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ARTICLE

Tacrolimus pharmacokinetics are influenced by CYP3A5, age, and concomitant fluconazole in pediatric kidney transplant patients

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Abstract

Tacrolimus, the most common immunosuppressant for organ transplant, has a narrow therapeutic range and is metabolized by CYP3A4/5. Trough concentration monitoring and dosing adjustments are used to reach a therapeutic range. CYP3A5 intermediate and normal metabolizers (*1 allele carriers; IM/NM) demonstrate faster tacrolimus metabolism than poor metabolizers (PM). We analyzed the electronic health records of 93 patients aged <21 years for the first 8 weeks after a kidney transplant between January 2010 and December 2021. The target tacrolimus trough was 10–15 ng/mL in the first 4 weeks and 7–10 ng/mL in the next 4 weeks. Banked DNA was collected and genotyped for CYP3A5*3, *6, *7, and *8 alleles. We found that CYP3A5 IM/NM ($n=21$) took longer than PM ($n=72$) to reach the therapeutic range (7 vs. 4 days, $p=0.048$). IM/NM had more dose adjustments (8 vs. 6, $p=0.025$) and needed >150% of the required daily dose compared with PM. The concentration/dose ratio was influenced by age and concomitant fluconazole ($p=0.0003$, $p=0.034$, respectively) and the average daily dose decreases with age in CYP3A5 PM ($p=0.001$). Tremors were more common in patients who ever had a trough concentration >15 ng/mL compared with those who never had a trough concentration >15 ng/mL (OR 3.31, 95% CI 1.03–8.98, $p=0.038$). Using standard dosing, CYP3A5 IM/NM took longer to reach the goal range and require more dose

adjustments and higher doses than PM. Preemptive genotyping could decrease the number of dose changes necessary to reach a therapeutic dose. We have implemented pre-transplant CYP3A5 testing at our institution.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The variability in tacrolimus PK is often studied months after transplant, usually in adult patients, where CYP3A5 variation contributes to ~50% of the variability. However, immediately after transplant is when the CYP3A5 genotype can be most helpful in making dose adjustments to reach the target trough concentration. A prior study in adult kidney transplant recipients showed that fluconazole influences tacrolimus pharmacokinetics only in CYP3A5 PMs.

WHAT QUESTION DID THIS STUDY ADDRESS?

How do CYP3A5, age, and concomitant fluconazole influence tacrolimus trough concentrations and dose requirements in the first eight weeks after kidney transplant in pediatric patients? What is the risk of toxicity when above the target trough range?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Early after kidney transplant in pediatric patients, CYP3A5, age, and fluconazole significantly affect the dose requirements for tacrolimus, with CYP3A5 and age explaining 20% and 12.6% of the variability in average dose-adjusted concentration. Fluconazole only influences tacrolimus pharmacokinetics in pediatric CYP3A5 PMs, not IM/NMs. Patients with trough concentrations above 15 ng/mL are at increased risk for toxicity.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

When adjusting doses of tacrolimus immediately after kidney transplant in pediatric patients, CYP3A5 genotype, age, and concomitant fluconazole could be combined in a precision dosing tool to help reach the target range faster and reduce tacrolimus toxicity.

INTRODUCTION

Tacrolimus, formerly known as FK506, is the most common immunosuppressant used for prophylaxis of organ rejection after solid organ transplantation. Tacrolimus binds to immunophilin, FK506 binding protein (FKBP), and forms a complex that inhibits calcineurin phosphatase.[1] As a result, T cell proliferation is inhibited. Tacrolimus has a narrow therapeutic index above which the drug exhibits adverse reactions more frequently and under which graft rejection is more likely; therefore, monitoring of tacrolimus concentration is a crucial part of post-transplant medical care. When taken orally, tacrolimus is metabolized by the CYP3A4 and CYP3A5 hepatic and intestinal enzymes. There are genetic variants in *CYP3A5* that affect tacrolimus pharmacokinetics, explaining up to 50% of the interpatient variability.[2] The presence of at least one *CYP3A5*1* allele (also known as CYP3A5 intermediate and normal metabolizers [IM/NM]) results in the expression of an active CYP3A5 enzyme.[2] Many studies have demonstrated that CYP3A5 IM/NM need a 50%–100% higher dose compared with poor metabolizers (PM).[2] Therefore, genotype-guided dosing is recommended by the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline to avoid graft rejection.[2,3] In cohort studies, genotype-guided dosing does not affect outcomes like graft survival; however, it influences therapeutic drug monitoring (TDM) outcomes and pharmacokinetic (PK) properties, such as time to target trough and concentration/dose ratio (C_0/D).[4,5] Recent trials showed that genotype-guided dosing of tacrolimus results in faster achievement of target concentrations (3.4 vs. 4.7 days in pediatrics and 2 weeks earlier in adults) compared with standard dosing.[5,6] Other retrospective cohorts showed that age and concomitant medications could also affect the TDM outcomes.[3,7,8] While there have been many studies associating *CYP3A5*

with tacrolimus C_0/D in adult patients, patients at stable doses months after transplant, and small studies on the influence of age or concomitant medications in combination with *CYP3A5* genotype,[9–17] studies of how age, fluconazole, and *CYP3A5* together contribute to variability in tacrolimus pharmacokinetics and toxicity using real-world data immediately after a kidney transplant in pediatric patients is sparse. There is one study assessing the influence of fluconazole and CYP3A5 on tacrolimus C_0/D in adult kidney transplant patients[7]; however, studies in pediatric kidney transplant recipients are lacking. Therefore, we studied pediatric patients within the first 8 weeks after kidney transplant to examine the combined effects of *CYP3A5* genotype, age, and fluconazole on tacrolimus trough concentrations, TDM outcomes, and toxicity.

