYP3A5 expressers with TTR  $\geq 30\%$ , 33.3% (95% CI: 15%–74.2%) for CYP3A5 nonexpressers with TTR < 30%, and 70.4% (95% CI 59.2%–83.7%) for CYP3A5 nonexpressers with TTR  $\geq 30\%$  (Figure S3). The results using TTR censored at the time of the event were similar to those obtained using TTR from all tacrolimus levels (Figure S4). The exploratory multivariable Cox proportional hazard model controlling for organ type and induction therapy further supports these findings; CYP3A5 phenotype was associated with an increased hazard of the composite outcome. The hazard ratio

**TABLE 2** Tacrolimus dosing, concentration data, and TTR by CYP3A5 phenotype

	Nonexpresser (n = 66)	Expresser (n = 19)	p Value
Tacrolimus dose, mg/kg			
Initial	0.072 (0.03)	0.077 (0.03)	0.490
At stable, therapeutic trough	0.10 (0.06)	0.18 (0.09)	<0.001
At 30 days	0.11 (0.07)	0.19 (0.11)	<0.001
At 90 days	0.11 (0.09)	0.19 (0.13)	0.003
At 180 days	0.11 (0.11)	0.18 (0.11)	0.034
At 365 days	0.10 (0.15)	0.19 (0.21)	0.192
Dose changes, median (IQR)			
0–30 days	6 (4–8)	7 (5–9)	0.123
31–90 days	4 (2–6)	5 (4–7)	0.005
91–180 days	4 (2–5)	4 (2–5)	0.913
181–365 days	3 (2–6)	4 (3–7)	0.383
Tacrolimus concentration, ng/ml			
Initial	6.5 (5.4)	4.1 (2.7)	0.0719
At stable, therapeutic trough	10.8 (1.98)	10.2 (1.4)	0.194
At 30 days	11.4 (4.4)	9.8 (3.8)	0.178
At 90 days	9.7 (2.9)	9.5 (2.8)	0.767
At 180 days	9.5 (3.9)	9.4 (2.7)	0.878
At 365 days	7.1 (3.5)	7.7 (4.1)	0.583
Number of levels, median (IQR)			
0–30 days	11 (8–13)	11 (10–15)	0.106
31–90 days	9 (4–10)	10 (9–12)	0.004
91–180 days	7 (5–10)	10 (8–12)	0.026
181–365 days	9 (6–12)	10 (8–15)	0.412
Time in therapeutic range, %			
0–90 days	38.0 (14.9)	27.8 (11.6)	0.003
91–180 days	39.2 (23.9)	39.5 (21.1)	0.959
181–365 days	45.7 (20.8)	39.1 (16.8)	0.173
0–365 days	42.5 (14.3)	36.5 (11.4)	0.082

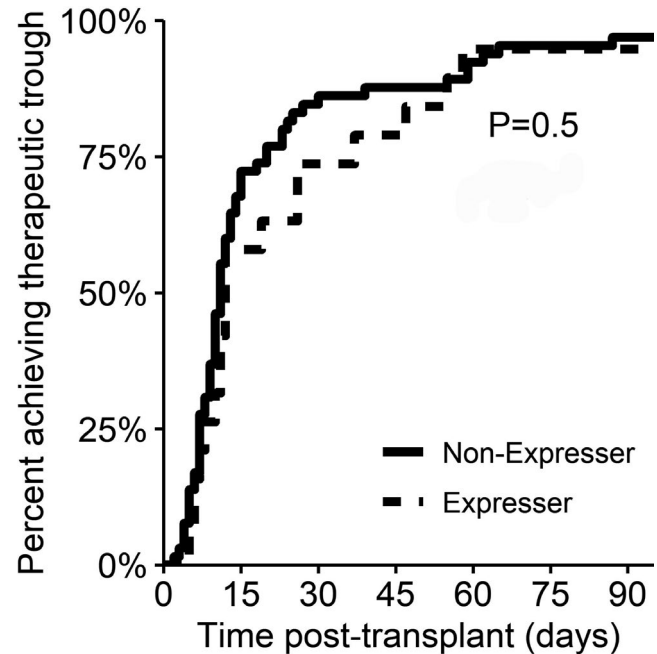
Note: Results reported as mean (SD) unless noted.

