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Gut Microbiome Meta-transcriptomics Associated with Mycophenolate Mofetil Enterohepatic Recirculation in Kidney Transplant Recipients.

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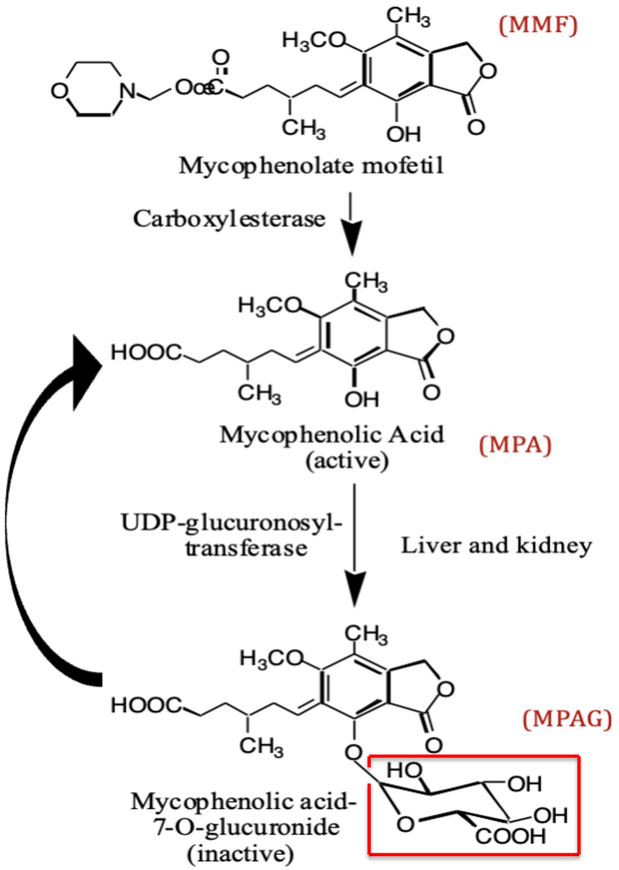
Introduction

Mycophenolic acid (MPA) area under the curve (AUC) has been associated with adverse events such as acute rejection, anemia, leukopenia, and diarrhea.

Bacterial β-glucuronidases (βGUS) in the β-D-glucuronidase pathway convert the inactivated mycophenolic acid glucuronide (MPAG) back to the active metabolite, mycophenolic acid (MPA).

This process, known as enterohepatic recirculation (EHR), enhances immunosuppression and toxicity in kidney transplant recipients (KTRs). EHR plays a key role in MPA variability and area under the curve (AUC).

We hypothesized that the abundance of transcripts for the β-D-glucuronidase pathways, are associated with EHR in kidney transplant recipients.



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Methods

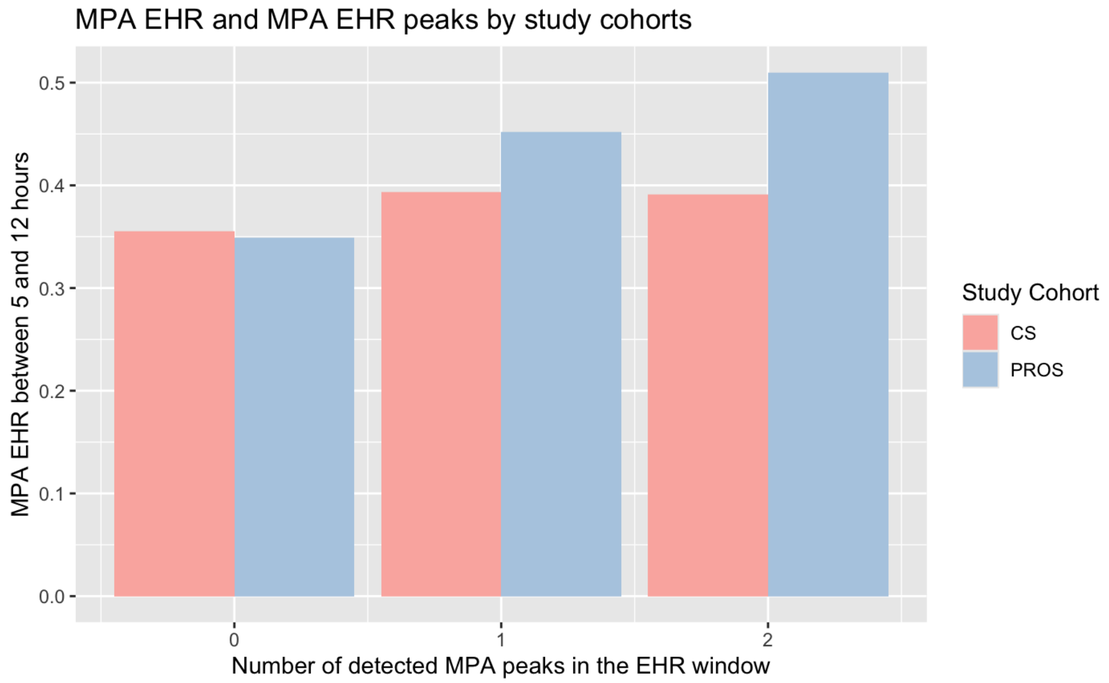
- Adult KTRs (34 in prospective cohort (<6 months post-transplant) and 47 in cross-sectional cohort (2+ years post-transplant)) underwent a 12hr MPA pharmacokinetic (PK) study with stool collection.

