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BRIEF REPORT

Comparison of P-glycoprotein function in peripheral blood mononuclear cells ex vivo in stable Black and White male and female kidney transplant recipients

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Abstract

Kidney allograft survival remains poorer in Black compared to White recipients due to racial differences in calcineurin inhibitor (CNI) pharmacology. P-glycoprotein (P-gp), an ABC efflux transporter expressed in peripheral blood mononuclear cells (PBMCs), modulates CNI pharmacokinetics and intracellular pharmacology. This study investigated P-gp function in PBMC ex vivo at 0 (trough), 4, 8, and 12 h in stable Black and White male and female kidney transplant recipients ($n = 67$) receiving tacrolimus and mycophenolic acid. Tacrolimus doses were adjusted to troughs of 4–10 ng/ml. P-gp function was quantified with flow cytometric measurement of cyclosporine (CYA; 2.5 μM)-reversible efflux of P-gp substrate, 3,3′-Diethyloxacarbocyanine iodide by determining the percentage change of mean fluorescent intensity (MFI) with CYA (% ΔMFI). The composite parameter of area under the concentration versus time $(AUC)_{0–12h}$ % ΔMFI estimated P-gp function. Data analysis examined race, sex, and race-sex associations to P-gp function. A secondary aim analyzed *ABCB1* genotypes: *1236C>T* (*rs1128503*), *2677G>T/A* (*rs2032582*), *3435C>T* (*rs1045642*), and P-gp function. P-gp function (% ΔMFI) was higher in White patients at troughs ($p = 0.031$) compared to Black counterparts with similar trends at 4 and 8 h. Reduced $AUC_{0–12h}$ % ΔMFI was noted in Black recipients ($N = 32$) compared with Whites ($N = 35$, $p = 0.029$) with notable pairwise adjusted differences between Black and White women ($p = 0.021$). Higher $AUC_{0–12h}$ % ΔMFI was associated with *ABCB1 2677 TT* compared to *GG* variants ($p = 0.035$). The $AUC_{0–12h}$ % ΔMFI was greater in White than Black subjects. P-gp function was higher at troughs in White subjects and differed between race-sex groups. P-gp function in PBMC may influence intracellular tacrolimus exposure and interrelating pharmacodynamic responses which may support race and sex pharmacologic differences.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Long-term kidney allograft survival remains poorer in Black recipients compared with White recipients and may be attributed to racial differences in calcineurin inhibitor pharmacology. P-glycoprotein (P-gp) mediate adenosine triphosphate-dependent removal of intracellular immunosuppressive drugs, such as tacrolimus. P-gp is expressed in the gastrointestinal tract, kidneys, liver, and peripheral blood mononuclear cells (PBMCs). It is unknown if P-gp function in PBMCs is different between Black and White male and female kidney transplant recipients (KTRs) and over the dosing interval of immunosuppression.

WHAT QUESTION DID THIS STUDY ADDRESS?

How does ex vivo P-gp function in PBMCs over a 12-h dosing interval during tacrolimus immunosuppression compare between Black and White male and female KTRs?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This study suggests that race-sex differences are present in P-gp function in PBMC between Black and White KTRs. Differences in the transporter function at specific times during the tacrolimus dosing interval were found.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY AND TRANSLATIONAL SCIENCE?

These findings provide insight into future approaches to characterize intracellular, pharmacodynamic responses to individualize tacrolimus therapy in the transplant population using this noninvasive marker. This may potentially enhance and broaden therapeutic monitoring of immunosuppression beyond measurement of trough concentrations.

INTRODUCTION

Black kidney transplant recipients (KTRs) have poorer long-term allograft survival than White counterparts reflecting immunosuppressive pharmacokinetic and pharmacodynamic variability, genomic variants, suboptimal medication prescribing, nonadherence, increased immunoreactivity, and donor to recipient immunologic mismatches.[1,2] Racial influences on tacrolimus, cyclosporine, and mycophenolic acid pharmacology have been reported.[2–6] Black KTRs require higher daily immunosuppressive doses to achieve comparable therapeutic concentrations to Whites KTRs.[2,7] Tacrolimus, a calcineurin inhibitor, exhibits interpatient variability in pharmacokinetics and pharmacodynamics modulated by cytochrome P-450 3A5 (CYP3A5) isoenzymes and P-glycoprotein (P-gp) efflux transport.[7–9] Investigations that elucidate P-gp transport within peripheral blood mononuclear cells (PBMCs) and examination of race and sex associations in KTRs are lacking and may assist in a noninvasive approach to evaluate maintenance immunosuppression in high-risk Black recipients.[10,11]

