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Tacrolimus Troughs and Genetic Determinants of Metabolism in Kidney Transplant Recipients: A comparison of four ancestry groups

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Abstract

Tacrolimus trough and dose requirements vary dramatically between individuals of European and African American ancestry. These differences are less well described in other populations. We conducted an observational, prospective, multi-center study from which 2595 kidney transplant

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by *the American Journal of Transplantation.*

Data Availability Statement

The data with participant consent, that support the findings of this study are openly available in dbGaP at https://www.ncbi.nlm.nih.gov/gap.

Supporting Information

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

recipients of European, African, Native American, and Asian ancestry were studied for tacrolimus trough, doses and genetic determinants of metabolism. We studied the well-known variants and conducted a *CYP3A4*/5 gene wide analysis to identify new variants. Daily doses, and dose-normalized troughs were significantly different between the four groups ($p<0.001$). *CYP3A5*3* (rs776746) was associated with higher dose-normalized tacrolimus troughs in all groups but occurred at different allele frequencies and had differing effect sizes. The *CYP3A5**6 (rs10264272) and *7 (rs413003343) variants were only present in African Americans. *CYP3A4*22* (rs35599367) was not found in any of the Asian ancestry samples. We identified seven suggestive variants in the *CYP3A4*/5 genes associated with dose-normalized troughs in Native Americans ($p=1.1\times10^{-5}$ to 8.8×10^{-6}) and one suggestive variant in Asian Americans ($p=5.6\times10^{-6}$). Tacrolimus daily doses and dose-normalized troughs vary significantly among different ancestry groups. We identified potential new variants important in Asians and Native Americans. Studies with larger populations should be conducted to assess the importance of the identified suggestive variants.

1. Introduction

The incidence of end stage kidney disease is increasing worldwide and kidney transplantation is the optimal treatment option due to better outcomes relative to dialysis [1]. Tacrolimus is a potent immunosuppressant that is used in >90% of transplants to prevent acute rejection and maintain graft function [1]. Tacrolimus has a narrow therapeutic index and troughs are therapeutically monitored to reduce toxicity and improve efficacy [2]. Tacrolimus has high inter-individual pharmacokinetic variability [3]. It is well known that tacrolimus troughs and dose requirements vary between recipients of European and African ancestry, African Americans have significantly lower tacrolimus trough concentrations in comparison with European Americans and require higher tacrolimus doses to achieve similar troughs concentrations [4–6]. Little is known about tacrolimus trough and dose requirements in other populations although some data suggest that Native American transplant recipients require lower tacrolimus doses possibly due to decreased oral clearance [7,8], others found that there were no difference between tacrolimus doses between European Americans and Native Americans [9].

Cytochromes P450 (CYP) 3A4 and 5 are the main drug metabolizing enzymes for tacrolimus and the genes encoding for these proteins contain important genetic variants [10–12]. These variants differ by ancestry and for some significantly different minor allele frequencies (MAF) [13,14]. The *CYP3A5* variant, *CY3A5*3* (rs776746), is a loss of function variant and has been well-studied with tacrolimus pharmacokinetics [15,16]. *CYP3A5*6* (rs10264272) and *7 (rs413003343) are reduced or loss of function variants which are observed exclusively in individuals of African ancestry [17]. *CYP3A4*22* (rs35599367) is also a reduced function variant which occurs primarily in European ancestry and is associated with variation in tacrolimus pharmacokinetics [18–22]. We have shown in our work that up to 50% of variability in tacrolimus pharmacokinetic is explained by *CYP3A* genetic variants and clinical factors [23–25]. Identifying the influential variants in each population is important in developing accurate precision medicine dose models for tacrolimus therefore we previously conducted genome wide association studies (GWAS) of tacrolimus troughs in

kidney transplant recipients of European and African ancestry to identify ancestry specific genetic variants [24,25]. We were able to find only one study in Native Americans[7] on the pharmacogenomics of tacrolimus.

