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

utmb Health

Department of Internal Medicine – Nephrology

G. Onyeaghala[1], D. Vo[1], K. Gupta[1], M. Mohamed[2], A. Saqr[2], J. Sekela[3], L. Teigen[2], C. Dorr[2], C. Staley[2], W. Guan[2], G. Golovko[1], R El-Rifai[2], A. Matas[2], R. Remmel[2], W. Oetting[2], M. R. Redinbo[3], P. Jacobson[2], A. Israni[1]

[1]University of Texas Medical Branch, Galveston, [2]University of Minnesota, Minneapolis, [3]University of North Carolina, Chapel Hill

Introduction

- Mycophenolate mofetil (MMF) is an important immunosuppressant commonly prescribed post organ transplantation (tx). Once ingested, MMF is bioactivated by the esterase enzymes in the GI tract and liver into mycophenolic acid (MPA) (Figure 1A). This drug poses a challenge in dosing because of its narrow therapeutic index and the unpredictable concentration of its active form MPA. MPA is metabolized in the liver through glucuronidation, producing mycophenolate glucuronide (MPAG), a more soluble metabolite that is excreted primarily in the bile.
- This metabolite undergoes extensive enterohepatic recirculation (EHR) via bacterial β-glucuronidase (βGUS) enzymes which reactivate MPA from MPAG within the GI tract. βGUS enzymes remove the glucuronide from MPAG to create MPA, which in turn is recirculated. MMF causes severe gastrointestinal toxicity in about 45% of recipients, so dosage is carefully controlled and can be affected by this EHR.
- We hypothesize that the abundance of transcripts for the β-D-glucuronidase and its isomeric analog β-D-galacturonidase pathways, is associated with EHR in kidney transplant recipients.

Methods and Materials

MISSION Study

- 84 patients who had undergone kidney transplant received MMF via their treatment plan and stool samples were collected at various time points for microbiome research.
- 47 patients were in the cross-sectional cohort (2+ years post-transplant) and 35 were in the prospective cohort (<6 months post transplant) (Table 1).
  - Prospective cohort: Patients were enrolled **<u>at the time of transplant</u>** between 2020 and 2023. Stool samples were collected at 1 week post-transplant, during PK sampling (30–90 days), and again at 4 and 6 months post-transplant.
  - Cross-sectional cohort: Participants were those who received a kidney transplant between January 1, 2012 and 2019. Stool samples were collected only at the time of MMF PK sampling.
- A 12-hour pharmacokinetics (PK) analysis was performed on the samples from both cohorts to determine drug concentrations.
- Enterohepatic recirculation (EHR) was calculated via the area under the curve (AUC) as the ratio of partial AUC between 5 to 12hr to total AUC 0 to 12hr.

Bioinformatics

- Microbiome meta-transcriptomics data were processed with HUMAnN3. In each cohort, MaAsLin2 was used to identify functional pathways associated with MPA EHR.
- In an exploratory correlation network analysis, we implemented the Louvain Modularity Maximization algorithm on the correlation between our transcripts, PK parameters and the relative abundance of 7 βGUS producing bacteria present in more than 10% of the samples (Threshold R=0.3).

Demographics				
Variable	**N (CS/PS)**	**CS**	**PS**	**p-value**
Male, n(%)	47/37	36 (76.6)	25 (67.57)	0.3569
White, n(%)	47/37	33 (70.21)	26 (70.27)	0.6941
Hispanic or Latino, n(%)	47/37	2 (4.26)	4 (10.81)	0.6139
Age at PK in years, mean(sd)	47/37	56.85 (12.81)	53.49 (14.15)	0.257
Weight at PK in kg, mean(sd)	47/37	87.94 (20.67)	86.36 (16.64)	0.706
Height at PK in cm, mean(sd)	47/37	172.91 (10.79)	171.88 (11.14)	0.6706
Medications				
PPI use, n(%)	47/37	5 (10.67)	11 (29.73)	0.027
Antifungal use, n(%)	47/37	1 (2.13)	3 (8.11)	0.2013
Antibiotic use, n(%)	47/37	26 (55.32)	34 (91.89)	0.0002
Antiviral use, n(%)	47/37	3 (6.38)	36 (97.3)	<0.001
Steroid use, n(%)	47/37	7 (14.89)	22 (59.46)	<0.001
Lab Values				
eGFR (ml/min/1.73m2), mean(sd)	47/37	60.95 (11.66)	54.94 (10.95)	0.0184
eGFR, Epi_RF_Cr2021, mean(sd)	47/37	69.79 (18.18)	59.21 (15.31)	0.0058
cbc_hgb, mean(sd)	47/37	13.48 (1.171)	11.53 (1.73)	<0.001
cbc_hct, mean(sd)	47/37	41.61 (4.74)	36.15 (5.32)	<0.001
Dietary Values				
Energy in kcal, mean(sd)	41/24	1939.99 (747.23)	2077.14 (594.91)	0.4458
Total fat in grams, mean(sd)	41/24	83.51 (46.17)	92.72 (32.44)	0.3936
Total carbohydrates in grams, mean(sd)	41/24	229.18 (121.76)	224.77 (78.22)	0.8596
Total protein in grams, mean(sd)	41/24	72.53 (32.03)	91.41 (29.02)	0.0208
Total dietary fiber in grams, mean(sd)	41/24	17.12 (9.49)	16.09 (6.47)	0.6388
HEI 2015 total score, mean(sd)	41/24	47.95 (10.27)	48.99 (10.99)	0.7041

Table 1. Mission study PK cohorts descriptive statistics. Outlines the demographics, medications, lab values, and dietary values of all 84 participants in both the cross-sectional (CS) as well as prospective (PS) cohorts. P-values were calculated based on the difference in values between the cohorts.

