Donor CYP3A5 Expression Decreases Renal Transplantation Outcomes in White Renal Transplant Recipients

Karola Warzyszyńska
Michał Zawistowski
Eryta Karpeta
Agnieszka Jałbrzykowska
Maciej Kosieradzki

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Corresponding Author: Karola Warzyszyńska, e-mail: karola.warzyszyńska@gmail.com
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Background: After renal transplantation, immunosuppressants should be administered to prevent organ rejection and prolong graft survival. One of them is tacrolimus, which is metabolized by the CYP3A enzyme family. The variability of the CYP3A5 gene in renal transplant recipients has been previously studied for its correlation with acute rejection and allogeneic kidney function. CYP3A5 enzyme is also present in the renal tissue, and its relevance has not yet been extensively investigated. This study aimed to evaluate the effect of donor and recipient CYP3A5 expression status on early and long-term transplant outcomes.

Material/Methods: Single-nucleotide polymorphism in CYP3A5 (rs776746) was analyzed in 95 kidney transplant recipients and their grafts. The effect of donor and recipient genotypes on the primary endpoint, which was the loss of the renal graft over 5-year follow-up, was assessed. The secondary endpoints were biopsy-proven acute rejection, proteinuria, delayed graft function, and renal function.

Results: Patients who received a CYP3A5*1 allele-carrying kidney (n=16) were at greater risk of graft loss (adjusted hazard ratio, 95% CI: 10.61, 2.28-49.42, P=.003) than those with the CYP3A5*3/*3 genotype (n=79). Renal CYP3A5 expression was also a predictor of acute rejection between the 2nd and 12th post-transplant months (adjusted odds ratio, 95% CI: 4.36; 1.08-17.6, P=.038) and proteinuria at different time intervals. No effect of the recipient CYP3A5 genotype was observed.

Conclusions: The donor CYP3A5 genotype is associated with inferior transplantation outcomes. Local renal tacrolimus metabolism is a potential target for improving long-term transplantation outcomes.

Keywords: CYP3A5 Protein, Human •Delayed Graft Function •Graft Rejection •Graft Survival •Kidney Transplantation •Proteinuria

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Background

Calcineurin inhibitors are among the most important groups of immunosuppressants, with tacrolimus (TAC) being the first-choice agent for the treatment of renal transplant recipients (RTRs) [1]. When TAC was placed on the market, it reduced biopsy-proven acute rejection (BPAR) occurrence at 12-month follow-up to 10-20%. Early BPAR is a crucial factor affecting overall patient and allogeneic graft survival [2,3].

TAC dosage adjustment remains a challenge owing to its narrow therapeutic index and high variability in pharmacokinetics. Underexposure favors allograft rejection, whereas overexposure results in severe adverse effects. Treatment requires therapeutic drug monitoring using whole-blood trough TAC concentration (C0) measures [1,4].

It is primarily metabolized by CYP3A cytochromes localized in the liver and gut mucosa. The function of CYP3A5 is well documented. The expression of CYP3A5 enzymes, which accelerate TAC metabolism and elimination, depends on the CYP3A5*1 coding allele, which is found in 10-15% of the White population. The common allelic polymorphism CYP3A5*3 (rs776746; 6986A>G) causes aberrant splicing and produces a protein lacking enzymatic function [4,5]. Thus, people who are CYP3A5 expressors are expected to have lower TAC blood concentrations than non-expressors. Numerous reports have confirmed that RTRs carrying at least 1 CYP3A5*1 allele (*1/*1 or *1/*3) have a lower TAC concentration-to-dose ratio than non-expressors (CYP3A5*3/*3). Genetic factors seem to play an important role in the dose and blood concentration variability of TAC [6-8].

Therefore, it was expected that the recipient’s CYP3A5 genotype may have an impact on renal function after transplantation, yet numerous studies and meta-analyses refuted that assumption [8-11]. On the other hand, CYP3A5 expression in renal tubular cells is well documented [12]. The significance of this phenomenon remains unclear, but an in vitro study showed that the concentrations of inactive metabolites of TAC in the kidney tissue of CYP3A5 expressors were significantly greater than in CYP3A5 non-expressors [13]. In addition, some reports have confirmed that RTRs receiving allogeneic kidney without CYP3A5 expression were at increased risk of TAC nephrotoxicity [14-17], emphasizing the role of its local clearance. Very few authors have investigated the influence of donor CYP3A5 expression on post-transplant allograft function, and the available studies present conflicting results [10,11,18-21].

Our cohort study aimed to assess whether the CYP3A5 genotype of the renal graft affects kidney survival and transplantation outcomes in the short- and long-term post-transplant periods.