The evidence for the association between genetic variation and tacrolimus disposition is strong and a Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline provides recommendations on tacrolimus directed dosing using the *CYP3A5*3*, **6*, and **7* variants [26]. Individuals who carry one or no loss of function alleles are *CYP3A5* protein expressers and should receive significantly higher tacrolimus doses. The guideline does not however address the effect of clinical factors, the *CYP3A4*22* variant, and variants that may be present in populations other than European Americans and African Americans.

In the current study, we compared tacrolimus daily dose requirements and troughs in European American, African American, Native Americans and Asian American transplant recipients. Additionally, we evaluated the association of well-known tacrolimus variants (*CYP3A5*3*, **6, *7* and *CYP3A4*22*) with troughs in the four populations. We also conducted a *CYP3A4* and *CYP3A*5 gene wide analysis to identify new variants possibly present in transplant recipients of Asian or Native American ancestry.

2. Methods

2.1 Study Design and Patient Selection

We studied transplant recipients enrolled in the Deterioration of Kidney Allograft Function (DeKAF) and GEN03 genomic studies. These are multicenter, observational studies, which prospectively followed kidney transplant recipients from 2005 to 2016 at seven study sites in the United States and Canada. They are registered at www.clinicaltrials.gov (NCT00270712 and NCT01714440). Participants were enrolled at time of transplant and signed informed consents approved by the institutional review boards of the enrolling centers. Transplant recipients were selected for this analysis if they were >18 years old, received oral immediate release tacrolimus as maintenance immunosuppression, had tacrolimus trough concentrations measured as part of clinical care in the first 6 months posttransplant and genome wide association (GWA) genomic data available. Ancestry of each individual was determined using principal components (PC) of ancestry computed from the GWAS panel and through knowledge of self-reported ancestry. When using top 3 ancestry PCs, patients were classified as either European, African, Native, or Asian American ancestry (Supplemental Figures 1 and 2). In the first group, most self-identified as Caucasian/European American. In the second, most self-identified as Black/African American. In the third, most self-identified as Asian/Asian American. In the fourth cluster, most self-identified as Native American or as Hispanic/Latino ethnicity. The Native Americans (as per self-report) and Hispanic/Latino ethnicity (as per self-report) were indistinguishable by PC and were therefore analysed as one group. There was concordance with self-reported ancestry and PC defined ancestry, however, when discordance was raised, PC defined ancestry was used in that individual to reduce genetic confounding.

2.2 Tacrolimus Trough Concentrations and Doses

Tacrolimus troughs and corresponding doses in the first 6 months posttransplant were obtained as part of routine clinical care and taken from the medical record for analysis. Two tacrolimus troughs were obtained per week in the first 8 weeks and two troughs were obtained per month in months 3, 4, 5 and 6 for a maximum of 24 troughs per patient. Largely, the target trough concentrations were 8 to 12 ng/mL in the first 3 months, then 6 to 10 ng/mL in 3 to 6 months posttransplant. Dose normalized trough concentrations were determined by the ratio of trough concentration (ng/ml) and total daily dose (mg).

2.3 Genotyping

Recipient DNA obtained at time of transplant from peripheral blood lymphocytes. DNA was isolated from lymphocytes by centrifugation after red blood cell lysis. Genotyping was conducted on an exome-plus Affymetrix Transplant Array chip (Affymetrix, Santa Clara, CA) with ~800,000 high quality genomic markers after quality control and >34M markers after imputation using the 1000 Genomes phase 3 and Genome of the Netherlands v5 [27–31]. Data quality control was carried out with PLINK software (version 1.90b1a) [32]. Genotypes were phased using SHAPEIT2 [33]; and imputed with IMPUTE2[34]. Imputed variants with information score more than 0.8 were considered of good quality and used in the analysis. Genotypes were subjected to a 95% call rate threshold. Samples with very high heterozygosity and suspected contamination were confirmed and removed if high heterozygosity could not be resolved. Individual variants were excluded if they were monomorphic or had low MAF (<0.5%). Approximately, 49,000 measured and imputed variants from the *CYP3A4* and *CYP3A*5 genes were taken from the GWA panel and used in this analysis. After removing variants in linkage disequilibrium (LD), the effective number of variants was ~10,000. *CYP3A4* and *CYP3A5* region was defined between positions 95,000,000 and 105,000,000 on chromosome 7.

