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Age and *CYP3A5* genotype affect tacrolimus dosing requirements after transplant in pediatric heart recipients

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Abstract

BACKGROUND—Tacrolimus is one of the commonly used immunosuppressive drugs for pediatric heart transplants. Large variation exists in pharmacokinetics during the direct post-transplant period, resulting in an increased risk of adverse events. Limited data are available on the interaction of age, *CYP3A5* and *ABCB1* genotype, and disease severity on the variation in disposition and outcome in pediatric heart transplant recipients.

METHOD—We studied the relationship between age and *CYP3A5* and *ABCB1* genotype and the Pediatric Risk of Mortality (PRISM) score on tacrolimus dose (mg/kg), steady-state trough concentrations, and concentration/dose ratio, as well as rejection and renal function for 14 days after heart transplant in children.

RESULTS—Tacrolimus was administered to 39 children (median age, 6.0 years) after transplant. A correlation was found between the age at the time of transplant and the tacrolimus dosing requirements ($r_s = -0.447$, $p = 0.004$) and the concentration/dose ratio ($r_s = 0.351$, $p = 0.029$). *CYP3A5* expressors required median (interquartile range) higher doses of tacrolimus (0.14 [0.09] vs 0.06 [0.04] mg/kg/12 hours, $p = 0.001$), and had lower concentration/dose ratios (45.34 [44.54] vs 177.78 [145.38] ng/ml per mg/kg/12 hours, $p < 0.0001$). This relationship was not seen with the *ABCB1* genotype. Age and *CYP3A5* genotype predicted the tacrolimus dosing requirements as well as the concentration/dose ratio ($R^2 = 0.351$, $p = 0.001$ and $R^2 = 0.521$, $p < 0.001$). No relationship was found between any of the *CYP3A5* or *ABCB1* genotypes and the estimated glomerular filtration rate.

CONCLUSION—Younger age and *CYP3A5* expressor genotype were independently associated with higher dosing requirements and lower tacrolimus concentration/dose ratios.

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Disclosure statement

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript or other conflicts of interest to disclose.

Keywords

pharmacogenetics; tacrolimus; heart transplantation; children

After its introduction into clinical use in 1997, tacrolimus became one of the most commonly used drugs for immunosuppressive treatment of solid-organ transplant recipients. In heart transplant recipients, it is often preferred to cyclosporin¹ for its lower incidence of hypertension, dyslipidemia, and fewer cosmetic adverse effects, such as hirsutism and gingival hypertrophy.¹⁻³ A narrow therapeutic window complicates tacrolimus dosing, however.

The first weeks after transplantation are generally marked by the highest risk for organ rejection. During this period, considerable variability in drug concentrations and pharmacokinetics can contribute to rejection risk with underdosing and drug toxicity (eg, nephrotoxicity, neurotoxicity) with overdosing. Sub-therapeutic tacrolimus concentrations confer a risk for biopsy-proven rejection in adult and pediatric heart transplant recipients.⁴⁻⁶ Furthermore, in 112 adult cardiac transplant recipients, early renal insufficiency, defined as 10% rise in serum creatinine and a serum creatinine above 1.5 mg/dl on Day 3 after transplantation, was associated with tacrolimus levels.⁷

The pharmacokinetics of tacrolimus have been extensively studied in adults.^{8,9} However, limited data exist on the sources of large interindividual and intraindividual variability of tacrolimus pharmacokinetics in children. Faster tacrolimus clearance rate in children aged younger than 6 years and higher tacrolimus doses per kilogram of body weight to achieve the target concentrations in this age group have been reported in liver, renal, and hematopoietic stem cell transplant recipients.¹⁰⁻¹³ The causes for these differences are presently unknown but may be due to age-related changes in *CYP3A* activity and to the large size of the liver allograft relative to body size in children aged younger than 6 years.¹⁴

The relationship between the *CYP3A5* genotype and higher tacrolimus clearance has been well established in adult cardiac transplant patients^{9,15} but only limited data are available in pediatric cardiac transplant recipients. A recent study¹⁶ reported a relationship in 65 pediatric patients between *CYP3A5* genotype and tacrolimus clearance at 3, 6, and 12 months after transplantation. This study suggested that *CYP3A5* expressors (*CYP3A5**1/*3) need higher drug doses to maintain the same blood concentration at 3, 6 and 12 months after transplant. However, the effects of mutations in *ABCB1* genotypes on tacrolimus disposition in liver and kidney transplant recipients were inconsistent, with reports both supporting¹⁷⁻²⁰ or refuting such an association.^{17,20,21} The study in pediatric heart transplant recipients demonstrated an association between *ABCB1* C3435T and G2677T/A and tacrolimus dosing requirements at 6 and 12 months after transplantation.¹⁶

