Identification of novel genetic variants associated with tacrolimus metabolism in kidney transplant recipients using next generation sequencing and extreme phenotype sampling

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INTRODUCTION

- Tacrolimus (Tac) is an immune suppressant used in >90% of solid organ transplantation and is a substrate of CYP3A4 and CYP3A5
- Tac has narrow therapeutic index and troughs, routinely monitored, are associated with efficacy and toxicity
- Tac trough interpatient variability partly due to genetics
- Genome Wide Association Studies (GWAS) resulted in 30-50% of variability explained by common genetics variants, in *CYP3A5* and *CYP3A4*, and clinical factors

HYPOTHESIS

Tac trough interpatient variability is partially due to variants, absent in GWAS array in Tac related genes (**Table 1**) and can be identified through next generation sequencing (NGS) and extreme phenotype sampling (EPS).

Table 1: Full Genes in Targeted Sequencing Strategy

Sequence entire length of 28 genes and extended ~20kb up and downstream of these genes results in 70 partial genes.

Metabolism	Co-enzymes	Transporters	Transcription Factors	
CYP3A4	*POR*	*ABCB1/MDR1*	*VDR*	*CEBPB*
CYP3A5	*CYB5A*	*ABCC1/MRP1*	*NR3C1/GR*	*PPARA*
CYP3A43	*CYB5R1*	*ABCC2/MRP2*	*NR1I2/PXR*	*FOXA2*
CYP3A7	*CYB5R2*	*ABCG2*	*NR1I3/CAR*	*NCOR1*
CYP3AP1	*CYB5R3*	*ABCE1/RNS41*	*HNF4A*	*YY1*
CYP2J2	*CYB5R4*	*SLCO1B3*	*CEBPA*	
	CYB5RL			
	CYB5D1			

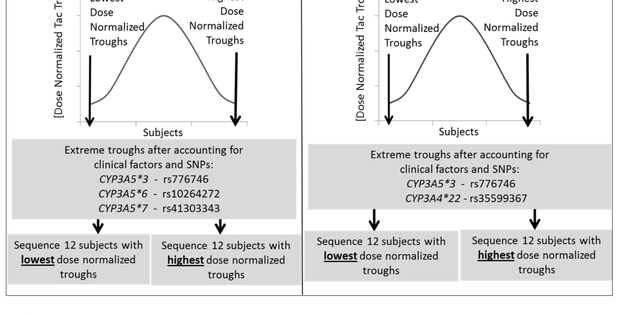
METHODS

- Subject DNA was genotyped. Linear mixed-effects models were used to test for associations between ln–transformed dose-normalized Tac troughs and LoF genotypes
- European American (EA) and African American (AA) subjects were selected with the EPS model (**Figure 1**) accounting for genetic variants and clinical factors (**Table 2**)
- NimbleGen targeted sequencing of gDNA was followed by alignment to GRCH37/hg19 and variant calling with GATK
- Variants were annotated with snpEff tool and VEP

METHODS

Figure 1: Extreme Phenotype Sampling (EPS) Model

48 kidney transplant recipients with the 12 highest and 12 lowest Tac troughs from the EA or AA cohorts, after accounting for clinical factors and known common genetic variants were selected

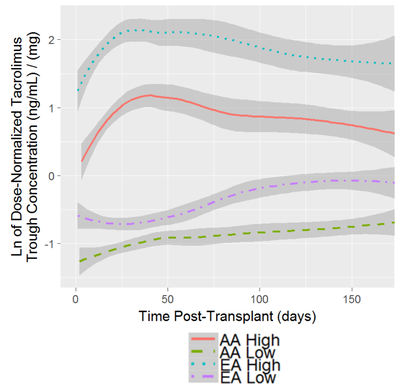


- Logistic regression as low vs. high (case-control) or Linear regression of dose normalized Tac troughs (continuous) tests were performed in two ways
  - A) Association test for single variants
  - B) Gene based tests: burden test, SKAT and SKATO

RESULTS

Figure 2: EPS model with Ln of dose-normalized Tac trough groups in the first 6-months posttransplant

Data represent smoothed conditional means and gray areas are 95% confidence intervals. The 12 EA subjects with the highest (0.8%) or 12 with the lowest (0.8%) Tac troughs were used for NGS from a cohort of 1,443 total subjects. The 12 AA subjects with the highest (3.5%) or 12 with the lowest (3.5%) Tac troughs were used for NGS from a cohort of 345 total subjects.



RESULTS

Table 2: Clinical and Genetic Characteristics of the Extreme Phenotype Subjects in African American (AA) and European American (EA) Groups.

The High and Low groups had the highest and lowest dose-normalized Tac troughs, AA cohort N=345 and EA cohort N=1443.

