Published in final edited form as:

Clin Transplant. 2018 December ; 32(12): e13424. doi:10.1111/ctr.13424.

Tacrolimus Trough and Dose Intra-Patient Variability and CYP3A5 Genotype: Effects on Acute Rejection and Graft Failure in European American and African American Kidney Transplant Recipients

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Conflict of Interest: The authors of this manuscript have no conflicts of interest to disclose.

Clinical Trial: Genomics of Transplantation, clinicaltrials.gov (NCT00270712)

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Abstract

Background: Suboptimal immunosuppression after kidney transplantation contributes to toxicity and loss of efficacy. Little is known regarding the impact of intra-patient variability of tacrolimus (TAC) doses and troughs in the early post-transplant period or the influence of genetic variants on variability.

Methods: Coefficients of variation (CV) of TAC troughs and doses of 1226 European American (EA) and 246 African American (AA) adult recipients enrolled in DeKAF Genomics were compared for association with acute rejection and graft failure. Additionally, the influence of recipients' number of CYP3A5 loss-of-function alleles was assessed.

Results: Acute rejection was associated with greater CV of dose in AA ($p < 0.001$) and EA recipients ($p = 0.012$). Graft failure was associated with a greater CV of dose ($p = 0.022$) and trough ($p < 0.001$) in AA, and higher CV of trough ($p = 0.024$) in EA recipients. In EA, CYP3A5 loss-of-function alleles were associated with decreased CV of trough ($p = 0.0042$) and increased CV of dose ($p < 0.0001$).

Conclusion: CYP3A5 loss-of-function alleles influence intra-patient TAC trough and dose variability. High variability of TAC dose increases risk of acute rejection. High variability of TAC trough increases risk of graft failure. Early clinical recognition of TAC dose and trough variability may improve patient management and outcomes.

Keywords

Kidney Transplant; Tacrolimus; Intra-Patient Variability; Acute Rejection; Graft Failure

Introduction

Tacrolimus has a narrow therapeutic window with high inter-patient pharmacokinetic variability, requiring close monitoring of blood trough concentrations (initial goal 8–12 ng/mL) and frequent early dose adjustments to reduce the risk of poor outcomes. Concentrations above the therapeutic range are associated with adverse effects such as nephrotoxicity, while sub-therapeutic concentrations may increase the risk of acute rejection (AR) and graft failure (GF).¹ Many factors have been identified contributing to TAC inter-patient pharmacokinetic variability such as drug-drug interactions, drug-food interactions, changes in hepatic metabolism, and nonadherence.²

Genetic factors are also well-known to account for TAC pharmacokinetics differences between patients and several single-nucleotide-polymorphisms (SNPs) have been associated with TAC troughs, including CYP3A5*3, CYP3A5*6, CYP3A5*7, CYP3A4*22, and possibly POR*28.^{3–6} CYP3A5*3, *6, and *7 variants are loss-of-function alleles and significantly reduce the metabolic clearance of TAC.³ The CYP3A5*3 allele occurs more frequently in European Americans (EA) (~95%) than in African Americans (AA) (~31%), while the *6 and *7 alleles are common in AA (5–12%), but absent in most EA.⁷

Studies on the impact of TAC trough intra-patient variability (mostly obtained in the first year post-transplant) have not been consistent, but generally point towards worse outcomes at higher levels of variability.^{8–20} Older studies showed that intra-patient variability in cyclosporine exposure and trough concentration was associated with poorer outcomes.^{21–28} Borra et al. in 2010 were the first to describe an association between high intra-patient variability in TAC clearance in months 6–12 and a composite endpoint of graft failure.¹⁰ Rodrigo et al. reported that individuals with TAC trough CV >30% at 4–12 months post-transplant were at greater risk of death-censored graft loss and development of de novo donor specific antibodies.¹¹ Vanhove et al. showed that TAC trough CV >22% between 6–12 months post-transplant was associated with more moderate to severe fibrosis and tubular atrophy on biopsy by 2 years.¹⁹ Similar studies showed association between high intra-patient TAC trough variability and acute rejection, decline in glomerular filtration rate, graft loss, or composites thereof.^{12,17,18} A study by Taber et al. of TAC troughs beginning at one month post-transplant showed that high trough variability was associated with a higher risk of acute rejection and graft loss.²⁰ To our knowledge, no study has previously investigated the impact of intra-patient variability in TAC dose on kidney transplant outcomes.