2.4 Statistical Analysis

An ANOVA was used to assess the difference in tacrolimus doses and troughs among the populations and a p-value <0.05 was considered statistically significant. The association between natural log (ln) transformed dose-normalized tacrolimus troughs and known variants, *CYP3A5*3*, *6, *7 and *3A4*22*, were tested in each of the 4 populations using linear mixed-effects models. A log transformation was used to ensure that the outcome was normally distributed. Dose-normalized trough concentrations initially started low and rose quickly until day 9 after transplant and then plateaued in the early weeks after transplant as we have previously described [35,36]. Therefore, a simple spline method was used to model the effect of time on all trough concentrations, with the change in slope occurring at day 9. The models included a random intercept and slope for days posttransplant and were adjusted for age, gender and enrolling center. An interaction analysis was then conducted to compare the effect sizes of *CYP3A5*3* among different groups in relation to the European American group. We also conducted a gene wide association analysis of the *CYP3A4* and *CYP3A*5 genes in the Native and Asian American populations. The analysis was adjusted for age, gender, enrolling centre, and *CYP3A5*3*, and the level of significance was set at a p-value of $<5\times10^{-6}$. The p-value for gene wide association test was adjusted based on a Bonferroni

correction using the effective number of single nucleotide polymorphisms (SNPs) (~10,000) which took into account SNPs in linkage disequilibrium [37]. In our previous studies, the known genetic and clinical variables explained ~50% of variation of tacrolimus concentrations. Given the 77 recipients in the Native American group and the significance level of 5×10^{-6}, we have 78% power to identify a variant that can explain 20% additional variation; for the 91 Asians American, we have 82% power to identify a variant explaining 18% additional variation. Analyses were conducted with R software version 3.5 (www.r-project.org) and SAS version 9.4 (SAS Institute, Cary, NC, USA).

3. Results

We studied 1966 European American, 461 African American, 77 Native American and 91 Asian American ancestry transplant recipients (Table 1). Tacrolimus total daily doses, trough concentrations and dose-normalized trough concentrations by ancestry are shown in Table 2 and were significantly different in the four populations ($p < 0.001$). Native Americans and European Americans received the lowest median tacrolimus daily dose (5 mg) and the African Americans received the highest median daily dose (8 mg) ($p < 0.0001$). Moreover, Native Americans had the highest dose-normalized tacrolimus trough concentration (1.73 ng/ml per total daily dose), and the African Americans had the lowest troughs (0.78 ng/ml per total daily dose) ($p < 0.0001$). Tacrolimus troughs over the 6 months were generally similar between the populations except for African Americans who had significantly lower median trough concentrations ($p < 0.0001$).

Alleles frequencies (AF) of the four well-known variants, *CYP3A5*3*, *6, *7, and *CYP3A4*22*, are shown in Figure 1. The *CYP3A5*3* variant, was present in all four groups, and was most common in European ancestry (AF=0.93) followed by Native American (AF=0.84) and Asian American (AF=0.72), while the African Americans had the lowest frequency (AF=0.30). The *CYP3A5* *6 and *7 variants were exclusively present in African Americans. The *CYP3A4*22* variant allele frequency was ≤ 0.05 and was not observed in those of Asian ancestry.