These studies did not evaluate the influence of genotype in relation to other clinical factors, such as age and comorbidity, on dose requirements in the early period after transplantation. A large variation in tacrolimus pharmacokinetics may occur in the early post-transplantation period due to critical illness-related factors, such as mechanical ventilation, altered cardiac output and consequent altered liver and kidney blood flow, body fluid, and plasma protein changes. Hence, we speculated that in patients with a higher severity of illness, as defined by the risk of mortality at intensive care unit admission by the Pediatric Risk of Mortality (PRISM) score, lower tacrolimus requirements would be observed. Our objective was to determine the effects of age, recipient *CYP3A5* and *ABCB1* genotypes, and PRISM score on tacrolimus disposition in the first 14 days after transplant in pediatric heart allograft recipients. In addition, we wanted to investigate the association between recipient *CYP3A5*

and *ABCB1* genotypes and tacrolimus levels on transplant outcomes such as rejection and renal function.

Patients and methods

This study was approved by the Institutional Research Ethics Board, and informed consent was obtained from parents and/or children during enrollment. Pediatric heart transplant recipients (aged <18 years at the time of transplant) who received oral tacrolimus during the first 14 days after transplant between 1995 and 2008 at the Hospital for Sick Children, Toronto, Ontario, were eligible for study entry. DNA samples were derived from a cohort of patients prospectively enrolled in the Sickkids Heart Centre Biobank.

Immunosuppressive protocol

Induction therapy with anti-thymocyte globulin was started peri-operatively and given up to 2 to 5 days after transplantation in all patients. Tacrolimus was started at Day 2 to 3 after transplantation, with a starting dose of 0.2 mg/kg/day orally, divided twice daily. The treating physician used therapeutic drug monitoring to adjust the tacrolimus dose to a target level of 10 to 12 ng/ml. Additional immunosuppressive therapy consisted of a maintenance dose of mycophenolate mofetil and steroids.

Pharmacokinetic and pharmacodynamics outcome measures

As dependent variables, we collected tacrolimus dose (mg/kg/12 hours) and tacrolimus trough concentrations from patient health records. Dose-corrected tacrolimus concentrations were calculated by dividing the tacrolimus trough concentration by the weight-adjusted dose.

Data were collected on the occurrence of rejection and renal function in our population. Rejection was graded according to the International Society of Heart and Lung Transplantation's (ISHLT) grading system.²² Rejection was defined as an ISHLT grade 2R or higher.

Creatinine clearance was estimated with the Schwartz formula, using the last available serum creatinine level during the study period.

Covariates

Patient sex, age, weight, the comedication received, *CYP3A5* and *ABCB1* genotype, and PRISM score were collected as independent variables.

Tacrolimus concentrations

Tacrolimus blood trough concentrations were determined in ethylenediaminetetraacetic acid (EDTA)-treated whole blood (0.25 ml) on the day of sampling, using liquid chromatography tandem mass spectrometry (LC-MS-MS) as previously described, as part of clinical care.²³

Genotyping

Blood for genotyping was collected in EDTA-containing tubes, and DNA was extracted using a Magna-Pure LC (Roche Diagnostics GmbH, Mannheim, Germany). Polymerase chain reaction (PCR)-restriction fragment length polymorphism for *CYP3A5**3 and *ABCB1* C3435T, G2677T, and C1236T were performed as described previously.²⁴⁻²⁶ Patients not carrying the *CYP3A5**3 allele were assigned the *CYP3A5**1/*1 genotype by default.

Statistical analyses

Data are presented as mean \pm standard deviation or median and interquartile range (IQR) when the data were skewed. Differences were compared using the Mann-Whitney test or the Kruskal-Wallis test. The Spearman ρ test was used to test possible correlations. A linear multivariate analysis was performed to test the influence of the predictors on all dependent variables. All data analyses were performed using SPSS 16.0 software (SPSS, Chicago, IL). The Hardy-Weinberg equilibrium was calculated by using the method from Rodriguez et al.²⁷

Results

Patient characteristics

The study comprised 39 eligible pediatric heart transplant recipients (25 boys, 14 girls) who were a median age of 6.0 (IQR, 13.75) years and a median weight of 13.1 (IQR, 25.5) kg. A detailed list of the patients' demographics can be found in Table 1.

