Validation of Genetic Variants Identified in GWAS for Tacrolimus Troughs

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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 for metabolism. The CYP3A4/5 genes are highly polymorphic and variants significantly influence the metabolism of TAC. The *CYP3A5*3, *6 and *7*, and *CYP3A4*22* variants are common and important variants. These variants in combination with clinical factors account for 30-50% of variability in TAC trough concentrations. We performed a genome wide association study (GWAS) to identify additional variants that may explain more of the variability in TAC troughs. Defining factors related to variability will improve precision medicine approaches to genotype-guided TAC dosing.

OBJECTIVES

To validate genetic variants associated with TAC troughs identified in the DeKAF Genomics GWAS in a new cohort of adult kidney transplant (tx) recipients enrolled in GEN03.

METHODS

Study Design

- Genetic variants associated with dose-normalized TAC troughs were identified in a discovery cohort and then replicated in a confirmatory cohort. Patients were selected for the study if they received TAC as maintenance immunosuppression, TAC trough concentrations were measured and were enrolled in the DeKAF Genomics study or the GEN03 study. 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).
- A GWAS was conducted in recipients enrolled in the DeKAF Genomics study (discovery cohort; n=1791). Association analysis was conducted to identify variants associated with dose-normalized TAC troughs. The identified variants were then replicated in a second cohort, GEN03 (confirmatory cohort; n=780).

TAC Troughs

- TAC troughs and corresponding doses in the first 6 months posttx were obtained from the medical record and analyzed. TAC trough concentrations were obtained at the respective study sites as part of routine clinical care.
- Two TAC troughs were obtained 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 first 3 months, then 6 to 10 ng/mL for 3 to 6 months post-transplant.

Genotyping

- Genotyping was conducted on an exome-plus Affymetrix Tx Array chip with ~800,000 high quality 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.
- The significant variants identified in the discovery cohort were then taken from the GWAS chip and replicated on the confirmatory cohort.
- Race was determined in each cohort using principal components using ancestry informative markers from the GWAS panel.

METHODS CONT…D

Statistical Analysis

- A GWAS was performed in the discovery cohort on TAC dose-normalized troughs from the first 6 months posttx using repeated measures regression adjusting for center, age, gender and top 4 principal components. Analyses were conducted separately for Caucasians and African Americans.
- In the Caucasian and African American recipients in the discovery cohorts, variants significant towards TAC troughs with a p-value of 1x10-6 or less were identified. These variants were then filtered and those with imputation quality of 0.8 or higher and minor allele frequency ≥5% or ≤95% were then tested in the confirmatory cohorts using a p-value with a Bonferroni correction threshold.
- **Table 1** shows the recipient characteristics of the discovery and confirmatory cohorts.

Table 1: Recipient Characteristics

	Discovery Cohort (n=1791)	Validation Cohort (n=780)
Caucasian (n)	1446	609
African American (n)	345	171
Age, median (range)	51 (18-83)	51 (18-81)
SPK transplant, no. (%)	136 (7.6%)	35 (4.5%)
Receiving dialysis at time of transplant no. (%)	1233 (68.8%)	542 (69.5%)
Diabetes at transplant, no. (%)	689 (38.5%)	233 (29.9%)
Living donor, no. (%)	1064 (59.4%)	494 (63.3%)
Prior kidney transplant, no. (%)	268 (15.0%)	114 (14.6%)
Primary cause of kidney disease		
Diabetes, no. (%)	536 (29.9%)	166 (21.3%)
Glomerular nephritis, no. (%)	373 (20.8%)	218 (28.0%)
Hypertension, no. (%)	247 (13.8%)	94 (12.1%)
Polycystic kidney disease, no. (%)	252 (14.1%)	116 (14.9%)
Other, no. (%)	326 (18.2%)	139 (17.8%)
Unknown, no. (%)	57 (3.2%)	47 (6.0%)
Anti-CMV drug use at time of trough	17,488 (56.3%)	7447 (51.9%)
Steroid use at time of trough	19,149 (61.4%)	9203 (64.2%)
TAC troughs (n)	31,207	14,338
TAC trough, median (IQR)	8.1 (6.1-10.1)	8.1 (6.4-10.0)
TAC dose, median (IQR)	6 (4-8)	6 (4-9)

RESULTS

- 661 and 225 variants were identified in the Caucasian and African American, respectively, in the GWAS analysis of the discovery cohorts.
- After removing variants with poor imputation quality, low minor allele frequency and those in LD with *CYP3A5*3* (well-known to be associated with TAC) there were 13 and 20 variants remaining, in the Caucasian and African American groups, respectively. These variants were then replicated in the confirmatory cohort.
- The variants in **Table 2** remained significant in the replication analysis.

Table 2: Variants Successfully Replicated Towards Dose Normalized TAC Troughs in the Confirmatory Cohort

	rs number	Variant	Effect Size (SE)	P-Value	MAF
Caucasian	rs776747_G	*CYP3A5*3*	0.628 (0.05)	2.3E-35	0.9417
	rs35599367_A	*CYP3A4*22*	0.274 (0.05)	2.4E-07	0.0459
African American	rs776747_G	*CYP3A5*3*	0.389 (0.06)	6.7E-10	0.2956
	rs10264272_T	*CYP3A5*6*	0.307(0.07)	3.3E-05	0.1345
	rs41303343_TA	*CYP3A5*7*	0.458(0.08)	4.1E-08	0.1082

CONCLUSION AND NEXT STEPS

We identified in GWAS and then replicated the *CYP3A5*3, *6, *7* and *CYP3A4*22* variants. No other common variants are likely to be influential towards TAC troughs in Caucasians and African Americans. Future research should identify variants important in other populations, factors that regulate gene expression and rare variants.

ACKNOWLEDGEMENTS

- We acknowledge the dedication and hard work of our coordinators: Nicoleta Bobocea, Tina Wong, Adrian Geambasu, Alyssa Sader, Myrna Ross, Kathy Peters, Danielle Berglund, Mandi DeGrote, Monica Myers, Lisa Berndt, Tom DeLeeuw, Wendy Wallace, Tammy Lowe, Catherine Barker, and Tena Hilario.
- We also acknowledge the dedicated work of our research scientists: Marcia Brott, Becky Willaert and Amutha Muthuswamy.
- The work by Michelle Cogrove, RHO, Inc and Helena Diop and Yvonne Morrison of NIAID is gratefully acknowledged.
- This work was supported by NIAID Grants 5U19-AI070119 and 5U01-AI058013.