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Impact of donor *CYP3A5* genotype on pharmacokinetics of tacrolimus in South African paediatric liver transplant patients

C Wheeler,[1,2] MSc; **C Masimirembwa,**[3,4] PhD; **B Mthembu,**[4] MSc; **J Botha,**[5] MB ChB; **J Scholefield,**[1,2*] PhD; **J Fabian,**[6*] MB ChB, PhD

[1] *Division of Human Genetics, National Health Laboratory Service and School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[2] *Bioengineering and Integrated Genomics Group, NextGen Health, Council for Scientific and Industrial Research, Pretoria, South Africa*
[3] *African Institute of Biomedical Science and Technology, Harare, Zimbabwe*
[4] *Sydney Brenner Institute of Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[5] *Transplantation Services, Intermountain Medical Centre, Salt Lake City, Utah, USA*
[6] *Wits Donald Gordon Medical Centre, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[*] *These authors contributed equally to this work*

Corresponding author: *C Wheeler (cawheeler02@gmail.com)*

Background. In the paediatric liver transplant programme in Johannesburg, South Africa (SA), tacrolimus is the calcineurin inhibitor of choice, comprising an essential component of the immunosuppression regimen. It is characterised by a narrow therapeutic index and wide interpatient variability, necessitating therapeutic drug monitoring of whole-blood concentrations. Pharmacogenetic research, although not representative of SA population groups, suggests that single-nucleotide polymorphisms within the cytochrome P450 3A5 (*CYP3A5*) gene contribute to the variability in tacrolimus dosing requirements. The rs776746 polymorphism, *CYP3A5*3*, results in a splice defect and a non-functional enzyme. Clinically, to reach the same tacrolimus concentration-to-dose ratio (CDR), expressors (*CYP3A5*1/*1* and **1/*3*) require a higher tacrolimus dose than non-expressors (**3/*3*).

Objectives. To compare the pharmacokinetics of tacrolimus in paediatric liver transplant recipients with their donors' *CYP3A5* genotypes, considering both donor and recipient characteristics.

Methods. Blood samples from 46 living liver donors were collected, their genomic DNA was extracted, and their *CYP3A5* genotype was established (polymerase chain reaction and restriction fragment length polymorphism analysis, validated by Sanger sequencing). The relationship of donor and recipient characteristics with the mean tacrolimus CDR was analysed using a general linear model. Non-confounding significant variables were included in a multiple regression model.

Results. The study showed that all expressor donors genotyped as *CYP3A5*1/*1* were of black African self-reported race and ethnicity. During the first 15 days post-transplant, we found that children who received grafts from donor *CYP3A5* expressors (*CYP3A5*1/*1* and **1/*3*) had significantly lower mean tacrolimus CDRs compared with those who received grafts from donor *CYP3A5* non-expressors (**3/*3*); the recipients of *CYP3A5* expressor grafts therefore require higher doses of oral tacrolimus to achieve the same therapeutic target range. In addition, graft-to-recipient weight ratio and the *CYP3A5* donor genotypes were independent factors that significantly ($p<0.05$) affected mean tacrolimus CDRs in recipients.

Conclusion. In this study, we showed that all *CYP3A5*1* homozygote donors were of black African self-reported race and ethnicity, and tacrolimus CDRs in paediatric living-donor liver transplant recipients were significantly affected by donor graft size and donor *CYP3A5* genotypes. Information from this study may inform the development of an Afrocentric tacrolimus precision-medicine algorithm to optimise recipient safety and graft outcomes.

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Liver transplantation is the standard of care for children with acute and chronic end-stage liver disease (ESLD), with organs sourced from living and deceased donors.[1,2] The Wits Transplant Unit in Johannesburg, South Africa (SA), hosts a paediatric liver transplant programme where children with chronic ESLD are waitlisted and those with the most severe disease are prioritised for transplantation.[1,3] In response to pervasive deceased-donor organ shortages and high waitlist mortalities, a living-donor liver transplant (LDLT) programme was initiated in 2013.[2] The backbone of immunosuppression regimens includes a combination of corticosteroids, calcineurin inhibitors (CNIs) and mycophenolic acid.[4] In the Wits Transplant Unit, children are weaned off corticosteroids within 6 months of their transplant, while the CNI, tacrolimus, remains the primary immunosuppressive drug with addition of mycophenolic acid as needed.[2] Dosing schedules for tacrolimus aim to achieve optimal therapeutic blood levels to avoid organ rejection, while reducing the potential for drug toxicity and adverse drug reactions.[4] Tacrolimus is characterised by a well-established narrow therapeutic index and high interpatient variability, which makes achieving blood target levels difficult.[5,6] Currently, there is no personalised dosing schedule for initiating tacrolimus in paediatric liver transplant patients. All children are started on a standard weight-based tacrolimus dose within 24 hours of their liver transplant, and thereafter daily doses are titrated based on trough concentrations.[7,8] The aim is to achieve blood concentrations

between 12 and 15 ng/mL in the immediate postoperative period.[5,7] Anecdotally, clinicians have observed that children of black African ancestry require much higher doses to achieve therapeutic target drug levels compared with other population groups.[7]

The wide interpatient variability in tacrolimus response has been attributed to cytochrome P450 3A5 (*CYP3A5*) genetic variation and drug-drug interactions (such as fluconazole), as well as patient and graft characteristics.[5,9-12] Specifically relating to liver transplantation, significant factors include graft-to-recipient weight ratio (GRWR), recipient age and recipient body weight.[5,11,13]