METHODS

Study design

This retrospective, single-center, cohort study was approved by the Cincinnati Children's Hospital Medical Center (CCHMC) Investigational Review Board and a waiver of informed consent was given. All patient data access complied with relevant data protection and privacy regulations, in compliance with the Declaration of Helsinki. DNA samples were provided by the CCHMC Discover Together Biobank, a College of American Pathology accredited biorepository in which patients voluntarily consented to their DNA being used for CCHMC research studies.

Standard treatment

During the time of the data collection, all patients were started on enteral tacrolimus 0.1 mg/kg/dose (max of 5 mg) twice daily immediately post-transplant, based on the pre-transplant weight. Trough concentrations were measured daily while inpatient and twice a week while outpatient. Dose adjustments to tacrolimus were made at the discretion of the transplant team. The target trough for the first 4 weeks after transplant was 10–15 ng/mL and 7–10 ng/mL for weeks 5–8. Nystatin is routinely used as fungal prophylaxis for the first 3 months; however, fluconazole may be substituted in those unable to tolerate nystatin or in those who have persistently subtherapeutic tacrolimus troughs despite multiple dose adjustments. Two patients included in the study had *CYP3A5* testing ordered clinically prior to the transplant (one PM, one IM). The IM patient received 0.1 mg/kg/dose for the first 4 days after transplant when the genotype result was not yet known.

Data collection

We analyzed the electronic health records (EHR) of 150 patients at CCHMC for the first 8 weeks following kidney transplantation. Data collected included demographics, the number of tacrolimus dose adjustments/changes, tacrolimus doses and concentrations, time to therapeutic dose, transplant dates, other organ transplants in addition to kidney, and total daily dose and weight for each patient at the time of the tacrolimus concentration measurement. We investigated whether patients were prescribed moderate or strong CYP3A4 inducers or inhibitors after transplant. The only moderate CYP3A4 inhibitor that was used in the first 8 weeks after transplant in a subset of our study patients was fluconazole. Therefore, we documented fluconazole start and end dates, Patients were grouped according to their race as identified in the medical record. Those who identified as more than one race were grouped as Other, as well as those not identified as Black/African American or White.

Inclusion/exclusion criteria

Patients were included if they received a kidney transplant between January 1, 2010 and April 1, 2021, consumed immediate release enteral tacrolimus after transplant, were 21 years old or younger at the time of transplant, and had a stored DNA sample in the institutional biobank for analysis. Exclusion criteria were as follows: (a) previous transplants (since there was previous tacrolimus exposure or DNA sample was donor-derived after a bone marrow transplant); (b) concomitant transplant of other organs; (c) comorbidities that affect tacrolimus clearance (e.g., liver cirrhosis, cystic fibrosis); and (d) conditions treated previously with tacrolimus (e.g., focal segmental glomerulosclerosis).

Genotyping

Banked DNA was collected, and genotyping was performed in a clinical laboratory certified by the College of American Pathologists and Clinical Laboratory Improvement Amendments (CAP/CLIA) for the *CYP3A5* gene using the MASSArray platform (Agena Biosciences). For *CYP3A5*, *1 (the normal function allele), *3, *6, *7, and *8 alleles were detected. Patients were then classified into two phenotypic categories of CYP3A5 IM/NM or CYP3A5 PM as defined in the CPIC guidelines.[2] Poor metabolizers (PM) are those with no *1 alleles detected, intermediate metabolizers (IM) have one functioning allele, and normal metabolizers (NM) have two functioning alleles.

Tacrolimus toxicity

Adverse reactions related to tacrolimus were assessed by manual chart review including central nervous system (CNS) toxicity (e.g., tremor, headache, insomnia, shaking), gastrointestinal (e.g., diarrhea, constipation, abdominal pain, nausea, vomiting), and others (e.g., rashes, edema, hypertension). Troughs closest to the first documentation of the adverse event were collected. If the patient had a trough concentration measured on the day the event was documented and the day before, the higher trough was documented. Events with no trough collected on the day or the day before the event were excluded. Hypertension, muscle cramps, and edema within the first 3 days after transplant were excluded due to the likelihood of being related to factors other than tacrolimus.

