Tacrolimus Troughs and Doses in African American, Asian, European American and Hispanic/Native American Kidney Transplant (tx) Recipients

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INTRODUCTION

Tacrolimus (TAC) is an immune suppressant with a narrow therapeutic index and high pharmacokinetic variability leading to uncertainty in blood concentrations. TAC is dependent on CYP3A4/5 enzymes for metabolism. The CYP3A4/5 genes are highly polymorphic and variants significantly influence the metabolism of TAC. The *CYP3A5*3, *6, *7*, are important variants in African Americans (AA) whereas CYP3A5*3 and *CYP3A4*22* variants are common and important variants influencing pharmacokinetics in European American (EA) subjects. These variants in combination with clinical factors account for 30-50% of variability in TAC trough concentrations. Little is known about important genetic variants in other populations. We performed a targeted genome wide association study (GWAS) to identify variants that may be associated with TAC troughs in other populations. Defining variants for all populations will improve precision medicine approaches to genotype-guided dosing.

OBJECTIVES

To define the differences in TAC daily doses requirements and troughs in EA, AA, His/Nat and AsA tx recipients. To evaluate the association of well-known variants (CYP3A*3, 6, 7 and CYP3A4*22) towards TAC troughs in four populations. To identify new and novel genetic variants associated with TAC troughs in tx recipients of Asian and Hispanic/Native American ancestry.

METHODS

Study Design

- A genomic association analysis of log dose-normalized TAC troughs was conducted in patients enrolled in the DeKAF Genomics study and GEN03 studies. These are multicenter, observational studies which prospectively followed kidney tx recipients from 2005 to 2016 at 7 study sites in the United States and Canada. Registered at www.clinicaltrials.gov (NCT00270712). Tx recipients were selected for this study if they received TAC as maintenance immunosuppression, had TAC trough concentrations measured and GWAS data available.

TAC Troughs

- TAC troughs and corresponding doses in the first 6 months posttx were obtained as part of routine clinical care and taken from the medical record for analysis. Two TAC 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.
- Generally, the target trough concentrations were 8 to 12 ng/mL in the first 3 months, then 6 to 10 ng/mL for 3 to 6 months post-transplant.

Genotyping and Genetic Association Analysis

- Genotyping was conducted on an exome-plus Affymetrix Tx Array chip with ~800,000 high quality SNP markers after QC and >34M markers after imputation using the 1000 Genomes phase 3 and Genome of the Netherlands v5. Both cohorts were genotyped on this array. Approximately, 45,000 SNPs from the CYP3A4 and CY3A5 genes were taken from the chip and analyzed.
- Race was determined in each cohort using principal components with ancestry informative markers from the GWAS panel. Patients identified as Hispanics/Native Americans (His/Nat), Asian Americans (AsA), EA or AA were selected for the analysis.
- We tested the associations between CYP3A4 and 5 gene variants and log dose-normalized TAC troughs in tx recipients of the four populations. Longitudinal Mixed Effect models for known and top identified variants were constructed adjusting for center, age and gender for all populations. For the CYP3A4 and 5 gene wide association analysis a p-value of $<1.1e^{-6}$ was considered significant after adjusting for multiple testing.

RESULTS

TAC troughs, dose-normalized troughs and daily doses by population are shown in Table 1. His/Nat received the lowest TAC daily dose (5 mg) and the AA received the highest dose (8 mg). His/Nat also had the highest dose normalized TAC trough and the AA the lowest levels (P <0.001, ANOVA).

Table 1: Tacrolimus troughs, total daily doses by population and dose-normalized troughs

	His/Nat American (N=77, obs= 1370)	Asian American (N=91, obs=1747)	European American (N=2052, obs=34594)	African American (N=516, obs= 8187)
Trough (ng/ml)	8.3 (6.5-10.3)	8.4 (6.7-10.6)	8.4 (6.5-10.3)	6.9 (5-9)
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)
Trough Dose-Normalized (ng/ml/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)

N: number of patients, Obs: number of TAC troughs collected in each population
Numbers are represented as median (Interquartile range)

Minor allele frequencies of the four known variants important towards TAC are shown in Figure 1. The CYP3A5*3 variant was most common in the EA population followed by the His/Nat, AsA and the least common in AA. The CYP3A5*6 and CYP3A5*7 variants were rare in the EA population and were not found in any of the His/Nat or AsA participants.