Studies in North America, Europe, Asia and Africa have investigated the association between *CYP3A5* polymorphisms and tacrolimus pharmacokinetics in liver, kidney, lung and heart transplants.[6,14-17] Paediatric patients tend to be the focus of this research owing to the high interpatient pharmacokinetic variability observed at a younger age.[5,12,13,18] Three *CYP3A5* single-nucleotide polymorphisms (SNPs) are significantly associated with tacrolimus metabolism: rs776746 C (*CYP3A5*3*), rs10264272 T (*CYP3A5*6*), and rs41303343 AA (*CYP3A5*7*).[10,19] These SNPs result in non-functional CYP3A5 enzyme products, thus reducing the metabolism and clearance of tacrolimus.[10,20,21] *CYP3A5* expressors are individuals with at least one copy of the *CYP3A5*1* allele, and this group is further classified into normal (**1/*1*) or intermediate metabolisers (**1/other*).[10,19,21] Non-expressors have two non-functional alleles (e.g. **3/*3*) and are classified as poor metabolisers.[10,19,21] Tacrolimus metabolism occurs in the liver and small intestine, with the most significant pharmacokinetic contribution from the liver.[5,14,18]

Allele frequency distributions for *CYP3A5*, curated by the Pharmacogenomics Knowledgebase (PharmGKB), illustrate that the biogeographical group of European cohorts (as defined by the database) have the highest frequency of *CYP3A5*3* (93%), which contrasts with sub-Saharan African cohorts, where *CYP3A5*1* allele frequency is highest (48%).[19] Similarly, a study replicated these findings in a sub-Saharan African cohort where the *CYP3A5*3* allele was observed at higher frequencies in non-black African populations in comparison with lower frequencies in black African populations.[22] It is noteworthy that *CYP3A5*6* and **7* have been observed in sub-Saharan African cohorts, but have rarely been reported in European cohort studies.[19]

Clinically, the consequences of the *CYP3A5* polymorphisms are that expressors are predisposed to having low therapeutic drug levels as a result of rapid metabolism of tacrolimus, which can lead to rejection of the transplanted graft.[5,10,18] In contrast, non-expressors are at risk of exceeding target therapeutic drug levels, predisposing them to various tacrolimus-related toxicities (most commonly neuropsychiatric and renal).[4,5] The current standard starting dose is set in patients of European ancestry, who are mostly non-expressors. *CYP3A5* expressors are in the majority in the black African population. They are therefore likely to require higher tacrolimus doses to reach the same therapeutic level, and may be at higher risk of graft rejection compared with non-expressors.[10,19,23] Implementation of preoperative living liver donor genotyping analysis for *CYP3A5* could therefore allow transplant physicians, specifically those treating patients of black African ancestry, to stratify tacrolimus dosing schedules to achieve therapeutic drug targets more accurately.[5,6,8]

In the setting of paediatric liver transplantation in southern Africa, no studies have investigated the relationship between *CYP3A5* genotype and the pharmacokinetics of tacrolimus.[1] In this study of a cohort of children undergoing LDLT in Johannesburg, SA, we aimed to: (*i*) determine living-donor liver *CYP3A5* genotype frequencies limited to rs776746; (*ii*) assess the association between *CYP3A5* donor genotype and recipient tacrolimus dose and therapeutic drug levels after transplantation; and (*iii*) determine the impact of clinical factors such as GRWR, age, weight, sex, and self-reported race and ethnicity on the mean tacrolimus concentration-to-dose ratio (CDR) over the first 15 days post-transplant.

Methods

This was a single-centre study of living liver donors with their paired paediatric liver transplant recipients comprising a retrospective and a prospective arm.

Retrospective arm

The retrospective arm was performed using the African Liver Tissue Biorepository (ALTBio), a collaboration between the Council for Scientific and Industrial Research and the University of the Witwatersrand, which included the Wits Transplant Unit, the Sydney Brenner Institute for Molecular Biosciences (SBIMB) and the African Institute of Biomedical Science and Technology. The ALTBio was initiated in 2019 with the aim of creating a repository of pharmacogenomic profiles from LDLT donors. Existing genotype data records from participants already consented to the ALTBio study were accessed for this analysis.

Prospective arm

For the prospective arm, written informed consent was obtained from living liver donors who had undergone transplant procedures at the Wits Transplant Unit prior to the start of the ALTBio in 2019. Eligibility criteria for recruitment to this study were: (*i*) any donor who underwent a living-donor hepatectomy procedure in the Wits Transplant Unit from inception of the LDLT programme; (*ii*) the recipient was a first-time liver transplant recipient; and (*iii*) the recipient was <18 years of age at the time of the transplant. Exclusion criteria were: (*i*) simultaneous liver and kidney transplants; (*ii*) repeat transplantation; and (*iii*) successful recruitment to the ALTBio study.

A 6 mL whole-blood sample was collected in ethylenediaminetetraacetic acid (EDTA) blood collection tubes after written informed consent had been received from each living liver donor. All blood samples were frozen at –20°C until DNA extraction.

Data collection

For the retrospective and prospective arms, the study variables were accessed from the Wits Donald Gordon Medical Centre Paediatric Liver Transplant Research Database. Recipient variables included age, weight, sex, and self-reported race and ethnicity; donor variables included age, weight, sex, self-reported race and ethnicity, and graft weight. The GRWR was calculated by dividing the graft weight (kg) by the recipient weight (kg). In this study, the guidelines set out in the *Updated Guidance on the Reporting of Race and Ethnicity in Medical and Science Journals*[24] have been followed when reporting on ancestry, race and ethnicity. Ancestry is defined as an individual's country/region of origin or a common genealogical line. Race and ethnicity is a multifaceted construct based on common descent or having common national or cultural attributes and is reported within the guidelines set out by Flanagin *et al.*[24] In this study, self-reported race and ethnicity was categorised as black African, coloured, Indian, Asian or white in accordance with SA population group categories published in the mid-year population estimates for 2022 by Statistics South Africa.[25]

Donor genetic analysis

Two methods of genotyping were used to assign *CYP3A5* allelic status.