Abbreviations: TTR, time in therapeutic range.

(HR) was similar regardless of TTR over the first year in CYP3A5 expressers compared to nonexpresser with TTR > 35% (Expresser/TTR ≥ 35%: HR 3.69 (95% CI 1.33–10.19) vs. Expresser/TTR < 35%: HR 3.62 (95% CI 1.08–12.13)). The full results of the Cox regression models are in Table 3.

## 4 | DISCUSSION

In this study, pediatric transplant recipients who are CYP3A5 expressers displayed lower tacrolimus TTR than CYP3A5 nonexpressers in

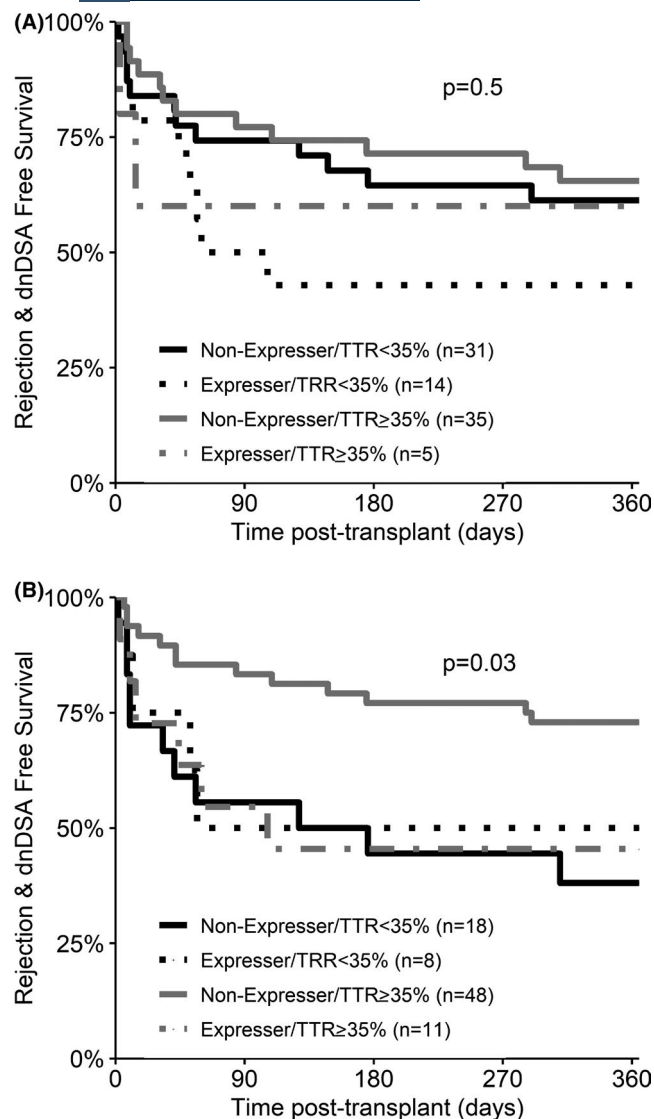


**FIGURE 2** Time to achieve stable therapeutic trough blood concentrations by CYP3A5 phenotype. The median time to achieve stable therapeutic concentrations was 11 days (IQR 7–20 days) for CYP3A5 nonexpressers (solid line) compared to 12 days (IQR 7–31 days) for CYP3A5 expressers (dashed line)

the early post-transplant period. This is a novel finding as existing literature has been limited to adults. An abstract by Salah et al. found similar results in adult heart recipients with a mean TTR of 30.5% in CYP3A5 expressers and 39.9% in CYP3A5 nonexpressers during the first year post-transplant when adjusting for serum creatinine ( $p = 0.09$ ).<sup>29</sup> Interestingly, these results contradict the original hypothesis that CYP3A5 nonexpressers would be expected to have greater variability in trough concentrations and correspondingly a lower TTR.<sup>30,31</sup> An alternative explanation may be that CYP3A5 expressers have a lower TTR in the early period after transplantation because of a longer time to achieve therapeutic concentrations. However, our study did not identify a difference in time to the first therapeutic trough blood concentrations by CYP3A5 phenotype.