Early acute rejection occurs in less than 10% of kidney transplant recipients by 6 months and in less than 15% by 2 years,²⁹ but is an important risk factor for graft loss.^{30–32} To effectively test the impact of intra-patient variability of TAC dose or trough on rejection, a sufficiently large population is required. We aimed to identify whether early intra-patient TAC variability in troughs and doses is associated with acute rejection and graft failure in kidney transplant recipients enrolled in the Deterioration of Kidney Allograft Function (DeKAF) Genomics study. We also sought to evaluate if CYP3A5 loss-of-function alleles contribute to intra-patient TAC variability. Understanding how much intra-patient variability is tolerable may provide opportunities to improve short- and long-term outcomes.

Methods

Study Population

Subjects were kidney allograft recipients, receiving TAC maintenance in the DeKAF Genomics study, which is a prospective, observational study that enrolled kidney and simultaneous pancreas-kidney transplant recipients transplanted between 2005 and 2011 at five U.S. and two Canadian centers.³³ The study is registered at clinicaltrials.gov (NCT00270712) and approved by the institutional review boards of each center. Informed consent was obtained from all participants. Recipients were included if they were 18 years of age or older at the time of transplant, received TAC maintenance therapy and had at least 5 TAC trough concentrations and corresponding TAC daily doses in the first 6 months after transplant. All recipients were initiated on TAC at time of transplant except for 26 recipients who initially received cyclosporine but were subsequently switched to TAC post-transplant. For another small group of 21 recipients, no baseline calcineurin inhibitor use could be determined but all later receive maintenance TAC immunosuppression.

Participants received immediate release oral TAC and mycophenolate maintenance with varying durations of steroid therapy per transplant center standard-of-care protocols. Induction therapy mainly consisted of Thymoglobulin (Genzyme, Cambridge, MA), Campath (Genzyme, Cambridge, MA) or Simulect (Novartis, East Hanover, NJ). High risk recipients were more likely to receive Thymoglobulin. TAC trough concentrations were measured by liquid chromatography-mass spectrometry from whole blood by each site's clinical laboratory using a Clinical Laboratory Improvement Amendments approved assay. TAC trough measurements and corresponding daily doses were obtained from the medical record. Two measurements were obtained in each of weeks 1 through 8, and two in each of months 3, 4, 5 and 6 for a maximum of 24 trough concentrations per recipient in the first 6 months post-transplant. The mean number of TAC measurements was 17.7 per recipient. These data included inpatient and outpatient measurements. TAC oral doses were adjusted based on trough concentrations to reach institution-specific trough goals (generally 8–12 ng/mL in months 0 to 3 and 6–10 ng/mL thereafter). There were 4292 and 21,807 TAC troughs and corresponding doses in the first 6 months post-transplant for the AA and EA analyses, respectively. Donor and recipient characteristics, clinical factors, and outcomes were obtained from medical records. Acute rejection was defined as biopsy proven or clinically treated T-cell or antibody mediated rejection occurring within the first 6 months post-transplant. All cause graft failure was obtained from the United States Renal Data System and was defined as a composite of return to dialysis therapy, re-transplantation, or death with function.

Genotyping

Pre-transplant recipient DNA was obtained at the time of transplant from peripheral blood lymphocytes after red blood cell lysis and isolation by centrifugation. A customized exome-plus Affymetrix TxArray SNP chip (Affymetrix, Santa Clara, CA) was used to genotype DNA for genetic variants.³⁴ This chip contains approximately 782,000 genetic markers including 168,000 exonic or coding variants and over 16,000 putative loss-of-function variants. The CYP3A5*3 (rs776746), CYP3A5*6 (rs10264272), and CYP3A5*7

(rs41303343) loss-of-function variants were used in this study and taken from this chip. Patients were assigned to either AA or EA subgroups after principal component analysis of ancestry.³⁵

Statistical Analysis

The coefficient of variation (CV) was used as the measure for intra-patient TAC dose and trough variability. The CV was defined as the ratio of standard deviation (SD) to the mean in percent and was calculated for both TAC trough concentrations and corresponding TAC doses. Because most acute rejection events occurred early and within the first 6 months and because the number and timing of obtained measurements was different among recipients, CV for the acute rejection analyses was calculated for each available set of 5 consecutive TAC measurements in the first 6 months after transplant. For example, a recipient with 5 measurements would only contribute one CV value (calculated from measurements 1–5), whereas a recipient with 7 measurements would contribute three CV values (calculated from measurements 1–5, 2–6, and 3–7). For time trends, the date of each calculated CV was set to be the date of the last TAC measurement in the set of 5. For recipients experiencing an acute rejection in the first 6 months post-transplant, none of the TAC doses or troughs after the event were used to calculate any CV. This method resulted in a total of 2975 CV values for TAC trough concentrations in 246 AAs (mean of 12.1 CV values per AA recipient) and 15883 CV values for TAC trough concentrations in 1226 EAs (mean of 13.0 CV values per EA recipient), with equal numbers of CV values for TAC dose in each respective group. Because graft failure events occurred at a time very distant from the TAC measurements, we calculated a single CV per person using all collected TAC measurements for the graft failure analyses. Analyses were performed separately for AA and EA recipients.

