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# Novel Polymorphisms Associated with Tacrolimus Trough Concentrations: Results from a Multicenter Kidney Transplant Consortium

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## Abstract

**Background—**The CYP4503A5*1 genotype is associated with lower tacrolimus concentrations. Although its effect is important, it incompletely explains the variability in tacrolimus concentrations and has a relatively low minor allele frequency in Caucasians relative to African Americans (AA).

**Methods—**We studied clinical and recipient genetic correlates of dose-normalized tacrolimus troughs (n=12,277) in the first 6 months posttransplant using a customized single nucleotide polymorphism chip with 2,722 variants in a large, ethnically diverse (144 AA and 551 non-AA) adult kidney transplant population through a 7-center consortium.

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**Results—**Over the 6 month study, AAs had consistently lower median (interquartile range)

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troughs than non-AAs, 6.2 (4.4–8.4) vs 8.3 (6.4–10.4) ng/mL ($p<0.0001$), in spite of 60% higher daily doses, 8 (5–10) vs 5 (4–7) mg ($p<0.0001$). The median tacrolimus trough concentration in week one posttransplant was particularly low in AAs [2.1 (1.2–3.5)] compared to non-AAs [5.0 (3.1–8.2) ng/mL]($p<0.0001$) despite similar initial doses. In single variant analysis, CYP3A5*3 (rs776746) was the top variant ($p=2.4x10^{-33}$) associated with troughs. After adjustment for CYP3A5*3, clinical factors and race, thirty-nine additional variants were identified ($p<0.01$, not significant at FDR 20%). In the final multivariant, regression models beginning with these variants and clinical factors, 7 variants were identified in the non-AA and 7 variants in the AA group towards the first trough concentrations. Rs776746 (CYP3A5), rs2239393 (COMT) and diabetes were the only factors common in both populations.

**Conclusion—**We identified variants beyond CYP3A5*3 which may further explain pharmacokinetic variability of tacrolimus and demonstrated that important variants differ by race.

### Keywords

tacrolimus; pharmacogenetics; cytochrome P450; pharmacokinetics

## INTRODUCTION

Effective immunosuppression is essential for organ transplantation and recent improvements in outcomes have been due largely to advances in drug therapy. However, immunosuppression still fails in some while causing toxicity in others. Studies have reported that genetic variation is associated with immunosuppressant pharmacokinetics, toxicity and outcomes after transplantation.(1–6) Pharmacogenetic findings have not been adopted into clinical practice, in part due to the unclear impact on clinical outcome, conflicting findings and modest variant effects.

Tacrolimus is the most commonly used calcineurin inhibitor.(7) It is metabolized by CYP3A in the gut and liver, and transported in the gut by P-glycoprotein (P-gp), an efflux pump, which is encoded by the multidrug resistant protein (MDR1)/ABCB1 gene.(8–12) Tacrolimus has a narrow therapeutic index necessitating therapeutic monitoring of blood concentrations.(13–15) There is high interpatient variability in tacrolimus concentrations and the dose required to achieve the therapeutic range; therefore, there is intensive interest in using genetics to optimize dosing.

The influence of variants on tacrolimus metabolism has been extensively studied and recently reviewed.(16–17) One or more CYP3A5*1 alleles (rs776746) results in higher clearance, lower concentrations, higher dose requirements and delayed time to therapeutic concentrations.(18–30) Despite the clear differences between the CYP3A5*1 and *3 genotypes, they explain up to 45% of the variability in dose(31) and 30% of clearance variability(32) suggesting additional determinants of drug disposition. CYP3A4 variants have been studied with conflicting results.(18, 21, 23–24) Most studies have shown that the MDR1 variants have small or no effects on tacrolimus pharmacokinetics.(18, 21, 23–25, 29, 31, 33–36) To date these variants do not explain enough variation in metabolism thereby limiting the enthusiasm for clinical use. To directly address these issues we conducted an evaluation of 2,722 genetic variants towards tacrolimus pharmacokinetics in 695 kidney transplant recipients. Identification of the important variants will allow for the development of dosing equations to individualize therapy.

