Multi-gene Pharmacogenomics of Tacrolimus Troughs in Kidney Transplant Recipients

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INTRODUCTION and OBJECTIVES

Tacrolimus is the calcineurin inhibitor immunosuppressant of choice in kidney transplantation. Tacrolimus has a narrow therapeutic index and a high degree of interpatient variability. Achieving optimal tacrolimus blood concentrations is important to achieving good transplant outcomes. Patients traditionally receive an initial dose of tacrolimus based on weight followed by therapeutic drug monitoring and titration of exposure into a therapeutic trough range. Though this iterative process eventually leads to the optimal dose, many patients spend time during the early transplant period outside of the optimal therapeutic range. To achieve improved clinical outcomes we developed a genotype guided dosing algorithm through our seven center consortium - DeKAF Genomics. (Passey et al, Br J Clin Pharmacol 2011)

Although our genetically guided dosing equation represents an improvement over standard weight-based dosing protocols, it explains approximately half of the variability in systemic tacrolimus exposure. Additional variability may be explained by other polymorphisms within genes encoding metabolic enzymes and transporter proteins.

Since development of the algorithm, additional genetic polymorphisms have been shown to affect tacrolimus trough levels. We choose to focus on three of these polymorphisms for this work: CYP3A4*22, POR*28, and an ABCC2 haplotype. Our objective is to validate the effect these polymorphisms on tacrolimus exposure in our large cohort of kidney transplant patients (n=2008) with the hope that they may explain residual variability in tacrolimus troughs. Significant polymorphisms will be added to the dosing algorithm. The goal of our work is to personalize the tacrolimus dose in kidney recipients whereby the blood target will be more quickly achieved.

STUDY DESIGN and METHODS

A total of 2008 adult recipients of living or deceased donor kidneys receiving tacrolimus were studied. Patients were enrolled in the DeKAF Genomics study. Demographic and clinical characteristics of our patient population are shown in Table 1. Trough concentrations (n=35,043) in the first six months post-transplant were analyzed. In general, two troughs were obtained in weeks 1-8, and in each of months 3, 4, 5 and 6, for a maximum of 24 trough concentrations per patient. Tacrolimus doses were adjusted based on trough concentrations to reach institution specific trough goals based on time post-transplant (generally 8-12 ng/mL in months 0 to 3 and 6-10 ng/mL in months 4 to 6). Whole blood tacrolimus concentrations were measured by each institutions preferred analytical technique. Liquid chromatography-mass spectrometry was used to measure 92.5% of concentrations.

Tacrolimus doses, troughs, and concomitant medications at time of trough are shown in Table 2. Pre-transplant DNA from patients were genotyped for POR*28, CYP3A4*22, CYP3A5*3, and ABCC2 (Table 3). ABCC2 haplotypes were determined by combining three polymorphisms within the ABCC2 gene locus using the HAPLOSTAT program. Patients were then stratified into 2 groups - high or average/low/undetermined ABCC2 expression groups (Table 4).

All variants were evaluated against log dose normalized tacrolimus trough concentrations using repeated measures multivariate regression analysis using SAS, with and without stratification by CYP3A5*3 genotype. Analyses were adjusted for center, race and clinical covariates.

RESULTS

Younger recipient age and increasing weight were associated with lower troughs. Whereas increasing time post-transplant, diabetes at time of transplant, calcium channel blocker use and antiviral drug use were associated with increasing troughs.