Each of the tested variants was associated with higher dose-normalized trough concentrations (Table 3). The *CYP3A5*3* variant was associated with higher dose-normalized tacrolimus troughs in all populations ($p = 3\times10^{-9}$ to 5×10^{-121}). The size of the effect varied where the largest effect of the *3 allele was in European Americans. Native Americans, Asian Americans, and African Americans had similar effect sizes for *CYP3A5*3* (Table 3) but were significantly different to the European Americans ($p<0.001$). In European Americans, each additional copy of *3 allele resulted in 1.86 times increase in dose-normalized troughs. In the other ancestry groups, the magnitude of increase in dose-normalized troughs of one *3 allele was similar to each other (1.6 in Native Americans, 1.55 in Asian American, and 1.54 in African Americans). In African Americans the *6 and *7 variants also were associated with an increase (each *6 and *7 allele increased dose-normalized troughs by 1.35 and 1.58 times, respectively) in dose-normalized troughs. Additionally, *CYP3A4*22* was associated with higher dose-normalized tacrolimus troughs but only in the European Americans. The effect size of *CYP3A4*22* variant was about one-

half that of the *CYP3A5*3* variant and was associated with 1.34 times increase in dose-normalized troughs.

In the *CYP3A4* and *CYP3A5* gene wide analysis in the Native American and Asian American recipients, no additional new variants were associated with tacrolimus dose-normalized trough concentrations (Table 4) after correcting for multiple testing. However, we found seven suggestive variants in the Native American group with (minor allele frequency) MAF > 0.05 ($p=1.1\times10^{-5}$ to 8.8×10^{-6}). Six of those variants were located near to each other and 4 were in complete LD (D'=1). In Asian Americans, one variant, rs6950190 with a MAF of 0.384, had a suggestive association with tacrolimus dose normalized dose troughs ($p=5.6\times10^{-6}$). The suggestive variants were imputed SNPs with a notable imputation quality (information score = 0.861–0.986).

4. Discussion

In the present study we evaluated the differences between tacrolimus troughs, doses and dose normalized troughs between four populations; European Americans, African Americans, Native Americans, and Asian Americans. African Americans received significantly higher doses, but achieved lower trough concentrations when compared to the 3 other populations. Our findings are in accordance with previous studies, including our own, which reported that African American patients require higher tacrolimus doses than European patients to achieve similar therapeutic trough concentrations [35,38–43]. Two studies reported that Native Americans required lower tacrolimus doses than European Americans [7,8], however, in our current analysis of Native Americans tacrolimus doses and troughs were not different although they had the highest dose-normalized troughs of all groups. Our findings are supported by a small study that also found no difference between doses required to maintain therapeutic tacrolimus troughs in Native Americans, Hispanics and Non-Hispanic whites[9]. Direct comparisons of our trough and dose data to published work is difficult since targeted concentrations are generally higher than contemporary trough targets, doses are reported in mg/kg, and few studies report dose-normalized troughs, which would allow for direct comparison.

We evaluated the four variants (*CYP3A5*3*, *6, *7 and *CYP3A4*22*) known to be strongly associated with tacrolimus metabolism and their frequency in the four populations. The *CYP3A5*3* loss of function variant was present in all four populations but the AFs were significantly different (Figure 1). The African Americans had the lowest AF of *CYP3A5*3* (0.30) whereas the European population had the highest AF (0.93). African Americans also carried two additional loss of function variants (*CYP3A5**6 and *7) which were not observed in our other populations. Despite African Americans carrying three common *CYP3A5* loss of function variants they still cumulatively carried fewer loss of function CYP3A variants than the other populations, which accounts for their significantly higher rate of tacrolimus metabolism, lower troughs and higher dose requirements compared to other groups. The AFs of *CYP3A5*3*, *6, and *7 in our study are similar to previous reports for African ancestry, European, and Asian populations [44,45]. Although infrequent, others have reported that individuals of Latin American ancestry also carry *CYP3A5**6 (AF=0.037) and *CYP3A5**7 (AF=0.025) as do individuals from the Middle East who carry *CYP3A5**6

(AF=0.019) [45]. In a study of 94 Native Americans in Montana, United States, by Fohner et al,[46] the AF of *CYP3A5*3* was 0.92 which is slightly higher than what we observed (0.84). They also found that *CYP3A5**6, and *7 were not present [46]. In another study of 24 adult Native Americans kidney transplant recipients, *CYP3A5*3* had an a higher AF (0.94) [7].