# RESULTS

## Transplant Recipients and Tacrolimus Trough Measurements

Recipient characteristics are shown in Table 1. A total of 12,277 tacrolimus trough concentrations were studied (Table 1) in 7 centers in an ethnically diverse population. Tacrolimus oral doses and troughs varied between centers. The median (interquartile range) daily dose (mg) over the 6 month study period in centers 1–7 were 8 (5–11), 5 (4–6), 4 (3–6), 6 (4–8), 6 (4–8), 6 (4–8) and 6 (4–8), respectively. The trough concentrations (ng/mL) in centers 1–7 were 9.5 (8.0–11.4), 10.5 (7.4–13.0), 7.9 (6.1–10.1), 9.7 (7.5–12.2), 8.9 (7.1–10.6), 9.1 (7.0–11.3) and 6.6 (4.7–8.7), respectively. Troughs increased over the first 2 weeks and then stabilized (Figure 1). The first trough concentration, measured within 4 days after transplantation, was <5 ng/mL in most subjects.

Figure 1 shows the differences in troughs between African Americans (AA) and non-AAs in the first 6 months posttransplant. The first trough in the AA group was a median (interquartile range) of 2.1 (1.2–3.5) ng/mL with a daily tacrolimus dose of 4.0 (4.0–5.0) mg/day. In contrast, the non-AA group achieved a higher first trough (5.0 [3.1–8.2] ng/mL) despite a similar daily dose (4.0 [4.0–6.0] mg/day). Although the first trough was low in both groups, more AAs had troughs <8 ng/mL (96.6%) than non-AA (73.9%)($p<0.0001$). Over the 6 month study period, AAs received a 60% higher median daily dose ($p<0.0001$) but yet achieved median troughs that were 2.1 ng/ml lower ($p<0.0001$) than non-AA recipients (Table 1).

## Clinical Factors Associated With Tacrolimus Trough Concentrations

Clinical factors (AA vs non-AA, enrolling center, recipient gender, age, donor type, diabetes at transplant, time of trough posttransplant, calcium channel blocker (CCB), ACE inhibitor and corticosteroid use at time of trough, creatinine clearance (CrCl) nearest trough and acute rejection±14 days of trough) were explored for their association with log transformed dose-normalized tacrolimus trough concentrations. Race($p<0.0001$), center($p<0.0001$), gender($p<0.0001$), age($p<0.0001$), diabetes($p=0.003$), CCB($p=0.04$) use and time of trough measurement($p<0.0001$) were associated with troughs and ACE inhibitor use neared significance ($p=0.07$). AA race and the first three troughs measurements were each associated with lower trough concentrations whereas male gender, diabetes, and CCB use were associated with higher troughs. AA race was associated with a reduction in log transformed dose-normalized trough of 0.04 relative to non-AAs. Male gender, diabetes and CCB use each were associated with an increase in log transformed dose-normalized troughs of 0.17, 0.12 and 0.03, respectively. The effect of age was quadratic, with higher troughs between 45 and 75 years with a maximum at 60 years.

## Single Variant Analysis For Association with Tacrolimus Trough Concentrations

The CYP3A5 variant (rs776746, A=*1, G=*3) was the most important variant associated with troughs after adjustment for clinical factors. Dose requirements were higher and troughs lower in individuals with one or two A alleles. The presence of one A allele was associated with a 36% reduction in log transformed dose-normalized troughs (−0.44±0.03 [SE]) and a 59% reduction if two A alleles were present ($p=2.4x10^{-33}$, Figure 2). Median (interquartile range) tacrolimus daily doses and troughs for the 3 genotype groups were as follows: 8(6–10) mg and 5.8(4.1–7.9) ng/mL for *1/*1, 7(5–10) mg and 7.1(5.1–9.3) ng/mL for *1/*3, and 4.5(3–6) mg and 8.4(6.5–10.5) ng/mL for the *3/*3 genotype. Dose-normalized troughs (ng/mL per mg/kg) were 54.3(37.9–80.3), 77.8(53.1–114.6) and 141.1(91.7–214.5) in those with CYP3A5*1/*1, *1/*3 and *3/*3 genotypes, respectively. The CYP3A5*1 allele was more frequent in AA (minor allele frequency (MAF)=0.65) than non-AA recipients (MAF=0.08). Fifty variants, besides CYP3A5*1, were significant

towards dose-normalized troughs after controlling for the false discovery rate (FDR) at 20% and were primarily variants in CYP3A5, CYP3A7, CYP3A4 and CYP3A43 genes (variants not shown). These variants are in the same chromosomal region with linkage disequilibrium with rs776746; therefore, all further analyses were adjusted for rs776746.