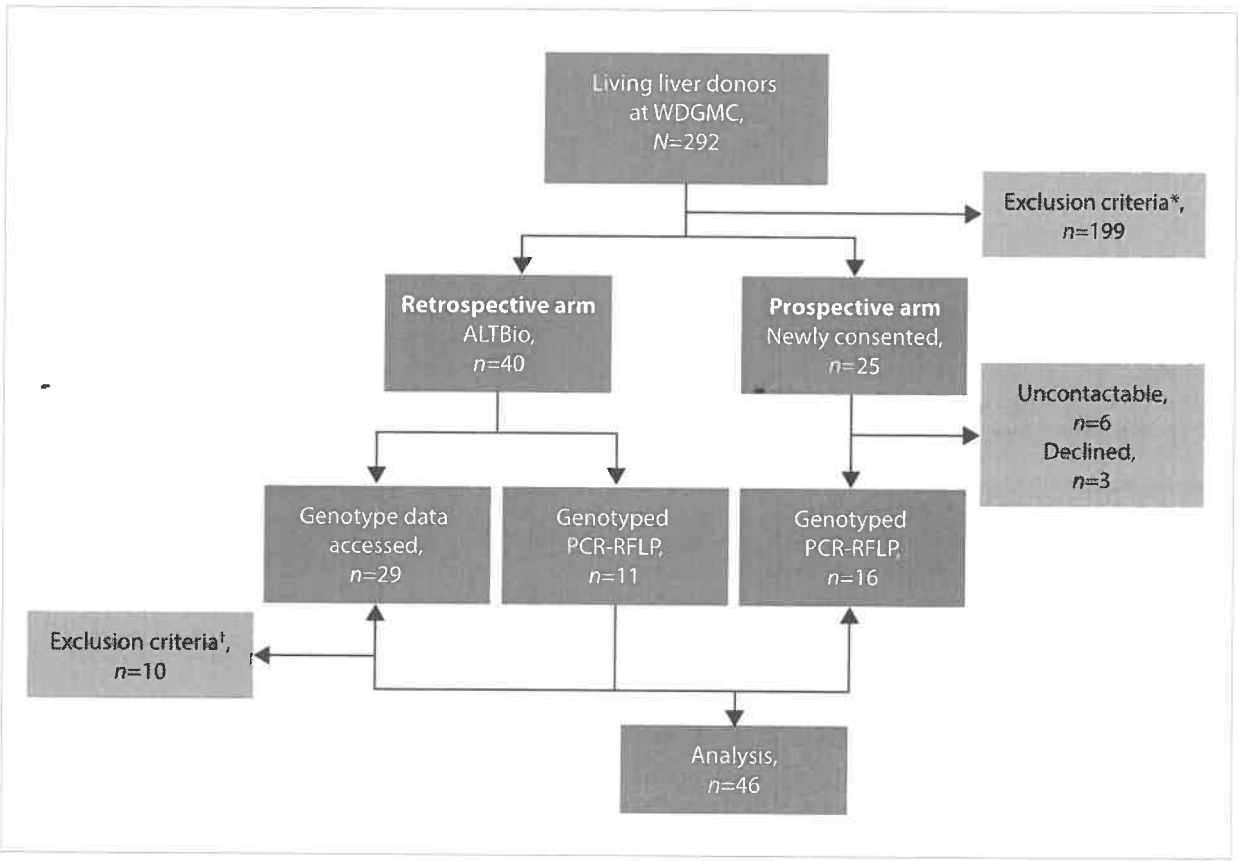


*Fig. 1. Flow diagram of the patient recruitment process and exclusion criteria. (WDGMC = Wits Donald Gordon Medical Centre; PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism; Inclusion criteria: any donor who underwent a living-donor hepatectomy operation with available tacrolimus trough levels, the recipient was a first-time liver transplant recipient, and the recipient was aged <18 years at the time of the transplant; *Exclusion criteria: simultaneous liver and kidney transplants, and repeat transplants; †Exclusion criteria: samples called as CYP3A5*6 or *7 by GenoPharm array were excluded from this analysis of CYP3A5 rs776746.)*

confounded variables and were therefore represented by donor genotype in the multivariable regression analysis. Strong correlations were also identified between recipient age, recipient pretransplant weight and GRWR, and GRWR was therefore chosen as the most meaningful representative variable. The independent variables in the multivariable analysis were GRWR, donor genotype and donor age. Only GRWR and donor genotype remained significantly associated with mean tacrolimus CDR in the multivariable analysis (Table 3).

Discussion

This study demonstrated that donor *CYP3A5* genotype varied significantly between groups of self-reported race and ethnicity. All homozygote **1/*1* expressors and the majority of heterozygote **1/*3* expressors were black African individuals. Tacrolimus CDR was found to be significantly lower in the expressor donor *CYP3A5* genotype groups compared with the non-expressor group, and multivariable analysis showed that GRWR was significantly associated with tacrolimus CDR. Although these findings are preliminary, this is the first study to investigate the associations between living liver donor *CYP3A5* genotype and tacrolimus pharmacokinetics in a paediatric liver transplant cohort from southern Africa.

This study included extensive clinical phenotype data with associated genotype from a well-characterised population-representative cohort. However, there are several limitations. Part of the study design was retrospective, the sample size was small, and it was a single-centre study, so findings may not be generalisable. The tacrolimus CDR determined is representative of both intestinal (recipient) and hepatic (donor) metabolism. Post-transplantation, the recipient's liver graft hepatic *CYP3A5* genotype may differ from their native intestinal genotype. While considered relevant, the influence of recipient *CYP3A5* genotype on the intestinal metabolism of tacrolimus has not been accounted for in this study.[5,18,29] Liver donor genotype is of note, as the patient's diseased liver is completely replaced with the donated liver graft, which would contribute to alteration of tacrolimus trough levels in the recipient.

