Gut microbiome signature associated with mycophenolate mofetil enterohepatic recirculation.

Guillaume Onyeaghala[1], Duy Vo[1], Bryan Sanchez[2] , Abdelrahman Saqr[3], Moataz Mohamed[3], Christopher Staley[3], Levi Teigen[3], Casey Dorr[1,2], Weihua Guan[3], Rasha El-Rifai[3], Arthur Matas[3], Rorry Remmel[3], William Oetting[3], Pamala Jacobson[3], Ajay Israni[1]

[1]University of Texas Medical Branch, Galveston, [2]Hennepin Healthcare Research Institute, Minneapolis, [3]University of Minnesota, Minneapolis

INTRODUCTION

- Mycophenolate mofetil (MMF) is used in >90% of kidney transplant recipients (KTRs) for immunosuppresion.
- MMF is bio-transformed to mycophenolic acid (MPA), the active metabolite, and MPA glucuronide (MPAG), an inactive metabolite (MPAG). MPAG is metabolized by gut microbiota, particularly by beta-glucuronidase (β-GUS) producers, and MPA is reabsorbed into the blood in a process know as enterohepatic recirculation (EHR).
- EHR leads to a secondary MPA peak, increasing MPA blood concentrations, enhancing immunosuppression and possibly toxicity in KTRs.

HYPOTHESIS

- We hypothesized that KTRs with extensive EHR in-vivo would have a distinct gut microbiome signature associated with EHR.

METHODS

- Participants (n=84, 37 prospective and 47 cross-sectional) underwent a pharmacokinetic (PK) study and microbiome stool collection post-kidney-transplant in the Microbiome and Immunosuppression in Kidney Transplantation (MISSION) study.
- A stool sample and 24-hour food recall was collected at the time of the PK study.
- Shotgun sequencing data from the stool samples were processed using HUMAnN 3.7 and analyzed with MaAsLin2. Zero inflated Poisson regression models were used for a-priori univariate analyses.
- Our main outcome was the MPA % EHR, defined as MPA $AUC_{5\text{-}12}$ / $AUC_{0\text{-}12}$ x 100.
  - In a secondary analysis, we also investigated the following PK parameters: MPA % EHR stratified in tertiles, MPAG AUC, MPA AUC to MPAG AUC between 5 and 12 hrs (window of secondary peak)

RESULTS

Table 1. Participant demographic and baseline characteristics

Variable	Cohort		
	Prospective (N=37)	Cross-sectional (N= 47)	Full Cohort (N=84)
Age at PK assessment, yr, mean (SD)	53.7 (14.1)	57.3 (12.8)	55.4 (13.4)
Gender, n (%)			
Female	12 (32.4)	11 (23.4)	23 (27.4)
Male	25 (67.6)	36 (76.6)	61 (72.6)
Ancestry, n (%)			
European	26 (70.2)	33 (70.2)	59 (70.2)
Black or African American	8 (21.6)	10 (21.3)	18 (21.4)
Asian or Pacific Islander	2 (5.4)	1 (2.1)	3 (3.6)
Native American	N/A	2 (4.2)	2 (2.4)
Unreported	1 (2.7)	1 (2.1)	2 (2.4)
eGFR, ml/min/1.73m2, mean (SD)*	59.2 (15.3)	69.8 (18.2)	65.13 (17.69)
Total bilirubin, mg/dL, mean (SD)	0.46 (0.57)	0.61 (0.32)	0.54 (0.45)
MMF daily dose, mg, mean (sd)	1234.0 (411.8)	1405.4 (302.5)	1309.5 (375.5)
MPA AUC0-12 hr, mg.h/L, mean (SD)	47.0 (15.2)	42.6 (17.9)	44.6 (16.8)
MPA EHR (AUC5-12 hr/AUC0-12 hr), mean (SD)	0.44 (0.10)	0.38 (0.07)	0.41 (0.09)

*eGFR, estimated glomerular filtration rate. Calculated using race-free eGFR equation.

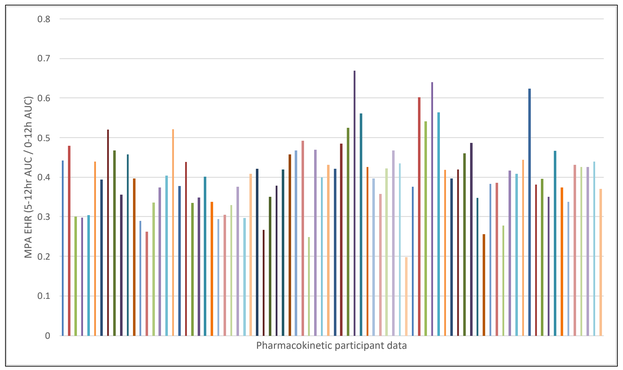


Figure 1: Enterohepatic recirculation variability among KTRs. Enterohepatic recirculation (EHR) was calculated as the ratio of MPA area under the concentration curve (AUC) for hours 5-12 to AUC for hours 0-12.

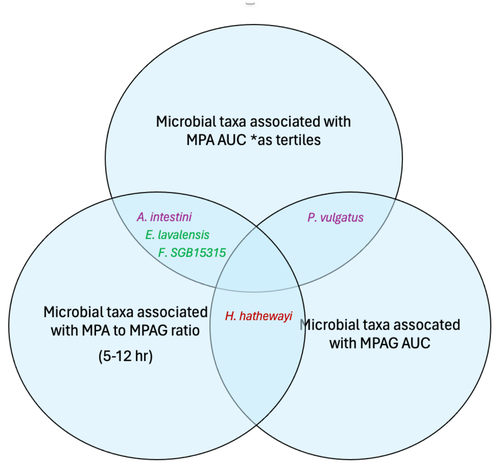
Table 2. Association between bacterial taxa and MPA EHR in the full cohort (n=84) at the time of PK

Bacterial taxa (species level)	Organism prevalence in full cohort	p-value
Faecalibacillus intestinalis	13.10%	0.004
Ruminococcus bromii*	16.70%	0.005
Blautia obeum*	63.10%	0.008
Parabacteroides distasonis*	36.90%	0.015
Gordonibacter pamelaeae	23.80%	0.024
Acidaminococcus intestini	11.90%	0.029

*β-GUS producing organisms

†The p-value for the association was generated with MaAsLin2, adjusting for the cohort variable (prospective vs cross-sectional).

Figure 2. Shared microbiome taxa across multiple PK parameters



The microbial taxa in purple represent associations with the full cohort (n=84), whereas the microbial taxa in green and red represent associations with the prospective (n=37) and cross-sectional (n=47) cohorts, respectively. All associations were generated with MaAsLin2.

- MPA EHR was highly variable within KTRs, among both early (<6 months) KTRs and stable KTRs who have had a transplant for more than 2 years (Figure 1).
- Our microbiome association analysis identified several taxa which are possibly associated with MPA % EHR. However, the findings were not statistically significant after multiple hypothesis testing correction (FDR, Table 2).
- We did not find strong evidence of a consistent group of bacterial taxa associated with multiple PK parameters in our secondary analysis. The the reported taxa were not statistically significant after FDR (Figure 2).

CONCLUSIONS

- Our preliminary findings suggest that the relative abundance of gut taxa is associated with MPA % EHR in KTRs, some of which (*R. bromii, B. obeum and P. distasonis*) have been previously reported as β-GUS producing organisms.
- Larger studies including ascertainment of β-GUS activity are needed to understand the interplay between the gut microbiome and MPA EHR.

Acknowledgements: This study was supported by NIH/NIAID grant **5R01AI140303.**