We examined the association between CV of TAC doses and TAC trough concentrations with acute rejection occurring in the first 6 months post-transplant in multivariable Cox proportional hazard models, treating CVs as time-dependent variables. Only the CVs prior to rejection were used in subjects with acute rejection. Time to acute rejection was assessed for each patient in a right censored fashion, censoring at acute rejection, death, graft failure, loss to follow-up, or 6 months post-transplant. CV of dose and trough were modeled as bivariate variables (highest quartile vs lower three quartiles). Due to the small number of acute rejection events in the AA group, model selection was based on the EA population and applied to both groups. Other covariates tested were prior kidney transplant, prior non-kidney transplant, simultaneous pancreas-kidney transplant, preemptive transplant, number of HLA mismatches (0, 1 or 2, 3 or 4, 5 or 6), B- or T-cell crossmatch, steroid use at day 14 post-transplant, type of antibody induction, recipient gender, recipient age at transplant, recipient weight at transplant, smoking status, primary cause of kidney disease, living donor status, CMV status, donor age, donor gender, and number of CYP3A5 loss-of-function alleles (0, 1 or 2). After backwards selection with a retention p-value of 0.10, the included covariates for both CV of dose and CV of trough models were simultaneous pancreas-kidney transplantation, number of HLA mismatches, B- or T-cell crossmatch, donor age at transplantation, and number of CYP3A5 loss-of-function alleles. Additionally, preemptive transplantation remained in the CV of dose model, while recipient gender remained in the

CV of trough model. Cox models were stratified by transplant center to control for potentially center difference.

Secondly, we tested for association between CV of TAC doses and trough concentrations with all cause graft failure using Cox proportional hazard models. Mean time to all cause graft failure in EA and AA was 4.21 and 4.58 years, respectively. Time to graft failure was assessed for each patient in a right censored fashion, censoring at death or loss to follow-up. After backwards selection with a retention p-value of 0.10, the included covariates for both CV of dose and CV of trough models were prior kidney transplant, preemptive transplant, and donor age. Additionally, recipient gender remained in the CV of trough model. Cox models were stratified by transplant center.

We also tested for the influence of number of CYP3A5 loss-of-function alleles on both CV of TAC trough and CV of TAC dose. Longitudinal mixed effects models adjusted for transplant center and time post-transplant were used to test for associations between CVs and number of loss-of-function alleles. Random effects for intercept and time trend were used in order to account for correlation among observations from the same recipient as calculated in the acute rejection analysis. Analyses were performed with SAS Statistical Software v9.4 (The SAS Institute, Cary, NC) using $p = 0.05$ as the indication for statistical significance.

Results

TAC Trough Concentration and Dose Variability

We studied 246 AA and 1226 EA transplant recipients. Demographics and clinical factors are shown in Table 1. AA recipients received higher median daily TAC doses and achieved lower median TAC trough concentrations (dose: 8.00 mg/day; trough: 6.80 ng/ml) than EA recipients (dose: 5.60 mg/day; trough: 8.94 ng/ml). The variability of TAC troughs, variability of TAC dose, and acute rejection events by ethnicity and CYP3A5 loss-of-function alleles are summarized in supplemental Table S1. The variability of TAC troughs, variability of TAC dose, and graft failure events by ethnicity and CYP3A5 loss-of-function alleles are summarized in Table S2. CV of TAC troughs was larger for AA recipients (AA 30.17% vs EA 25.18% in AR analyses; AA 42.21% vs EA 33.44% in GF analyses; p -values<0.0001) while the mean CV of TAC dose was larger among EA recipients (AA 11.46% vs EA 16.60% in AR analyses; AA 24.93% vs EA 30.23% in GF analyses; p -values<0.0001). The correlation (r^2) between TAC dose and TAC troughs was weak (EA $r^2=0.033$, $p<0.0001$; AA $r^2=0.0048$, $p=0.0002$).