Tacrolimus disposition

During the 2-week post-transplant period, 258 tacrolimus concentrations were available for analysis. A median of 6 concentration measurements were available for each patient during the study period. The median tacrolimus trough concentration was 9.6 (IQR, 2.08) ng/ml, and the median dose requirement was 0.06 (IQR, 0.06) mg/kg/12 hours. The median concentration/weight-adjusted dose ratio (as a surrogate for estimated clearance) was 150.79 (IQR, 173.3) ng/ml/dose. Of all analyzed concentrations, 32.0% were above the target range and 53.7% were below the target range (Figure 1). On Day 7, 28% were above the target range and 51.3% were below the target range.

Outcome

None of the patients were diagnosed with a grade 2R or higher of rejection in the first 14 days after transplantation. The median estimated glomerular filtration rate (eGFR) at the last available creatinine level was 130.29 (IQR 66.27) ml/min/1.73 m².

Relationship with genotype

DNA for *CYP3A5* genotyping was available for 37 of the 39 patients. Only 1 patient carried the *CYP3A5**1/*1 genotype, 7 carried the *CYP3A5**1/*3 genotype, and 29 carried the *CYP3A5**3/*3 genotype. *CYP3A5* genotypes did not deviate from the Hardy-Weinberg equilibrium (chi-square = 0.49, $p = 0.5$). *CYP3A5* expressors (*CYP3A5**1/*1 and *CYP3A5**1/*3) required significantly higher doses of tacrolimus than the nonexpressors, at 0.14 (IQR, 0.09) vs 0.06 (IQR, 0.04) mg/kg/12 hour ($p = 0.001$; Figure 2). Expressors also had significantly lower tacrolimus trough concentrations, at 7.7 (IQR, 5.85) vs 9.8 (IQR 3.05) ng/ml ($p = 0.032$), and lower concentration/ dose ratios of 45.34 (IQR, 44.54) vs 177.78 (IQR, 145.38) ng/ml per mg/kg/12 hours ($p < 0.0001$; Table 2).

DNA for *ABCB1 C3435T*, *ABCB1 G2677T/A*, and *ABCB1 C1236T* genotyping was available for 37 of the 39 patients. The frequencies of each of the genotypes are described in Table 3. None of the *ABCB1* genotypes deviated from the Hardy-Weinberg equilibrium. No relationship was found between tacrolimus dosing requirements, tacrolimus trough concentrations, or concentration/dose ratio and *ABCB1 3435*, *2677*, and *1236* genotypes (Table 4).

Relationship with age and PRISM scores

Tacrolimus dosing requirements were higher in younger than older children ($r_s = -0.447$, $p = 0.004$; Figure 3). Concentration/dose ratios were lower in younger children ($r_s = 0.351$, $p = 0.029$); however, the tacrolimus trough concentrations were not significantly correlated with age ($r_s = 0.052$, $p = 0.752$). No significant correlation was found between the PRISM score and tacrolimus dosing requirements ($r_s = -0.29$, $p = 0.09$), concentration/dose ratios ($r_s = 0.20$, $p = 0.25$), or tacrolimus trough concentrations ($r_s = -0.150$, $p = 0.38$).

Interplay of age and CYP3A5 genotype

Age and *CYP3A5* genotype both appeared to be associated with tacrolimus dosing requirements or the concentration/ dose ratio. The contribution of both parameters was assessed with multivariate linear regression. Age and *CYP3A5* genotype were independently associated with the tacrolimus dosing requirements ($R^2 = 0.351$, $p = 0.001$) and with the concentration/dose ratio ($R^2 = 0.521$, $p < 0.001$). This was reflected by the observation that in *CYP3A5* expressors younger than 6 years, the dosing requirements were more than 1.5 times higher than in *CYP3A5* expressors older than 6 years (0.15 [IQR, 0.08] vs 0.09 [IQR, 0.04] mg/kg/12 hours). *CYP3A5* non-expressors younger than 6 years also needed 1.5 times higher doses than *CYP3A5* non-expressors older than 6 years (0.07 [IQR, 0.18] vs 0.047 [IQR, 0.25] mg/kg/12 hours). In addition, the dosing requirements of *CYP3A5* expressors younger than 6 years were 3 times higher than *CYP3A5* non-expressors older than 6 years (0.15 [IQR, 0.08] vs 0.04 [IQR, 0.25] mg/kg/12 hours).

When the analysis excluded 1 patient who received fluconazole and 2 patients who received amiodarone, which are CYP3A inhibitors, the results were similar for the relationship between *CYP3A5* and *ABCB1* genotype and age and tacrolimus disposition.

Relationship between genetic variation, tacrolimus levels, and outcomes

We did not find a relationship between eGFR at the last available creatinine level and median or highest tacrolimus trough level ($r_s = 0.128$, $p = 0.439$; $r_s = -0.005$, $p = 0.975$). We also did not find a relationship between eGFR at the last available creatinine level and *CYP3A5* genotype for expressors (median eGFR, 125.37 [IQR, 56.77] ml/min/ 1.73 m²) vs non-expressors (130.43 [IQR, 72.65] ml/min/ 1.73 m², $p = 0.941$).