*CYP3A5*3* is the most studied *CYP3A5* variant in association with tacrolimus and has been evaluated in Europeans[18,21,47–52] African Americans[5,24,35,53], Asian [52,54–60], and Native Americans[7]. Most studies have compared dose-normalized troughs in carriers of only the *CYP3A5*3* allele to those who are not carriers. It is well established that carriers of *CYP3A5*3* have significantly higher dose-normalized trough concentrations. We also found that the *CYP3A5*3* variant had a large effect size in all four populations. However, the magnitude of the *CYP3A5*3* effect varied by ancestry with the largest effect in the European American population. The effect size was lower but similar in the other three populations compared with European Americans in this study. This effect size may be explained by additional, yet unrecognized, variants that are important in the other populations; thereby reducing the overall effect of the *CYP3A5*3* allele. The different effect sizes among the ancestry groups suggests that dosing models for each group will need to be developed for each population and that generalizability of an effect size will likely not be possible. We previously developed a tacrolimus dosing model for African Americans [61] and propose that dosing models such as this will be needed for each ancestry population for precise dosing predictions.

Tacrolimus is also metabolized by the *CYP3A4* enzyme and is likely the predominant enzyme in those of European ancestry since they commonly carry the *CYP3A5*3*/*3 genotype and therefore do not express functional *CYP3A5* enzyme. The *CYP3A4*22* is the only variant in the *CYP3A4* gene that has been consistently associated with tacrolimus metabolism[18–20,62–65]. *CYP3A4*22* has a low MAF and an effect size about one-half relative to the *CYP3A5*3* variant which is most likely because *CYP3A4*22* is not a complete loss of function variant with a smaller reduction on tacrolimus clearance [62]. We observed *CYP3A4*22* in our European American, African American and Native American ancestry recipients (MAF≤0.05) whereas it was not found in our Asian American group, which is consistent with other published data [46,66,67]. *CYP3A4*22* was only significantly associated with tacrolimus in our European ancestry population. The lack of effect in our other populations may be due to its low AF and it will require a larger sample size to identify the effect. Because of the smaller effect size some propose that *CYP3A4*22* may not be important clinically [68,69] which may be true when the variant occurs alone or in the absence of other variants. We recently described 4 patients who carried both *CYP3A5*3*/*3 and *CYP3A4*22*/*22 genotypes who had significantly reduced tacrolimus metabolism and very low dose requirements[26]. Therefore, when it is combined with other reduced or loss of function alleles, the effect can be quite profound. The current tacrolimus CPIC guidelines do not include recommendations regarding the *CYP3A4*22* variant however, future updates should consider adding. The association of tacrolimus with ABCB1 variants is conflicting. [53,70] In one study carriers of CYP3A4*22 the effect of ABCB1 was associated with a small but strong effect on tacrolimus in renal transplant patients[71].