The top variants, after adjustment for rs776746 and the significant clinical factors, towards troughs are shown in Table A, Supplemental Digital Content 4, http://links.lww.com/TP/A325. No variants met significance after controlling the FDR at 20% although 39 variants achieved a p-value<0.01. The most frequently represented variants were from the CYP, COMT, MSH, ATF, XRCC5 and FMO genes. Variants within the CYP3A gene, CYP3A4 (rs7801671, rs12114000, rs2687117), CYP3A7 (rs2687080, rs776740) and CYP3A5 (rs10264272) were associated with 0.18 to 0.28 increase in log transformed dose-normalized troughs (20–32 % increase in dose-normalized trough). The CYP3A variants had higher MAF in the AA population (9–32%) than the non-AA (≤0.2%) and large effects on trough concentrations (0.18–0.28 for one allele). We previously described no association between variants and acute rejection, graft survival and survival.(37)

### Multiple Variant Analysis For Association with Initial Tacrolimus Trough Concentrations

Multiple regression models for the first trough in week one were developed using the important clinical factors, CYP3A5 (rs776746) and the 39 variants from Table A (Supplemental Digital Content 4, http://links.lww.com/TP/A325). Final models were developed separately for all subjects, for non-AA and for AA (Table 2). The models explained 45.71%–52.54% of total variability. Center was important, explaining 19.19%–37.27% of variation. CYP3A5 was significant, accounting for 2–6% of variation. Six additional variants were significant in each of the AA and non-AA groups each explaining 2–6% of variation. CYP3A5 and COMT (rs2239393) variants overlapped between the groups. In AAs three variants (genes CYP3A4, CNAP1, FASTK) had large effect sizes (≥0.38). Diabetes was an important factor explaining ~2% of the variation. The were confirmed by bootstrapping and support their association with trough concentrations (see Appendix 1, Supplemental Digital Content 1, http://links.lww.com/TP/A322) although the AA model was relatively less stable compared to the full data.

## DISCUSSION

Tacrolimus is metabolized by CYP3A enzymes to form active and inactive metabolites.(11, 38–40) In vitro, the CYP3A5 enzyme has twice the intrinsic tacrolimus clearance of CYP3A4.(41) In hepatic enzymes from CYP3A5*1 allele carriers, 60% is metabolized through CYP3A5. Transplant recipients carrying CYP3A5*1 allele(s) have higher clearance, lower concentrations and delayed time to therapeutic concentrations.(18, 20, 22–23, 25–32) We confirmed CYP3A5*1 is an important variant and is associated with a reduction in troughs and explained only 2–6% of total variation in troughs. Other variants (including CYP3A4) explained equal or more of total variation (Table 2). This is consistent with previous data where variants each accounted for 3–9% of variability in tacrolimus clearance.(32) Variants in the COMT and CYP3A4 genes were important in our analysis. Catechol-O-methyltransferase (COMT) enzymes are responsible of O-methylation of dopamine, epinephrine and norepinephrine; and involvement in tacrolimus metabolism has not been described whereas variants in the CYP3A4 are relevant, particularly in AAs.(42) Additional variants (MSH, ATF, XRCC5 and FMO) are also potentially involved. Multigene involvement in may explain the findings of the prospective CYP3A5*1 guided tacrolimus dosing trial where genotype adapted dosing resulted in only 43.2% of subjects achieving the trough target (vs 29.1% control group, p=0.03)(43) Additional variants in the dosing model may improve genotype dosing. We found no association between the ABCB1 variants and troughs; consistent with published data.(28–29, 31)

The variants were different in the AA and non-AAs (Table 2) and were only identifiable when the races were analyzed separately. Several variants identified in the AAs had large effect sizes and may in part explain the differences in troughs between races. Warfarin pharmacogenetic studies have shown that clinical factors, effect sizes and pertinent genotypes differ by race.(44–45) We found that early troughs were low in both groups. The non-AA group quickly achieved troughs >8 ng/mL whereas the AAs median trough remained <8 ng/mL(Figure 1) despite 60% higher doses. Lower troughs and requirement for higher doses in AAs have been described.(38, 46–48) The low troughs may be due to high clearance due to CYP3A5*1 and/or other variants and inadequate starting dose; although, this does not sufficiently explain the persistently low troughs in AAs over the first 6 months. It is possible that the high degree of genetic diversity in the CYP3A or others, and the large effect size may result in longer periods of trial and error dosing. In a recent study, the intersubject pharmacokinetic variability in tacrolimus area under the curve was 41.1% higher in patients with CYP3A5*3/*3 compared to *1/*1 or *1/*3 suggesting that patients with variants are more difficult to manage.(49)