Study and Methods

Study Cohort

We conducted a retrospective cohort study in 95 deceased donor kidney recipients who were selected from among 595 patients who underwent transplantation between January 2010 and January 2017 at a single center. Patients were considered eligible for inclusion based on the following criteria: 1) >18 years old; 2) donation after brainstem death donor; 3) White ethnicity of both donor and recipient; 4) receiving twice-daily TAC formula (Prograf®, Astellas Pharma, Warsaw, Poland) in post-transplant immunosuppressive treatment; 5) availability of donor DNA; 6) completeness of the follow-up (patients who were managed at our outpatient transplantation clinic); and 7) written informed consent to participate in the study. Exclusion criteria were: 1) multiorgan transplantation and 2) primary non-functioning kidney transplant. The participants, transplanted 1-7 years prior to recruitment and with stable transplant function, were consecutively enrolled during their follow-up visits at our outpatient transplantation clinic between December 2018 and March 2019 until the required sample size was reached. Recipient blood samples were collected during routine check-up visits after obtaining informed consent. Donor deoxyribonucleic acid (DNA) was obtained from the tissue typing laboratory of the Department of Immunology, where samples were isolated and stored at -20°C after routine crossmatching before transplantation. Demographic data of the RTRs and their corresponding donors, drug doses, TAC C0 results, and clinical outcomes of transplantation were obtained from medical records. Donors were evaluated using the non-scaled US Kidney Donor Risk Index (KDRI) [22]. No deaths were noted in the observed population, and no patients were lost to follow-up.

The analyzed subjects received triple immunosuppressive therapy consisting of:

1) a twice-daily TAC formula (Prograf®, Astellas Pharma, Warsaw, Poland) after an initial body weight-adjusted dosage of TAC (0.1-0.2 mg/kg/day), and the doses were TAC C0-corrected; 2) mycophenolate mofetil at a dose 1.5-2 g/day; 3) steroids – an initial single intravenous infusion of 500 mg methylprednisolone was given intraoperatively, tapered to 30 mg of prednisone on day 4, and then reduced gradually to a final dose of 5-10 mg/d.

In those patients who had positive panel reactive antibody test (PRA) or underwent re-transplantation, the induction therapy was implemented: either anti-thymocyte globulin (Thymoglobulin) or basiliximab. The immunosuppressive regimen was conducted in line with the Polish Transplantation Society guidelines [23].
Whole-blood C₉ TAC concentrations were assayed using the chemiluminescence microparticle method and measured routinely during the post-transplant check-ups. This study conforms to the STREGA statement [24].

**Genotyping**

Real-time polymerase chain reaction (RT-PCR) was used to determine the genotype of CYP3A5 (rs776746; g.99672916C>T) single-nucleotide polymorphism (SNP) in deceased donors and RTRs. RTR DNA was extracted from whole blood using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany). The quality and quantity of the DNA samples were verified by electrophoresis using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific). Analysis was performed using the rs776746 TaqMan® SNP Genotyping Assay (Thermo Fisher Scientific, Vilnius, Lithuania), according to the qPCR protocol for SNP Genotyping (Thermo Fisher Scientific, Carlsbad, US) [25]. RT-PCR was performed using the QuantStudio™ 12 K Flex Real-Time PCR System (Thermo Fisher Scientific, Carlsbad, US) and repeated 3 times to minimize any genotyping errors. Data analysis was performed using TaqMan® Genotyper software (Thermo Fisher Scientific).

**Outcome Measures**

We compared subgroups defined by (1) donor and (2) recipient CYP3A5 expressor status. The primary outcome was graft loss observed within 5 post-transplant years. It was defined as a loss of renal function resulting in the need for long-term dialysis, graftectomy, or re-transplantation [26]. Secondary outcomes included:

1) any BPAR episode within the first post-transplant year, diagnosed in line with the Banff classification [27]. Biopsies were indicated when ≥25% elevation of serum creatinine level was observed or when it was advised by a transplant nephrologist [1];

2) proteinuria occurrence within 3-year follow-up, defined as a loss of more than 300 mg of protein per day [28];

3) DGF defined as the need for dialysis within the first post-transplant week [29];

4) estimated glomerular filtration rate (eGFR) was calculated from the Modification of Diet in Renal Disease (MDRD-4) equation and assessed for 3 post-transplant years [30].

**Ethics**

The study protocol was reviewed and approved by the institutional review board of the Medical University of Warsaw (IRB protocol number: KB/203/2018). This study conforms to the Declaration of Helsinki, Council for International Organizations of Medical Sciences Guidelines, and International Conference on Harmonization of Good Clinical Practice.