Table 1: Recipient and Donor Demographics and Clinical Factors

	Median (IQR) or No. (%)
Recipient Age (yrs)	
18-34	276 (13.8%)
35-64	1447 (72.1%)
65-84	285 (14.2%)
Recipient weight at transplant (kg)	81.8 (69.0-95.5)
Recipient male gender	1261 (62.8%)
Race (self reported)	
White	1533 (76.3%)
Black or African American	373 (18.6%)
Asian	55(2.8%)
Native American / Aleutian Islander	31(1.5%)
Multiracial	10(0.5%)
Hawaiian / Pacific Islander	4 (0.2%)
Not Specified	2 (0.1%)
Primary Cause of End Stage Renal Disease	
Diabetes Mellitus	606 (30.2%)
Glomerulonephritis	441 (22.0%)
Other (Including unknown)	424 (21.1%)
Hypertension	275 (13.7%)
Polycystic kidney disease	262 (13.1%)
Diabetes at time of transplant	780 (38.8%)
Prior Kidney Transplant	295 (14.7%)
Donor Age (yrs)	
0-34	696 (34.7%)
35-64	1254 (62.5%)
65-84	58 (2.9%)
Deceased Donor	838 (41.7%)
Inductionin Immunosuppression	
Combination	54 (2.7%)
IL-2 antagonists	410 (20.4%)
Monoclonal antibodies	402 (20.0%)
None	72 (3.6%)
Polyclonal antibody	1070 (53.3%)
Dialysis after transplant	179 (8.9%)

Table 2: Tacrolimus doses, troughs and concomitant medication use

	Median (IQR) or No. (%)
Number of trough concentrations	35043
Total daily dose (mg)	6.0 (4-8)
Trough concentrations (ng/mL)	8.1 (6.1-10.1)
Trough dose-normalized (ng/mL per mg/d)	1.38 (0.87-2.18)
Dosing interval	
Twice daily	34553 (98.6%)
Once Daily	414 (1.2%)
Three times a day	75 (0.2%)
Troughs with ACE inhibitor	4592 (13.1%)
Troughs with CCB	14002 (40.0%)
Troughs with corticosteroids	21379 (61.0%)
Troughs with antiviral drug	19658 (56.1%)

Table 3: Minor Allele Frequencies in Subjects by Race

Variant	All Subjects	Non AA*	AA*
POR*28 rs1057868, 503C>T	0.262	0.265	0.254
CYP3A4*22 rs35599367, c522-191 C>T	0.039	0.040	0.036
CYP3A5*1 rs776746, 6986 G>A, *1=A, *3=G	0.20	0.08	0.70
ABCC2			
rs717620, 24 C>T	0.16	0.19	0.06
rs2273697, 1249 G>A	0.20	0.21	0.15
rs3740066, 3972 C>T	0.34	0.35	0.26

AA = African American

In a multivariate model adjusting for CYP3A5*1 status, carrying one or two POR*28 alleles was associated with a 4.5% reduction in dose normalized tacrolimus troughs. For every day in the first 9 days post-transplant there is a 7.4% increase in dose normalized tacrolimus troughs (Table 5 and Figure A). There was no association between CYP3A4*22 and ABCC2 haplotypes and troughs (Figures B and C).

Table 4: Predicted Haplotypes of ABCC2 Variants

Haplotype	rs717620	rs2273697	rs3740066	No	Diplotype	No.
H1 – WT Average	C	G	C	1876	H1/H1	460
H2 High	C	A	C	779	H1/H2	348
H9 Low	C	G	T	685	H1/H9	87
H10 Low	T	G	C	11	H1/H10	6
H12 Low	T	G	T	639	H1/H12	293
HX[a]	T	A	C	2	H2/H2	71
HY[a]	C	A	T	24	Other	743

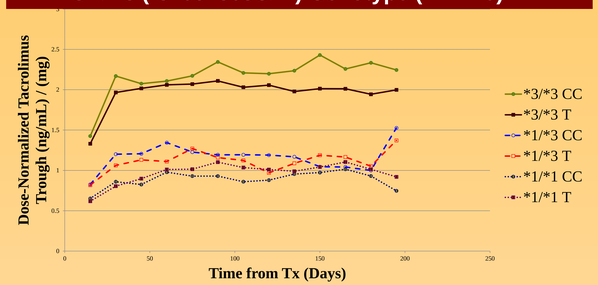
Haplotypes were assembled using software PHASE. Haplotype identification numbers H1, H2, H9, H10 and H12 were assigned according to previous reports in the literature. [a]-expression is undetermined.