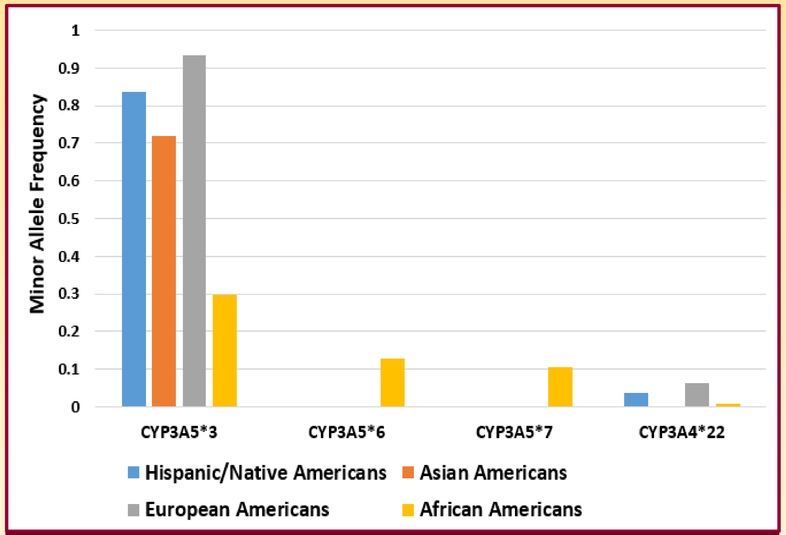

Figure 1: Minor Allele Frequency by Population

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RESULTS CONT..D

The effect of CYP3A5*3 on log dose-normalized TAC troughs was highly significant in all four groups (p = 3.3E-09 to 4.9E-127). CYP3A5*6 and *7 were associated with dose-normalized TAC troughs in the AA group (p=2.0E-12 and 1.3E-24) and CYP3A4*22 in the EA group (2.2xE-22).

In the CYP3A4/5 gene wide analysis, no SNPs were significantly associated with dose-normalized TAC troughs in His/Nat and AsA using a GWAS p-value cutoff. However, when increasing the P-value to $5x10^{-4}$, we found 7 SNPs with suggestive association with dose-normalized TAC troughs in His/Nat group, and 1 SNP suggestive in the AsA group. All of these SNP increased TAC troughs suggesting they may be reduced or loss of function variants (Table 2).

Table 2: SNPs with Suggestive Associations with Dose-normalized TAC Troughs in Asian Americans and Hispanic/Native Americans

SNP	Effect size	P-value (95%CI)	MAF
Hispanic/Native Americans			
rs73238872_G#	0.269 (0.14-0.40)	6.6E-05	0.188
rs878502_C#	0.269 (0.14-0.40)		
rs28369152_C#	0.269 (0.14-0.40)		
rs28413832_C#	0.269 (0.14-0.40)		
rs139190940_TG	0.306 (0.17-0.44)	8.8E-06	0.178
rs2158498_G	0.302 (0.17-0.436)	1.1E-05	0.192
rs151269855_G	0.527 (0.27-0.789)	8.5E-05	0.066
Asian Americans			
rs6950190_C	0.3837 (0.2187-0.5487)	5.60E-06	0.384

SNP: single nucleotides polymorphism; CI: confidence intervals; MAF: minor allele frequency
#SNPs in linkage disequilibrium, r2 > 0.8. Only SNPs with MAF≥ 5% were include.

CONCLUSIONS

There are differences in TAC daily doses and dose-normalized troughs by race. Native American/Hispanics had highest dose adjusted TAC trough levels and AAs had significantly lower dose-adjusted TAC troughs than other groups due to higher prevalence of CYP3A5 expressors. Genetic variants that influence TAC metabolism are highly significant and vary across populations. CYP3A5*3 is an important predictor of TAC exposure in all populations. Two additional variants are important in AA, CYP3A5*6 and *7, whereas one additional SNP, CYP3A4*22, is important in EA. No additional gene wide significant SNPs were identified for AsA or His/Nat kidney tx recipients but several were suggestive. Larger populations of the AsA and His/Nat recipients are needed to assess importance of suggestive variants and low frequency variants.