Center was an important factor towards troughs explaining 19–37% of the total variability. The effect of center is confounded by genotype (more AAs carry CYP3A5*1) and race (AAs were mostly from one center). Center effects are also possible given the heterogeneity in concomitant medications, timing of troughs, environment and diet. We found that diabetes at was important towards troughs with an effect as large as some variants. However, in a small study of 11 diabetics and 9 nondiabetics the rate of tacrolimus absorption was slowed in diabetics but the extent of absorption and troughs were unchanged. (50) Other factors influence tacrolimus pharmacokinetics including route of administration, hematocrit and hepatic function and should be evaluated in future studies.(8, 32, 51–52)

Drug interactions pose a dilemma in pharmacogenetic studies.(53–59) We found that the concomitant administration of CCBs, ACE inhibitors and corticosteroids were not significant towards troughs in the final models. Only class of concomitant medication was collected in our study. CCBs are not equipotent in their inhibitory activity towards tacrolimus and it is possible that the effect of CCBs with potent CYP3A inhibitory effects, such as diltiazem, are underestimated. The interaction between corticosteroids and tacrolimus is controversial.(53, 56, 58, 60–61) It is possible that high doses of corticosteroids may induce metabolism.(53) We did not collect corticosteroid dosing information and are unable test this possibility. We have no information on concomitant administration of antifungals which may be pertinent.(62–63)

To our knowledge, this is first pharmacogenetic study to explore a broad panel of variants (n=2722) and the largest group of AAs studied towards tacrolimus troughs. We confirmed that CYP3A5 is important and identified additional variants with effects as large or larger than CYP3A5. Trough concentrations are lower in AAs compared to non-AA subjects and therefore require higher tacrolimus doses. Variants important towards troughs differed between the AA and non-AA subjects. This study provides the data necessary to now develop dosing equations that use genotype and relevant clinical variables instead of weight to determine initial doses. These variants can now be validated in independent groups.

## MATERIALS AND METHODS

### Study Design

This is a 7-center, prospective, observational study in 695 subjects to identify novel variants associated with tacrolimus troughs using a customized SNP chip. Transplant recipients were recruited from the first 1000 subjects of the prospective arm of the Long-term Deterioration of Kidney Allograft Function (DeKAF) study, which is designed to characterize the causes

of late allograft failure.(64–66) The study is registered at www.clinicaltrials.gov (NCT00270712). Transplant recipients were eligible if they were undergoing kidney or simultaneous kidney-pancreas transplantation, ≥18 years of age and to receive tacrolimus. Subjects were enrolled at time of transplant and signed informed consents approved by the Institutional Review Boards.

Tacrolimus trough concentrations, measured prior to an oral dose, were obtained as part of clinical care for the first 6 months posttransplant. Two measurements, if available, were obtained in each of weeks 1–8 and in each of months 3, 4, 5 and 6 for a maximum of 24 measurements per patient. Tacrolimus doses were adjusted to achieve individual institutional trough targets but in general were 8–12 ng/mL in months 0–3 and 6–10 ng/mL in months 4–6. Troughs were normalized for dose by taking the ratio of the trough concentration (ng/ml) by the total daily tacrolimus dose (mg/kg). Dose in mg/kg was used since this is the common clinical method for dose determination. Tacrolimus concentrations (n=12,277) were measured from whole blood by each institutions preferred analytical technique. Liquid chromatography-mass spectroscopy was used to measure 97.1% concentrations.

All participants received a tacrolimus based immunosuppressive regimen with mycophenolate, steroids, with and without antibody induction per transplant center preference. Donor and recipient characteristics, race, serum creatinine (SCr), and estimated CrCl(67), concomitant medications at time of each trough, acute rejection as diagnosed by the treating physician were obtained from the medical record.

## Genotyping

Pretransplant recipient DNA was isolated from lymphocytes obtained from blood after RBC lysis. Genotyping was done using a customized Affymetrix GeneChip (Affymetrix, Santa Clara, CA).(68–69) Additional variants were genotyped using SNPlex (Applied Biosystems, Foster City, CA) and Sequenom (Sequenom, San Diego, CA). The variants were within genes in pathways associated with immunity, cell cycle, signaling, growth, proliferation, differentiation, movement, structure and death, inflammation, hematologic systems, and ~700 variants related to drug absorption, disposition, metabolism and excretion. Validated, functional polymorphisms including non-synonymous variants with a MAF >5%, and variants within conserved (in humans and mouse) transcriptional regulatory regions were chosen. In the absence of functional variants, intragenic tagging variants were used. Genotyping is described in (see Appendix 3, Supplemental Digital Content 3, http://links.lww.com/TP/A324).