Table 5: Multivariate models for the association of POR*28 with log transformed dose-normalized tacrolimus troughs (n=1429)

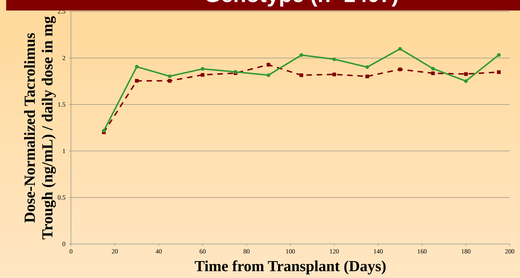
Variable	Effect on tacrolimus troughs (95% CI)	p-value
POR*28	-0.046 (-0.090 - -0.0027)	0.037
Days since transplant	0.071 (0.064 - 0.078)	$1.9x10^{-80}$
Days since day 9 post-transplant	-0.071 (-0.077- -0.064)	$1.9x10^{-83}$
CYP3A5*1	-0.428 (-0.474- -0.380)	$9.2x10^{-75}$
Age, recipient (yrs)		
18—34 vs. 65-84	-0.295 (-0.385 - -0.206)	$6.6x10^{-10}$
35-64 vs. 65-84	-0.139 (-0.206- -0.071)	
Age, donor (yrs)		
0-34 vs. 65-84	0.111 (-0.025-0.206)	0.25
35-64 vs. 65-84	0.112 (-0.021-0.245)	
African American	0.078 (-0.007 – 0.164)	0.073
Male recipient gender	-0.011 (-0.059-0.037)	0.66
Diabetes at transplant	0.097 (0.051-0.144)	$4.0x10^{-5}$
Deceased donor vs. living donor	0.034 (-0.016-0.085)	0.19
Steroid use*	-0.020(-0.052-0.011)	0.21
Calcium channel blocker use*	0.050 (0.033-0.066)	$4.1x10^{-9}$
Antiviral use*	0.055 (0.043-0.066)	$1.8x10^{-21}$
Induction Immunosuppression		
Combination vs. Polyclonal	-0.162 (-0.294- -0.030)	$1.8x10^{-5}$
Monoclonal vs. Polyclonal	0.049 (-0.006 – 0.105)	
None vs. Polyclonal	0.279 (0.148-0.410)	
Recipient weight (kg)	-0.0023 (-0.003- -0.001)	$1.6x10^{-5}$

*Concomitant drug use is at time of trough

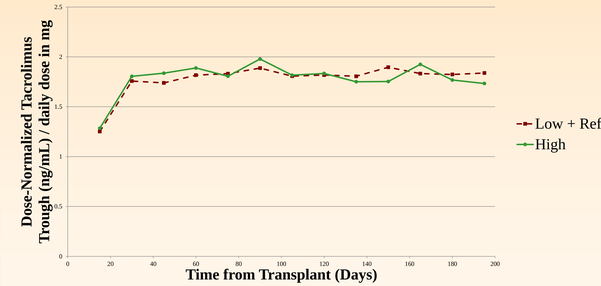
A. Tacrolimus Troughs by CYP3A5*3 (rs776746) and POR*28 (rs1057868C>T) Genotype (n=1429)



B. Tacrolimus Troughs by CYP3A4*22 (rs35599367) Genotype (n=1407)



C. Tacrolimus Troughs by ABCC2 Activity Haplotype (n=2008)



Plots of troughs are shown in 15 day intervals

CONCLUSIONS

After adjustment for clinical factors, POR*28 was associated with reduced trough concentrations although the effect was small. The POR effect was observed only in CYP3A5*3/*3 carriers suggesting that POR primarily regulates the CYP3A4 enzyme. We could not replicate the previously observed associations between CYP3A4*22 and ABCC2 haplotypes. The large size of our cohort allows us to evaluate genetic associations for infrequent variants such as CYP3A4*22 with greater confidence. However, we cannot rule out that differences in clinical practice or population specific variants not present in our population may account for positive association in other studies. These data demonstrate that tacrolimus disposition is influenced by clinical factors and more than just the CYP3A5 genotype.

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