Gut Microbiome Meta-transcriptomics and Mycophenolate Mofetil Enterohepatic Recirculation Variability in Kidney Transplant Recipients.

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INTRODUCTION

- Mycophenolate mofetil (MMF) is used in >90% of kidney transplant recipients (KTRs).
- Its inactive metabolite, MPAG, is de-glucuronidated by bacterial beta-glucuronidase (βGUS) enzymes in the gut and the active metabolite MPA is reabsorbed back into the blood in a process know as enterohepatic recirculation (EHR).
- EHR leads to a second MPA peak, increasing MPA blood concentrations, enhancing immunosuppression and possibly increased toxicity in KTRs.
- The numbers and diversity of βGUS these enzymes is not well defined in KTRs.

HYPOTHESIS

- We investigated the association between a sequencing panel of βGUS enzymes and MPA pharmacokinetic (PK) parameters in KTRs.
- We hypothesized that KTRs with extensive EHR in-vivo would have greater abundance of β GUS in their stool microbiome.

METHODS

- Adult KTRs (37 in a prospective cohort (<6 months post-transplant) and 47 in cross-sectional cohort (2+ years post-transplant)) underwent a 12hr MPA PK study with stool collection.
- Microbiome meta-transcriptomics data were processed and matched against a BLAST database panel of 279 βGUS enzymes from the human microbiome clustered gene indices (HMGC).
- The relative abundance of βGUS transcripts were associated with MPA %EHR ([MPA AUC5-12/AUC0-12] × 100) and the number of EHR peaks observed during the 12hr PK using linear regression models.

RESULTS

Table 1. Demographic characteristics of study participants

Characteristic	Cross-sectional cohort	Prospective cohort	p-value
Number of Participants, count (%)	47 (55.95%)	37 (44.05%)	
Time to PK from transplant (days), mean(sd)	2288 (699)	66.6 (16.1)	<0.05
Race, count (%)			0.69
White	33 (70.2%)	26(70.3%)	
Black or African American	10 (21.3%)	8 (23.5%)	
Asian	1 (2.13%)	2 (5.4%)	
Ethnicity, count (%)			0.61
Hispanic or Latino	2 (4.26%)	4 (10.8%)	
Not Hispanic or Latino	37 (78.7%)	29 (78.4%)	
Donor Status, count (%)			0.31
Living Donor	28 (59.6%)	17 (45.9%)	
Deceased Donor	19 (40.4%)	20 (54.1%)	
Gender, count (%)			0.5
Male	36 (76.6%)	25 (67.6%)	
Female	11 (23.4%)	12 (32.4%)	
Primary cause of Transplant, count (%)			0.68
Diabetes	11 (23.4%)	8 (21.6%)	
Glomerular Disease	13 (27.7%)	7 (18.6%)	
Hypertensive Nephrosclerosis	4 (8.51%)	5 (13.5%)	
Other	7 (14.9%)	10 (27.0%)	
Polycystic Kidney Disease	8 (17%)	5 (13.5%)	
Unknown	4 (8.51%)	2 (5.41%)	
Age at transplant, mean(sd)	56.8 (12.8)	53.5 (14.1)	0.26
eGFR (ml/min/1.73m2), mean(sd)	69.8 (18.2)	59.2 (15.3)	<0.05
Standardized measured creatinine clearance (ml/min/1.73m2), mean(sd)	73.4 (27.7)	65.0 (19.5)	0.12
Total bilirubin (mg/dL), mean(sd)	0.60 (0.33)	0.46 (0.57)	0.18
Albumin (g/dL), mean(sd)	4.2 (0.4)	3.9 (0.4)	<0.05
PPI Use, count(%)	5 (10.6%)	11 (29.7%)	0.06
Antifungal use, count(%)	1 (2.13%)	3 (8.11%)	0.45
Antiviral use, count(%)	3 (6.38%)	36 (97.3)	<0.05
Antibiotic use, count(%)	26 (55.3%)	34 (91.9%)	<0.05
Steroid use, count(%)	7 (14.9%)	22 (59.5%)	<0.05