- Microbiome meta-transcriptomics data were processed with HUMAnN3 to identify transcripts belonging to the β-D-glucuronidase pathway (GLUCUROCAT-PWY).
  - The number of GLUCUROCAT-PWY transcripts was adjusted for the total reads per samples to generate the relative abundance of transcripts.
  - Transcripts in the β-D-glucuronidase pathway were associated with MPA $AUC_{5\text{-}12}$, MPA $AUC_{0\text{-}12}$ and MPA %EHR (MPA $AUC_{5\text{-}12}/AUC_{0\text{-}12}\times100$).

- A random forest regressor was used to determine which bacterial species were most predictive of transcripts in the β-D-glucuronidase pathway.
  - MaAsLin2 was used to identify the association between the relative abundance of bacterial species identified by the random forest machine learning method and the abundance of transcripts in the β-D-glucuronidase pathway.

Study cohort & Pharmacokinetic (pk) characteristics

Mission Study			
Characteristic	**Cross-sectional cohort (CS)**	**Prospective cohort (PS)**	**p-value**
Number of Participants, count (%)	47 (58%)	34 (42%)	
Race (White), count(%)	33 (70%)	24 (70%)	0.68
Gender (Male) , count(%)	36 (76.6%)	22(64.7%)	0.35
Age at transplant, mean(sd)	56.8 (12.8)	52.7 (14.4)	0.18
eGFR (ml/min/1.73m2), mean(sd)	69.8 (18.2)	58.8 (15.7)	<0.05
Total bilirubin (mg/dL), mean(sd)	0.6 (0.3)	0.4 (0.6)	0.15
Albumin (g/dL), mean(sd)	4.2 (0.4)	3.9 (0.4)	<0.05
MPA AUC0-12 hr (mg.h/L), mean(sd)	42.6 (17.9)	46.2 (15.4)	0.33
MPA AUC5-12 hr (mg.h/L), mean(sd)	16.5 (8.0)	20.3 (8.2)	<0.05
MPA EHR (AUC5-12 hr/AUC0-12 hr), mean(sd)	0.4 (0.1)	0.4 (0.1)	<0.05



Differences in EHR levels	
Characteristic	**p-value**
Cohort (prospective vs cross-sectional)	<0.01
Number of MPA peaks during EHR	<0.05

Association between the relative abundance of BGUS pathway transcripts and PK parameters

Difference across all MPA EHR peaks groups

Overall Cohort (N=81)

Pharmacokinetic Parameter	t-value	P-value
Number of MPA Peaks	1.51	0.13
MPA $AUC0_{-12\ hr}$	-1.34	0.18
MPA $AUC_{5\text{-}12\ hr}$	-0.55	0.58
MPA $AUC_{5\text{-}12\ hr}$ / $AUC_{0\text{-}12\ hr}$	1.08	0.28

Prospective Arm (N=34)

Pharmacokinetic Parameter	t-value	P-value
Number of MPA Peaks	1.56	0.13
MPA $AUC0_{-12\ hr}$	-0.75	0.46
MPA $AUC_{5\text{-}12\ hr}$	0.54	0.6
MPA $AUC_{5\text{-}12\ hr}$ / $AUC_{0\text{-}12\ hr}$	2.05	0.04

Association between the relative abundance of BGUS pathway transcripts and PK parameters, subgroup analysis

Difference between lowest (0) and highest (2+) MPA EHR Peaks groups

Overall Cohort (N=35)