**Statistical Analysis**

Continuous variables were tested for normality using the Shapiro-Wilk test along with the Q-Q plot analysis and reported as mean (M) and standard deviation (SD) or median (Mdn) with the first and third quartile (interquartile range, IQR), as appropriate. Categorical data were reported as the number of events and percentages. Hardy-Weinberg equilibrium and the chi-squared test were used to evaluate the allele frequencies. Levene’s test was used to assess the equality of variances. Differences between subgroups were tested using the t test, Welch’s t test, or Mann-Whitney U test for continuous variables and the chi-squared or Fisher’s exact test for categorical variables. The primary outcome was evaluated using Kaplan-Meier curves, log-rank test, and Cox proportional hazards analysis. Logistic regression was used to estimate secondary endpoints. All multivariable models were created using backward elimination. Potential risk factors were selected based on a literature search [2,3,28,31,32]. Owing to the small sample size, we could only include a limited number of covariates in our models. Therefore, in some cases, 2 alternative models were created. Missing data were deleted pairwise. Statistical significance was defined as a 2-sided P value <.05. All tests and plots were created using the survival, survminer, transplantR, and CoxR2 packages in R 4.1.2 (R Core Team, 2021).

**Results**

**Genotyping, Donor, and Patient Characteristics**

The 95 recipients received kidneys from 63 donors. The call rate of the tested samples from both donors and recipients was 100% (n=158). CYP3A5 *1/*3 (TC) was identified in 22 (13.9%) and *3/*3 (CC) in 136 (86.1%) individuals. No wild-type homozygotes *1/*1 (TT) were found in the tested samples. The genotype distribution was consistent with the HWE \( \chi^2 \) (2, 158)=0.9, P=.642. The allele frequencies were T=0.070 and C=0.930, which is consistent with the data from the 1000 Genomes Project [5] for the European population (T=0.057 and C=0.943). The CYP34A5 genotypes were divided into 2 subgroups: expressors (CYP34A5 *1/*3) and non-expressors (CYP34A5 *3/*3). There were 8 (12.7%) and 14 (14.7%) CYP34A5 expressors among donors and recipients, respectively.

RTRs were divided into subgroups according to the presence or absence of CYP34A5 coding alleles in 1) donors (D+, donor expressor; D-, donor non-expressor) and 2) recipients (R+, recipient expressor; R-, recipient non-expressor). The demographic and clinical characteristics are provided in Table 1.
Table 1. Baseline demographics and clinicopathological characteristics of patients and brain-dead kidney donors.

| Variable                        | All patients N=95 | D+ (n=16) | D- (n=79) | R+ (n=14) | R- (n=81) |
|---------------------------------|-------------------|-----------|-----------|-----------|-----------|
| **Donor gender: male**          | 66 (69.5)         | 12 (75.0) | 54 (68.4) | 10 (10.0) | 56 (69.1) |
| **Donor age (years)**           | 45 [38-52]        | 37.5 [35-46.75] | 47 [40-52] | 45 [42.25-51] | 45 [38-52] |
| **Donor BMI (kg/m^2)**          | 25.5 [23.1-28.4]  | 23.1 [21.4-24.9] | 25.7 [23.7-28.7] | 25.0 [21.7-26.1] | 25.5 [23.6-28.4] |
| **Cause of death**              |                   |           |           |           |           |
| Brain injury                    | 31 (32.6)         | 4 (25.0)  | 27 (34.2) | 6 (42.9)  | 25 (30.9) |
| Cardiovascular event            | 54 (56.8)         | 8 (50.0)  | 46 (58.2) | 5 (35.7)  | 49 (60.5) |
| Other                           | 10 (10.5)         | 4 (25.0)  | 6 (7.6)   | 3 (28.6)  | 7 (8.6)   |
| Extended criteria donor         | 6 (6.3)           | 0 (0.0)   | 6 (7.6)   | 1 (7.1)   | 5 (6.2)   |
| Non-scaled US KDRI              | 2.58±0.48         | 2.51±0.45 | 2.59±0.49 | 2.60±0.46 | 2.57±0.49 |
| Serum creatinine (mg/dL)        | 1.04 [0.80-1.53]  | 0.98 [0.81-1.31] | 1.08 [0.80-1.71] | 1.04 [0.84-1.34] | 1.04 [0.80-1.65] |
| Patient gender: male            | 66 (69.5)         | 9 (56.3)  | 57 (72.2) | 8 (57.1)  | 58 (71.6) |
| Patient age (years)             | 43.8±11.1         | 46.7±10.9 | 43.3±11.2 | 39.6±10.9 | 44.6±11.1 |
| Patient BMI (kg/m^2)            | 24.3±3.8          | 26.2±3.9  | 23.9±3.7  | 23.9±3.3  | 24.4±3.9  |
| **Cause of CKD**                |                   |           |           |           |           |
| Glomerulonephritis              | 49 (51.6)         | 7 (43.8)  | 42 (53.2) | 9 (64.3)  | 40 (49.4) |
| ADPKD                           | 14 (14.7)         | 1 (6.3)   | 13 (16.5) | 1 (7.1)   | 13 (16.1) |
| Hypertensive nephropathy        | 9 (9.5)           | 1 (6.3)   | 8 (10.1)  | 0 (0.0)   | 9 (11.1)  |
| Diabetic nephropathy            | 5 (5.3)           | 2 (12.5)  | 3 (3.8)   | 1 (7.1)   | 4 (4.9)   |
| Other                           | 10 (10.5)         | 3 (18.3)  | 7 (8.9)   | 2 (14.3)  | 8 (9.9)   |
| Unknown                         | 8 (8.4)           | 2 (12.5)  | 6 (7.6)   | 1 (7.1)   | 7 (8.6)   |
| Retransplantation               | 16 (16.8)         | 5 (31.3)  | 11 (13.9) | 1 (7.1)   | 15 (18.5) |
| Maximal PRA >0%                 | 25 (26.3)         | 5 (33.3)  | 20 (25.6) | 5 (35.7)  | 20 (25.3) |
| Pre-transplant PRA >0%          | 14 (14.7)         | 2 (20.0)  | 11 (14.1) | 2 (14.3)  | 12 (15.2) |
| **HLA mismatches**              |                   |           |           |           |           |
| 0-2                             | 44 (46.3)         | 8 (50.0)  | 36 (45.6) | 5 (35.7)  | 39 (48.2) |
| 3-4                             | 45 (47.4)         | 8 (50.0)  | 37 (46.8) | 7 (50.0)  | 38 (46.9) |
| 5-6                             | 6 (6.3)           | 0 (0.0)   | 6 (7.6)   | 2 (14.3)  | 4 (4.9)   |
| Induction immunosuppression     | 43 (45.3)         | 11 (68.8) | 31 (39.7) | 7 (50.0)  | 35 (43.8) |