AUC= area under the curve; EHR = enterohepatic recirculation; PPI = proton pump inhibitor; pk = pharmacokinetic visit

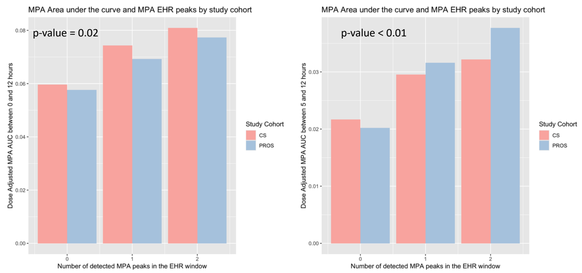

Figure 1: Distribution of MPA AUC $_{0\text{-}12}$ and MPA AUC $_{5\text{-}12}$ across MPA EHR peaks. Pharmacokinetic parameters were determined using noncompartmental analysis. The partial MPA AUC5-12 was selected to represent EHR because the secondary peaks associated with EHR were observed at ≥5 hours post-dose. Secondary peaks were defined as any increase in the plasma concentration after 5 hours post-dose of at least 1 mg/L following a disposition phase (i.e. decreasing concentration).

Table 2. Pearson correlations between βGUS transcripts and MPA PK parameters in the prospective cohort

Measure	Pearson correlation	
	r	p-value
βGUS transcripts - Number of PK Peaks	0.36	0.03
MPA % EHR - Number of PK peaks	0.52	0.001
βGUS transcripts - MPA % EHR	0.24	0.16

*MPA %EHR was calculated as (MPA $AUC_{5\text{-}12}/AUC_{0\text{-}12} \times 100$)

Table 3. Subgroup analysis of the association between the relative abundance of BGUS transcripts and PK parameters restricted to the lowest (0) and highest (2+) number of MPA EHR peaks.

Pharmacokinetic parameters	Overall cohort (n=36)			
	Univariate Model		Multivariate Model	
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
AUC 0-12 hrs	53.72 (-54.51, 161.95)	0.34	53.68 (-63.37, 170.72)	0.38
AUC 5-12 hrs	63.54 (9.92, 117.15)	0.38	61.25 (4.72, 117.79)	0.04
EHR	0.88 (0.33, 1.43)	0.04	0.82 (0.24, 1.41)	0.01
	Prospective cohort (n=17)			
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
AUC 0-12 hrs	76.40 (-48.98, 201.78)	0.25	59.05 (-86.17, 204.27)	0.44
AUC 5-12 hrs	86.80 (22.81, 150.79)	0.02	77.47 (10.39, 144.54)	0.04
EHR	1.13 (0.31, 1.96)	0.04	1.07 (0.17, 1.97)	0.04
	Cross-sectional cohort (n=19)			
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
AUC 0-12 hrs	3.24 (-201.05, 207.52)	0.98	33.49 (-182.98, 249.97)	0.77
AUC 5-12 hrs	10.49 (-83.69, 104.67)	0.83	27.09 (-68.09, 122.26)	0.59
EHR	0.24 (-0.41, 0.89)	0.48	0.35 (-0.33, 1.02)	0.33

*Multivariate model was adjusted for cohort, eGFR (ml/min/1.73m2), albumin, antibiotics use at the time of PK, and steroid use at the time of PK.

- In the prospective cohort, MPA % EHR during the 12-hour PK was associated with the relative abundance of βGUS transcripts (p=0.04) when comparing participants with the lowest (0) and highest (2+) number of MPA EHR peaks.
- In the cross-sectional cohort, no associations were found with PK and relative abundance of βGUS transcripts.

CONCLUSIONS

- These findings suggests that (βGUS) transcripts play a role in MPA variability, captured by the number of peaks during PK. Developing in-vitro panels for these specific βGUS microbial enzymes responsible for EHR will be necessary elucidate their clinical implications.

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