Study outcomes

The primary goal of this study was to compare the tacrolimus-related outcomes between CYP3A5 IM/NM and PM. Outcomes analyzed included the number of dose adjustments, number of measurements within the therapeutic range, and time to the target trough (10–15 ng/mL for the first 4 weeks or 7–10 ng/mL for weeks 5–8). The number of dose adjustments is the number of changes in the tacrolimus dose either by increasing or decreasing. The percentage of measurements within the range is the number of troughs within the range to the total number of troughs for each patient. The time to the target trough is defined as the number of days from starting tacrolimus to when the average trough concentration for the group was within the target range. Secondary goals were to measure the influence of concomitant fluconazole and age on tacrolimus trough concentration/dose ratio (C_0/D), and to study the association between tacrolimus trough and adverse reactions to tacrolimus. Study data were collected and managed using REDCap (Research Electronic Data Capture) tools hosted at Cincinnati Children's Hospital Medical Center.[18,19]

Data analysis

The number of dose adjustments, daily tacrolimus dose, average trough concentrations, and percentage of trough concentrations within the therapeutic range were compared between CYP3A5 IM/NM and PM using an unpaired *t*-test if the data were normally distributed or a Mann–Whitney test if the data were not normally distributed. The time to the first trough in the therapeutic range was calculated with the Gehan–Breslow–Wilcoxon test. We specifically chose this test because it weights early events more than later events. As the dose of tacrolimus is adjusted so that all patients eventually achieve a trough within the target range, we wanted to place more emphasis on those patients that reached it sooner. Average trough concentrations and daily doses among CYP3A5 PM were compared between age groups using the ANOVA test for trend. The variability in the average C_0/D was calculated by the change in R^2 using stepwise addition of variables (CYP3A5 PM vs. IM/NM, age as a continuous variable) to a general linear model. The C_0/D ratios in patients that were taking fluconazole were compared with those in the same patients when they were not taking fluconazole using a paired *t*-test. Trough concentrations prior to or on the day an adverse reaction was recorded in the EHR were compared with the average trough concentration in the same patient through the first 4 weeks if the event was recorded in the first 4 weeks or the average for weeks 5–8 if the event occurred during weeks 5–8. If the data were normally distributed, a paired *t*-test was used; if not normally distributed, the Wilcoxon matched-pairs signed rank test was performed. The statistical analysis was performed using Prism version 9 (GraphPad Software). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Patient demographics

Of the 150 patient charts reviewed, 57 were excluded, leaving 93 patients in this study (Figure S1). There were 21 patients that were CYP3A5 IM/NM and 72 PM (Table 1). Some 15% of CYP3A5 IM/NM identified as White, and

TABLE 1 Patient demographics by CYP3A5 intermediate and normal metabolizers (IM/NM) and CYP3A5 poor metabolizers (PM).

Demographic	IM/NM patients ($n = 21$) *n* (%)	PM patients ($n = 72$) *n* (%)	Total patients ($n = 93$) *n* (%)
Sex			
Female	3 (14.3)	31 (43.1)	34 (36.6)
Male	18 (85.7)	41 (56.9)	59 (63.4)
Race			
White	10 (47.6)	59 (81.9)	69 (74.2)
Black	6 (28.6)	4 (5.6)	10 (10.8)
Other	5 (23.8)	9 (12.5)	14 (15.0)
Age (years)			
Mean (SD)	6.8 (6.7)	9.2 (6.2)	8.6 (6.4)

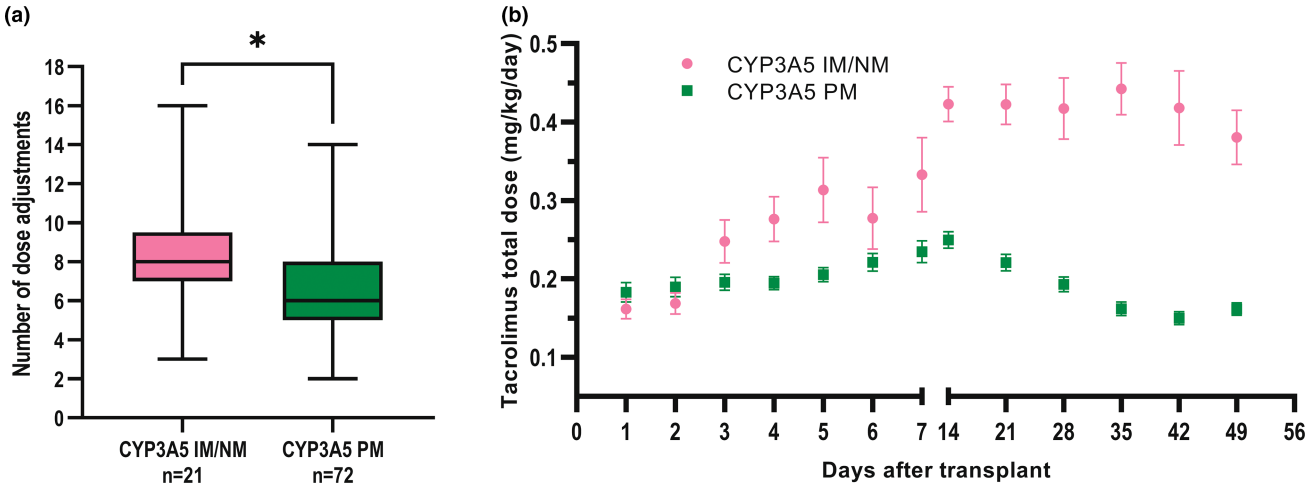

FIGURE 1 CYP3A5 intermediate and normal metabolizers require more dose adjustments and higher daily doses. (a) The number of dose adjustments within the first 8 weeks after kidney transplant (*$p = 0.025$; Mann–Whitney test). IM/NM, intermediate and normal metabolizers ($n = 21$); PM, poor metabolizers ($n = 72$). The upper line of the box represents the 75% percentile of the values, the middle line is the median, the lower line of the box represents the 25% percentile of the values, and the whiskers represent the minimum and the maximum values. (b) The tacrolimus total daily dose (mg/kg) within the first 8 weeks after kidney transplant. Doses where fluconazole was prescribed concomitantly have been excluded. Squares and circles represent the means, the lines are the standard error of the mean.