Categorical variables reported as number (%) and assessed using the chi-squared or Fisher’s exact test (expected n < 5). Continuous data reported as median [Q1-Q3] or mean±SD based on the normality of data, and evaluated using Mann-Whitney U or t test, as appropriate. D+ – donor CYP3A5 expressor; D- – donor CYP3A5 non-expressor; R+ – recipient CYP3A5 expressor; R- – recipient CYP3A5 non-expressor; KDRI – unscaled US Kidney Donor Risk Index; ADPKD – autosomal dominant polycystic kidney disease; PRA – panel reactive antibody; SD – standard deviation.
Association of Polymorphisms with TAC Exposure

The median TAC C\text{\textsubscript{0}} levels according to CYP3A5 expression in both recipients and donors within 3 years of follow-up are shown in Supplementary Figure 1.

In recipients who received kidneys from CYP3A5 expressors, TAC C\text{\textsubscript{0}} was significantly lower on the third day after transplantation than in non-expressors (Mdn [IQR], 6.0 [4.5-6.7] ng/mL vs 10.9 [6.8-16.5] ng/mL, respectively; U = 248.50, \( P < .001 \)). In RTRs, carrying the CYP3A5 *1/*3 genotype also resulted in lower TAC C\text{\textsubscript{0}} concentrations on the third (Mdn [IQR], 6.3 [4.9-7.6] ng/mL vs 10.9 [6.5-16.4] ng/mL, U=281.50, \( P = .003 \)), and fifth post-transplant days (Mdn [IQR], 7.5 [6.2-8.9] ng/mL vs 10.7 [8.4-14.8] ng/mL; U=151.50, \( P = .009 \)). No such correlation was observed during further follow-up when the drug doses were TAC C\text{\textsubscript{0}}-adjusted.

Association between Polymorphisms and Transplantation Outcomes

The parameters considered relevant for transplantation outcomes, such as graft loss, BPAR, proteinuria, and DGF, were assessed in CYP3A5 expressing grafts and recipients, and are summarized in Table 2 and Supplementary Figure 2.

### Table 2. Transplantation outcomes and group comparisons.

| Outcome        | All patients \( \text{N}=95 \) | Subgroup (by donor CYP3A5 expression status) | \( P \) value | Subgroup (by recipient CYP3A5 expression status) | \( P \) value |
|----------------|-------------------------------|-----------------------------------------------|--------------|-----------------------------------------------|--------------|
| **Graft loss** |                               | D\+ (n=16)                                    |              | R\+ (n=14)                                    |              |
|                |                               | D\- (n=79)                                    |              | R\- (n=81)                                    |              |
|                | 8 (8.4)                       | 5 (31.3)                                      | .003         | 1 (7.1)                                       | >.999        |
| **BPAR**       |                               |                                               |              |                                               |              |
| Within the 1\text{st} month | 5 (5.3)                      | 0 (0.0)                                       | .585         | 0 (0.0)                                       | >.999        |
| Within the 1\text{st} year | 12 (12.6)                     | 4 (25.0)                                      | .115         | 1 (7.1)                                       | .687         |
| **Proteinuria**|                               |                                               |              |                                               |              |
| Within the 1\text{st} month | 17 (17.9)                     | 4 (25.0)                                      | .476         | 5 (35.7)                                      | .122         |
| Within the 1\text{st} year | 13 (13.7)                     | 5 (31.3)                                      | .040         | 1 (7.1)                                       | .684         |
| In the 2\text{nd} and 3\text{rd} year | 18 (19.4)                     | 6 (42.9)                                      | .026         | 2 (14.3)                                      | >.999        |
| **Total**      |                               |                                               |              |                                               |              |
|                | 33 (34.7)                     | 8 (50.0)                                      | .160         | 6 (42.9)                                      | .549         |
| **DGF**        |                               |                                               |              |                                               |              |
|                | 30 (31.6)                     | 3 (18.8)                                      | .226         | 5 (35.7)                                      | .760         |
| Categorical variables reported as number (%). \( \chi^2 \)-squared or Fisher’s exact test (expected \( n<5 \)) was used. D\+ – donor CYP3A5 expressor; D\- – donor CYP3A5 non-expressor; R\+ – recipient CYP3A5 expressor; R\- – recipient CYP3A5 non-expressor; BPAR – biopsy-proven acute rejection; DGF – delayed graft function.