Regarding the *CYP3A5* genotype frequencies (limited to rs776746), our findings are generally consistent with a previous SA study in a kidney transplant cohort where the expressor recipient genotype revealed similar patterns across groups of race and ethnicity.[6] Similarly, a study in a North American renal transplant cohort showed that >90% of *CYP3A5* expressors were African American participants, compared with 5% who were individuals of white race and ethnicity.[30] These findings (although from kidney transplant cohorts) reinforce the association between high frequencies of the *CYP3A5*1* expressor allele in populations of black African ancestry. European studies conducted in liver transplant patients have rarely detected homozygous expressor genotypes in individuals of white race and ethnicity, which furthermore supports the association between European ancestry and *CYP3A5* non-expressor alleles such as **3*.[5,13,31]

Similar to previous studies in different biogeographical cohorts, the data presented here show that tacrolimus levels are significantly affected by the *CYP3A5* genotype.[5,6,11,18,32] The only other study in

1. ***Genotyping of rs776746 by polymerase chain reaction and restriction fragment length polymorphism (n=27)***
Genomic DNA was manually extracted from whole-blood samples as per a modified salting-out protocol established as the standard operating procedure used in the biobank laboratory at the SBIMB.[26,27] Optimised polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were performed to determine *CYP3A5*3* (rs776746) genotype. PCR was performed using a designed forward primer (5′-GACTTAGTAGACAGATGACACA-3′) and reverse primer (5′-GGTCCAAACAGGGAAGAAATA-3′) as published in Muller *et al.*[6] Phusion Green High-Fidelity DNA polymerase (Thermo Scientific, USA) was used as per the manufacturer's instructions with the following cycling conditions: step 1, 98°C for 30 seconds; step 2, 98°C for 10 seconds, 57.9°C for 15 seconds and 72°C for 5 seconds, repeated for 35 cycles; and step 3, 72°C for 10 minutes. The amplified PCR products were purified using the GeneJET PCR purification kit (Thermo Scientific, USA) according to the manufacturer's instructions, followed by RFLP analysis using SspI-High Fidelity (New England Biolabs, UK). Gel electrophoresis was used to assign genotypes. Selected samples with respective *CYP3A5*1/*1*, **1/*3* and **3/*3* genotypes identified through RFLP analysis were selected for Sanger sequencing validation. The designed forward primer was used to sequence the amplicons with the BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Scientific, USA) and on an ABI 3500xl genetic analyser (Thermo Scientific, USA).

2. ***Genotyping by GenoPharm SNP array (n=29)***
Thirty-nine samples had previously been genotyped on a GenoPharm array (African Institute of Biomedical Science and Technology (AiBST), Zimbabwe), and retrospective *CYP3A5* genotype data were accessed. This open array chip covers multiple variants including rs776746 (*CYP3A5*3*), rs10264272 (**6*) and rs41303343 (**7*). The data available for *CYP3A5*3* were included in this analysis. In this study, we defined individuals with the *CYP3A5*1/*1* (TT) or *CYP3A5*1/*3* (TC) genotype at rs776746 as expressors (ex) and individuals with the *CYP3A5*3/*3* (CC) genotype as non-expressors (non-ex).

Recipient tacrolimus CDR

Tacrolimus trough levels were monitored in hospital post-transplant until discharge. The therapeutic drug protocol for tacrolimus requires a trough level to be measured 2 hours before administration of the next dose. Tacrolimus was measured using a chemiluminescent microparticle immunoassay.[28] Available tacrolimus trough levels and dosages for each recipient were accessed retrospectively from the Paediatric Liver Transplant Research Database and from available clinical records. A CDR was calculated for each day post-transplant until discharge according to the following equation:

$$\text{CDR} = \frac{\text{tacrolimus trough level in blood (ng/mL)}}{\text{total daily tacrolimus dosage (mg)/recipient weight pretransplant (kg)}}$$

Statistical analysis

Possible deviations of the *CYP3A5* genotype frequencies from Hardy-Weinberg equilibrium (HWE) were investigated through an exact test for HWE using the genetics package in RStudio (v4.3.1; R Core Team, USA), using a 5% level of significance. Comparison of categorical study variables between donor genotype groups was performed using Fisher's exact test. Comparison of continuous study variables between donor genotype groups was performed using one-way analysis of variance (ANOVA) (or the Kruskal-Wallis test where the data did not meet the assumptions of the one-way ANOVA).

The relationship of each study variable with mean CDR over the first 15 days of tacrolimus therapy was analysed using a general linear model. Variables significant at $p<0.20$ were combined into a multiple regression model. Each pair of variables was first accessed for possible confounding effects as follows: for pairs of categorical variables, the χ^2 test (or Fisher's exact test for 2×2 tables) was used; a value of Cramér's V (or the phi coefficient for Fisher's exact test) >0.50 was regarded as too strong an association to include both variables in the multivariable model. Pairs of continuous variables were assessed similarly, using Spearman's correlation coefficient. Categorical-continuous variable pairs were assessed by one-way ANOVA or the Kruskal-Wallis test, using the corresponding effect size parameter (Cohen's d or the r-value) to assess confounding. Non-significant variables were removed sequentially until all remaining variables were significant at $p<0.05$. Data analysis was carried out using SAS version 9.4 for Windows (SAS Institute, USA). A 5% significance level was used.

Results

Donor *CYP3A5* genotype

A total of 46 living liver donors and their corresponding liver transplant recipients were included in this study (Fig. 1). Demographic information and clinical phenotype stratified by *CYP3A5* genotype group are summarised in Table 1.

The distribution of the *CYP3A5* genotype frequencies across donor self-reported race and ethnicity did not deviate from HWE ($p>0.05$) (Table 2).