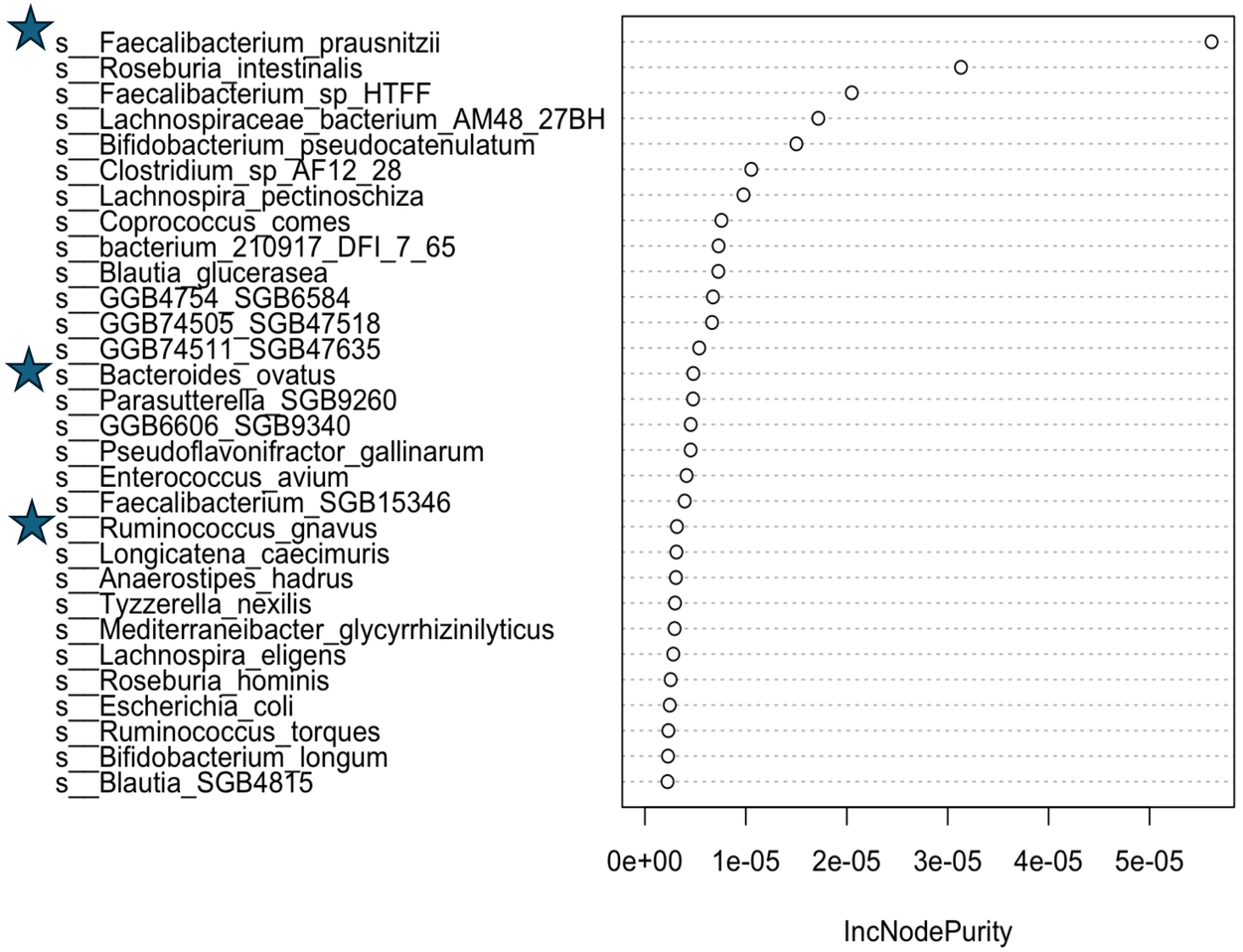
Pharmacokinetic Parameter	t-value	p-value
Number of MPA Peaks	1.43	0.16
MPA $AUC0_{-12\ hr}$	-0.95	0.34
MPA $AUC_{5\text{-}12\ hr}$	0.55	0.58
MPA $AUC_{5\text{-}12\ hr}$ / $AUC_{0\text{-}12\ hr}$	3.01	<0.01

Prospective Arm (N=16)

Pharmacokinetic Parameter	t-value	p-value
Number of MPA Peaks	1.24	0.23
MPA $AUC0_{-12\ hr}$	-0.85	0.41
MPA $AUC_{5\text{-}12\ hr}$	0.65	0.53
MPA $AUC_{5\text{-}12\ hr}$ / $AUC_{0\text{-}12\ hr}$	2.52	0.02

Association between the relative abundance of BGUS pathway transcripts and gut microbiome composition

Random forest classification of bacterial taxa most predictive of BGUS pathway transcript abundance



Differential expression analysis of species level bacterial taxa associated with BGUS pathway transcript abundance

Bacterial Taxa (Species level)	Prevalence in cohort	p-value
Faecalibacterium prausnitzii	82.50%	<0.01
bacterium 210917 DFI 7 65	15%	<0.01
Faecalibacterium sp HTFF	16.25%	<0.01
Coprococcus comes	27.50%	<0.01
Roseburia intestinalis	43.75%	<0.01
Anaerostipes hadrus	26.25%	<0.05
Bacteroides ovatus	52.50%	<0.05
Ruminococcus torques	62.50%	<0.05
Escherichia coli	17.50%	<0.05
Ruminococcus gnavus	40%	<0.05
Blautia glucerasea	40%	<0.05

**11 of the 30 top predictive species level bacterial taxa were also associated with BGUS pathway transcript abundance (p <0.05), although these associations did not hold after multiple adjustment.

Take-away

- MPA pharmacokinetics
  - MPA % EHR levels were significantly higher in the group of PK participants with 2 or more EHR peaks during PK than those with no EHR peak during PK.

- BGUS transcripts
  - MPA EHR was associated with BGUS transcripts in participants who were still in the early (<6 months) post transplant period.
  - The relative abundance of BGUS transcripts was statistically significantly associated with EHR in the group of PK participants with 2 or more EHR peaks during PK compared to those with no EHR peak during PK.

- Microbiome composition
  - 11 out of the top 30 most predictive gut microbial taxa were also associated with BGUS transcripts.
  - Several of these organisms can serve as model organisms to modulate BGUS metabolism of MPA EHR in-vitro.

- Future Directions
  - Stool-based BGUS proteomics levels.
  - Population PK models developed with microbiome covariates.

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