Data quality was assessed by negative controls and duplicate samples (3% on Affymetrix, 7% SNPlex, and 1% Sequenom). On Affymetrix, duplicate samples from 31 individuals were genotyped with >99% concordance. Variants with concordance <90% and calls <60% were excluded. Twenty variants were run on multiple platforms and had a concordance rate of >97% and with calls >82%. The Hardy-Weinberg equilibrium assumption was tested by $\chi^2$ analysis and variants that deviated (p-value < $1x10^{-6}$) were removed from the analysis. Variants were excluded from analysis if the MAF was <5% in both the AA and non-AAs. The final analysis included 2,552 variants from Affymetrix, 165 from SNPlex and 7 from Sequenom (see Appendix 2, Supplemental Digital Content 2, http://links.lww.com/TP/A323).

## Association Testing of Variants Towards Clinical Factors and Trough Concentrations

The importance of clinical factors towards log transformed dose-normalized troughs over the first 6 months posttransplant was evaluated, with no variants, using a general linear model with time trough obtained posttransplant (1 to 24) as a categorical variable and a

covariance structure to account for correlation between repeated measurements on the same person. Factors explored were recipient race (AA vs. non-AA), age, gender, donor type (living or deceased), diabetes at transplant and center. We explored time varying covariates such as CCBs, ACE inhibitor and corticosteroid use at time of trough, acute rejection episode±14 days of the trough and CrCl nearest the trough. A number of model covariance structures (autoregressive, Toeplitz and compound symmetry) were considered. The structure which best fit the data, by the Akaike information criterion, in a model with all of the potential covariates listed above, was the Toeplitz covariance structure, which allows the correlation to decline slowly and non-uniformly over time. This structure was used for all models. Factors with F-test $p<0.15$ in backward selection were retained.

Each variant was first tested for association with the log transformed dose-normalized troughs obtained over the 6 months using a general linear model with time posttransplant (1 to 24) as a categorical variable and a Toeplitz covariance structure, using an additive genetic model. The analyses were adjusted for center and the clinical factors identified above. For variants where the MAF was >5% in non-AA and AAs, all subjects were used to estimate the variant effect. For variants where the MAF in one race was <5%, potentially resulting in unreliable estimates, the effect was estimated using the other race where the MAF was >5%. Since the subgroup analyses have somewhat less power than the analysis in all subjects, this procedure improves reliability at the cost of underestimating the significance of those variants. Rs776746 was the top variant and all further analyses were adjusted for it. This study aimed to identify novel variants and multiple testing was taken into account by controlling the FDR at 20% which is common for these type of studies. P-values for variant association were ordered in increasing order and denoted by $P_{(1)}, \ldots, P_{(m)}$. They were considered significant if below a FDR of 20%, i.e., $P_{(k)} < 0.2k/m$. We used an effective number of variants $m = 2110$, which was computed based on linkage disequilibrium between all variants.(70)

Pharmacogenetic testing is most useful at the beginning of therapy and the effect of variants and clinical factors on the first log transformed dose-normalized trough (within the first 4 days posttransplant) was evaluated through multiple variant analysis using linear regression models. The significant factors identified initially (center, rs776746 and variants in Table A, Supplemental Digital Content 4, http://links.lww.com/TP/A325) were considered for entry into the model. Stepwise selection was used with the criterion for entry set at p-value <0.15 and removal at >0.10. The reliability of the final regression models were confirmed by bootstrap resampling (see Appendix 1, Supplemental Digital Content 1, http://links.lww.com/TP/A322).

Troughs and daily doses were compared between AA and non-AAs using nonparametric Wilcoxon-Mann-Whitney. Analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

**FDR** false discovery rate – statistical method to correct for multiple comparisons. For a FDR of 20%, as used in this paper, we would expect no more than 20% false positives among the variants that are declared as significant

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## Participating Centers

Participating transplant centers were University of Alberta, Edmonton, Canada; University of Manitoba, Winnipeg, Canada; University of Minnesota, Minneapolis, MN; Hennepin County Medical Center, Minneapolis, MN; Mayo Clinic, Rochester, MN; University of Iowa, Iowa City, IA; and University of Alabama, Birmingham, AL.