### Graft loss

We conducted survival analysis to evaluate the occurrence of graft loss in predefined subgroups (Figure 1). The number of events was 8 (8.4%) within the 5-year follow-up. We found that RTRs with grafts from CYP3A5 expressors were at greater risk of graft loss (Figure 1A), log-rank test, \( \chi^2(1)=10.8, \text{ } P<.001 \)). Cox proportional hazard analysis revealed donor CYP3A5 expressor status to be a risk factor for graft loss when adjusted for unscaled US KDRI (adjusted hazard ratio, 95% confidence interval [CI]; 10.61 (2.28-49.42), \( P = .003 \), Table 3). We also created a Cox model adjusted for BPAR episodes, which also significantly increased the risk of loss of graft function; however, the model was overfitted due to the small number of observations (Supplementary Table 1).

### Secondary Outcomes

BPAR episodes occurred in 12 patients (12.6%) within the first postoperative year. Logistic regression analysis showed that donor CYP3A5 expression status increased the risk of BPAR from the 2\text{nd} to the 12\text{th} post-transplant month, which occurred in 31.3% of kidneys carrying the CYP3A5*1 allele vs 7.6% of others (adjusted odds ratio [OR], 95% CI; 4.36, 1.08-17.6; \( P=.038 \)). No difference was observed within the 1\text{st} month and in total within the 1\text{st} year postoperatively.
Figure 1. Kaplan-Meier curves for graft loss in subgroups defined by (A) donor and recipient CYP3A5 expression status, and (B) recipient CYP3A5 expression status.
Donor CYP3A5 expressor status also increased the risk of proteinuria within the first post-transplant year (adjusted OR, 95%CI: 5.90, 1.40-24.82; \( P = .015 \), Table 4) and after that time, within the study follow-up (adjusted OR, 95%CI: 4.49, 1.28-15.72; \( P = .019 \)). No differences were detected in proteinuria within the first post-transplant month and in delayed graft function. Owing to the small sample size and number of events, analysis of other endpoints was unfeasible. No significant associations were detected regarding recipient CYP3A5 expressor status.

The function of the allogeneic kidney was evaluated using eGFR during 3 years of follow-up. No significant difference was observed, regardless of donor CYP3A5 expression, until the third post-transplant year, when recipient CYP3A5 expression was associated with lower eGFR (M [SD], 59.55 [21.01] ml/min/1.73 m\(^2\) vs 45.46 [16.73] ml/min/1.73 m\(^2\); t[87]=−2.37, \( P = .020 \) (Supplementary Table 2).

### Discussion

The main finding of our study was that donor CYP3A5 expression is strongly correlated with graft loss and other clinically relevant transplant outcomes, such as BPAR and proteinuria, which are also essential risk factors for allogeneic graft failure [2,3,33]. This observation is a novel finding that urges the reconsideration of intrarenal mechanisms of immunosuppressive treatment. Evidence regarding donor CYP3A5 expression is limited [10,11,18-21], especially when combined with data on recipients’ expression status [8-11,21]. To the best of our knowledge, proteinuria has never been studied in the context of donor CYP3A5 genetic variation. Some potentially useful conclusions can be drawn from these results.

There is no conclusion regarding the effect of donor CYP3A5 genetic variability on acute rejection and kidney function in RTRs, as few studies have reported conflicting results. Graft loss was assessed in a recent genome-wide association study.