Because over 40% of kidney allografts are received by women in the United States, sex differences in CYP3A4/5 isoenzymes and P-gp function also requires investigation.[1,10–14] P-gp in the liver and intestines is a source for interpatient variability in calcineurin inhibitor pharmacokinetics.[7–9] Studies comparing male and female hepatic P-gp expression have yielded mixed results, showing lower expression in female patients compared to male patients,[15] whereas more recent investigations observed no difference between sexes.[15–17] In contrast, intracellular P-gp function in PBMCs has been associated with more favorable clinical outcomes in women with B-cell chronic lymphocytic leukemia or HIV-infected individuals receiving treatment.[18,19] These findings suggest that investigating ex vivo P-gp function in PBMCs may provide a noninvasive pharmacodynamic marker to characterize intracellular tacrolimus immunosuppression, which impacts the clinical responses during maintenance therapy. PBMCs may fluctuate over a dosing interval and may impact overall P-gp function.[18–20] However, few studies in Black and White male and female transplant recipients have examined intracellular mechanisms for drug and immune mediator transport during maintenance tacrolimus immunosuppression. P-gp function in PBMCs may provide additional understanding of factors that contribute to tacrolimus pharmacodynamics in different populations.[8,12]

P-gp mediates adenosine triphosphate-dependent removal of intracellular drugs and is expressed in the small intestine, liver, renal proximal tubule, blood brain barrier, and PBMCs.[8,20] P-gp in PBMCs and lymphocyte subpopulations modulate intracellular concentrations of xenobiotics and cytokines (i.e., interleukin-2 [IL-2], IL-4, interferon-ɣ)

and contribute to immunomodulation.[20] *ABCB1* gene encodes P-gp with single nucleotide polymorphisms (SNPs) that include *ABCB1*: *1236C>T* (*rs1128503*), *2677G>T/A* (*rs2032582*), and *3435C>T* (*rs1045642*) and increase or decrease P-gp function and expression.[7,8,21] These SNPs have also been viewed as surrogate markers for P-gp function or expression, with conflicting outcomes reported for their influence on calcineurin inhibitor (CNI) pharmacokinetics.[7–9] *ABCB1* gene expression in PBMCs has been examined after liver and kidney transplantation and was associated with acute rejection or altered CNI pharmacokinetics.[7–9]

P-gp function ex vivo in PBMCs may serve as a potential noninvasive clinical marker of mononuclear cell responsiveness to tacrolimus immunosuppression in stable KTRs. To date, no studies have reported P-gp function in PBMCs over a tacrolimus dosing interval in stable Black and White male and female KTRs. This approach may provide insight into comparative pharmacodynamic and intracellular responses between these populations during maintenance immunosuppression. The primary aim of this analysis was to investigate ex vivo P-gp function in PBMCs at time 0 (trough) and 4, 8, and 12 h over a steady-state tacrolimus dosing period and compare P-gp function between Black and White male and female KTRs. A secondary objective was to examine the association of P-gp function ex vivo and *ABCB1* SNPs.

METHODS

Study population

Sixty-seven stable male and female Black and White KTRs receiving tacrolimus (Prograf; Astellas Pharma US, Chicago, IL) and mycophenolic acid as enteric-coated mycophenolate sodium (ECMPS; Myfortic; Novartis, Hanover, NJ) for ≥6 months participated in a 12-h clinical pharmacology study. Immunosuppressive pharmacokinetics from this study have been previously reported.[4,5] Participant clinical status was determined by physical examination, comprehensive metabolic panel, and complete blood count. Prior to the study, tacrolimus dosage had been adjusted to attain trough range of 4–10 ng/ml.

ECMPS was dose-adjusted based upon clinical response. Medication adherence was verified. Estimated glomerular filtration rate (e-GFR) was calculated using the Modification of Diet in Renal Disease equation[22] with adjustments for race and sex.

Study procedure

This was a cross-sectional, single center, open-label clinical pharmacology study in stable male and female Black and White KTRs conducted at the University at Buffalo (UB) Renal Research Center at the Erie County Medical Center. UB Health Sciences Institutional Review Board approved the study (IRB #PHP0599703-4), which was conducted in accordance with the ethical standards for human subjects and the 1964 Helsinki Declaration. Upon enrollment, patients provided written consent. Inclusion criteria were: (1) ≥6 months post-kidney transplant; (2) age 25–70 years; (3) first or second deceased-donor or living allograft recipient; (4) receipt of the tacrolimus and mycophenolic acid (MPA) immunosuppression for ≥3 months and the same doses for ≥7 days; (5) baseline eGFR >30 ml/min/1.73 m^2 with no change >20% from baseline during prior two clinic visits with clinical stability confirmation by nephrologist; and (6) leukocyte count ≥3000/mm^3 and hemoglobin ≥8.0 g/dl. Exclusion criteria were: (1) infection or acute rejection within 2 weeks; (2) drugs interfering with immunosuppressive absorption; (3) strong cytochrome P450 3A4/3A5 or P-gp inhibitors or inducers within 4 weeks; and (5) significant and unstable medical or psychiatric diseases.