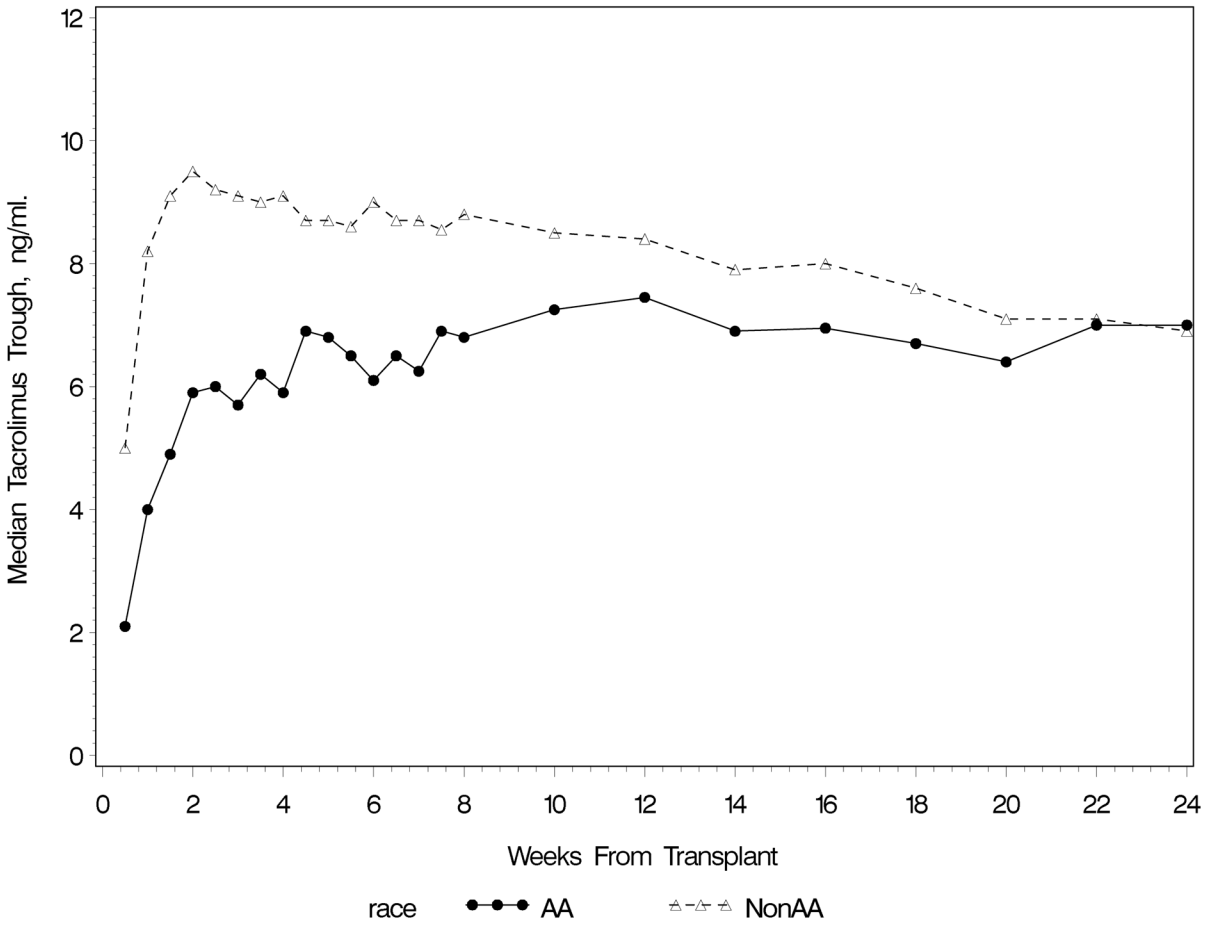


**Figure 1.**
Median Tacrolimus Trough Concentrations In the First 6 Months Posttransplant by Race

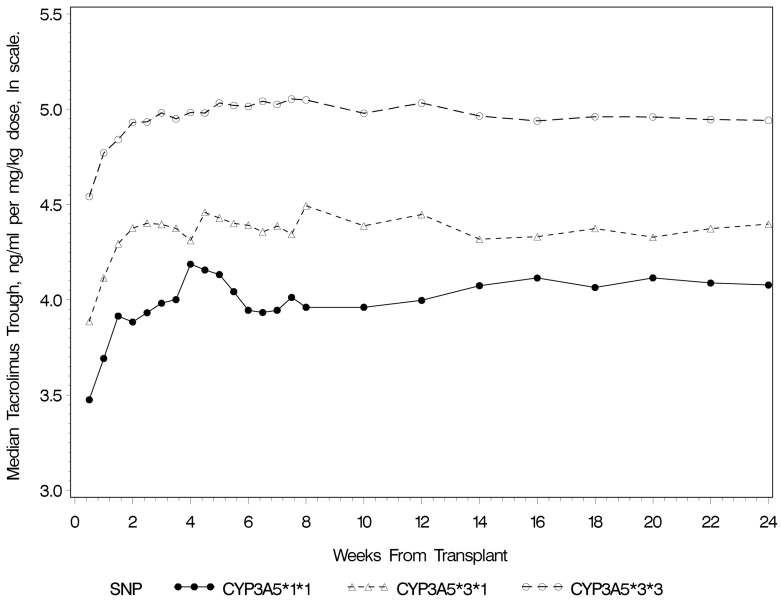


**Figure 2.**
Median Log Transformed Dose-Normalized Tacrolimus Trough Concentrations by CYP3A5 Genotype Status (rs776746)