### Table 3. Cox proportional hazards regression analysis for graft loss.

| Covariates | Univariable | Multivariable* |
|------------|-------------|----------------|
|            | HR (95% CI) | \( P \) value | HR (95% CI) | \( P \) value |
| Donor CYP3A5 expressor | 8.168 (1.898-35.140) | .005 | 10.610 (2.278-49.420) | .003 |
| Donor’s sex (male) | 0.476 (0.116-1.957) | .303 |
| Donor’s age | 1.048 (0.959-1.145) | .304 |
| Donor’s BMI | 0.920 (0.770-1.101) | .364 |
| Donor’s serum creatinine, mg/dL | 0.702 (0.232-2.128) | .532 |
| Machine perfusion | 0.186 (0.037-0.920) | .039 |
| KDRI | 3.128 (0.854-11.451) | .085 | 4.950 (1.065-23.020) | .041 |
| PRA max >0 | 0.702 (0.116-4.233) | .699 |
| Recipient CYP3A5 expressor | 0.725 (0.089-5.913) | .764 |
| Recipient’s sex (male) | 0.996 (0.218-4.546) | .996 |
| Recipient’s age | 0.953 (0.889-1.020) | .165 |
| Recipient’s BMI | 1.050 (0.868-1.269) | .615 |
| Retransplantation | 5.634 (1.398-22.699) | .015 |
| Induction immunosuppression | 3.155 (0.622-15.991) | .165 |
| Delayed graft function | 1.562 (0.368-6.623) | .545 |
| Biopsy-proven acute rejection within the first post-transplant month | 10.431 (2.012-54.079) | .005 |
| Biopsy-proven acute rejection within the first post-transplant year | 11.757 (2.775-49.811) | .001 |

HR – hazard ratio; CI – confidence interval; BMI – body mass index; KDRI – unscaled US Kidney Donor Risk Index; PRA – Panel Reactive Antibody; HLA – human leukocyte antigen. * Concordance (standard error), 0.866 (0.055); R2, 0.776; N, 95; number of events, 8.
Table 4. Logistic regression models to estimate secondary endpoints (biopsy-proven acute rejection, proteinuria, and delayed graft function) using donor CYP3A5 expression status as a predictive factor.

| OR   | 95% CI          | P value | AUC | R2N | Accuracy | Overall model test |
|------|-----------------|---------|-----|-----|----------|--------------------|
| BPAR within the 1st post-transplant year as a dependent variable |
| Crude | 2.96 | 0.77-11.38 | .115 | 0.59 | 0.04     | 87%                 | $\chi^2$: 2.28, df: 1, $P=131$ |
| Proteinuria within the 1st post-transplant month as a dependent variable |
| Crude | 5.53 | 1.44-21.24 | .013 | 0.66 | 0.12     | 88%                 | $\chi^2$: 5.77, df: 1, $P=0.016$ |
| Model 1 | 4.36 | 1.08-17.6 | .038 | 0.70 | 0.16     | 89%                 | $\chi^2$: 7.85, df: 2, $P=0.020$ |
| Delayed graft function |
| Crude | 1.69 | 0.47-6.08 | .420 | 0.54 | 0.01     | 82%                 | $\chi^2$: 0.62, df: 1, $P=432$ |

OR = odds ratio; CI = confidence interval; R² – Nagelkerke’s R²; AUC – area under the curve. **Model 1**: adjusted for patient’s body mass index. **Model 2**: adjusted for patient’s age and donor’s sex. **Model 3**: adjusted for donor’s sex. No statistically significant associations were observed for BPAR within the first post-transplant month.

by Woillard et al (2018, 2 populations of N=330 and N=369 RTRs) [21] and showed no effect of CYP3A family genes of either the donor or recipient; however, donor CYP3A5 (rs776746) was not determined or included in the study. In our study, which was conducted in a White population, a very strong correlation between donor CYP3A5 genotype and graft loss was observed in univariable and multivariable analyses. There was no difference, however, in recipient CYP3A5 expression, which is consistent with the findings of Woillard et al [21].

We were unable to find any literature concerning the effect of donor CYP3A5 expression on proteinuria after transplantation, which is a strong predictor of allogeneic kidney failure [33]. Our data showed that it is a significant risk factor for proteinuria within 3-year follow-up after transplant.

Most of the available literature refers to the association between donor CYP3A5 and acute rejection. In our study, BPAR episodes between the second and twelfth post-transplant months occurred in significantly greater number of RTRs with kidneys carrying the CYP3A5*1 allele than in those carrying the CYP3A5*3/*3 genotype (adjusted OR, 95% CI: 4.36; 1.08-17.6, $P=0.038$). Glowacki et al (2011) [11] did not find a correlation between CYP3A6 genotype and acute rejection in RTRs (N=209). Moreover, no such association was reported by Neansens et al (2009, n=252) [18] and Hu et al (2019, n=165) [19]. In contrast, Gervasini et al (2018, N=137) [10] found donor CYP3A5 expressor grafts to be at a higher risk of acute rejection than in non-expressors at 1-year follow-up (N=137, OR, 95% CI: 3.42, 1.06-11.01, $P=0.039$), which is consistent with our results. However, unlike our study, this conclusion was not supported by the multivariable analysis, unless analyzed together with the CYP3A4 genotype variant.