All expressors with *CYP3A5*1/*1* haplotypes were black African individuals. None of the individuals in this study who self-reported as black African were **3/*3* non-expressors. To contextualise our results, we compared the phenotype frequencies of expressors and non-expressors with those in African and European biogeographical cohorts adapted from PharmGKB (Fig. 2).[19]

Donor *CYP3A5* genotype effect on tacrolimus CDR

The distributions of tacrolimus CDRs for each of the first 15 days of tacrolimus immunosuppression post-transplant are shown in Fig. 3. Significant differences in the median CDR between the *CYP3A5*1/*1* and **3/*3* donor genotype groups were observed on days 2 - 9, 11 and 14 - 15 post-transplant. The **3/*3* donor genotype groups had significantly higher CDRs ($p<0.05$) compared with the **1/*1* donor genotype group. There was no significant difference between the **1/*1* expressor and **1/*3* partial-expressor donor genotype groups during the first 15 days post-transplant, except for day 4 ($p=0.034$). However, the CDRs reported in the **3/*3* non-expressor group were significantly higher than those reported in the **1/*3* partial-expressor group on days 3, 9, 11 and 14 - 15.

Fig. 4 demonstrates the effect of *CYP3A5* genotype on recipient mean CDRs for tacrolimus over the first 15 days of tacrolimus immunosuppression. The median (interquartile range) value was significantly higher for the *CYP3A5*3/*3* non-expressor group (184 (88 - 247)) compared with those of the **1/*3* expressor (73 (40 - 92)) and **1/*1* expressor groups (40 (33 - 99)).

Clinical factors associated with tacrolimus CDR

In the univariable analysis, GRWR, donor genotype, recipient age, recipient weight, recipient race and ethnicity, and donor race and ethnicity were significantly associated with mean tacrolimus CDR in the first 15 days post-transplant. Donor genotype, donor race and ethnicity, and recipient race and ethnicity were found to be strongly

Table 1. Demographic information and clinical phenotypes stratified by genotype for living liver donors and recipients (*N*=46)

Characteristic†	Donor *CYP3A5* genotype, *n* (%)*			Overall (*N*=46; 100%), *n* (%)*
	**1/*1* (ex) (*n*=18; 40%)	**1/*3* (ex) (*n*=14; 30%)	**3/*3* (non-ex) (*n*=14; 30%)	
Donors				
Age (years), median (IQR)	30 (26 - 41)	33 (29 - 39)	36 (31 - 41)	33 (28 - 40)
Sex				
Male	11 (61)	5 (36)	2 (14)	18 (39)
Female	7 (39)	9 (64)	12 (86)	28 (61)
Self-reported race and ethnicity				
Black African	18 (100)	6 (43)	0	24 (52)
Coloured	0	5 (36)	2 (14)	7 (15)
Indian	0	2 (14)	2 (14)	4 (9)
White	0	1 (7)	10 (71)	11 (24)
Weight (kg), median (IQR)‡	71 (63 - 82)	66 (59 - 75)	68 (63 - 73)	68 (62 - 80)
Recipients				
Age (years), median (IQR)	2.8 (1.5 - 3.3)	1.9 (0.9 - 3.1)	3.1 (0.9 - 8.9)	2.3 (1.4 - 3.4)
Sex				
Male	7 (39)	10 (71)	7 (50)	24 (52)
Female	11 (61)	4 (29)	7 (50)	22 (48)
Self-reported race and ethnicity				
Black African	18 (100)	6 (43)	0	24 (52)
Coloured	0	4 (29)	2 (14)	6 (13)
Indian	0	3 (21)	2 (14)	5 (11)
White	0	1 (7)	10 (71)	11 (24)
Weight (kg), median (IQR)‡	12.2 (10.5 - 16.0)	11.9 (7.5 - 15.0)	15.9 (9.2 - 28.0)	13.3 (9.2 - 16.9)
GRWR, mean (SD)	2.4 (0.8)	2.4 (1.0)	2.2 (1.4)	2.4 (1.0)

IQR = interquartile range, GRWR = graft-to-recipient weight ratio, SD = standard deviation
*Except where otherwise indicated.
†Missing data: GRWR was missing for 2 patients and donor weight pretransplant was missing for 1
‡Recipient and donor weight (kg) measured pretransplant.

Table 2. Frequency and distribution of *CYP3A5* genotypes across living-donor groups

Donor self-reported race and ethnicity	Donor *CYP3A5* genotype, *n* (%)			Overall (*N*=46), *n* (%)
	**1/*1* (ex) (*n*=18)	**1/*3* (ex) (*n*=14)	**3/*3* (non-ex) (*n*=14)	
Black African	18 (100)	6 (43)	0	24 (52)
Coloured	0	5 (36)	2 (14)	7 (15)
Indian	0	2 (14)	2 (14)	4 (9)
White	0	1 (7)	10 (71)	11 (24)

adult liver transplant patients from Africa, which was conducted in Egypt (*N*=48), showed that expressor *CYP3A5*1/*1* or **1/*3* genotypes resulted in lower CDRs in comparison with **3/*3* non-expressor genotypes.[32] This association between expressor genotype and low recipient tacrolimus CDR post-transplant was furthermore confirmed in recent SA and Egyptian kidney transplant studies.[6,33] A European study on paediatric liver transplant patients, notably lacking homozygous **1/*1* expressor representation, illustrated that the CDR was significantly lower in recipients of heterozygous expressor grafts in comparison with non-expressors.[5] In Chinese cohorts, the homozygous expressor genotype was observed at lower frequencies than the present study, yet donor *CYP3A5* genotype significantly affected recipient CDR post-transplant.[11,18]

The stratification of tacrolimus dosing has previously been proposed based on GRWR in addition to donor *CYP3A5* genotype.[5,11,34] Tacrolimus CDR has been shown to be significantly lower in recipients with higher GRWR in European and Chinese cohorts,[5,11] which is corroborated by our findings. It should be noted that, theoretically, higher graft weights are directly proportional to the liver tissue volume and therefore the amount of CYP3A5 enzymes present in the graft.[5,11]