60% of the patients identified as Black, which is comparable with the percentage of IM/NM in these populations (Table 1).[2] The median age of the cohort was 7 years, with an interquartile range of 2–15 years. There was no difference in the number of trough concentration measurements between CYP3A5 PM and IM/NM (median 20 for each group) (Figure S2).

Dose adjustments and dose requirements

The median number of dose adjustments was significantly higher in CYP3A5 IM/NM ($n = 21$) compared with PM ($n = 72$) within the first 8 weeks (8 vs. 6, $p = 0.025$; Figure 1a). Figure S3 shows the dose adjustments across all three CYP3A5 metabolizer groups. Though all patients started with equal weight-based dosing, the average total daily dose requirement was 70% higher in the IM/NM compared with the PM after 2 weeks of treatment (0.42 vs. 0.25 mg/kg/day, $p < 0.0001$; Figure 1b) and over 100% higher at 1 month (0.42 vs. 0.19 mg/kg/day, $p < 0.0001$).

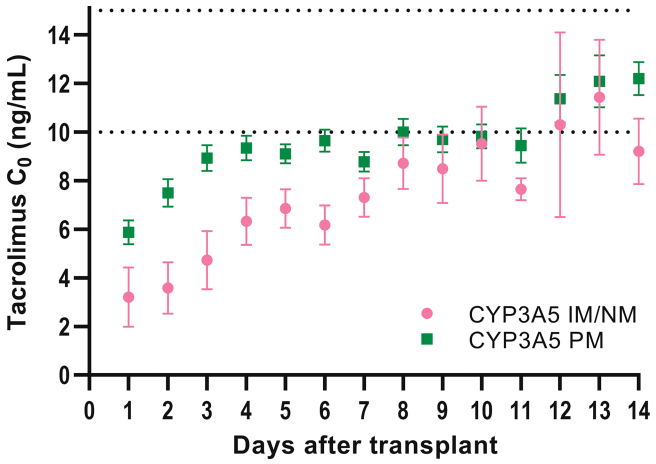

FIGURE 2 Average trough concentrations (C_0) within the first 2 weeks after kidney transplant are lower in CYP3A5 intermediate and normal metabolizers (IM/NM) ($n = 21$) than poor metabolizers (PM) ($n = 72$). Troughs where fluconazole was prescribed concomitantly have been excluded. Squares and circles represent the means, the lines are the standard error of the mean. The dotted lines represent the trough target range within the first 4 weeks (10–15 ng/mL).

Time to target trough and measurements in range

The average trough concentration in the PM was closer to the target (10–15 ng/mL) than the average trough concentration for IM/NM on postoperative day 4 (9.3 vs. 6.3 ng/mL, respectively, $p = 0.01$; Figure 2). The average trough concentration in the PM was within target on postoperative day 8, while the average trough concentration in the IM/NM was within target starting from day 12 (Figure 2). The IM/NM had fewer trough concentrations within the target range compared with the PM during the first 8 weeks (32.5% vs. 39.6%, $p = 0.029$; Figure 3a). In addition, the time to the first trough within the target range was shorter in CYP3A5 PM compared with IM/NM (median 4 days vs. 7 days, respectively, $p = 0.048$; Figure 3b).