Acute Rejection

Ninety-seven acute rejections (6.6%) in 1472 recipients occurred in the first 6 months. In AA, rejecters had a median TAC trough concentration of 5.71 ng/mL compared to 6.82 ng/mL in non-rejecters. In EA, rejecters had a median TAC trough concentration of 9.07 ng/mL compared to 8.92 in non-rejecters. Variability of TAC dose was higher in those who experienced acute rejection (Figure 1 and Table S1). In multivariate Cox regression analysis, the highest quartiles of CV of TAC dose (CV>19% for AA; CV>24% for EA) was a

significant risk factor for acute rejection compared to the lower three quartiles in AA (HR: 33.53, $p=0.0001$) and EA (HR: 1.81, $p=0.012$) (Table 3). After controlling for variability in TAC dose, each loss-of-function allele was associated with 84% lower hazard of acute rejection relative to those with no loss-of-function alleles in AA (HR 0.16, $p=0.0015$) while in EAs, the number of loss-of-function alleles was not associated with a change in the hazard of acute rejection (HR 1.51, $p=0.26$) (Figure 2 and Table 3). Variability in TAC troughs did not significantly influence the risk for acute rejection in either of our AA or EA cohorts (all $p>0.15$, data not shown).

Graft Failure

There were 54 (21.8%) graft failures in the AA recipients and 141 (12.8%) in the EA recipients. AAs with graft failure had a median TAC trough concentration of 6.3 ng/mL in the first 6 months compared to 6.6 ng/mL in those without failure. EAs with graft failure had a median TAC trough concentration of 8.2 ng/mL compared to 8.3 ng/mL in those without failure. In multivariate Cox regression analysis, the highest quartiles of CV of TAC dose ($CV>31\%$ for AA; $CV>38\%$ for EA) were significant risk factors for graft failure compared to the lower three quartiles in AA (HR: 2.06, $p=0.022$) but not in EA recipients (HR: 1.39, $p=0.097$) (Table 4a). Variability in the highest quartiles of TAC troughs ($CV>49\%$ for AA; $CV>38\%$ for EA) significantly increased the risk for graft failure in both the AA cohort (HR: 2.95, $p=0.0002$) and the EA cohort (HR: 1.53, $p=0.024$). The number of CYP3A5 loss-of-function alleles did not reach retention thresholds of $p<0.10$ in our final graft failure models.

TAC Variability and CYP3A5 Loss-of-Function Alleles

One or two loss-of-function CYP3A5 alleles were carried by 99.5% of EA recipients whereas 78% of AA carried one or two loss-of-function allele (Table 1). Highest variability in TAC troughs was observed in recipients with no loss-of-function alleles while higher variability in TAC dose was found in recipients with two loss-of-function alleles (Tables S1 and S2). In EA recipients, the number of CYP3A5 loss-of-function alleles was significantly associated with TAC trough variability and dose variability (Table 2b). The reduction in % absolute change of CV of trough with more loss-of-function alleles is due to additional CYP3A5 loss-of-function alleles decreasing the SD of TAC troughs more than trough means, therefore CVs of troughs decreasing overall. Conversely, additional CYP3A5 loss-of-function alleles decreased dose means more than the SD of doses, resulting in an overall increase in % absolute change of CV of dose. In AA recipients, the trends were similar but neither trough or dose variability was significantly associated with CYP3A5 loss-of-function alleles.

Discussion

This study investigated the influence of early intra-patient TAC pharmacokinetic variability in trough and dose on acute rejection and all cause graft failure in a large cohort of kidney transplant recipients. We also characterized the influence of common CYP3A5 loss-of-function variants on variability and acute rejection. Previously, we identified high variability between patients in TAC trough and dose in EA and AA subjects, and showed in a genome

wide association study that CYP3A5*3, *6, and *7 loss-of-function alleles and clinical factors accounted for 53.9% of the observed variability in troughs among AA recipients³ and ~40% of variability among EA recipients.³⁶ Other small studies have associated CYP3A5 genotype with TAC pharmacokinetic variability, although with mixed results.^{37–39} In this study we sought to understand if TAC trough and dose variability were important clinical factors that should be considered in developing tools to improve post-transplant care.