At 6:00 a.m., patients were admitted, vital signs documented, and an intravenous angiocatheter was inserted.

A 0-h sample (~15 ml) was collected prior to immunosuppressive dosing for drug concentrations and clinical chemistry profiles. Blood samples (12 ml) were collected at predose (0 h) and 4, 8, and 12 h after administration of immunosuppressives.

Analysis of P-gp function in PBMCs

Specimens were cryopreserved upon receipt at the flow cytometry laboratory and thawed according to standard procedures. P-gp function was analyzed as described previously with minor modifications.[23] A standardized concentration of 1×10^6 ficoll-enriched PBMCs per 1 ml was used for each timed specimen to normalize analysis and were incubated with 3 ng/ml of the fluorescent P-gp substrate 3,3-diethyloxacarbocyanine iodide (DiOC2(3)) in RPMI 1640 medium supplemented with 10% fetal bovine serum for 30 min at 37°C in the presence or absence of 2.5 μm cyclosporin A (CYA), an established inhibitor of P-gp function. Following the incubation, cells were washed with cold phosphate buffered saline and kept on ice until analysis. The flow cytometry data were analyzed using the *WinList* software program (*Verity*). P-gp function was quantified by determining the relative increase of cellular DiOC2(3) uptake in the presence of CYA compared to the uptake in the absence of CYA, expressed as percentage increase mean fluorescent intensity (MFI) with CYA = (mean fluorescence intensity with CYA – mean fluorescence intensity without CYA)/(mean fluorescence intensity without CYA).[23] Parameters were reported as percentage delta MFI

TABLE 1 P-glycoprotein function in PBMCs of stable kidney transplant recipients by race and sex ($N = 67$)

% ΔMFI mean (SD)[a]	White men [$N = 16$]	White women [$N = 16$]	Black men [$N = 22$]	Black women [$N = 13$]	*p* values	Pair wise comparisons (Tukey adjusted)
MFI at 0 h	88.4 (47.7) $n = 16$	120.6 (106.8) $n = 15$	82.0 (57.1) $n = 22$	44.2 (21.6) $n = 13$	**0.031**	**WF vs. BF; $p = 0.017$**
MFI at 4 h	83.8 (39.3) $n = 15$	108.4 (87.0) $n = 16$	83.6 (46.8) $n = 22$	52.2 (32.7) $n = 13$	*0.075*	**WF vs. BF; $p = 0.044$**
MFI at 8 h	98.4 (73.5) $n = 15$	131.7 (134.7) $n = 15$	71.0 (42.3) $n = 20$	52.6 (27.9) $n = 11$	*0.065*	*WF vs. BF; $p = 0.076$*
MFI at 12 h	91.3 (52.6) $n = 15$	95.6 (58.0) $n = 11$	81.2 (58.3) $n = 19$	70.4 (39.0) $n = 11$	0.673	–
MFI $AUC_{0–12h}$	1088.3 (600.8) $n = 16$	1393 (1071) $n = 16$	944.8 (440.3) $n = 22$	648.5 (280.8) $n = 13$	**0.029**	**WF vs. BF; $p = 0.021$**

Note: Data displayed as mean (SD). Significant *p* values are denoted by bold type. *p* values that depict a trend are denoted in italics.

Abbreviations: AUC, area under the concentration versus time curve; B, Blacks; F, Females; M, Males; W, White.

[a]All P-gp parameters were log transformed during statistical analysis.

(% ΔMFI) for each time and the area under the concentration versus time ($AUC_{0–12h}$) as a 12-h composite ($AUC_{0–12h}$ % ΔMFI). The $AUC_{0–12h}$ % ΔMFI was used as a 12-h exposure parameter for each subject. Healthy human specimens were utilized as controls.

The intra- and interassay technical reproducibility of the P-gp functional assay was conducted using an established cell line (HL60/VCR) with a high degree of P-gp expression. The intra-assay reproducibility was determined using triplicate independent measurements that were repeated on 5 consecutive days to determine the interassay reproducibility. The intra and interassay coefficient of variability was less than 15%.