Variable		Dose-Normalized Tac Trough Groups			
		AA High	AA Low	EA High	EA Low
N		12	12	12	12
Age	18-34	2	2	1	0
	35-64	9	10	8	10
	65-84	1	0	3	2
Diabetes	yes	6	9	9	7
	no	6	3	3	5
Donor Status	Living	2	8	11	7
	Deceased	10	4	1	5
Donor Gender	Male	7	4	7	10
	Female	5	8	5	2
Number of subjects with *CYP3A5 *3* Alleles rs776746_G	0	5	4	0	0
	1	6	7	1	2
	2	1	1	11	10
Number of subjects with *CYP3A5 *6* Alleles rs10264272_T	0	10	10	12	12
	1	1	2	0	0
	2	1	0	0	0
Number of subjects with *CYP3A5 *7* Alleles rs41303343_TA	0	8	11	12	12
	1	3	1	0	0
	2	1	0	0	0
Number of subjects with *CYP3A4 *22* Alleles rs35599367_A	0	11	12	10	11
	1	1	0	2	1
	2	0	0	0	0
Number of subjects with known CYP3A Loss of Function Alleles (*CYP3A5 *3,*6,*7,CYP3A4 *22*)	0	2	1	0	0
	1	4	10	1	2
	2	6	1	11	10
Estimated Glomerular Filtration Rate* (mL/min)	< 54.9	19.9%	9.1%	19.0%	31.6%
	54.9-67.9	11.7%	45.7%	28.8%	27.0%
	67.9-83.5	24.5%	17.8%	22.3%	20.9%
	>83.5	43.9%	27.4%	29.9%	20.5%
Weight (kg)*	< 69.4	26.5%	4.6%	56.5%	20.5%
	69.4-80.9	20.9%	12.8%	28.3%	49.3%
	80.9-94.6	32.7%	21.0%	12.0%	0.9%
	>94.6	19.9%	61.6%	3.3%	29.3%
Steroid Use in First 6 Months	Yes	11	11	12	12
	No	1	1	0	0
Simultaneous Pancreas and Kidney Transplant	Yes	1	0	0	1
	No	11	12	12	11
Antibody Induction	Monoclonal	8	5	5	3
	Polyclonal	4	7	7	8
Calcium Channel Blocker in First 6 Months	Yes	8	9	5	9
	No	4	3	7	3
ACE Inhibitor in First 6 Months	Yes	4	4	5	2
	No	8	8	7	10
Antiviral Use in First 6 Months	Yes	12	9	12	11
	No	0	3	0	1
Tac Daily Dose (mg) Median (range)		4.0 (.5 – 12)	14.0 (1.0 – 36)	1.0 (0.1 – 6.0)	14.0 (2.0 – 36.0)
Tac Trough Concentration (ng/mL) Median (range)**		7.5 (1– 21)	5.1 (1-18)	8.9 (2.4 – 26)	8.1 (1.3 – 29)
Dose Normalized Tac Trough Concentration (ng/mL) Median (range)		2.4 (0.3-31)	0.38 (.083-1.4)	7.7 (1.0-82)	0.57 (0.13-4.8)

* eGFR and Weight are for time point closest to the corresponding Tac trough measurement
**Tac troughs were measured periodically for each subject, up to 24 times per subject.

Figure 3: Variant Effect Predictor (VEP) results on identified genetic variants. Of 18,661 identified variants, 3,961 (21.2%) were unknown. A) VEP consequences of 18,661 genetic variants B) VEP gene expression on coding sequences

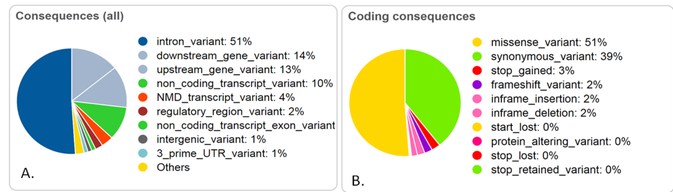
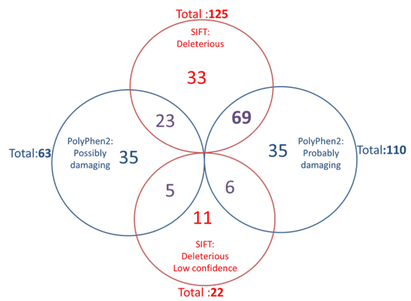


Figure 4: SIFT and PolyPhen2 results of all 18,661 variants in a Venn diagram



- 69 Variants were predicted by SIFT and PolyPhen2 to disrupt protein function
- A group of 125 deleterious variants, by SIFT analysis, were associated with Tac troughs in EAs (burden test, p=0.008) by case-control test
- By both logistic regression case-control test and linear regression continuous trait test
  - 15 Variants in *POR*, *ADIPOR1, OVCH2* and *CYB5R2* were associated with AA group (p<0.005)
  - 9 Variants in *ABCC1, ANAPC10, NR3C1 and OTUD4* were associated with EA group (p<0.005)
- Gene based test identified the association of *CYB5R2* with Tac and AAs (SKAT, p=0.00079)
- In *CYB5R2*, rs61733057 (elevated allele frequency in AAs) was predicted to disrupt protein function by SIFT & PolyPhen2

CONCLUSIONS

The identified variants in *CYB5R2* and other genes merit further validation and investigation relevant to Tac metabolism.

Acknowledgements: This study was supported in part by NIH/NIAID grants 5U19-AI070119, 5U01-AI058013 and K01AI130409