Discussion

Our data show that less than 15% of tacrolimus trough concentrations are within the (narrow) target range in the early post-transplant period in pediatric heart transplant recipients. Age and *CYP3A5* genotype, independently, both contribute to the variation in the tacrolimus dosing requirements in this cohort.

Limited data exist on pharmacogenetic influences in pediatric transplant recipients:

- Zheng et al¹⁶ reported similar results at 3, 6, and 12 months after transplantation in 65 pediatric heart transplant recipients. These investigators showed a significant difference in the tacrolimus concentration/dose ratio between *CYP3A5* expressors and non-expressors, with the expressors requiring higher doses to maintain the same tacrolimus blood concentration.
- A lower tacrolimus oral clearance was reported by Zhao et al²⁸ for pediatric kidney transplant recipients with the *CYP3A5**3/*3 genotype compared with those with the *CYP3A5**1/*3 genotype less than 2 months after transplant.

- Two other studies of pediatric liver transplant recipients found no relationship between recipient *CYP3A5* genotype and tacrolimus disposition; in contrast, the liver donor's *CYP3A5* genotype was a significant predictor.^{18,29}

Our study showed a *CYP3A5* recipient genotype-tacrolimus disposition relationship in the first 2 weeks after transplantation, arguably one of the most vulnerable periods. We did not find associations between *ABCB1* genotype and tacrolimus dosing requirements and disposition. This is consistent with a study in children done by Zheng et al,¹⁶ which also failed to find an association at 3 months after pediatric heart transplant. In contrast, at 6 and 12 months after transplant, they found lower concentration/dose ratios in patients with the GG and CC haplotype (*ABCB1 G2677T/A* and *C3435T*, respectively). They explained this by higher cytokines concentrations in the early post-transplant period (ie, 3 months) that may have contributed to increased variability in P-glycoprotein expression. Other studies also provide conflicting data about this association, with more studies showing positive associations late after transplant rather than the early period.^{17,29}

The effect of age on tacrolimus pharmacokinetics has been reported.³⁰ Studies of pediatric renal³¹ and liver^{12,13,32} transplant recipients have shown that pre-pubertal children need 2 to 3 times higher doses than adults. In pediatric bone marrow transplantation, a higher clearance rate, compared with adults, was reported.³³ Within the pediatric population, age-related differences between younger and older children in tacrolimus pharmacokinetics have been reported as well. Przepiorka et al¹⁰ documented a decreased tacrolimus clearance in the first 2 weeks after hematopoietic stem cell transplantation only for children aged older than 12 years. In addition, at steady state, the clearance rate was higher for those younger than 6 years than in older children. In pediatric renal transplant recipients, Kim et al¹¹ showed that the younger children (< 5 years and 5–12 years) required 2.7 and 1.9 times higher dosages, respectively, than older children (>12 years), and that a significant inverse correlation between dose/kg and age among all age groups was present. Naesens et al³⁴ reported that younger pediatric renal transplant recipients needed significantly higher doses to achieve comparable tacrolimus trough concentrations compared with older children. Our results show similar findings, with higher dose requirements and lower concentration/dose ratios (as surrogate marker for clearance) in younger pediatric heart transplant recipients.

Ontogeny in tacrolimus biotransformation may explain these findings. The hepatic metabolism of drugs is altered in younger children, with different ages for the different cytochromes to reach maturity, resulting in different metabolism rates¹⁴ and consequent clearance rates. For many *CYP3A4/5* substrates, it is widely established that clearance is increased in the age group between 6 months and 3 years. This has been attributed to higher *CYP3A4/5* activity compared with adults, but others have suggested this is due to a larger liver/body size ratio in children than in adults.^{14,35}

No patient experienced rejection within the 14 days after transplantation. Therefore, we were unable to test a relationship of genetic variation with outcomes. However, the potential influence of the pharmacokinetic variability in the first 14 days after transplantation on the long-term rejection risk still needs to be studied. In addition, we could not establish a relationship between genetic variability, tacrolimus levels, and renal function. Possible reasons could be the relatively small sample size as well as the limited 14-day interval. As an increase in serum creatinine is only apparent with a marked decrease in renal function, the 14-day interval may not have been big enough to see an effect of the high tacrolimus levels on causing a rise in serum creatinine. Factors other than tacrolimus-induced nephrotoxicity, such as comedication and altered hemodynamics, may affect renal function directly after transplant.