Genetic analysis

Blood was collected at 0 h in Cell Preparation Tubes (CPT; BD Vacationer, Franklin Lakes, NJ) for PBMCs separation at 25°C. The PBMCs were harvested and transferred to cryovial aliquots, immediately frozen in liquid nitrogen, and stored at −70°C. All samples were viable, and genotypes were determined using validated TaqMan allelic discrimination assays (Applied Biosystems) with a CFX96 Real-Time Polymerase Chain Reaction Detection System (Bio-Rad). De-identified specimens were assayed in duplicate for *ABCB1*: *1236C>T* (*rs1128503*), *2677G>T/A* (*rs2032582*), and *3435C>T* (*rs1045642*). Allele frequencies were confirmed in Hardy–Weinberg equilibrium (HWE) when adjusted for race.

Statistical methods

Patient demographic and clinical characteristics were summarized by race and sex groups using the mean and SD for continuous variables and frequencies for categorical variables. Comparisons were made using one-way analysis of variance or the Pearson chi-square tests, as appropriate. The clinical pharmacology study was originally designed based on tacrolimus clearance with a priori power calculation completed.[4] Samples were also collected to conduct this investigation of ex vivo P-gp function in PBMCs as a secondary objective and forms the basis for this report. Model assumptions of data normality for parametric analysis were verified by graphical representation and Box-Cox review with data transformations used when appropriate.

The % ΔMFI at each time point and $AUC_{0–12h}$ were modeled as a function of race and sex using a general linear model (GLM), with race-sex groups compared using Tukey adjusted pairwise comparisons. To evaluate the variability in P-gp parameters, R^2 was obtained from GLM analysis based upon race, sex, race-sex, and each *ABCB1* genotype.