We previously conducted GWAS on tacrolimus troughs and found in European Americans that the *CYP3A5*3* and *CYP3A4*22* variants were important determinants of metabolism and in African Americans the *CYP3A5*3*, *6 and *7 were important [24,25]. A recent study, using an extreme tacrolimus trough phenotype sampling model with targeted next-generation sequencing, identified potentially new variants associated with tacrolimus disposition. Numerous variants in the *CYB5R2* gene were associated with extreme tacrolimus troughs in African Americans, including the potential loss of function variant rs61733057[72]. We hypothesized that genetic variants beyond *CYP3A5*3* may also explain additional tacrolimus trough variability in Asian American and Native American transplant recipients. Therefore, we conducted an exploratory gene wide association analysis in the *CYP3A4* and *CYP3A5* genes and found no variants that were significantly associated at a gene wide significance level; however, several variants were suggestive and worthy of further investigation. Seven variants with modest MAF (0.07 to 0.19) were suggestive in the Native American group, of which 4 of the variants were in complete LD with each other. These variants were in intergenic regions so the mechanism of their effect is unclear. Genetic variants in strong LD (r2≥ 0.8) to those suggestive SNPs were identified, however, none of them were reported to have any function. Identifying the function of those variants might be aided by using gene-tissue expression or cell-line validation techniques that we have developed for tacrolimus metabolism[73]; however, that was beyond the scope of the present study. In the Asian Americans, one variant, rs6950190, in the dynein cytoplasmic 1 intermediate chain 1 gene (*DYNC1I1)* with a MAF of 0.384 also had a suggestive association with tacrolimus dose-normalized troughs. The *DYNC1I1* gene, is involved in limb development and its association with metabolism is uncertain [74]. One limitation to the current study is the lack of adherence and dietary information which could affect tacrolimus trough concentrations, however, when nonadherence was suspected, the trough concentration around that time was excluded. Because of the large number of variants studied, the power for discovery is low and these variants must be validated in other populations.

5. Conclusion

There are significant differences in tacrolimus daily doses and dose-normalized troughs by ancestry. Native American had highest dose normalized tacrolimus trough concentrations and African Americans had the lowest dose-normalized troughs due to higher frequency of *CYP3A5* expressers. Genetic variants that influence tacrolimus metabolism also differ across populations. *CYP3A5*3* is an important predictor of tacrolimus exposure in the four populations studied however its effect size on metabolism varied among the populations. In addition to *CYP3A5*3*, *CYP3A5**6 and *7 are important predictors of tacrolimus metabolism in African Americans, and *CYP3A4*22* is important in those of European ancestry. No additional variants at a gene wide significance level were identified for Asian or Native American kidney transplant recipients but several were suggestive and should be further evaluated. Larger populations of Asian and Native American ancestry recipients are needed to assess importance of suggestive variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations:

CYP	Cytochromes P450
MAF	Minor allele frequencies
GWAS	Genome wide association studies
CPIC	Clinical Pharmacogenetics Implementation Consortium
DeKAF	Deterioration of Kidney Allograft Function
GWA	genome wide association
AF	Alleles frequencies
LD	Linkage disequilibrium
DYNC1I1	Dynein cytoplasmic 1 intermediate chain 1 gene

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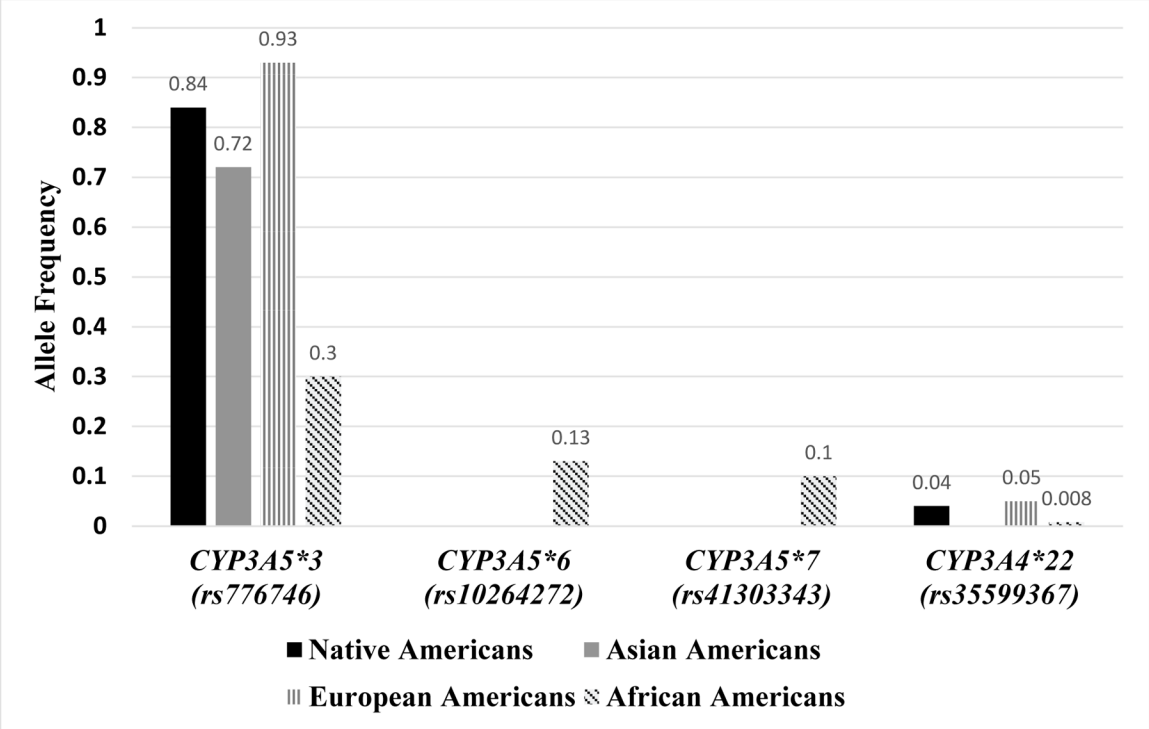