Min et al. investigated the frequency of out-of-range concentrations among pediatric patients with renal, heart, or liver transplant randomized to CYP3A5 genotype-based versus standard dosing in the first 30 days post-transplant.<sup>16</sup> The odds of at least 1 out-of-range concentration were significantly lower in the genotype-based dosing arm (OR = 0.6, 95% CI = 0.44, 0.83,  $p = 0.002$ ). However, 60% of patients in the genotype-dosing arm still had an out-of-range concentration. The time to stable therapeutic concentration in the genotype-based dosing arm was 18 days (IQR 14–27 days), with 69% achieving therapeutic concentrations by 30 days. Median could not be calculated in the standard dosing arm, as only 44% had achieved stable therapeutic concentrations. In our study, both groups reached stable therapeutic levels in a similar timeframe without genotype-based dosing. While studies have found mixed results regarding the impact of CYP3A5 on time to therapeutic concentrations, these data





**FIGURE 3** Time-to-event analysis for first rejection episode or dnDSA development by CYP3A5 phenotype and time in therapeutic range. Panel A shows the results for TTR calculated using trough concentrations in the first 90 days. Panel B depicts the results for TTR calculated during the first year. dnDSA, de novo donor-specific antibody; TTR, time in therapeutic range

suggest CYP3A5 phenotype may still play a role in the time spent in the therapeutic range during this early period.<sup>10,32</sup>

Other studies focusing on CYP3A5 and inpatient variability (IPV) of tacrolimus concentration in adults have found conflicting results.<sup>30–36</sup> The largest evaluation of IPV and CYP3A5 genotype from the DeKAF Genomics cohort found a statistically significant decrease in CV among individuals of European descent with at least one CYP3A5 no function allele, but the effect size was small (1.82% for each no-function CYP3A5 allele).<sup>36</sup> Again, these findings oppose the theory that CYP3A5 nonexpressers experience greater variability due to reliance on CYP3A4 for tacrolimus metabolism. The DeKAF investigators offer another hypothesis that may also explain our findings; providers are less familiar with dose-response patterns in CYP3A5 expressers due to the lower prevalence in

**TABLE 3** Multivariable Cox regression models for the impact of CYP3A5 phenotype and time in therapeutic range in (1) the first 90 days and (2) the 1st year on biopsy-proven acute rejection and de novo DSA development at 1 year

	Hazard ratio (95% CI)	p Value
Model 1: CYP3A5 phenotype/TTR in the 1st 90 days		
Nonexpresser/TTR ≥ 35%	Reference	
Nonexpresser/TTR < 35%	1.36 (0.59–3.12)	0.465
Expresser/TTR ≥ 35%	2.78 (0.55–14.1)	0.216
Expresser/TTR < 35%	2.84 (1.12–7.22)	0.028
Heart transplant	5.26 (2.24–12.35)	<0.001
Rabbit antithymocyte globulin induction	1.94 (0.82–4.57)	0.130
Model 2: CYP3A5 phenotype/TTR in the 1st year		
Nonexpresser/TTR ≥ 35%	Reference	
Nonexpresser/TTR < 35%	2.75 (1.20–6.29)	0.016
Expresser/TTR ≥ 35%	3.69 (1.33–10.19)	0.012
Expresser/TTR < 35%	3.62 (1.08–12.13)	0.037
Heart transplant	5.52 (2.22–13.7)	<0.001
Rabbit antithymocyte globulin induction	2.45 (1.02–5.92)	0.047

Abbreviations: DSA, donor-specific antibody; TTR, time in therapeutic range.

their population and, therefore, may introduce iatrogenic variability through dose adjustments. Interestingly, CYP3A5 expressers in our study underwent significantly more dose adjustments than CYP3A5 nonexpressers (13.7 vs. 9.9,  $p = 0.007$ ), but the study design does not allow elucidation of the causal pathway.

We found that the influence of CYP3A5 phenotype on TTR was significant early. However, the difference by phenotype was no longer significant after 90 days post-transplantation, consistent with the existing literature evaluating the impact of CYP3A5 phenotype on tacrolimus dose and concentration, which suggests the effect wanes by 6 months.<sup>37,38</sup> These findings reinforce the recommendation to incorporate pharmacogenetic information but also highlight a potential role for subsequent dose titrations. Each center or provider participating in the various studies cited here may have different practices, possibly contributing to the conflicting results. There is a need to identify methods to enhance dosing and increase the stability of trough concentrations beyond selecting an initial dose. Our finding that CYP3A5 phenotype is associated with TTR, but not CV, is compelling, suggesting CYP3A5 is unlikely a major contributor to the general fluctuation in levels around the mean but may make it harder to maintain a level in the narrow therapeutic range. Differences in response to dose changes by CYP3A5 phenotype remain a logical hypothesis for this finding.