The findings of the present study are clinically relevant for physicians transplanting solid organs from black African individuals. *CYP3A5* hepatic expressors will have low tacrolimus blood concentrations, making it difficult to achieve therapeutic levels, which places recipients at significant risk for graft rejection. Doses required are therefore much higher, which substantially increases the cost of treatment; however, this increase would be significantly outweighed by patients avoiding graft rejection and potential secondary surgeries. In contrast, non-expressors are susceptible to an increased risk of tacrolimus toxicity and associated adverse side-effects. Preoperative living liver donor genotyping analysis for *CYP3A5* could therefore allow transplant physicians to initiate personalised tacrolimus dosing schedules at the time of the transplant to allow for more accurate therapeutic drug monitoring in the immediate post-transplant period. This preoperative strategy is entirely feasible for an LDLT setting, as there is sufficient time prior to the transplant to conduct genotyping analysis.[1] Financially, in a resource-limited setting a preoperative genotype test would be beneficial considering the high expense associated with the transplant procedure. Recommended dosing would need to be adjusted postoperatively in accordance with the

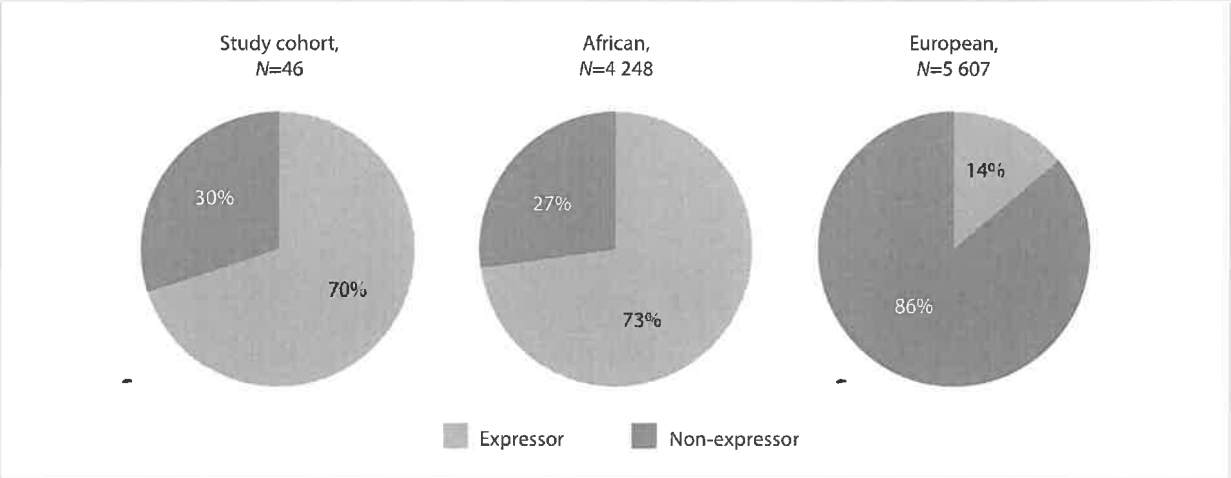


*Fig. 2. CYP3A5 expressor and non-expressor frequencies comparing the present study with PharmGKB African and European biogeographical cohorts. For the purpose of the present study cohort, CYP3A5 expressors are classified as liver donors with *1/*1 or *1/*3 genotype status, and non-expressors as donors with *3/*3 genotype status. Phenotype frequencies for the African and European biogeographical cohorts were adapted from PharmGKB[19] (from which the definitions of biogeographical cohorts are taken), where expressors included normal and intermediate metabolisers and non-expressors were classified as poor metabolisers. Phenotype frequencies in the PharmGKB database estimated using the equation describing Hardy-Weinberg equilibrium based on reported CYP3A5 allele frequencies from different cohorts in the biogeographical areas.[19]*