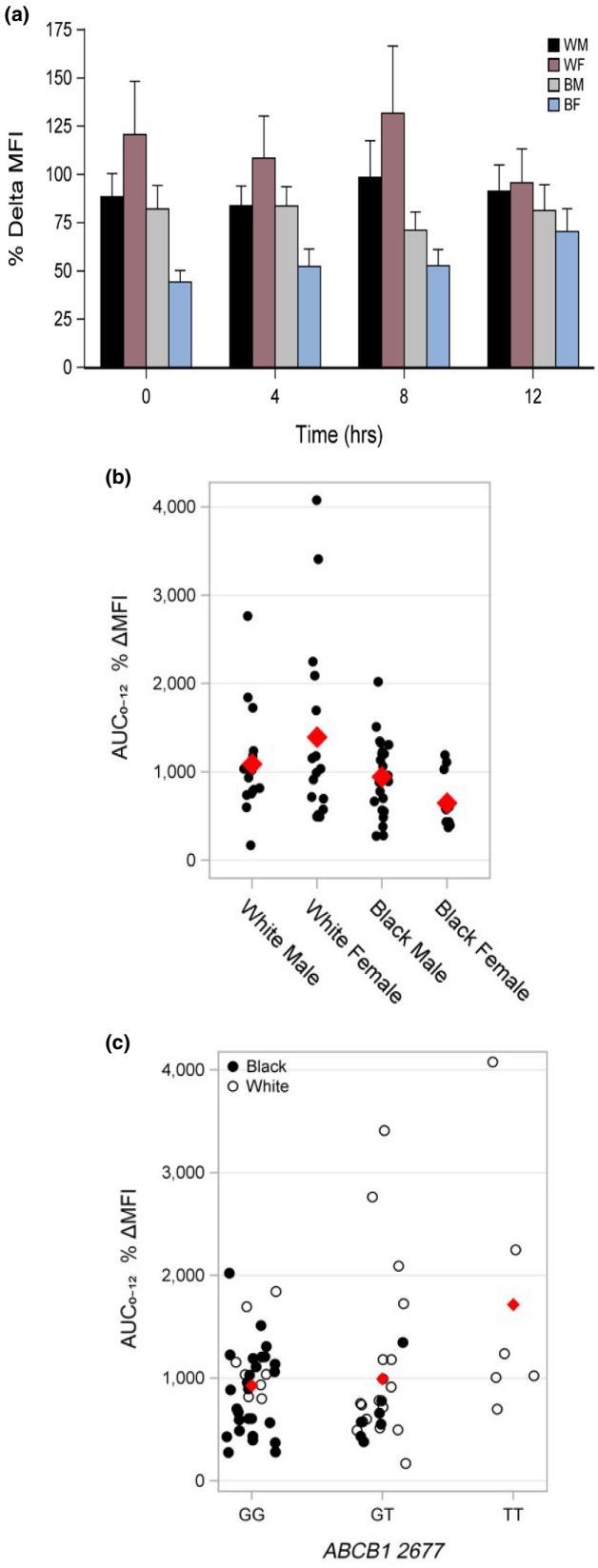

FIGURE 1 (a) P-gp function (% ΔMFI] in PBMC by time and race-sex groups during steady-state tacrolimus dosing interval in stable kidney transplant recipients (KTRs; $N = 67$). Greater P-gp function in PBMCS was found in White female and male recipients compared to Black recipients at time 0 (trough, $p = 0.031$) with similar trends noted at time four ($p = 0.075$; Pairwise $p = 0.044$ in White female subjects vs. Black female subjects) and 8 h ($p = 0.065$; Pairwise $p = 0.076$ in White female subjects vs. Black female subjects). For additional comparisons, see Table 1. (b) P-gp function in PBMC ex vivo represented as AUC_{0-12h} stratified by race-sex groups of stable KTRs. White female and male KTRs exhibit greater P-gp function in PBMCs represented as AUC_{0-12h} % ΔMFI compared to Black patients ($p = 0.029$). The White subjects demonstrate intersubject variability in P-gp function with the AUC_{0-12h} % ΔMFI as a composite parameter (see Table 1). (c) P-gp function in PBMCs stratified by *ABCB1 2677* SNP and race: P-gp function in PBMCs over the 12-h dosing interval during steady-state tacrolimus are represented as AUC_{0-12h} % ΔMFI and stratified by *ABCB1 2677* genotypes which exhibits significant associations. The White transplant recipients (open circles) exhibit greater P-gp function in PBMCs with associations to *ABCB1 2677 TT and GT* variants than Black KTRs (closed circles; $p = 0.035$). The majority of the Black participants exhibit the wild-type (GG). Inter-subject variability in AUC_{0-12h} % ΔMFI are present with White recipients exhibiting TT (pairwise; $p = 0.027$) or GT SNPs (pairwise; $p = 0.055$; see Table S2). BF, Black female; BM, Black male; MFI, mean fluorescent intensity; PBMC, peripheral blood mononuclear cell; WF, White female; WM, White male

The P-gp function in PBMC was also determined as AUC_{0-12h} % ΔMFI as a composite parameter over 12-h calculated using the trapezoid method. P-gp function was determined ex vivo on a standardized concentration of 1×10^6 cells in 1 ml of specimen and represents a summary of P-gp function over a dosing interval normalizing the measurement. If a % ΔMFI value for a timepoint was missing, that value was replaced with the respective race-sex average for calculation of the AUC_{0-12h} % ΔMFI. In order to assess the effect of possible confounding variables on each P-gP parameter, linear mixed models were considered with the P-gP parameter as a function of race, gender, and each demographic or clinical covariate in a one-at-a-time manner. P-gp function was modeled relative to genotypes using GLMs, with Tukey adjusted pairwise comparisons. Analyses were performed in SAS version 9.4 (Cary, NC) at a significance level of 0.05.

RESULTS

Patients

Sixty-seven recipients (13 Black women, 22 Black men, 16 White women, and 16 White men) completed the study with no differences in age, body mass index, diabetes, or time post-transplant with enrollment scheme previously published.[4] Demographics and clinical characteristics are summarized in Table S1. Hematology and liver function

tests were within normal range with no group differences. The e-GFR in Black recipients was more rapid than in White recipients ($p = 0.055$). MPA doses were not different among groups. Tacrolimus doses were ~40% higher in Black recipients ($p < 0.001$).

P-gp function

P-gp function (% ΔMFI) in PBMC is summarized in Table 1. Figure 1 depicts the P-gp function (% ΔMFI) in PBMC at 0, 4, 8, and 12 h and is higher for White recipients compared to Black recipients. A race-sex difference was noted at 0 h ($p = 0.031$). Figure 1b depicts P-gp $AUC_{0–12h}$ %ΔMFI, as a composite parameter, which is higher in White recipients ($p = 0.029$) with intersubject variability noted. Table S3 summarizes R^2 to describe the variability due to race, sex, race-sex, and genotypic groups relative to P-gp function in PBMCs. Combined race-sex accounted for R^2 of 12% of variability for $AUC_{0–12h}$ % ΔMFI and <10% for all timed measurements. In summary, race, sex, and the race-sex groups accounted for a small amount of variability.

ABCB1 genotypes

Table S2 and Figure S1 summarizes *ABCB1* genotype distribution for *1236C>T* (*rs1128503*), *2677G>T/A* (*rs2032582*), and *3435C>T* (*rs1045642*) by race-sex groups. For two alleles, *ABCB1 2677* and *3435*, there were differences in allele frequencies between race determined by the goodness-of-fit test (*G* test). Figure 1c depicts P-gp function in PBMCs stratified by *ABCB1 2677* SNPs. The transplant recipients exhibited greater P-gp function in PBMC with associations to *ABCB1 2677 TT* when compared to the *GG* variants ($p = 0.035$). More than 70% of the Black participants exhibited the wild-type (GG) and the group exhibiting the TT variant were White participants.