Importantly, our study shows that *CYP3A5* genotype and age are independently associated with tacrolimus disposition. As major changes occur in drug disposition during development, the effect of genetic variation in drug disposition should be studied in the context of this age-related variation. We observed that all *CYP3A5* expressors, independent of age, had higher tacrolimus dosing requirements than non-expressors. Taking age into account further amplified the genotype effect, with younger *CYP3A5* expressors needing, on average, tacrolimus doses that were 3 times higher than those needed by older *CYP3A5* non-expressors.

One limitation of our study is the relatively small sample size, which affected the amount of confounders we could look at. In addition, the drug dosing data in our study were retrospectively collected, and this may have introduced unknown variation in the noted vs the actually administered dose, such as inaccuracies with drug dispensing and vomiting with repeated dosing. Although we only studied oral doses of tacrolimus, most of the children do receive tacrolimus orally. Some children, however, cannot tolerate oral tacrolimus and receive intravenous tacrolimus. These children need to be studied separately because differences exist between oral doses and intravenous doses. The systemic exposure is different with intravenous tacrolimus because the first-pass metabolism (eg, metabolism by *CYP3A5* and *ABCB1* transport before absorption) is bypassed, although liver metabolism would still occur.

In conclusion, we showed that in the first 14 days after heart transplantation, younger age and *CYP3A5* expressor status were independently associated with higher tacrolimus dosing requirements and concentration/dose ratio (as surrogate marker for clearance). Drug dosing algorithms need to be developed to guide initial dosing that is individualized based on age and genotype, with the goal of optimizing the ability to safely and rapidly achieve therapeutic targets.

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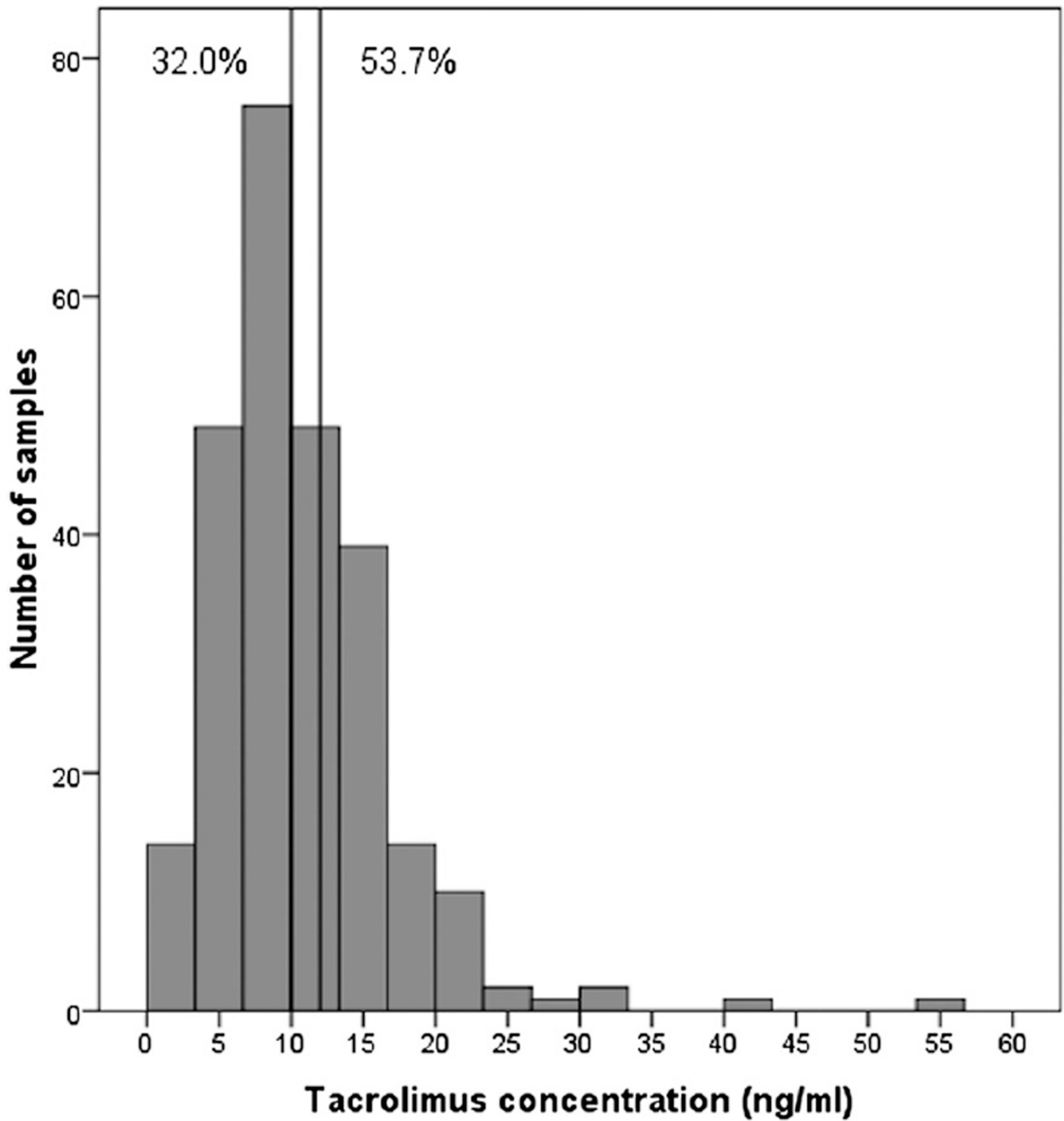