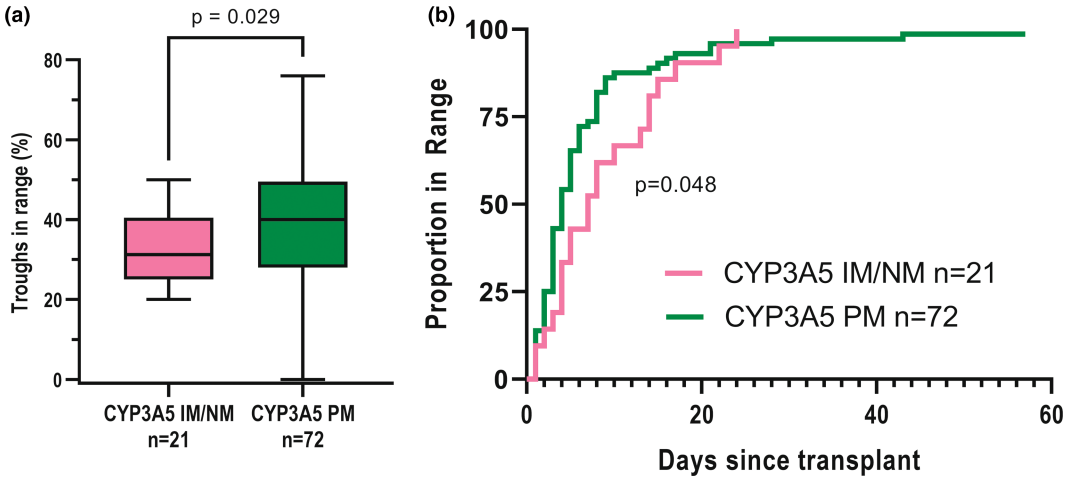

FIGURE 3 CYP3A5 intermediate and normal metabolizers have fewer troughs in the therapeutic range during the first 8 weeks of treatment and take longer to reach the therapeutic range. (a) The percentage of trough concentrations within the range for each patient within the first 8 weeks after kidney transplant ($p = 0.029$; Mann–Whitney test). IM/NM, intermediate and normal metabolizers ($n = 21$); PM, poor metabolizers ($n = 72$). Each patient's percentage in range was calculated, then the upper line of the box represents the 75% percentile of these values, the middle line is the median, the lower line of the box represents the 25% percentile of these values, and the whiskers represent the minimum and the maximum values. The range was defined as 10–15 ng/mL in the first 4 weeks and 7–10 ng/mL thereafter. (b) The time to the first trough within the therapeutic range ($p = 0.048$; Gehan–Breslow–Wilcoxon test).

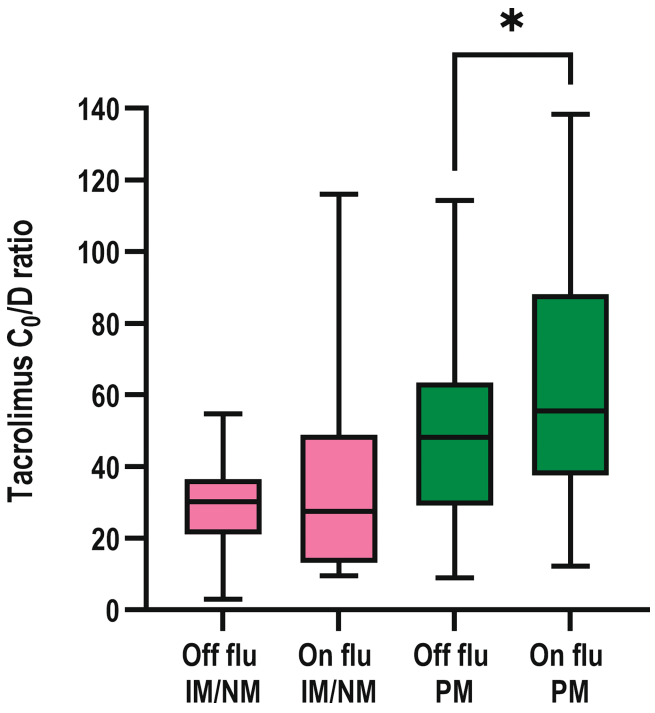

FIGURE 4 Effect of fluconazole and CYP3A5 expression on the concentration/dose (C_0/D) ratio within the first 8 weeks after kidney transplant (*$p = 0.034$; paired t-test). The upper line of the box plots represents the 75% percentile of the values, the middle line is the median, the lower line of the box represents the 25% percentile of the values, and the whiskers represent the minimum and the maximum values. The Y axis is for trough concentration C_0 (ng/mL)/total daily dose (mg/kg/day) ratio. IM/NM, intermediate and normal metabolizers; Off flu, patients before taking fluconazole; On flu, the same patients after taking fluconazole; PM, poor metabolizers.

Concomitant fluconazole

Fluconazole was mainly prescribed for prophylaxis of fungal infections as a substitution for nystatin. There were 15 CYP3A5 IM/NM and 25 PM that had troughs both on and off fluconazole. We found that the C_0/D ratio was influenced by concomitant fluconazole in the PM only (median 48.2 when off fluconazole to 55.5 when on fluconazole, $p = 0.034$; Figure 4). The median change for individual non-expresser patients was a 34% increase when on fluconazole.

Age effect

The average C_0/D ratio increased with age in CYP3A5 PM ($p = 0.0003$, Figure 5a), while the average daily dose decreased with age in CYP3A5 PM ($p = 0.001$; Figure 5b). Overall, 20% of the variability in the average C_0/D ratio was explained by the CYP3A5 status, while 12.6% was explained by age.

Tacrolimus toxicity

We assessed tacrolimus concentrations prior to four tacrolimus-related adverse reactions: tremor, edema, diarrhea, and rash (Figure 6a–d). Tacrolimus concentrations were higher just before reporting diarrhea or rash compared with the patient's average trough concentration. Patients who ever had troughs over 15 ng/mL were more