Figure 1.
Allele Frequencies of Known Variants among the Four Ancestry Groups

Table 1.

Recipient Demographics and Characteristics by Ancestry

	Native Americans (N=77)	Asia Americans (N=91)	African Americans (N=461)	European Americans (N=1966)
Male Gender, n (%)	41 (53.25%)	56 (61.54%)	287 (62.26%)	1232 (62.67%)
Age at transplant (years), mean (s.d.)	49.44 (±14.84)	45.23 (±14.29)	47.25 (±11.95)	51.24 (±13.20)
Weight (kg), mean (s.d.)	78.57 (±16.63)	67.37 (±13.38)	85.72 (±18.64)	83.03 (±19.55)
SPK, n (%)	6 (7.79%)	1 (1.10%)	17 (3.69%)	148 (7.53%)
Diabetes before transplant, n (%)	38 (49.35%)	22 (24.18%)	163 (35.36%)	704 (35.83%)
Living donor, n (%)	38 (49.35%)	44 (48.35%)	144 (31.24%)	1336 (67.96%)
Antibody induction				
Monoclonal	27 (35.06%)	39 (42.86%)	209 (45.34%)	746 (37.95%)
Polyclonal	46 (59.74%)	47 (51.65%)	240 (52.06%)	1109 (56.41%)
Combination	2 (2.60%)	2 (2.20%)	7 (1.52%)	53 (2.70%)
None	2 (2.60%)	3 (3.30%)	5 (1.08%)	58 (2.95%)
Steroid present at day 14	51 (66.23%)	59 (64.84%)	269 (58.35%)	1266 (64.43%)

SPK: simultaneous pancreas-kidney transplant

Table 2.

Tacrolimus Doses and Concentrations by Ancestry in the First 6 Months Posttransplant

	Native American (N=77, obs= 1370)	Asian Ancestry (N=91, obs=1747)	European Ancestry (N=1966, obs=34594)	African American (N=461, obs= 8187)	P-value
Trough Concentration (ng/ml)	8.3 (6.5–10.3)	8.4 (6.7–10.6)	8.4 (6.5–10.3)	6.9 (5–9)	<0.0001 *
Total Daily Dose (mg)	5.0 (3.0–8.0)	6.0 (3.5–8.0)	5.0 (4.0–8.0)	8.0 (6.0–12.0)	<0.0001 *
Dose-Normalized Trough Concentration (ng/ml per total daily dose in mg)	1.73 (1.06–2.67)	1.50 (0.98–2.53)	1.56 (1.02–2.40)	0.78 (0.53–1.2)	<0.0001 *

N=number of patients; Obs = number of tacrolimus troughs; Numbers are represented as median (interquartile ranges);

* ANOVA test was used for comparison

Table 3.