Figure 1. Histogram of tacrolimus concentrations. The vertical lines denote therapeutic range in first 2 weeks after heart transplant. The percentages designate the samples outside of therapeutic window.

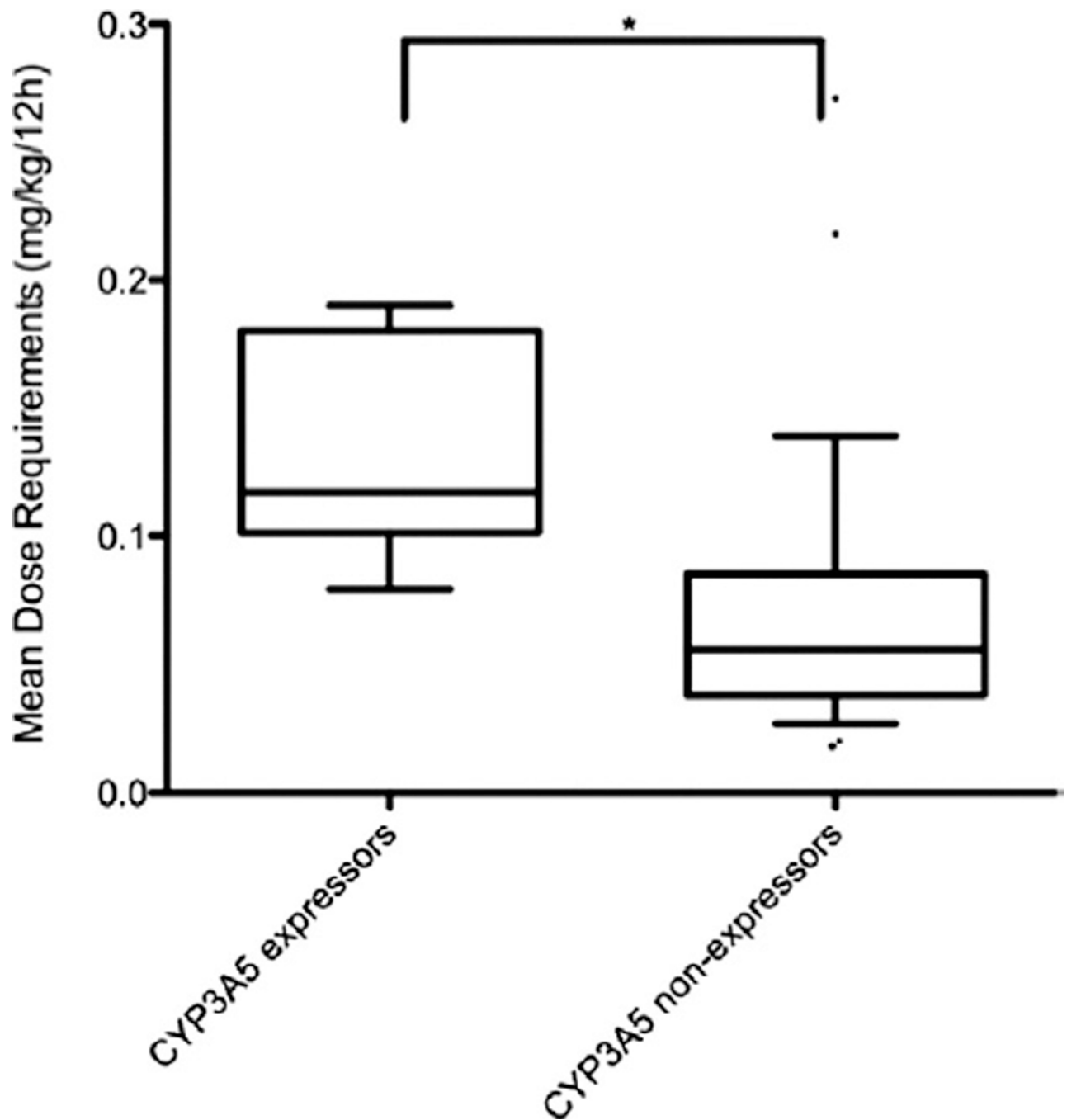


Figure 2. Box and whisker plots show the relationship between *CYP3A5* genotype and tacrolimus dosing requirements. The horizontal line in the middle of each box indicates the median; the top and bottom borders of the box mark the 75th and 25th percentiles, respectively, the whiskers mark the 90th and 10th percentiles, and • designates the outliers. * $p < 0.05$ expressors vs non-expressors.