Several papers have been published studying TAC variability. These studies generally had smaller sample sizes ($n < 400$), focused on later TAC measurements (6–12 months post-transplant), did not evaluate variability in TAC dose, and, with the exception of a study by Ro et al., did not assess genetic variables.^{8–20} Previous studies have mostly excluded early TAC measurements because it is presumed that therapeutic drug monitoring is aggressive during this period and that close contact is maintained with the provider, resulting in more stable immunosuppression. In comparison, our study evaluated a larger cohort and focused on early TAC troughs and doses. A more recent study by Taber et al. also included earlier intra-patient TAC trough variability starting at one month post-transplant in a larger cohort and showed that trough variability was higher in AA (CV 39.9%) compared to non-AA recipients (CV 34.8%), which is consistent with our findings.²⁰

Our first aim was to investigate the influence of TAC trough variability on post-transplant outcomes. In our study, we did not find an association between intra-patient TAC trough variability and acute rejection in either cohort, possibly due to our focus on early TAC troughs within the first 6 months post-transplant when induction therapy immunosuppressive effects persist. This is consistent with the findings by Taber et al. that TAC trough variability is not a risk factor for rejection in EA. Their group, however, did find that TAC trough variability was a risk factor for rejection in AA, possibly due to their larger number of AA recipients, where we may have had too few to detect the effect. Our results should not be considered contradictory, but rather complimentary to previous findings, as studies have been widely inconsistent regarding the association between TAC trough variability and AR. Pollock, Hsiao, Ro, Prytula, and Taber (AA cohort) showed association between TAC trough variability and acute rejection,^{13–16} while Schmid, Rodrigo, and Taber (EA cohort) did not.^{8,11,20}

We did, however, find a strong association between early TAC trough variability and all cause graft failure, which is consistent with previous studies.^{10–13} We found that EA recipients with TAC trough variability $>38\%$ and AA recipients with trough variability $>49\%$ were at increased risk (Table 4b). Poor immune control in the early post-transplant period may contribute to worse long-term outcomes. Early immune dysfunction and acute rejection events may mediate immunologic damage, resulting in a slow decline in organ function and graft failure. Additionally, patients who potentially experience high early variability due to poor adherence may remain poorly adherent beyond 6 months after transplant, causing sub-optimal long-term immunosuppression.

Our second aim was to investigate the effects of TAC dose intra-patient variability. We found that high TAC dose variability in the early post-transplant period is associated with a greater risk of acute rejection (Table 3, Figure 1). EA recipients with dose variability $>24\%$ and AA

recipients with dose variability >19% were at increased risk. The wide confidence interval in the AA analysis is likely due to the small number of only 14 rejection events in this group by 6 months post-transplant and should be interpreted with caution. Additionally, we found that high TAC dose variability (CV > 31%) was a significant risk factor for graft failure in the AA population (Table 4a). CVs were overall larger in the GF analyses compared to the AR analyses; however, this stems from the difference in calculation methods rather than from a biological cause.

While close monitoring of troughs and dose adjustments are generally viewed as beneficial, our data suggest that large dose changes may have adverse consequences. High TAC dose variability is likely due to many factors including adherence, drug-drug interactions, toxicities, changing TAC formulations and capsule strengths, and discrepancies in drug regimen. Sicker patients with more comorbidities may require more dose adjustments due to physiological changes or the addition of concomitant medications. In this case, high dose variability may not be causal of rejection, but rather a consequence of a complex post-transplant course. Narrow therapeutic targets (i.e. 8–10ng/mL) relative to a wider target (i.e. 8–12 ng/mL) are often difficult to maintain and likely require repeated dose adjustments to stay within range and consequently result in more dose changes and higher dose variability. Clinicians may also have a tendency towards over-adjusting small fluctuations in observed TAC concentrations. Observed concentrations falling slightly outside the therapeutic target range may not negatively affect a patient's graft, but could trigger dose changes which lead to dose over-adjustments and periods of even greater sub- or supra-therapeutic troughs that occur in the days or weeks before the next trough measurement.

Non-adherence has been described as a major risk for poor kidney transplant outcomes and is often proposed as a cause of high variability in TAC trough concentrations and dose adjustments.^{2,9–14,40–42} However, no easy and accurate methods of measuring compliance in an outpatient setting exist. Our data suggests that large dose changes themselves may lead to worse outcomes and should be made cautiously. A leading premise for the use of once daily TAC products is reduction in TAC variability. The ongoing Astellas ASTOUND trial (NCT02723591) is evaluating the effect of TAC variability of immediate vs extended release formulations on de novo donor specific antibody formation and immune activation in the first 2 years post-transplant and may be helpful in further understanding the role of TAC variability.