Known *CYP3A5* and *CYP3A4* Variants and Associations with Dose-Normalized Tacrolimus Trough Concentrations by Ancestry

	***CYP3A5**3* (rs776746)**		***CYP3A5**6 (rs10264272)**		***CYP3A5**7 (rs41303343)**		**CYP3AA*22 (rs35599367)**	
	Effect Size[1] (95%CI)	**P-value**	**Effect Size (95%CI)**	**P-value**	**Effect Size (95%CI)**	**P-value**	**Effect Size (95%CI)**	**P-value**
Native Americans[2]	0.47 (0.31–0.62)	3×10^{-9}	NE		NE		−0.07 (−0.36– 0.22)	0.63
Asian Americans[2]	0.44 (0.30–0.57)	2.1×10^{-10}	NE		NE		NE	
European Americans[3]	0.62[4] (0.57–0.67)	5.0×10^{-121}	NE		NE		0.28 (0.22–0.34)	3.3×10^{-22}
African Americans[3]	0.43 (0.36–0.49)	2.0×10^{-37}	0.30 (0.22 −0.39)	6.1×10^{-12}	0.46 (0.36–0.55)	9.6×10^{-22}	0.26 (−0.06–0.56)	0.11

Models were adjusted for time posttransplant, age, gender, and enrolling center and each variant was adjusted for the other known variants.

CI: confidence interval.

NE - not evaluable due to either absence or low allele frequency of the variant in the population

[1]Statistically significant difference between European American and other ancestry groups ($p<0.001$)

[2]Gene wide association significance set at p-value$< 5\times10^{-6}$

[3]Genome wide association significance set at p-value $< 5\times10^{-8}$

[4]Effect size is the effect of one variant allele on ln of dose-normalized troughs; for example, in European Americans, the effect size of *CYP3A5**3 is 0.62, which means that dose-normalized troughs are 1.86 [$e^{0.62}=1.86$] times greater if recipient carries one *3 allele than carriers of no *3 alleles, and 3.46 [($e^{0.62}$) x ($e^{0.62}$)=3.46] times greater if a carrier of two *3 alleles compared to those with no *3 alleles.

Table 4:

Variants with Suggestive Associations with Dose-Normalized Tacrolimus Troughs in Native Americans and Asian Americans[1]

Variant	Effect size	P-value (95%CI)	MAF	Location of SNP
Native Americans				
rs139190940_TG	0.31 (0.17–0.44)	8.8×10^{-6}	0.18	inter-genic region on chromosome 7, located downstream of SDHAF3 (succinate dehydrogenase complex assembly factor 3) and upstream of an uncharacterized gene LOC105375416
rs73238872_G[2]	0.27 (0.14–0.40)	6.6×10^{-5}	0.19	
rs878502_C[2]	0.27 (0.14–0.40)	6.6×10^{-5}	0.19	
rs28369152_C[2]	0.27 (0.14–0.40)	6.6×10^{-5}	0.19	
rs28413832_C[2]	0.27 (0.14–0.40)	6.6×10^{-5}	0.19	
rs2158498_G	0.30 (0.17–0.44)	1.1×10^{-5}	0.19	
rs151269855_G	0.53 (0.27–0.79)	8.5×10^{-5}	0.07	downstream of LOCLOC105375416 at position 97,215,572 on chromosome 7
Asian Americans				
rs6950190_C	0.38 (0.22–0.55)	5.6×10^{-6}	0.38	intronic SNP in DYNC1I1 (dynein cytoplasmic 1 intermediate chain 1) on chromsome 7 at 95,558,358

CI: confidence intervals; MAF: minor allele frequency; SNP: single nucleotide polymorphism

[1] Longitudinal linear mixed effects model included a random intercept and random slopes for days after transplant and adjusted for *CYP3A5*3*, age, gender and transplant center

[2] Variants are in linkage disequilibrium with each other, D' = 1.0, p <0.0001.