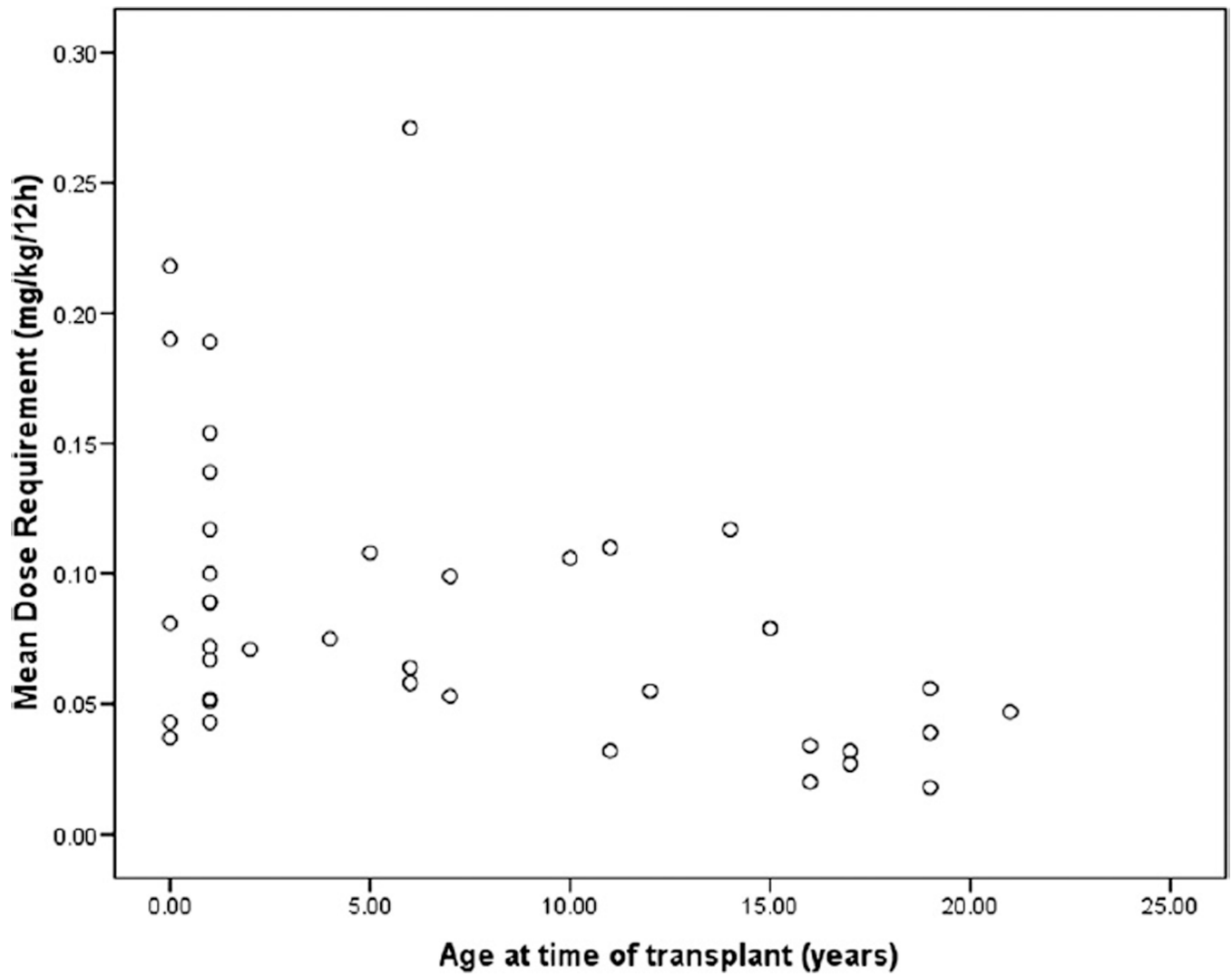


Figure 3.
The relationship between mean tacrolimus dosing requirements and the patient's age at the time of transplant