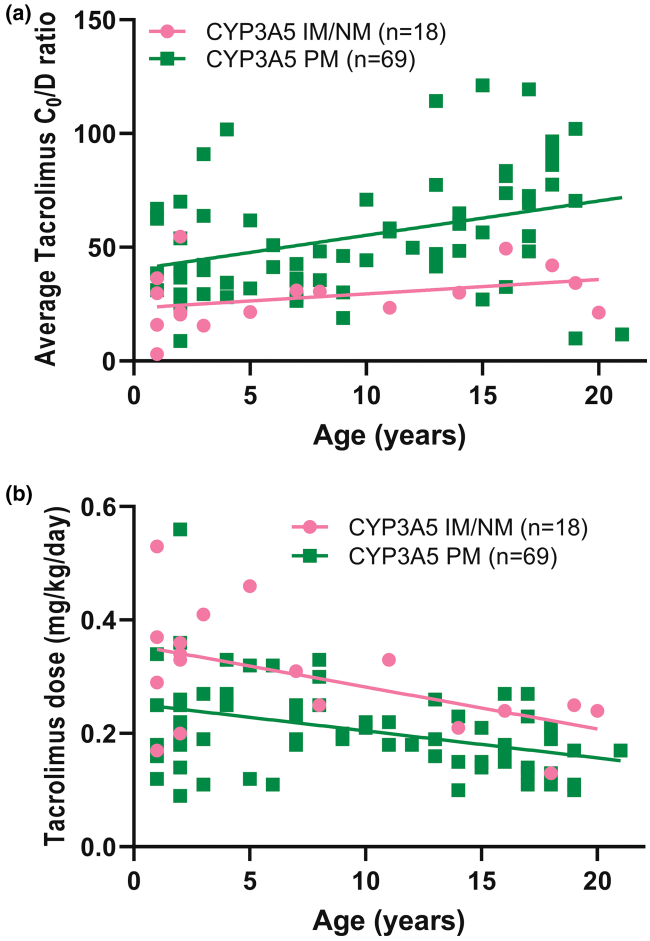

FIGURE 5 Effect of age on the concentration/dose ratio (C_0/D; panel a) and daily tacrolimus dose (panel b). In CYP3A5 poor metabolizers (PM), the C_0/D increases with age ($p=0.002$, linear regression, $R^2=0.13$, panel a) while the effect of age is not significant in intermediate and normal metabolizers (IM/NM) ($p=0.15$, linear regression). The tacrolimus daily dose decreases with age in both groups (PM, $p=0.001$, $R^2=0.15$; IM/NM, $p=0.03$, $R^2=0.26$, panel b). Troughs and doses where fluconazole was prescribed concomitantly have been excluded, which means 3 IM/NM patients and 3 PM patients have no data included.

likely to experience tremors and CNS toxicity compared with patients with troughs never over 15 ng/mL (tremor odds ratio [OR] 3.31, 95% CI 1.03–8.98, $p=0.038$; CNS toxicity OR 2.62, 95% CI 1.13–5.90, $p=0.034$; Figure 6e,f). Though likely underpowered, we did not find a difference in the incidence of tremor or CNS toxicities between CYP3A5 IM/NM and PM (Figure 6g,h), although the average trough concentrations were lower in CYP3A5 IM/NM than PM (median 8.1 vs. 10.11 ng/mL, $p=0.0003$).

DISCUSSION

This study identifies the clinical variables that affect the tacrolimus concentration within the first 8 weeks after kidney transplant in pediatric patients. *CYP3A5* genotype affects the number of dose adjustments, dose required to reach the target trough concentration, and time to reach the target concentration. Concomitant fluconazole and increasing age increase the concentration/dose ratio (C_0/D) significantly in CYP3A5 PM. We found that *CYP3A5* genotype explained more of the variability in the average C_0/D than age, but there is substantial variability unexplained, indicating that monitoring of tacrolimus concentrations is still required. Patients with trough concentrations above 15 ng/mL were more likely to experience tremors and CNS toxicity. This study is unique in that it focuses on the first 8 weeks of routine clinical care after transplant when there are many dose adjustments to reach the therapeutic range.

Effect of CYP3A5

Consistent with previous randomized controlled trials where genotype-guided dosing resulted in achieving the target concentration faster than standard dosing,[5,6] we found that CYP3A5 IM/NM achieved the target trough slower with fewer in-range concentrations compared with the PM. In addition, our findings replicate previous retrospective studies where the CYP3A5 IM/NM required significantly higher doses compared with PM.[10,11,15,20] Moreover, the number of dose adjustments was significantly higher in the CYP3A5 IM/NM compared with PM, which has also been described in previous cohorts.[3,5,21] The novelty in our study lies in the combined analysis of the contribution of *CYP3A5*, fluconazole, and age on tacrolimus pharmacokinetics in the first 8 weeks after a kidney transplant in a pediatric cohort.

Standard body weight-based dosing was used in our study patients, but preemptive genotype-guided dosing was implemented in March 2021 at our institution. Two of the patients included in the study had transplants after this date and had clinical *CYP3A5* genotyping prior to transplant. One was a PM and one was a IM. The usual dose of 0.1 mg/kg/dose was used until the genotyping result for the IM patient was returned on day 3 after transplant. We do not believe this patient has skewed the results of the study as the patient had eight dose changes and reached the target range on day 7 post-transplant, which is similar to the rest of the IM/NM patients. We did not analyze whether genotype-guided dosing helped the CYP3A5 IM/NM reach the target trough concentration sooner than when weight-based dosing was used. In addition, the number of dose adjustments was significantly higher in the IM/NM than the PM, which is consistent with findings from another retrospective cohort that showed significant dose adjustments in pediatric patients receiving a heart transplant.[3]