The evidence cited above is highly heterogeneous regarding the study groups, stemming from the inclusion of recipients and donors of different ethnicities (which is important due to the variability in allele prevalence in various populations), immunization risk factors, donor type, immunosuppressive therapy based on several regimens, and short follow-up. The advantage of our cohort is relatively the high homogeneity and long-term observation. All participants were recipients of deceased donor kidneys who underwent a transplant procedure using the same technique and were maintained on the same TAC-based immunosuppressive regimen. In addition, all the RTRs had stable graft function when entering the study and were successfully maintained within the therapeutic range of TAC.

Additionally, we evaluated the impact of donor and recipient CYP3A5 expression on TAC trough concentrations, as these could possibly affect BPAR episodes. As expected [6-8], recipients-CYP3A5 expressors had significantly lower TAC C₀ in the...
early postoperative period (the third- and the fifth-day measurements) when doses were adjusted to body weight only. However, patients who received kidneys from CYP3A5 expressors also had significantly lower TAC C₀ on the third post-transplant day, which might support the hypothesis that intrarenal metabolism plays a more important role than previously thought. This trend was not observed during the longer follow-up period, regardless of the CYP3A5 genotype in donors and recipients. The results of this study showed that even in patients with controlled exposure (equivalent C₀ in both groups), the donor genotype can have a clinical impact on transplantation outcomes. Tubular expression of CYP3A5 can influence the local metabolism and clearance of TAC, as confirmed by in vitro [13] and in vivo [14-16,20,34] studies. Rekers et al (2017, 2 cohorts of N=153 and N=66 RTRs) [34] observed that steroid resistance during BPAR is significantly lower in donor CYP3A5 expressors. The proposed mechanism involves CYP3A5-dependent enhancement of methylprednisolone metabolism and its conversion into a more effective metabolite, thereby increasing the immunosuppressive effect of the treatment. This is consistent with the observations of Joy et al (2007, N=59) [14], Udomkarnjananan et al (2018, n=50) [16], and Metadilis et al (2011, N=103) [15], who concluded that donor CYP3A5 expression status can increase the risk of calcineurin inhibitor nephrotoxicity. In contrast, Glowacki et al (2011, N=209) [11] and Yang et al (2018, N=232) [20] observed no correlation between donor CYP3A5 genotype and clinical features of TAC nephrotoxicity.

In our study, TAC nephrotoxicity was not covered due to the difficulties in differentiating this entity from other causes of kidney injury, regardless of biopsy evaluation [35]. Lack of a criterion standard for diagnosis is a serious problem, which disqualifies toxicity as a relevant endpoint [20,35]. Therefore, kidney function was described as serum creatinine level and eGFR in different follow-up periods. This has also been reported by other authors. Glowacki et al (2011) reported that kidney function at 2-year follow-up does not appear to be correlated with donor CYP3A5 polymorphisms [11]. Tavira et al (2015) observed no correlation between genetic variability and kidney eGFR within 12 months of follow-up [36]. Our study revealed no significant difference in short- and long-term kidney function (creatinine and eGFR), as well as in DGF occurrence, related to donor CYP3A5 expression status. These observations are concordant with other published reports suggesting that genetic variability has no influence on kidney function. However, one should be cautious when drawing such a conclusion from a retrospective study, as all patients are under therapeutic drug monitoring that aims to minimize drug concentration fluctuations to help maintain good renal function.

We also investigated the impact of recipient CYP3A5 expression on transplant outcomes, but no significant correlations were observed, except for a lower eGFR in the third post-transplant year in the CYP3A5 expressor group. Our observations are in line with some previous reports [8-11,21].

Limitations

This study has several limitations, among which the most important are its retrospective design and the small number of patients included in the analyses. As the funding was limited, we were only able to include a limited number of patients, which might have the same impact, mainly on the secondary analyses. Moreover, in our center, no protocol biopsies are routinely performed, resulting in possible underestimation of BPAR occurrence. We are aware that the method of choice in TAC C₀ determination is LC-MS/MS, and the use of immunoenzymatic assay may provide a measurement error, but it was not available in our center when the data were collected. Finally, the results of our study cannot be extrapolated to other populations owing to the different evidence of the evaluated polymorphisms worldwide.

Conclusions

In conclusion, our data showed that the donor CYP3A5 genotype is a risk factor for graft loss and other inferior transplantation outcomes (BPAR and proteinuria) in the White population. Therapeutic drug monitoring does not appear to help prevent this phenomenon. To explain this observation, new insights into the mechanisms underlying local TAC metabolism are required. It is a potential new drug target for improving transplant outcomes by individualizing immunosuppressive therapy. Prospective studies are required to evaluate the clinical relevance of our findings. Considering this evidence, we suggest that pharmacogenetic testing, particularly CYP3A5 genotyping, should be considered before renal transplantation to facilitate a personalized patient approach.