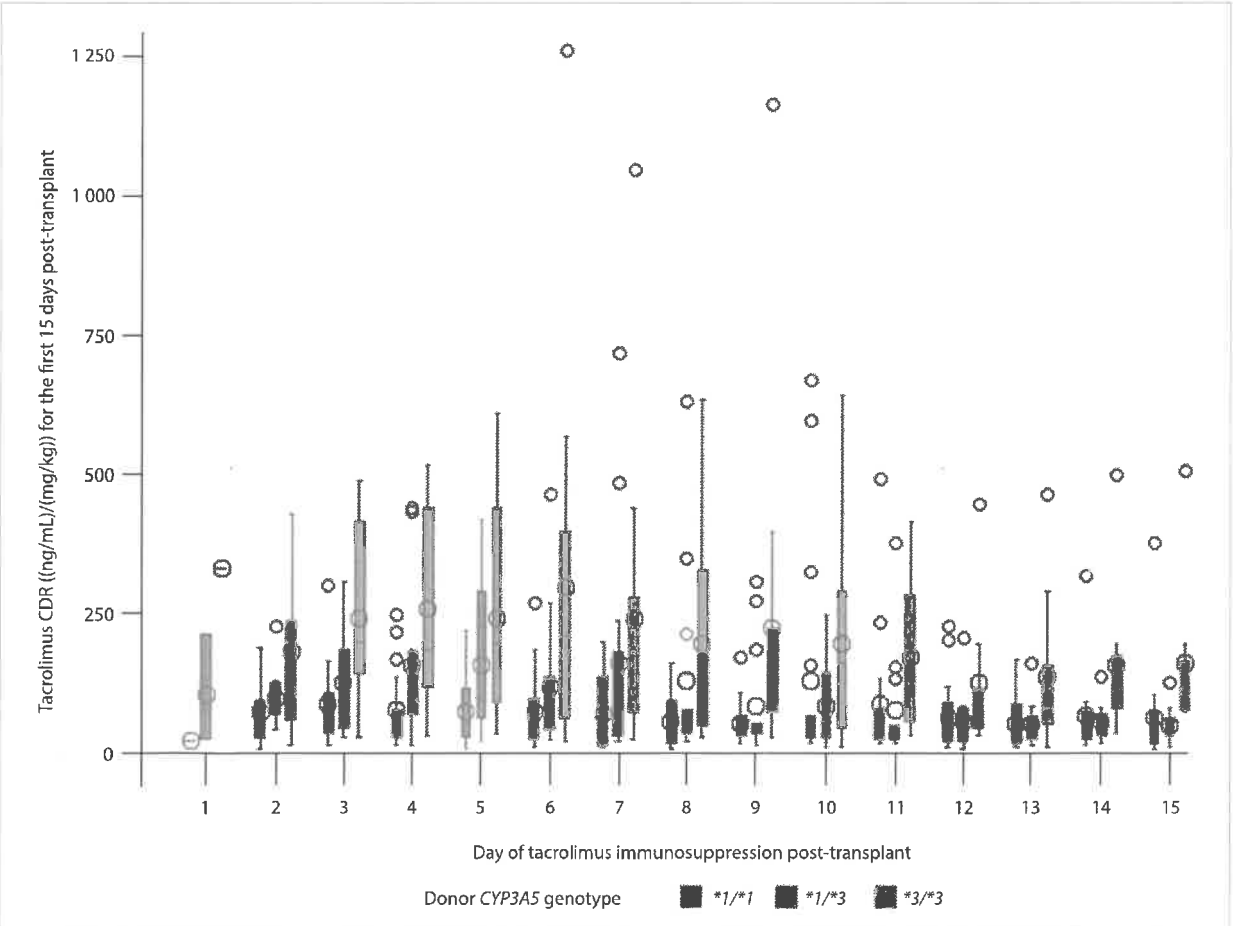


*Fig. 3. Box-and-whisker plot of the CDR for the first 15 days of tacrolimus therapy for donor CYP3A5 genotype. Box-and-whisker plots illustrate the IQR, mean and median for tacrolimus CDR. The median value is denoted by horizontal lines inside the boxes. The mean value is denoted by open circles inside the boxes. The whisker lines extend to ±1.5*IQR. Outlier values (1.5*IQR below the first quartile or 1.5*IQR above the third quartile) are shown as open circles outside the IQR. Significant differences ($p<0.05$) were observed in the median CDR value between the *1/*1 and *3/*3 donor genotype groups on days 2 - 9, 11 and 14 - 15. (CDR = concentration-to-dose ratio; IQR = interquartile range.)*