Lastly, we aimed to characterize the influence of CYP3A5 variants on variability and outcomes. The number of CYP3A5 loss-of-function alleles was significantly associated with TAC trough and dose variability in EA subjects. Each additional loss-of-function allele reduced the CV of TAC trough concentrations by 1.82% and increased CV of TAC dose by 2.57% (Table 2). Similar trends were observed in AA patients, although neither CV of trough nor CV of dose reached the significance threshold ($p=0.07$ and 0.13 , respectively), possibly due to the smaller size of the AA cohort. These observations, too, may be explained by clinician behavior. Over 90% of EAs carry two copies of the loss-of-function allele and can be described as 'poor metabolizers' of TAC. This "standard" phenotype may be what clinicians are familiar with and what dosing protocols have been developed for. It seems likely that prescribers feel comfortable managing these patients with appropriate dose

adjustments during the fine-tuning of regimens, preventing over- or under-shooting of trough concentrations. In patients without loss-of-function CYP3A5 alleles, TAC metabolism is significantly higher. This leads to the observation of very low TAC troughs despite high TAC doses. Clinicians may be less familiar with the dose-response patterns in these patients, may not “believe” low troughs and may be less inclined to increase the dose appropriately. It is reasonable to conclude that dose adjustments in moderation are needed to achieve optimal immunosuppression, while excessive changes are either reflective of post-transplant complications or of over-adjustments causing prolonged intervals outside the therapeutic range.

In our AA cohort, each CYP3A5 loss-of-function allele was significantly associated with a reduction in the risk of acute rejection (Table 3). Approximately 25% carried no loss-of-function alleles, 50% carried one loss-of-function allele, and 25% carried two loss-of-function alleles. AAs that carry two loss-of-function alleles, have higher TAC troughs and show dose-response patterns more similar to the typical EAs recipient. CYP3A5 loss-of-function alleles were not associated with the risk for graft failure. Determining CYP3A5 loss-of-function status may be important for rejection risk assessment and individualized management of dosing in the early post-transplant course, but less important for long term outcomes.

There are limitations to this work. One of the challenges in the field are the differences in the methods of calculating intra-patient variability, the time periods over which they are studied, and the number of troughs or doses used to estimate the variability, making direct comparisons of variability across published studies difficult. Previous studies have used standard deviation (SD), mean absolute deviation, or coefficient of variation (CV), although Hsiau et al. argue that CV is the superior measurement, as it is more robust than SD calculations.¹⁵ Additionally, previous studies have looked exclusively at single variability values per person. We decided to calculate multiple CVs per person based on each set of five consecutive measurement points in order to better capture time trends of variability in the early post-transplant course when acute rejection is most likely to occur. Conversely, we calculated a single CV per person for our graft failure models, as these events usually happen at much later time points in the post-transplant course, often months after the last measurement is collected.

Our study has several additional limitations. This analysis is based on data from an observational study in which trough measurements and doses were not overseen by study staff. The number and timing of measured TAC trough concentrations and corresponding doses were not standardized to a specific day among recipients, which could have artificially lowered CVs for patients with fewer measurements. However, calculating CV values from each set of five consecutive measurements and accounting for correlation among observations from the same patient in our models allows us to minimize this potential for bias in the AR analyses. Additionally, the relatively small size of our AA cohort and the low frequency of acute rejection events in this group may have influenced some of our results, specifically inflating the hazard ratio of high variability in TAC dose on acute rejection risk and limiting the statistical significance of the association trends between CVs and CYP3A5 loss-of-function alleles. Drug-food interactions, diarrhea, and non-adherence have been

reported to contribute to intra-patient variability in TAC trough concentrations.² These factors were not included in our models and may have affected variability. Drug-drug interactions are also known to affect variability and we controlled for steroid use. However, the goal of this study was not to identify all the factors related to variability but to study if variability in trough concentration and dose adversely affects transplant outcomes. Lastly, our study did not consider rarer genetic variants, such as CYP3A4*22 or POR*28, which have been shown to influence TAC metabolism to a lesser extent.⁴⁻⁶

Conclusion

This is a large study assessing the influence of early intra-patient TAC pharmacokinetic and dose variability on acute rejection and all cause graft failure in a cohort of kidney transplant recipients. We showed that intra-patient trough variability is higher in AAs compared to EAs. High CV of dose >24% and >19% is associated with greater hazard of early acute rejections in EA and AA, respectively. CV of TAC trough >49% and >38% is associated with greater hazard of graft failure in AA and EAs recipients, respectively. In AA, CYP3A5 loss-of-function variants are associated with lower risk of rejection but not with graft failure. While a well designed and implemented therapeutic drug monitoring plan should be standard practice for transplant patients, our findings illustrate a need for the development of new and proactive tacrolimus dosing strategies that minimize extreme dose and trough variation. Assessment for variability should be incorporated into routine therapeutic drug monitoring and relevant genotypes should be considered. Recipients with high variability could be offered more surveillance and additional patient education, and identifying the source of variability could improve clinical outcomes. Future studies should evaluate the effect of TAC variability on BK virus development, donor specific antibody formation, and nephropathy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge the contributions of our generous patients. We also acknowledge the dedication and hard work of our coordinators at each of the DeKAF Genomics clinical sites: University of Alberta, Nicoleta Boboccea, Tina Wong, Adrian Geambasu and Alyssa Sader; University of Manitoba, Myrna Ross and Kathy Peters; University of Minnesota, Mandi DeGrote, Monica Meyers and Danielle Berglund; Hennepin County Medical Center, Lisa Berndt; Mayo Clinic, Tom DeLeeuw; University of Iowa, Wendy Wallace and Tammy Lowe; University of Alabama, Jacquelin Vaughn, Valencia Stephens and Tena Hilario. We also acknowledge the dedicated work of our research scientists Marcia Brott and Amutha Muthusamy. This study was supported in part by NIH/NIAID grants 5U19-AI070119 and 5U01-AI058013.