## Table 1

Characteristics, Tacrolimus Concentrations and Doses of Study Subjects

| Characteristics | All subjects (n=695) | nonAfrican American (n=551) | African American (n=144) |
|---|---|---|---|
| Male recipient, n (%) | 439 (63.2%) | 346 (62.8%) | 93 (64.6%) |
| Age at transplant (yrs)[a] | 50.4 (20.1–81.9) | 50.9 (201.-81.9) | 47.2 (20.3–72.9) |
| Weight at time of transplant (kg)[a] | 80.9 (37.7–151.7) | 80.7 (37.7–151.7) | 81.0 (42.3–133.0) |
| Living donor | 405 (58.3%) | 361 (65.5%) | 44 (30.6%) |
| Diabetes at time of transplant | 276 (39.7%) | 222 (40.3%) | 54 (37.5%) |
| Race/Ethnicity[b] (n) | | | |
| Caucasian | 513 | 512 | 1 |
| African-American | 144 | | 144 |
| Asian | 22 | 22 | -- |
| Indian | 13 | 13 | -- |
| Hawaiian | 2 | 2 | -- |
| Hispanic Ethnicity | 9 | 8 | 1 |
| Primary cause of kidney disease (n) | | | |
| Diabetes | 225 | 183 | 42 |
| Glomerular disease | 136 | 124 | 12 |
| Hypertensive nephrosclerosis | 100 | 37 | 63 |
| Polycystic kidney disease | 85 | 76 | 9 |
| Tubular and interstitial disease | 19 | 17 | 2 |
| Other | 130 | 114 | 16 |
| Acute rejections in first 6 months | | | |
| No. of subjects | 130 | 116 | 14 |
| Time to first rejection (days)[b] | 22.5 (7–176) | 22.5 (7–176) | 21.5 (9–130) |
| **Tacrolimus Trough Concentrations, Doses and Concomitant Medications and Events** | | | |
| No. trough concentrations | 12,277 | 9,881 | 2,396 |
| Total daily dose (mg)[c] | 5.5 (4–8) | 5 (4–7) | 8 (5–10)[d] |
| Trough concentration (ng/mL)[c] | 7.9 (5.9–10.1) | 8.3 (6.4–10.4) | 6.2 (4.4–8.4)[d] |
| Trough dose-normalized (ng/ml per mg/kg/day)[c] | 116.5 (70.7–186.3) | 131.4 (83.0–201.8) | 68.3 (46.1–103.6)[d] |
| Dosing interval[e] (n) | | | |
| Twice daily | 12,150 | 9,767 | 2,383 |
| Once daily | 112 | 100 | 12 |
| Three times daily | 10 | 10 | 0 |
| % of trough concentrations with ACE inhibitor[f] | 10.1% | 9.5% | 12.3% |
| % of trough concentrations with calcium channel blockers[f] | 43.0% | 41.0% | 51.2% |
| % of trough concentrations with corticosteroids[f] | 52.7% | 55.5% | 41.2% |

| Characteristics | All subjects (n=695) | nonAfrican American (n=551) | African American (n=144) |
|---|---|---|---|
| % of trough concentrations around an acute rejection episode[g] | 4.6% | 5.0% | 2.7% |

[a] Data are median (range).

[b] Race and ethnicity are self-identified. Totals do not always match number of subjects, because people could identify as no race or as more than one race. Patients identified as multiracial were classified as African American (AA) if one of the races was AA.

[c] Data are median (interquartile range) over the first 6 months.

[d] p-value is <0.0001 for comparison of AA and non-AAs.

[e] Number of trough concentrations for each dosing interval. The numbers of dosing intervals do not match the number of trough concentrations since dosing interval was not known in all individuals.

[f] Concomitant drug was in use at the visit nearest in time to the trough measurement.

[g] % of subjects where acute rejection episode occurred ±14 days of the trough measurement.