Including organ type and time to therapeutic trough in the regression model predicting TTR increased the  $R^2$  from 0.08 to 0.17 and the adjusted  $R^2$  from 0.07 to 0.14. The low  $R^2$  of the models provides evidence that only a small portion of TTR variability is

explained by the CYP3A5 phenotype, suggesting additional factors must also be considered. It also should be noted that the significant difference in TTR is based on the mean, and patients of both CYP3A5 phenotypes experienced a wide range of TTR values during the first year following transplantation. Taken together, our results and the existing literature indicate that in clinical practice, factors such as frequent dose modification, nonadherence, changes in GI function, and miss-timed levels may have stronger direct influences on TTR when compared to CYP3A5 phenotype. This theory is consistent with evidence that models that include clinical variables in addition to pharmacogenetics better predict outcomes.<sup>39</sup>

The 9%–10% difference in TTR between CYP3A5 phenotypes identified in the linear regression models has been associated with clinically meaningful outcomes. In adult lung recipients, each 10% increase in TTR was associated with a lower likelihood of acute rejection, chronic lung allograft dysfunction (CLAD), and mortality at 1 year with HRs of 0.67, 0.31, and 0.44, respectively.<sup>28</sup> The impact of CYP3A5 phenotype on clinical outcomes has been more widely studied but is less clear. A recent meta-analysis of kidney transplant recipients found no association of CYP3A5 phenotype with rejection at various time points within the first year, but between 3 to 5 years post-transplant, CYP3A5 expressers were more likely to experience rejection.<sup>40</sup> Consistent with existing literature, in our study, early TTR was not associated with clinical outcomes during the first year providing further support that not all causes of variable exposure early post-transplant are associated with long-term outcomes.<sup>41,42</sup> Interestingly, the survival curves separate within the first 90 days when using TTR calculated over the first year. We hypothesize this suggests variability associated with negative outcomes can be present early, but the signal is weakened by an increased number of sources of variability, which may not be related to graft outcomes. As a result, the survival analysis further supports that maintaining an appropriate TTR may attenuate poor long-term outcomes, particularly among CYP3A5 nonexpressers, and may contribute to the discrepancies found in previous studies.

The main limitations of this study are its single-center nature and limited sample size. The sample size prevented the exploration of additional clinical variables, which may substantially contribute to TTR, such as modifying interacting medications. In particular, we were unable to analyze kidney and heart recipients separately. Further, our analysis did not explore the relationship between CYP3A5 expression and adverse events to evaluate the impact on TTR. While the use of individualized tacrolimus goals included reductions for adverse events, we could not capture reductions in TTR associated with nonadherence resulting from experiences of toxicity. As this is the first study to explore the relationship between TTR and outcomes in pediatric transplant recipients, it provides data to improve the design of future studies, but the results of this work should be interpreted with caution, particularly given the small sample size.

The low mean TTR of 35% in pediatric kidney and heart recipients is concerning, particularly given the association with BPAR and dnDSA. As a result, the ability to increase tacrolimus TTR

through more precise individualized dosing has the potential to improve clinical outcomes. These results demonstrate CYP3A5 phenotype is associated with TTR early post-transplant beyond the known increase in time to the therapeutic concentrations, but the relationship diminishes with time. CYP3A5 phenotype may offer an opportunity to further risk stratify or target interventions for patients with low TTR. Future work should continue to examine the optimal TTR threshold and explore the benefit of ongoing genotype-directed dosing to reduce tacrolimus trough variability.

## ACKNOWLEDGMENTS

National Center for Advancing Translational Sciences, Grant/Award Number: UL1TR002240.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTIONS

Abbie Leino: concept/design, data analysis/interpretation, drafting and final approval of the article. Jeong Park: concept/design, data collection, data analysis/interpretation, critical revision of article, and final approval of the article. Amy Pasternak: concept/design, data collection, data analysis/interpretation, critical revision of article, and final approval of the article.