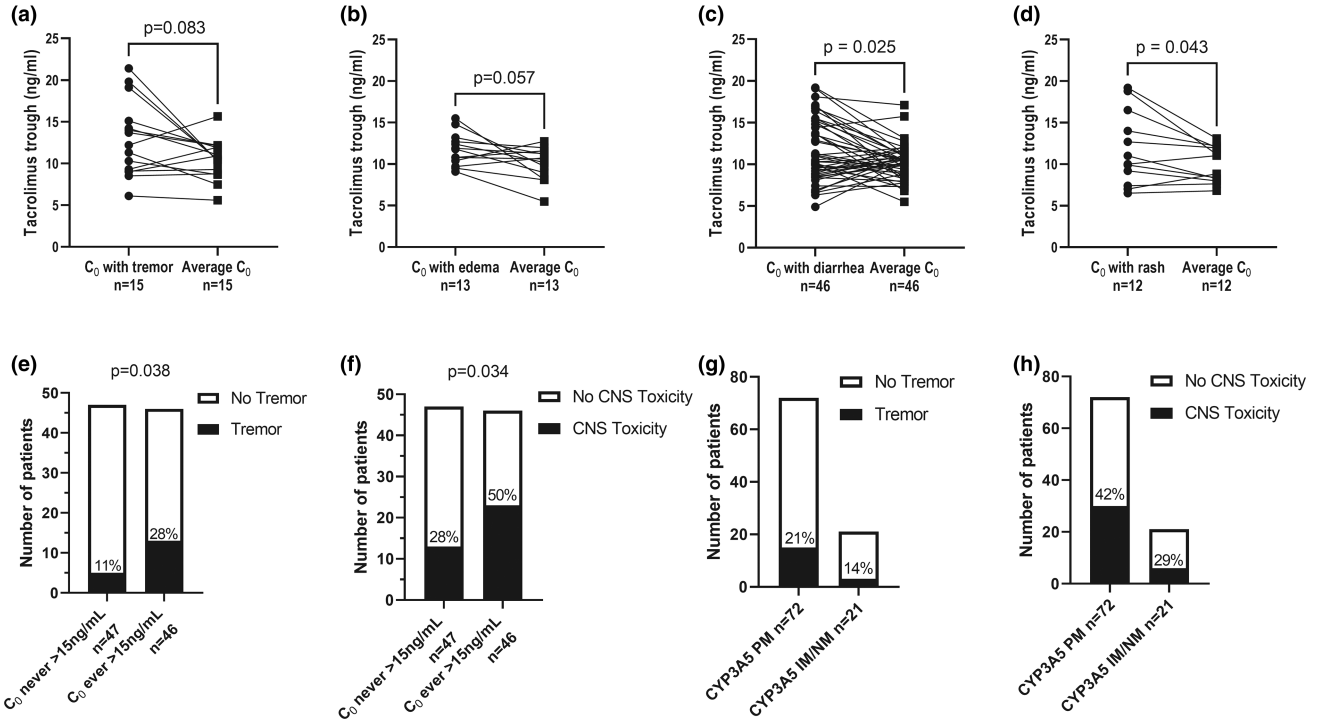

FIGURE 6 Relationship between tacrolimus trough concentrations and toxicity. Tacrolimus trough concentrations were higher than a patient's average trough concentration just before noting tremor (panel a), edema (panel b), diarrhea (panel c), and rash (panel d). *P* values were calculated with paired *t*-tests for those with normal distributions (rash) or a Wilcoxon test (tremor, edema, diarrhea) when not normally distributed. Patients that ever had a trough (C_0) concentration >15 ng/mL were at increased risk for tremor (panel e, $p = 0.038$, odds ratio [OR] 3.31) and central nervous system (CNS) toxicity (panel f, $p = 0.034$, OR 2.6). Frequency of tremor or CNS toxicity did not differ by CYP3A5 status (panels g and h, respectively). IM/NM, intermediate and normal metabolizers; PM, poor metabolizers.

An increased number of dose adjustments in the CYP3A5 IM/NM could indicate longer hospitalizations, higher costs, and increased potential for medication errors.[3] At CCHMC we have implemented pre-transplant *CYP3A5* genotyping with recommendations to initiate tacrolimus at 1.5 times the standard dose or 0.15 mg/kg/dose twice a day with a maximum of 7 mg per dose. Implementing genotype-guided dosing may result in faster achievement of therapeutic concentration with a smaller number of dose adjustments and blood samples required for TDM, though we do not have enough patients to evaluate this yet. Future studies will examine the impact of implementing CYP3A5-guided dosing in routine clinical care in our institution and compare this to the cohort described here.

Effect of fluconazole

Fluconazole is a moderate CYP3A4 inhibitor that is often used for fungal prophylaxis in patients after a kidney transplant. Additionally, in our clinical practice, fluconazole was sometimes used with the goal of increasing the tacrolimus concentration in cases where dose escalation was unsuccessful in reaching the target trough. Our findings are consistent with an adult study showing that fluconazole increases the tacrolimus C_0/D ratio only in the CYP3A5 PM indicating an effect on CYP3A4 that is not present when CYP3A5 is active in the IM/NM.[7] That increase in the C_0/D ratio indicates close monitoring of the tacrolimus concentration is necessary following the addition of fluconazole. In CYP3A5 PM patients, who are already within the therapeutic goal range, they may ultimately need their tacrolimus decreased by approximately 25% in the days to weeks following fluconazole initiation. This difference in trough concentrations on versus off fluconazole is consistent with studies in adult transplant patients demonstrating a 25%–40% dose reduction for tacrolimus was necessary when starting fluconazole, though these studies did not account for CYP3A5 status.[22–24]