## Table 2

Final Models for First Tacrolimus Trough Concentration[a]

| Variable[b] | % Cumulative Contribution to Variation in Trough | Effect on Trough[c] (Estimate ± SE) | p-value[c] |
|---|---|---|---|
| **All subjects**[d] | | | |
| Enrolling Center | 37.27 | | <0.0001[e] |
| rs776746 (CYP3A5) | 42.38 | −0.33 ± 0.05 | <0.0001 |
| Diabetes | 44.68 | 0.23 ± 0.05 | <0.0001 |
| Age[f] | 45.88 | 0.008 ± 0.002 | 0.0001 |
| rs2608555 (GAN) | 46.87 | 0.15 ± 0.04 | 0.0003 |
| rs2239393 (COMT) | 47.90 | −0.28 ± 0.08 | 0.0002 |
| rs2687117 (CYP3A4) | 48.86 | 0.26 ± 0.08 | 0.0019 |
| rs3734354 (SIM1) | 49.78 | 0.19 ± 0.06 | 0.0013 |
| rs3135506 (APOA5) | 50.62 | −0.23 ± 0.07 | 0.0009 |
| rs17567 (EPS15) | 51.01 | 0.10 ± 0.04 | 0.0141 |
| rs4646312 (COMT) | 51.42 | 0.22 ± 0.08 | 0.0075 |
| rs4926 (SERPING1) | 51.81 | −0.09 ± 0.04 | 0.0311 |
| rs2072374 (CNAP1) | 52.13 | −0.08 ± 0.04 | 0.0542 |
| rs3448 (GPX1) | 52.34 | 0.07 ± 0.04 | 0.0680 |
| rs13321 (TNC) | 52.54 | 0.06 ± 0.04 | 0.0823 |
| % total variation explained | 52.54 | | |
| **Non-African American subjects only** | | | |
| Enrolling Center | 34.04 | | <0.0001[e] |
| Age[f] | 36.52 | 0.010 ± 0.002 | <0.0001 |
| rs776746 (CYP3A5) | 38.90 | −0.31 ± 0.07 | <0.0001 |
| Diabetes | 40.55 | 0.21 ± 0.06 | 0.0004 |
| rs2608555 (GAN) | 42.09 | 0.17 ± 0.04 | 0.0001 |
| rs3734354 (SIM1) | 43.74 | 0.20 ± 0.06 | 0.0008 |
| rs3135506 (APOA5) | 45.03 | −0.23 ± 0.08 | 0.0024 |
| rs4926 (SERPING1) | 45.96 | −0.12 ± 0.04 | 0.0053 |
| rs17567 (EPS15) | 46.78 | 0.12 ± 0.05 | 0.0122 |
| rs1800822 (FMO3) | 47.41 | 0.20 ± 0.09 | 0.0248 |
| rs2239393 (COMT) | 47.75 | −0.07 ± 0.04 | 0.0676 |
| % total variation explained | 47.75 | | |
| **African American subjects only** | | | |
| Enrolling Center | 19.19 | | <0.0001[e] |
| rs776746 (CYP3A5) | 22.97 | −0.25 ± 0.08 | 0.0025 |
| rs2687117 (CYP3A4) | 29.41 | NA[g] | NA[g] |
| rs3136228 (MSH6) | 33.57 | −0.20 ± 0.08 | 0.0238 |
| rs2288648 (FASTK) | 35.62 | −0.41 ± 0.17 | 0.0210 |
| Diabetes | 37.95 | 0.45 ± 0.12 | 0.0003 |
| rs2072374 (CNAP1) | 40.09 | −0.45 ± 0.17 | 0.0110 |

| Variable[b] | % Cumulative Contribution to Variation in Trough | Effect on Trough[c] (Estimate ± SE) | p-value[c] |
|---|---|---|---|
| rs2239393 (COMT) | 41.74 | −0.19 ± 0.08 | 0.0246 |
| rs2280789 (CCL5) | 43.64 | 0.23 ± 0.10 | 0.0262 |
| rs12114000 (CYP3A4) | 45.63 | 0.38 ± 0.11 | 0.0008 |
| rs2687117 removed | 45.71 | NA[g] | NA[g] |
| % total variation explained | 45.71 | | |

[a] Multiple linear regression models on the log transformed dose-normalized tacrolimus trough concentration at the first measurement posttransplant using stepwise variable selection.

[b] Variables are listed in order of entry into the model.

[c] Effect estimates on log transformed dose-normalized troughs, standard errors (SE) and p-values are for the final model.

[d] Model is adjusted for race.

[e] p-value for enrolling center is for the overall F-test.

[f] Age is in years, mean-centered at 50.2 years.

[g] variant rs2687117 was removed during variable selection, so it is not in the final model.