## ORCID

Abbie D. Leino  <https://orcid.org/0000-0003-4310-4697>

Jeong M. Park  <https://orcid.org/0000-0002-7961-494X>

Amy L. Pasternak  <https://orcid.org/0000-0002-3162-5498>

## REFERENCES

- Hart A, Smith JM, Skeans MA, et al. OPTN/SRTR 2018 annual data report: kidney. *Am J Transplant*. 2020;20(s1):20-130. <https://doi.org/10.1111/ajt.15672>
- Colvin M, Smith JM, Hadley N, et al. OPTN/SRTR 2018 annual data report: heart. *Am J Transplant*. 2020;20(Suppl s1):340-426. <https://doi.org/10.1111/ajt.15676>
- Lind M, Fahlén M, Kosiborod M, Eliasson B, Odén A. Variability of INR and its relationship with mortality, stroke, bleeding and hospitalisations in patients with atrial fibrillation. *Thromb Res*. 2012;129(1):32-35. <https://doi.org/10.1016/j.thromres.2011.07.004>
- Phillips KW, Ansell J. Outpatient management of oral vitamin K antagonist therapy: defining and measuring high-quality management. *Expert Rev Cardiovasc Ther*. 2008;6(1):57-70. <https://doi.org/10.1586/14779072.6.1.57>
- Björck F, Renlund H, Lip GY, Wester P, Svensson PJ, Själander A. Outcomes in a Warfarin-treated population with atrial fibrillation. *JAMA Cardiol*. 2016;1(2):172-180. <https://doi.org/10.1001/jamacardio.2016.0199>
- Kuyppers DRJ. Inpatient variability of tacrolimus exposure in solid organ transplantation: a novel marker for clinical outcome. *Clin Pharmacol Ther*. 2020;107(2):347-358. <https://doi.org/10.1002/cpt.1618>
- Kuyppers DR, de Jonge H, Naesens M, Vanrenterghem Y. Effects of CYP3A5 and MDR1 single nucleotide polymorphisms on drug interactions between tacrolimus and fluconazole in renal allograft recipients. *Pharmacogenet Genomics*. 2008;18(10):861-868. <https://doi.org/10.1097/FPC.0b013e328307c26e>