Table 1

Demographics of the Population

Variable	Median (IQR) or No. (%)
Total patients	39
Age in years	6.0 (13.75%)
< 1 year	15/39 (38.5%)
Sex	
Female	14
Male	25
Weight, kg	13.1 (25.5)
Ethnicity	
White	28
African American	2
Asian	4
Unknown	5
Diagnosis	
Dilated cardiomyopathy	22
Congenital heart disease	15
Unknown	2
PRISM score ^a	9.50 (10)
Need for mechanical ventilation	
Pre-transplant	7/39 (17.9%)
Post-transplant	29/39 (74.4%)
Days ventilation required	2.56 (3.6) ^b
Tacrolimus oral dose ^c	0.06 (0.06)
Tacrolimus trough level ^c	9.6 (2.08)
Concentration/dose ratio ^c	150.79 (173.3)

IQR, interquartile range; PRISM, Pediatric Risk of Mortality.

^aThe PRISM score is based on variables collected during the first 24 hours of intensive care unit admission after transplantation.

^b $p < 0.05$ expressors vs non-expressors.

^cThe tacrolimus dose (mg/kg/12 hours), tacrolimus trough level (ng/ml), and concentration/dose ratio (ng/ml per mg/kg/12 hours) are averages of all values obtained during the 14 day post-transplant period.

Table 2

Relationship of *CYP3A5* Genotype With Tacrolimus Disposition

Variable	Tacrolimus trough levels (ng/ml)		Tacrolimus dosing requirements (mg/kg/12 hours)		Concentration/dose ratio (ng/ml per mg/kg/12 hours)		<i>p</i> -value
	No.	Median (IQR)	<i>p</i> -value	No.	Median (IQR)	No.	
<i>CYP3A5</i>	37		0.032 ^a	37		37	<0.0001 ^a
Expressors	8	7.70 (5.85)		8	0.139 (0.09)	8	45.34 (44.54)
Non-expressors	29	9.80 (3.05)		29	0.055 (0.04)	29	177.78 (145.38)

IQR, interquartile range.

^a *p* < 0.05 expressors vs non-expressors.

Table 3*ABCB1* Genotype Frequencies

Genotype	Frequency (%)	Hardy-Weinberg equilibrium	
		Chi-square	<i>p</i> -value
<i>ABCB1</i>		1.29	0.2
3435 <i>CC</i>	12 (32)		
3435 <i>CT</i>	15 (41)		
3435 <i>TT</i>	10 (27)		
<i>ABCB1</i>		0.02	0.9
2677 <i>GG</i>	11 (30)		
2677 <i>GA</i>	1 (3)		
2677 <i>GT</i>	17 (46)		
2677 <i>TT</i>	8 (21)		
<i>ABCB1</i>		0.29	0.5
1236 <i>CC</i>	10 (27)		
1236 <i>CT</i>	20 (54)		
1236 <i>TT</i>	7 (19)		

Table 4

Relationship of *ABCB1* Genotype With Tacrolimus Disposition

Genotype	Tacrolimus trough level (ng/ml)			Tacrolimus dosing requirements (mg/kg/12 hours)			Concentration/dose ratio (ng/ml per mg/kg/12 hours)			<i>p</i> -value
	No.	Median (IQR)	<i>p</i> -value	No.	Median (IQR)	<i>p</i> -value	No.	Median (IQR)	<i>p</i> -value	
<i>ABCB1</i> 3435	37		0.466	37		0.899	37		0.823	
<i>CC</i>	12	9.85 (5.07)		12	0.062 (0.11)		12	150.16 (301.16)		
<i>CT</i>	15	9.50 (2.75)		15	0.055 (0.08)		15	150.79 (175.33)		
<i>TT</i>	10	10.03 (1.97)		10	0.073 (0.06)		10	145.10 (156.82)		
<i>ABCB1</i> 2677	37		0.318	37		0.963	37		0.531	
<i>GG</i>	11	9.80 (5.70)		11	0.067 (0.09)		11	154.55 (331.46)		
<i>GA/GT</i>	18	9.50 (3.68)		18	0.073 (0.08)		18	139.54 (151.79)		
<i>TT</i>	8	10.17 (1.88)		8	0.072 (0.05)		8	174.12 (138.62)		
<i>ABCB1</i> 1236	37		0.413	37		0.835	37		0.776	
<i>CC</i>	10	10.20 (5.40)		10	0.061 (0.06)		10	179.01 (283.16)		
<i>CT</i>	20	9.50 (2.88)		20	0.074 (0.08)		20	139.54 (178.71)		
<i>TT</i>	7	10.20 (2.30)		7	0.081 (0.04)		7	170.46 (144.19)		

IQR, interquartile range.