Effect of age

In other studies, the tacrolimus dose requirement was higher in younger children and the C_0/D ratio was positively correlated with age.[8,11,25] Our findings are consistent with previous studies, which could be explained by a decrease in activity/gene expression of the CYP3A enzymes with increasing age. In vitro data show increasing activity of CYP3A4 with age, while in vivo data show CYP3A

enzyme activity decreases with age, which is consistent with our observation of increased C_0/D ratio and decreased daily dose of tacrolimus in CYP3A5 PM.[26] Knowledge about how in vivo CYP3A enzyme activity changes during development is sparse due to ethical concerns about liver biopsies on healthy children.[26–29] In patients less than 6 years of age, there are often challenges related to the administration of tacrolimus given patients inability to swallow tacrolimus capsules or their dependence on medication administration via a feeding tube. This variability in administration likely contributes to variability in tacrolimus absorption and therefore tacrolimus concentrations. Since food plays a role in tacrolimus absorption, the timing of the dosing related to tube feeds could also play a role. Another explanation could be the need for allometric scaling of the dosing which we did not perform in this study. As a result, the allometric scaling of dosing and changes in the metabolizing enzyme with age should be investigated more thoroughly in pediatric, adult, and geriatric populations that are prescribed tacrolimus.

Effect of other gene variants

Many studies have identified genetic variants other than those in *CYP3A5* that could affect the tacrolimus pharmacokinetics.[30–36] *CYP3A4* is increasingly being recognized as having an influence on tacrolimus pharmacokinetics. Specifically, the *CYP3A4*22* allele affected the tacrolimus dosing and dose requirements in one study.[37] Another polymorphism is *POR*28*, which was significantly associated with lower tacrolimus dose requirements and higher C_0/D ratio in the first month post-transplant.[30,31,33] Moreover, the gene *NR1I2* has been shown to impact tacrolimus pharmacokinetics.[32] Variants in transporter genes *ABCB1* and *ABCC2* were associated with changes in tacrolimus pharmacokinetics in other studies.[34,35,38] However, a large cohort of 1923 kidney allograft recipients were studied to identify the association between these genes and tacrolimus concentrations, but nothing was identified to have a significant influence other than *CYP3A5*.[36] Therefore, we did not investigate any of these variants in our study.

Tacrolimus toxicity

Adverse reactions to tacrolimus present as CNS toxicity (e.g., tremors, shaking, insomnia, headache, and dizziness), gastrointestinal toxicity (e.g., diarrhea, constipation, abdominal pain, nausea, and vomiting), and other toxicities such as muscle cramps, edema, rashes, and hypertension. Though there have been studies associating tacrolimus trough concentrations with toxicity, a clear threshold concentration for specific toxicities has not been defined in pediatric kidney transplant patients.[39] The target trough range for tacrolimus in pediatric kidney transplant patients varies by center, usually based on empirical decisions and clinicians' observations.[37] At CCHMC, the target tacrolimus trough is 10–15 ng/mL in the first 4 weeks after transplant and 7–10 ng/mL through 6 months' post-transplant. A global consensus report recommends that the target in pediatric kidney transplant patients be 10–20 ng/mL during the first 2 months after transplantation and between 5 and 10 ng/mL thereafter.[37] We investigated toxicity using a trough threshold of 15 ng/mL as this is the upper limit of the target range at our center. We found that patients with any level exceeding this threshold had a 3.3 times higher incidence of tremors.

Study limitations

The findings of this study are limited by the retrospective design and sample size, particularly of the CYP3A5 IM/NM. There are many clinical factors that could influence the pharmacokinetics of tacrolimus that we were unable to capture in this retrospective study, specifically related to absorption. These include consistency in the timing and technique for administering tacrolimus (especially for patients unable to swallow capsules) and adherence to medications in the outpatient setting. As this study used real-world clinical data, we were unable to confirm timing of previous doses prior to tacrolimus level analyzed. There is a possibility that not all of the tacrolimus measurements were true trough concentrations.

CONCLUSIONS

Tacrolimus blood concentration is affected by many variables such as CYP3A5, concomitant fluconazole, and age. CYP3A5 IM/NM had fewer tacrolimus concentration measurements in the goal range and took longer to reach the goal range. They require more dose adjustments and higher doses than PM. The tacrolimus concentration/dose ratio increases approximately 25% when fluconazole is prescribed concurrently in CYP3A5 PM, indicating these patients may need a dose decrease. Additionally, the tacrolimus metabolizing capability seemed to decrease with age. Therefore, the consideration of age and fluconazole in dosing tacrolimus must be further studied and appropriate dose adjustments implemented. Moreover, CNS toxicity is associated with tacrolimus trough concentration; patients with troughs above 15 ng/mL are more likely to have CNS toxicity symptoms.

AUTHOR CONTRIBUTIONS

A.A., S.S., D.K.H., C.V., L.D., T.M., D.L., and L.B.R. wrote the manuscript. A.A., D.L., and L.B.R. designed the research. A.A and S.S. performed the research. A.A. and L.B.R. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

D.K.H. serves as a consultant for Hive Networks, Magnolia Innovation, Bioporto, Kaneka, and Alnylam. D.L. is now an employee of Eurofins Transplant Genomics. L.B.R. has received grants and serves as a consultant for BTG Specialty Pharmaceuticals. All the other authors declared no competing interests for this work.

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