8. Chandel N, Aggarwal PK, Minz M, Sakhuja V, Kohli KK, Jha V. CYP3A5\*1/\*3 genotype influences the blood concentration of tacrolimus in response to metabolic inhibition by ketoconazole. *Pharmacogenet Genomics*. 2009;19(6):458-463. <https://doi.org/10.1097/FPC.0b013e32832bd085>
9. Passey C, Birnbaum AK, Brundage RC, et al. Validation of tacrolimus equation to predict troughs using genetic and clinical factors. *Pharmacogenomics*. 2012;13(10):1141-1147. <https://doi.org/10.2217/pgs.12.98>
10. Thervet E, Lorient MA, Barbier S, et al. Optimization of initial tacrolimus dose using pharmacogenetic testing. *Clin Pharmacol Ther*. 2010;87(6):721-726. <https://doi.org/10.1038/clpt.2010.17>
11. De Meyer M, Haufroid V, Kanaan N, et al. Pharmacogenetic-based strategy using de novo tacrolimus once daily after kidney transplantation: prospective pilot study. *Pharmacogenomics*. 2016;17(9):1019-1027. <https://doi.org/10.2217/pgs-2016-0005>
12. Yanik MV, Seifert ME, Locke JE, et al. CYP3A5 genotype affects time to therapeutic tacrolimus level in pediatric kidney transplant recipients. *Pediatr Transplant*. 2019;23(5):e13494. <https://doi.org/10.1111/ptr.13494>
13. Pasternak AL, Marshall VD, Gersch CL, Rae JM, Englesbe M, Park JM. Evaluating the impact of CYP3A5 genotype on post-transplant healthcare resource utilization in pediatric renal and heart transplant recipients receiving tacrolimus. *Pharmacogenom Personal Med*. 2021;14:319-326.
14. Hanauer DA. EMERSE: the electronic medical record search engine. *AMIA Annu Symp Proc*. 2006;2006:941.
15. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briët E. A method to determine the optimal intensity of oral anticoagulant therapy. *Thromb Haemost*. 1993;69(3):236-239.
16. Min S, Papaz T, Lafreniere-Roula M, et al. A randomized clinical trial of age and genotype-guided tacrolimus dosing after pediatric solid organ transplantation. *Pediatr Transplant*. 2018;22(7):e13285. <https://doi.org/10.1111/ptr.13285>
17. Haas M, Sis B, Racusen LC, et al. Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am J Transplant*. 2014;14(2):272-283. <https://doi.org/10.1111/ajt.12590>
18. Berry GJ, Angelini A, Burke MM, et al. The ISHLT working formulation for pathologic diagnosis of antibody-mediated rejection in heart transplantation: evolution and current status (2005–2011). *J Heart Lung Transplant*. 2011;30(6):601-611. <https://doi.org/10.1016/j.healun.2011.02.015>
19. Billing H, Höcker B, Fichtner A, et al. Single-nucleotide polymorphism of CYP3A5 impacts the exposure to tacrolimus in pediatric renal transplant recipients: a pharmacogenetic substudy of the TWIST Trial. *Ther Drug Monit*. 2017;39(1):21-28. <https://doi.org/10.1097/ftd.0000000000000361>
20. de Wildt SN, van Schaik RH, Soldin OP, et al. The interactions of age, genetics, and disease severity on tacrolimus dosing requirements after pediatric kidney and liver transplantation. *Eur J Clin Pharmacol*. 2011;67(12):1231-1241. <https://doi.org/10.1007/s00228-011-1083-7>
21. Kausman JY, Patel B, Marks SD. Standard dosing of tacrolimus leads to overexposure in pediatric renal transplantation recipients. *Pediatr Transplant*. 2008;12(3):329-335. <https://doi.org/10.1111/j.1399-3046.2007.00821.x>
22. Calvo PL, Serpe L, Brunati A, et al. Donor CYP3A5 genotype influences tacrolimus disposition on the first day after paediatric liver transplantation. *Br J Clin Pharmacol*. 2017;83(6):1252-1262. <https://doi.org/10.1111/bcp.13219>
23. Montini G, Ujka F, Varagnolo C, et al. The pharmacokinetics and immunosuppressive response of tacrolimus in paediatric renal transplant recipients. *Pediatr Nephrol*. 2006;21(5):719-724. <https://doi.org/10.1007/s00467-006-0014-9>
24. Gijzen V, Mital S, van Schaik RH, et al. Age and CYP3A5 genotype affect tacrolimus dosing requirements after transplant in pediatric heart recipients. *J Heart Lung Transplant*. 2011;30(12):1352-1359. <https://doi.org/10.1016/j.healun.2011.08.001>
25. Shemesh E, Duncan S, Anand R, et al. Trajectory of adherence behavior in pediatric and adolescent liver transplant recipients: the medication adherence in children who had a liver transplant cohort. *Liver Transpl*. 2018;24(1):80-88. <https://doi.org/10.1002/lt.24837>
26. Pollock-Barziv SM, Finkelstein Y, Manlihot C, et al. Variability in tacrolimus blood levels increases the risk of late rejection and graft loss after solid organ transplantation in older children. *Pediatr Transplant*. 2010;14(8):968-975. <https://doi.org/10.1111/j.1399-3046.2010.01409.x>
27. de Oliveira JTP, Kieling CO, da Silva AB, et al. Variability index of tacrolimus serum levels in pediatric liver transplant recipients younger than 12 years: non-adherence or risk of non-adherence? *Pediatr Transplant*. 2017;21(8):e13058. <https://doi.org/10.1111/ptr.13058>
28. Ensor CR, Isella CJ, Harrigan KM, et al. Increasing tacrolimus time-in-therapeutic range is associated with superior one-year outcomes in lung transplant recipients. *Am J Transplant*. 2018;18(6):1527-1533. <https://doi.org/10.1111/ajt.14723>
29. Salah LM, Oreschak K, Ambardekar AV, Page RL, Lindenfeld J, Aquilante CL. Effect of CYP3A genetic variants on different measures of tacrolimus variability in heart transplant recipients. *J Heart Lung Transplant*. 2020;39(4 Suppl.):S212. <https://doi.org/10.1016/j.healun.2020.01.838>
30. Yong Chung J, Jung Lee Y, Bok Jang S, Ahyoung Lim L, Soo Park M, Hwan KK. CYP3A5\*3 genotype associated with intrasubject pharmacokinetic variation toward tacrolimus in bioequivalence study. *Ther Drug Monit*. 2010;32(1):67-72. <https://doi.org/10.1097/FTD.0b013e3181c49a4c>
31. Pashae N, Bouamar R, Hesselink DA, et al. CYP3A5 genotype is not related to the inpatient variability of tacrolimus clearance. *Ther Drug Monit*. 2011;33(3):369-371. <https://doi.org/10.1097/FTD.0b013e31821a7aa3>
32. Shuker N, Bouamar R, van Schaik RH, et al. A Randomized Controlled Trial Comparing the efficacy of Cyp3a5 genotype-based with body-weight-based tacrolimus dosing after living donor kidney transplantation. *Am J Transplant*. 2016;16(7):2085-2096. <https://doi.org/10.1111/ajt.13691>
33. Ro H, Min SI, Yang J, et al. Impact of tacrolimus intraindividual variability and CYP3A5 genetic polymorphism on acute rejection in kidney transplantation. *Ther Drug Monit*. 2012;34(6):680-685. <https://doi.org/10.1097/FTD.0b013e3182731809>
34. Spierings N, Holt DW, MacPhee IA. CYP3A5 genotype had no impact on inpatient variability of tacrolimus clearance in renal transplant recipients. *Ther Drug Monit*. 2013;35(3):328-331. <https://doi.org/10.1097/FTD.0b013e318289644d>
35. Cheng CY, Wu MJ, Lin CC, Hou YC, Liou WS. Intervention of on-line percent coefficient of variation reporting system reduces the variability of tacrolimus trough concentration in kidney transplant recipients. *Transplant Proc*. 2018;50(8):2401-2403. <https://doi.org/10.1016/j.transproceed.2018.03.097>
36. Seibert SR, Schladt DP, Wu B, et al. Tacrolimus trough and dose intra-patient variability and CYP3A5 genotype: Effects on acute rejection and graft failure in European American and African American kidney transplant recipients. *Clin Transplant*. 2018;32(12):e13424. <https://doi.org/10.1111/ctr.13424>
37. Genvigir FDV, Campos-Salazar AB, Felipe CR, et al. CYP3A5\*3 and CYP2C8\*3 variants influence exposure and clinical outcomes of tacrolimus-based therapy. *Pharmacogenomics*. 2020;21(1):7-21. <https://doi.org/10.2217/pgs-2019-0120>
38. Hendijani F, Azarpira N, Kaviani M. Effect of CYP3A5\*1 expression on tacrolimus required dose for transplant pediatrics: a systematic