DISCUSSION

This investigation is the first report of P-gp function in PBMC measured at tacrolimus trough (0 h), post peak (4 h), and during elimination (8 and 12 h) over a steady-state dosing interval in stable Black and White KTRs. P-gp function was different at timepoints over the 12-h dosing interval noted at 0 h and the composite $AUC_{0–12h}$ % ΔMFI. Differences in race-sex groups were also observed with higher P-gp function in PBMCs in White KTRs than Black KTRs.

Comparison of timepoints within the same dosing interval and race-sex differences in P-gp function in PBMCs

We investigated P-gp function in stable Black KTRs, who are at high-risk for long-term allograft rejection compared to White KTRs. Black recipients often exhibit greater immunoreactivity pre- and post-transplant.[2] This may impact pharmacodynamic responses to long-term immunosuppression. We observed a race-sex difference at 0-h with changes in P-gp function with trends noted at the 4-h and 8-h timepoints. These findings suggest mechanisms that regulate the efflux transporter and intracellular immunosuppression may not be constant over a 12-h dosing interval. Studies in healthy individuals and participants with rheumatologic disease or lung transplant evaluated P-gp function using only a single timepoint which limits the ability to detect pharmacodynamic differences over time from these data.[10,24–27] Although past investigations have reported mixed outcomes while studying P-gp expression and sex,[15,16,18,19] our study observed that White male and female KTRs exhibited greater P-gp function in PBMCs at time 0 h and with the composite parameter, $AUC_{0–12h}$ % ΔMFI, compared to Black men and women.[12,25] The pair-wise comparison for each significant P-gp parameter was noted between White and Black women which reinforces the combined race-sex association. Note that White women exhibited P-gp function that was approximately two-fold greater than Black women, as summarized in Table 1.

We also report race and sex differences with reduced $AUC_{0–12h}$ % ΔMFI representing composite P-gp function over dosing interval in Black subjects compared to White subjects. This finding may have clinical implications for calcineurin inhibitor individualization or minimization between Black and White subjects because it reflects a quantitative composite measure of efflux transporter function in PBMCs over the 12-h dosing interval. P-gp also modulates IL-2, Il-4, or interferon-ɣ transport with implications for modulating immunoreactivity between the recipient and transplant organ.[11,20,21] Further investigations are needed to examine race and sex differences in P-gp function in PBMCs and lymphocyte subpopulations in stable transplant recipients and healthy participants as well as the application of this research blood tests as a routine liquid biopsy to guide drug therapy adjustments post-transplant.[12,25,26]

Recently, liquid biopsies have been utilized to quantify proteins, such as P-gp, and efforts have been made to correlate such expression to activity (e.g., function) with patient response. Liquid biopsies have successfully detected variability in P-gp expression, and expression has been moderately and strongly correlated to P-gp activity.[21,24,28]

Although promising for general pharmacokinetic outcomes, measuring P-gp activity in target cells, such as PBMCs, may provide a noninvasive surrogate marker for a pharmacodynamic response within lymphocytes during maintenance immunosuppressive therapy. This approach may add to our understanding of observed pharmacokinetic differences of tacrolimus between patient groups based upon race and sex.

P-glycoprotein function and association to ABCB1 genotype

The influence of common *ABCB1* SNPs, including *1236C>T* (*rs1128503*), *2677G>T/A* (*rs2032582*), and *3435C>T* (*rs1045642*) have focused on tacrolimus pharmacokinetics or renal pharmacodynamics that includes acute rejection and nephrotoxicity.[7–9] Interestingly, these *ABCB1* SNPs are inherited as a haplotype[21,29] that exhibit distinct racial frequencies. We found significant genotype frequencies between race for two SNPs (*ABCB1 2677* and *3435*) but not for *ABCB1 1236* despite linkage. The *ABCB1 2677G>T* SNP is part of the *1236T-2677T-3435T* (*TTT*) haplotype that is associated with significant reductions in P-gp function compared to the wild type.[21] Figure 1c depicts the association of the single SNP, ABCB*1 2677G>T*, to P-gp function represented as AUC_{0-12h} % ΔMFI. *ABCB1* 2677 is the only SNP that results in a change in the amino acid sequence resulting in a serine to threonine substitution.[21,29] This missense mutation may contribute to the observed reduced P-gp activity associated with *ABCB1* haplotypes.[7,9,21,29] This utility of *ABCB1* variant to assess P-gp function in PBMCs as a pharmacodynamic marker requires additional research. The greater P-gp function in PBMCs associated with White KTRs manifesting the *ABCB1 TT* variant provides intriguing preliminary findings that require extension of our study to a larger Black and White population that includes covariate analysis of age and sex.