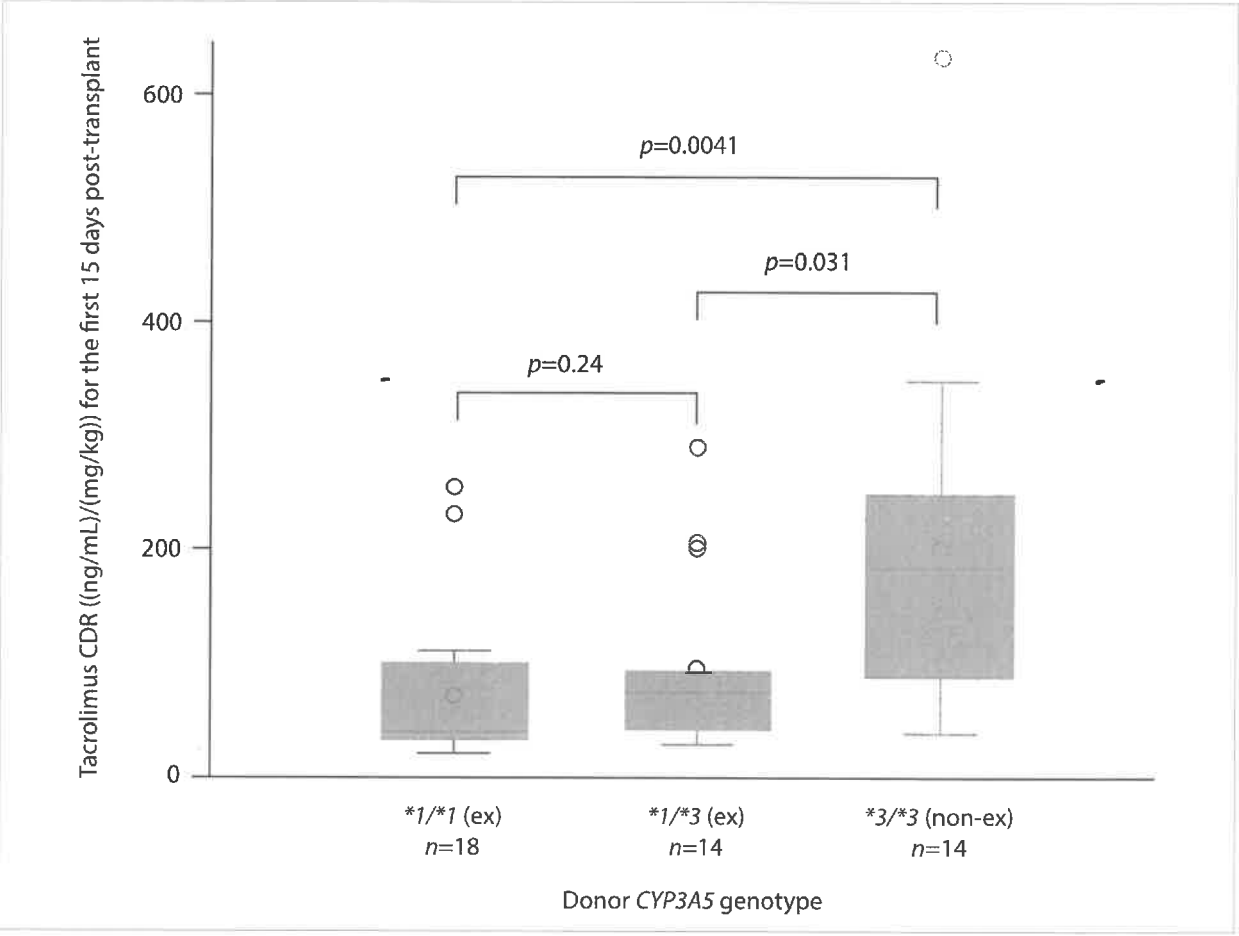


*Fig. 4. Box-and-whisker plot of mean tacrolimus CDR for donor CYP3A5 genotype groups. Box-and-whisker plots illustrate the IQR, mean and median for CDR (mean CDR over the first 15 days post-transplant). The median value is denoted by horizontal lines inside the boxes. The mean value is denoted by open circles inside the boxes. The whisker lines extend to ±1.5*IQR. Outlier values (1.5*IQR below the first quartile or 1.5*IQR above the third quartile) are shown as open circles outside the IQR. The p-value between each donor genotype group is indicated. Significant differences ($p<0.05$) were observed in the median CDR value between the CYP3A5*3/*3 (non-ex) donor genotype group in comparison with the *1/*3 (ex) and *1/*1 (ex) groups. (CDR = concentration-to-dose ratio; IQR = interquartile range.)*

results of the genotyping analysis. However, more investigations need to be conducted into the association between the highly polymorphic *CYP3A5* gene and tacrolimus dosing requirements in order to develop a clinical precision-medicine approach to tacrolimus.

Future studies should consider analysis of the function obliterating *CYP3A5*6* and **7* alleles owing to the higher allele frequency observed in African populations in comparison with their European counterparts.[19] Analysis of additional alleles would possibly shed further light on the study by Muller *et al.*,[6] who did not observe *CYP3A5*6* in an SA renal study population similar in size and ethnic distribution to the present study. The *CYP3A5*8* allele should also be of interest for future work to investigate the impact of *CYP3A5* missense variants in black African populations. Further investigation is therefore needed to assess the impact of these *CYP3A5* SNPs, both individually and as part of a haplotype, in a tacrolimus dosing requirement algorithm. The wide interpatient variability in tacrolimus response has been attributed to several other factors that should be investigated further. Future work should consider investigating the impact of the cytochrome P450 3A4 isoenzyme, which has overlapping specificities with the CYP3A5 enzyme for tacrolimus. In a clinical research setting, the impact of drug-drug interactions between tacrolimus and fluconazole should also be considered during the complex post-transplant management of paediatric patients in transplant units.

Conclusion

While aspects of this study have been published previously, this is the first dataset in a paediatric liver transplant cohort to have predominant representation of homozygous *CYP3A5* expressors, prevalent in the black African population, with tacrolimus CDR data in association with GRWR. The findings from this study, while preliminary, suggest that a stratified tacrolimus precision-medicine approach could be developed from future work, which would better inform immunosuppression treatment schedules, especially in black African patients undergoing solid-organ transplantation.

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Table 3. Results of univariable and multivariable regression analysis to determine factors associated with mean CDR post-transplant

Characteristic	Overall (N=46), n (%)[†]	Univariable regression model[‡]: Estimated coefficient (p-value)	Multivariable regression model[§]: Estimated coefficient (p-value)
Intercept	-	Varies by model	275.0 (p<0.0001)
GRWR ratio, mean (SD)	2.4 (1.0)	−48.0 (p=0.0002*)	−46.2 (p<0.0001*)
Donor *CYP3A5* genotype			
**3/*3* (non-ex)	14 (30)	Ref	Ref
**1/*3* (ex)	14 (30)	−72.8 (p=0.028*)	−65.0 (p=0.023*)
**1/*1* (ex)	18 (40)	−95.9 (p=0.0028*)	−93.0 (p=0.0009*)
Recipient age (years), median (IQR)	2.3 (1.4 - 3.4)	14.8 (p<0.0001*)	-
Recipient weight pretransplant (kg), median (IQR)	13.3 (9.2 - 16.9)	8.6 (p<0.0001*)	-
Recipient sex			
Male	24 (52)	Ref	-
Female	22 (48)	−4.7 (p=0.86)	-
Recipient self-reported race and ethnicity			
Black African	24 (52)	Ref	-
Coloured	6 (13)	46.4 (p=0.23)	-
Indian	5 (11)	44.8 (p=0.29)	-
White	11 (24)	98.1 (p=0.0035*)	-
Donor age (years), median (IQR)	33 (28 - 40)	3.1 (p=0.056)	-
Donor weight pretransplant (kg), median (IQR)	68 (62 - 80)	−0.9 (p=0.46)	-
Donor sex			
Male	18 (39)	Ref	-
Female	28 (61)	35.5 (p=0.20)	-
Donor self-reported race and ethnicity			
Black African	24 (52)	Ref	-
Coloured	7 (15)	33.9 (p=0.35)	-
Indian	4 (9)	66.2 (p=0.15)	-
White	11 (24)	98.1 (p=0.0034*)	-

CDR = concentration-to-dose ratio, GRWR = graft-to-recipient weight ratio, SD = standard deviation, IQR = interquartile range
*Statistically significant (p<0.05)
†Except where otherwise indicated
‡The relationship of each study variable with mean CDR over the first 15 days of tacrolimus therapy in paediatric liver transplant recipients was analysed by a general linear model. Variables significant at p<0.20 were combined into a multiple regression model, after examining each pair of variables for possible confounding.
§Non-significant variables were removed sequentially until all remaining variables were significant at p<0.05.

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Conflicts of interest. None.

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