- review and meta-analysis. *Pediatr Transplant*. 2018;22(6):e13248. <https://doi.org/10.1111/ptr.13248>
39. Miano TA, Flesch JD, Feng R, et al. Early tacrolimus concentrations after lung transplant are predicted by combined clinical and genetic factors and associated with acute kidney injury. *Clin Pharmacol Ther*. 2020;107(2):462-470. <https://doi.org/10.1002/cpt.1629>
  40. Khan AR, Raza A, Firasat S, Abid A. CYP3A5 gene polymorphisms and their impact on dosage and trough concentration of tacrolimus among kidney transplant patients: a systematic review and meta-analysis. *Pharmacogenomics J*. 2020;20(4):553-562. <https://doi.org/10.1038/s41397-019-0144-7>
  41. Baker WL, Steiger S, Martin S, et al. Association between time-in-therapeutic tacrolimus range and early rejection after heart transplant. *Pharmacotherapy*. 2019;39(5):609-613. <https://doi.org/10.1002/phar.2262>
  42. Taber DJ, Hirsch J, Keys A, Su Z, McGillicuddy JW. Etiologies and outcomes associated with tacrolimus levels out of typical range that lead to high variability in kidney transplant recipients. *Ther Drug*

*Monit*. 2021;43(3):401-407. <https://doi.org/10.1097/ftd.0000000000000863>

#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Leino AD, Park JM, Pasternak AL. Impact of CYP3A5 phenotype on tacrolimus time in therapeutic range and clinical outcomes in pediatric renal and heart transplant recipients. *Pharmacotherapy*. 2021;41:649-657. <https://doi.org/10.1002/phar.2601>