A limitation to consider is that the original study in which the samples were collected was powered using tacrolimus clearance and association to race-sex groups in order to determine the sample size.[4] This investigation of P-gp function ex vivo in PBMCs was a secondary objective to the main pharmacokinetic tacrolimus study to compare transporter responses between Black and White male and female KTRs at 4-h periods during a 12-h tacrolimus dosing interval. Although the cohort was smaller, rigorous inclusion and exclusion criteria were used to confirm participant clinical stability, and batched analysis was conducted with internal controls to minimize technical variability. A follow-up investigation is ongoing with a similar study design in a larger KTR population that utilized this preliminary data to generate a power calculation and sample size estimate for a larger group of Black and White male and female KTRs. Parametric statistics were conducted and accounted for varying P-gp at each timepoint and among race, sex, and race-sex groups to generate valid differences in P-gp function.

CONCLUSION

This study reports P-gp function ex vivo at selected times over a steady-state tacrolimus dosing interval in stable Black and White KTRs. Despite therapeutic tacrolimus troughs, clinically stable Black KTRs had lower P-gp function in PBMC (i.e., AUC_{0-12h} % ΔMFI) compared to White recipients. Varying P-gp function was also noted between race and sex groups. This report utilized a novel study design to characterize the P-gp function in PBMCs over a dosing interval and observed notable race-sex differences during tacrolimus immunosuppression. These findings may assist future investigations to characterize intracellular pharmacologic differences between race and sex post-transplant.

AUTHOR CONTRIBUTIONS

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

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

REFERENCES

1. Hart A, Lentine KL, Smith JM, et al. OPTN/SRTR 2019 annual data report: kidney. *Am J Transplant*. 2021;21(Suppl 2):21-137. doi:10.1111/ajt.16502
2. Young CJ, Kew C. Health disparities in transplantation: focus on the complexity and challenge of renal transplantation in African Americans. *Med Clin North Am*. 2005;89(5):1003-1031, ix. doi:10.1016/j.mcna.2005.05.002
3. Mancinelli LM, Frassetto L, Floren LC, et al. The pharmacokinetics and metabolic disposition of tacrolimus: a comparison across ethnic groups. *Clin Pharmacol Ther*. 2001;69(1):24-31. doi:10.1067/mcp.2001.113183
4. Tornatore KM, Meaney CJ, Attwood K, et al. Race and sex associations with tacrolimus pharmacokinetics in stable kidney transplant recipients. *Pharmacotherapy*. 2022;42(2):94-105. doi:10.1002/phar.2656
5. Tornatore KM, Meaney CJ, Wilding GE, et al. Influence of sex and race on mycophenolic acid pharmacokinetics in stable African American and Caucasian renal transplant recipients. *Clin Pharmacokinet*. 2015;54(4):423-434. doi:10.1007/s40262-014-0213-7
6. Tornatore KM, Sudchada P, Dole K, et al. Mycophenolic acid pharmacokinetics during maintenance immunosuppression in African American and Caucasian renal transplant recipients. *J Clin Pharmacol*. 2011;51(8):1213-1222. doi:10.1177/0091270010382909
7. Knops N, Levtchenko E, van den Heuvel B, Kuypers D. From gut to kidney: transporting and metabolizing calcineurin-inhibitors in solid organ transplantation. *Int J Pharm*. 2013;452(1–2):14-35. doi:10.1016/j.ijpharm.2013.05.033
8. Brunet M, van Gelder T, Asberg A, et al. Therapeutic drug monitoring of tacrolimus-personalized therapy: second consensus report. *Ther Drug Monit*. 2019;41(3):261-307. doi:10.1097/FTD.0000000000000640
9. Vanhove T, Annaert P, Kuypers DR. Clinical determinants of calcineurin inhibitor disposition: a mechanistic review. *Drug Metab Rev*. 2016;48(1):88-112. doi:10.3109/03602532.2016.1151037
10. Donnenberg VS, Burckart GJ, Griffith BP, Jain AB, Zeevi A, Berg AD. P-glycoprotein (P-gp) is upregulated in peripheral T-cell subsets from solid organ transplant recipients. *J Clin Pharmacol*. 2001;41(12):1271-1279. doi:10.1177/00912700122012850
11. Kock K, Grube M, Jedlitschky G, et al. Expression of adenosine triphosphate-binding cassette (ABC) drug transporters in peripheral blood cells: relevance for physiology and pharmacotherapy. *Clin Pharmacokinet*. 2007;46(6):449-470. doi:10.2165/00003088-200746060-00001
12. Bebawy M, Chetty M. Gender differences in p-glycoprotein expression and function: effects on drug disposition and outcome. *Curr Drug Metab*. 2009;10(4):322-328. doi:10.2174/138920009788498996
13. Hickson LJ, Balls-Berry JE, Jaffe AS, Rule AD. Biomarkers associated with progression of diabetic kidney disease: do they hold the same meaning for blacks and women? *J Am Soc Nephrol*. 2018;29(6):1781. doi:10.1681/ASN.2017121249
14. Momper JD, Misel ML, McKay DB. Sex differences in transplantation. *Transplant Rev (Orlando)*. 2017;31(3):145-150. doi:10.1016/j.trre.2017.02.003
15. Schuetz EG, Furuya KN, Schuetz JD. Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms. *J Pharmacol Exp Ther*. 1995;275(2):1011-1018.
16. Prasad B, Evers R, Gupta A, et al. Interindividual variability in hepatic organic anion-transporting polypeptides and P-glycoprotein (ABCB1) protein expression: quantification by liquid chromatography tandem mass spectroscopy and influence of genotype, age, and sex. *Drug Metab Dispos*. 2014;42(1):78-88. doi:10.1124/dmd.113.053819
17. Couto N, Al-Majdoub ZM, Achour B, Wright PC, Rostami-Hodjegan A, Barber J. Quantification of proteins involved in drug metabolism and disposition in the human liver using label-free global proteomics. *Mol Pharm*. 2019;16(2):632-647. doi:10.1021/acs.molpharmaceut.8b00941
18. Chandler B, Detsika M, Khoo SH, Williams J, Back DJ, Owen A. Factors impacting the expression of membrane-bound proteins in lymphocytes from HIV-positive subjects. *J Antimicrob Chemother*. 2007;60(3):685-689. doi:10.1093/jac/dkm230
19. Steiner H, Polliack A, Kimchi-Sarfaty C, Libster D, Fibach E, Rund D. Differences in rhodamine-123 efflux in B-type chronic lymphocytic leukemia suggest possible gender and stage variations in drug-resistance gene activity. *Ann Hematol*. 1998;76(5):189-194. doi:10.1007/s002770050387
20. Yakusheva EN, Titov DS. Structure and function of multidrug resistance protein 1. *Biochemistry (Mosc)*. 2018;83(8):907-929. doi:10.1134/S0006297918080047
21. Hodges LM, Markova SM, Chinn LW, et al. Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). *Pharmacogenet Genomics*. 2011;21(3):152-161. doi:10.1097/FPC.0b013e3283385a1c
22. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of diet in renal disease study group. *Ann Intern Med*. 1999;130(6):461-470. doi:10.7326/0003-4819-130-6-199903160-00002
23. Minderman H, O'Loughlin KL, Pendyala L, Baer MR. VX-710 (biricodar) increases drug retention and enhances chemosensitivity in resistant cells overexpressing P-glycoprotein, multidrug resistance protein, and breast cancer resistance protein. *Clin Cancer Res*. 2004;10(5):1826-1834. doi:10.1158/1078-0432.ccr-0914-3
24. Achour B, Gosselin P, Terrier J, et al. Liquid biopsy for patient characterization in cardiovascular disease: verification against