Funding:

This project was supported by grants (U19-AI070119 and U01-AI058013) from the National Institute of Allergy and Infectious Disease (AM, PJ, AI, WO) and by a Melendy Student Research Scholarship, University of Minnesota, College of Pharmacy (SS). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or National Institute of Allergy and Infectious Disease.

Abbreviations:

AA	African American
AR	Acute Rejection
CI	Confidence Interval
CMV	Cytomegalovirus
CV	Coefficient of Variation
CYP	Cytochrome P450
DeKAF	Deterioration of Kidney Allograft Function
EA	European American
GF	Graft Failure
HLA	Human Leukocyte Antigen
HR	Hazard Ratio
IQR	Inter-Quartile Range
POR	P450 Oxidoreductase
SD	Standard Deviation
SNP	Single-Nucleotide Polymorphism
TAC	Tacrolimus

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Figure 1.

TAC trough and dose variability in patients with and without acute rejection. A) CV of TAC trough concentrations in AA. B) CV of TAC trough concentrations in EA. C) CV of TAC dose in AA. D) CV of TAC dose in EA.

Figure 2.

Kaplan-Meier curves for acute rejection in AA patients (A) and EA patients (B) according to number of loss-of-function alleles. Only six European Americans had no loss-of-function variants and none had an acute rejection event.

Table 1.

Recipient Demographics and Clinical Characteristics

Variable	Number with Characteristic or Median (% or range)	
	African American (n = 246)	European American (n = 1226)
Male Recipient	162 (65.9)	774 (63.1)
Age at Transplant, years	48 (20 – 72)	52 (18 – 83)
Weight at Transplant, kg	84.0 (44.2 – 137.0)	83.7 (39.0 – 158.0)
Cause of Kidney Disease		
Diabetes	75 (30.5)	382 (31.2)
Hypertension	107 (43.5)	81 (6.6)
Glomerular Disease	23 (9.3)	280 (22.8)
Polycystic Kidney Disease	11 (4.5)	205 (16.7)
Other (or unknown)	30 (12.2)	278 (22.7)
Donor Age at Transplant, years	35 (6 – 70)	42 (1 – 76)
Deceased Donor Status	180 (73.2)	426 (34.7)
Preemptive Transplant	11 (4.5)	464 (37.8)
Simultaneous Kidney/Pancreas	13 (5.3)	109 (8.9)
Previous Transplant		
Kidney	20 (8.1)	207 (16.9)
Non-Kidney	14 (5.7)	206 (16.8)
HLA Mismatches		
0	17 (6.9)	156 (12.7)
1 or 2	19 (7.7)	218 (17.8)
3 or 4	110 (44.7)	478 (39.0)
5 or 6	100 (40.7)	372 (30.3)
Unknown	0 (0)	2 (0.2)
PRA Positive	89 (36.2)	626 (51.2)
B- or T-Cell Crossmatch	19 (7.7)	85 (6.9)
Antibody Induction[†]		
Monoclonal	122 (49.6)	463 (33.7)
Polyclonal	116 (47.2)	673 (54.9)
Combination	4 (1.6)	34 (2.8)
None	4 (1.6)	56 (4.6)
Calcineurin Inhibitor at Baseline		
Tacrolimus	232 (94.3)	1193 (97.3)
Cyclosporine [‡]	3 (1.2)	23 (1.9)
None (or unknown)	11 (4.5)	10 (0.8)
Steroids at Day 14	119 (48.4)	795 (64.9)
CMV		
Neg. Recipient, Neg. Donor	15 (6.1)	302 (24.6)