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Declaration of Figures’ Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.
Supplementary Materials

Supplementary Figure 1. Tacrolimus trough concentration in renal graft recipients who received kidneys from CYP3A5 expressors compared with non-expressors during three-year follow-up.

Supplementary Table 1. Cox proportional hazards regression analysis for graft loss adjusted for biopsy-proven acute rejection episodes.

| Covariates                                      | Univariable HR (95% CI) | P value | Multivariable* HR (95% CI) | P value |
|-------------------------------------------------|--------------------------|---------|-----------------------------|---------|
| Donor CYP3A5 expressor                          | 8.168 (1.898-35.140)     | .005    | 23.22 (2.654-203.200)       | .004    |
| Donor’s sex (male)                              | 0.476 (0.116-1.957)      | .303    |                             |         |
| Donor’s age                                     | 1.048 (0.959-1.145)      | .304    |                             |         |
| Donor’s BMI                                     | 0.920 (0.770-1.101)      | .364    |                             |         |
| Donor’s serum creatinine, mg/dL                 | 0.702 (0.232-2.128)      | .532    |                             |         |
| Machine perfusion                               | 0.186 (0.037-0.920)      | .039    |                             |         |
| KDRI                                            | 3.128 (0.854-11.451)     | .085    |                             |         |
| PRA max >0                                      | 0.702 (0.116-4.233)      | .699    |                             |         |
| Recipient CYP3A5 expressor                      | 0.725 (0.089-5.913)      | .764    |                             |         |
| Recipient’s sex (male)                          | 0.996 (0.218-4.546)      | .996    |                             |         |
| Recipient’s age                                 | 0.953 (0.889-1.020)      | .165    |                             |         |
| Recipient’s BMI                                 | 1.050 (0.868-1.269)      | .615    |                             |         |
| Retransplantation                               | 5.634 (1.398-22.699)     | .015    |                             |         |
| Induction immunosuppression                     | 3.155 (0.622-15.991)     | .165    |                             |         |
| Delayed graft function                          | 1.562 (0.368-6.623)      | .545    |                             |         |
| Biopsy-proven acute rejection within the first posttransplant month | 10.431 (2.012-54.079) | .005 | 3.851 (4.235-522.900) | .002 |
| Biopsy-proven acute rejection within the first posttransplant year | 11.757 (2.775-49.811) | <.001 |                             |         |

HR – hazard ratio; CI – confidence interval; BMI – body mass index; KDRI – unscaled US Kidney Donor Risk Index; PRA – Panel Reactive Antibody; HLA – human leukocyte antigen. * Concordance (standard error), 0.918 (0.026); R2, 0.880; N, 95; number of events, 8.
Supplementary Figure 2. Estimated glomerular filtration rate (eGFR) values in different post-transplant periods (three-year follow-up) in patients with grafts from CYP3A5 expressors and non-expressors.

Supplementary Table 2. Estimated glomerular filtration rate (eGFR) within three-year follow-up depending on the presence of the renal or recipient CYP3A5*1 allele variant.

| Outcome       | All patients (N=95) | Subgroup (by donor CYP3A5 expression status) | P value | Subgroup (by recipient CYP3A5 expression status) | P value |
|---------------|---------------------|---------------------------------------------|---------|-------------------------------------------------|---------|
|               |                     | D+ (n=16)                                  |         | R+ (n=14)                                      |         |
| eGFR [ml/min/1.73 m²] |                     |                                             |         |                                                 |         |
| 7th day       | 26.59 [13.65-41.83] | 23.86 [12.65-35.35]                       | .920    | 27.79 [13.71-43.25]                            | .920    |
| 14th day      | 43.44 (19.23)       | 44.92 (20.75)                              | .737    | 43.14 (19.03)                                  | .737    |
| 1st month     | 51.03 (17.72)       | 50.27 (19.46)                              | .386    | 51.74 (17.39)                                  | .386    |
| 6th month     | 56.00 [43.27-69.70] | 50.27 [43.25-65.33]                       | .859    | 54.53 [44.08-70.19]                            | .859    |
| 1st year      | 56.41 [47.32-68.17] | 57.88 [46.18-70.89]                       | .921    | 57.03 [47.34-68.17]                            | .921    |
| 2nd year      | 57.29 (21.23)       | 64.27 (29.51)                              | .200    | 56.02 (19.35)                                  | .200    |
| 3rd year      | 57.33 (20.96)       | 59.27 (32.44)                              | .820*   | 57.03 (18.86)                                  | .820*   |

Continuous data reported as median [Q1-Q3] or mean±SD, as appropriate. D+ – donor CYP3A5 expressor; D– – donor CYP3A5 non-expressor; R+ – recipient CYP3A5 expressor; R– – recipient CYP3A5 non-expressor; eGFR – estimated glomerular filtration rate calculated from the MDRD formula; SD – standard deviation. * Welch’s t-test was used due to violation of the assumption of equal variances (Levene’s test, P=.021).
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