markers of cytochrome P450 and P-glycoprotein activities. *Clin Pharmacol Ther*. 2022;111(6):1268-1277. doi:10.1002/cpt.2576
25. Cortada CM, Escobar A, Bramuglia GF, Niselman AV, Carballo MA, Rubio MC. Lymphocyte P-glycoprotein variability in healthy individuals. *Medicina (B Aires)*. 2009;69(6):619-624.
26. Szeremy P, Tauberne Jakab K, Barath S, et al. Determination of reference values of MDR-ABC transporter activities in CD3+ lymphocytes of healthy volunteers using a flow cytometry based method. *Cytometry B Clin Cytom*. 2019;96(6):469-474. doi:10.1002/cyto.b.21729
27. Tsujimura S, Tanaka Y. Disease control by regulation of P-glycoprotein on lymphocytes in patients with rheumatoid arthritis. *World J Exp Med*. 2015;5(4):225-231. doi:10.5493/wjem.v5.i4.225
28. Minderman H, Brooks TA, O'Loughlin KL, Ojima I, Bernacki RJ, Baer MR. Broad-spectrum modulation of ATP-binding cassette transport proteins by the taxane derivatives ortataxel (IDN-5109, BAY 59-8862) and tRA96023. *Cancer Chemother Pharmacol*. 2004;53(5):363-369. doi:10.1007/s00280-003-0745-2
29. Brazeau DA, Attwood K, Meaney CJ, et al. Beyond single nucleotide polymorphisms: CYP3A5(*)3(*)6(*)7 composite and ABCB1 haplotype associations to tacrolimus pharmacokinetics in black and white renal transplant recipients. *Front Genet*. 2020;11:889. doi:10.3389/fgene.2020.00889

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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