Variable	Number with Characteristic or Median (% or range)	
	African American (n = 246)	European American (n = 1226)
Neg. Recipient, Pos. Donor	28 (11.4)	225 (18.4)
Pos. Recipient	192 (78.0)	657 (53.6)
Unknown	11 (4.5)	42 (3.4)
No. of CYP3A5 LOF Alleles (*3, *6, *7)		
0	54 (22.0)	6 (0.5)
1	126 (51.2)	163 (13.3)
2	66 (26.8)	1057 (86.2)
TAC in Months 0–6 posttransplant		
Daily Dose (mg/day)	8.00 (1.00 – 28.00)	5.60 (0.42 – 34.00)
Trough Concentration (ng/mL)	6.80 (1.00 – 23.50)	8.94 (1.04 – 37.22)

[†] Monoclonal antibodies included muromonab-CD3, alemtuzumab, rituximab, basiliximab, and daclizumab. Polyclonal antibodies included antithymocyte globulin (rabbit or equine).

[‡] Subjects later converted to tacrolimus.

HLA – Human Leukocyte Antigen, PRA – Panel Reactive Antibody, CMV – Cytomegalovirus, LOF – Loss-of-Function

Table 2.

Change in TAC Trough and Dose Variability per Additional CYP3A5 Loss-of-Function Allele (*3, *6 or *7)[†]

Variability	African American Recipients		European American Recipients	
	Absolute Change, % (95% CI)	P	Absolute Change, % (95% CI)	P
CV of Trough Concentration	−1.61 (−3.36 to 0.14)	0.07	−1.82 (−3.06 to −0.57)	0.0042
CV of Dose	1.09 (−0.30 to 2.49)	0.13	2.57 (1.31 to 3.84)	< 0.0001

[†] Adjusted for enrolling center and time post-transplant. CV was calculated as described in methods for acute rejection.

Table 3.

Multivariate Model of CV of TAC Dose for Acute Rejection

Variable	African American		European American	
	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
CV of TAC Dose (Highest Quartile) [†]	33.53 (5.54 to 202.85)	0.0001	1.81 (1.14 to 2.86)	0.012
Number of CYP3A5 Loss-of-Function Alleles	0.16 (0.05 to 0.49)	0.0015	1.51 (0.74 to 3.11)	0.26
No. of HLA mismatches		0.85		0.0073
1 or 2	0.30 (0.01 to 6.42)		3.15 (0.87 to 11.37)	
3 or 4	0.37 (0.04 to 3.71)		3.64 (1.11 to 11.96)	
5 or 6	0.39 (0.03 to 4.30)		5.95 (1.82 to 19.41)	
B- or T-Cell Crossmatch	3.01 (0.46 to 19.65)	0.25	2.25 (1.13 to 4.50)	0.022
Donor Age at Transplant	1.01 (0.97 to 1.06)	0.65	1.02 (1.00 to 1.04)	0.039

[†]Highest CV quartile is >19% for AA and >24% for EA. CV was calculated as described in methods for acute rejection.

Table 4a.

Multivariate Model of CV of TAC Dose for Graft Failure

Variable	African American		European American	
	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
CV of TAC Dose (Highest Quartile) [†]	2.06 (1.11 to 3.84)	0.022	1.39 (0.94 to 2.04)	0.097
Prior Kidney Transplant	1.00 (0.39 to 2.57)	0.99	1.84 (1.24 to 2.72)	0.0025
Preemptive Transplant	N.D. [‡]		0.53 (0.37 to 0.78)	0.0012
Donor Age at Transplant	1.00 (0.98 to 1.02)	0.99	1.02 (1.01 to 1.03)	0.0024

[†] Highest CV quartile is >31% for AA and >38% for EA. CV was calculated as described in methods for graft failure.

[‡] Insufficient numbers for analysis.

Table 4b.

Multivariate Model of CV of TAC Trough for Graft Failure

Variable	African American		European American	
	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
CV of TAC Trough (Highest Quartile) [†]	2.95 (1.67 to 5.23)	0.0002	1.53 (1.06 to 2.20)	0.024
Prior Kidney Transplant	0.92 (0.35 to 2.39)	0.86	1.87 (1.26 to 2.78)	0.0018
Preemptive Transplant	N.D. [‡]		0.56 (0.38 to 0.82)	0.0026
Donor Age at Transplant	1.00 (0.98 to 1.03)	0.72	1.02 (1.01 to 1.04)	0.001
Male Gender Recipient	0.61 (0.34 to 1.09)	0.095	1.46 (1.01 to 2.11)	0.0042

[†]Highest CV quartile is >49% for AA and >38% for EA. CV was calculated as described in methods for graft failure.

[‡]Insufficient numbers for analysis