Results

- β-D-glucuronidase and β-D-galacturonidase transcripts were highly prevalent in the prospective and cross-sectional cohorts (>90%).
- Our exploratory correlation network analyses suggest that the relative abundances of *Faecalibacterium prausnitzii* and *Blautia wexlerae* were strongly correlated with increased β-D-glucuronidase transcripts (Figure 1B).
- In the prospective cohort, higher abundances of β-D-glucuronidase transcripts were associated with increased EHR (p=0.04) and higher abundances of β-D-galacturonidase transcripts were associated with low MPAG AUC (p=0.02) (Table 2). In the cross-sectional cohort, no associations were found with PK parameters and βGUS meta-transcriptomics.

Association between gut microbiome transcriptomics and PK parameters

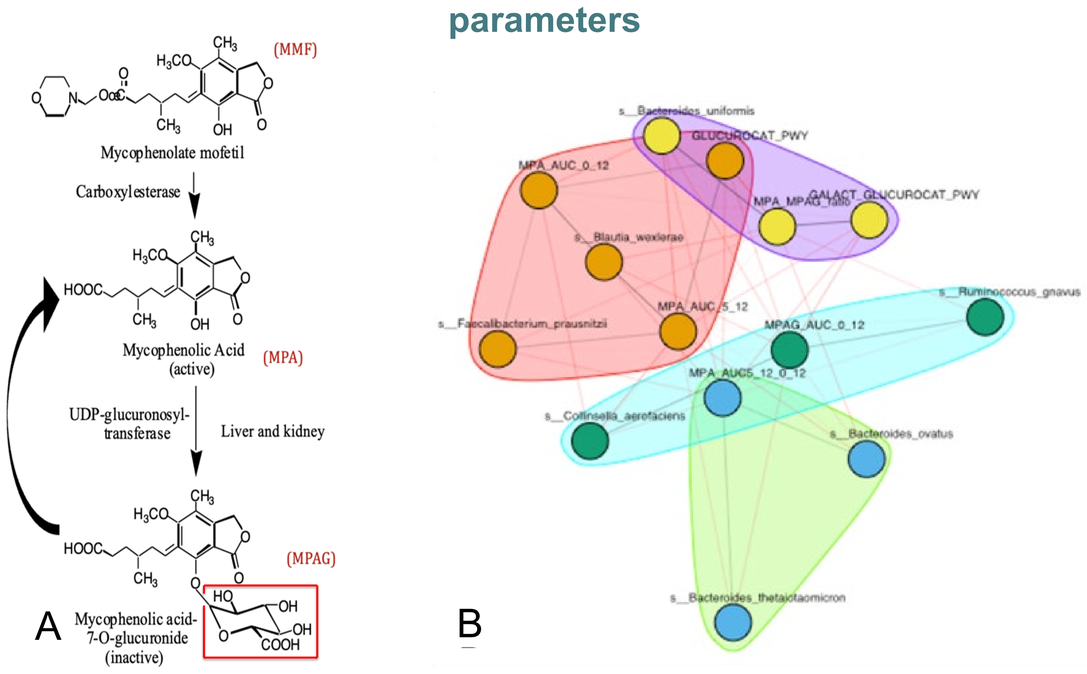

Figure 1A: Metabolic pathway of Mycophenolate (MMF). Glucuronide moiety in red box.
Figure 1B: Correlation network between known βGUS producers and βGUS transcriptomic pathway in the prospective cohort

Prospective Cohort				
Functional Pathway	**Prevalence**	**Pharmacokinetic Parameter**	**Coefficient**	**p-value**
GLUCUROCAT-PWY	91.40%	MPA $AUC_{5\text{-}12\,hr}$ / $AUC_{0\text{-}12\,hr}$	0.71	0.04
GALACTUROCAT-PWY	97.10%	MPA $AUC_{5\text{-}12\,hr}$ / $AUC_{0\text{-}12\,hr}$	0.46	0.14
GLUCUROCAT-PWY	91.40%	MPAG $AUC_{0\text{-}12\,hr}$	-0.54	0.13
GALACTUROCAT-PWY	97.10%	MPAG $AUC_{0\text{-}12\,hr}$	-0.70	0.02
GLUCUROCAT-PWY	91.40%	MPAG $AUC_{0\text{-}12\,hr}$ / MPA $AUC_{0\text{-}12\,hr}$	-0.14	0.71
GALACTUROCAT-PWY	97.10%	MPAG $AUC_{0\text{-}12\,hr}$ / MPA $AUC_{0\text{-}12\,hr}$	-0.47	0.14
Cross-sectional Cohort				
Functional Pathway	**Prevalence**	**Pharmacokinetic Parameter**	**Coefficient**	**p-value**
GLUCUROCAT-PWY	95.70%	MPA $AUC_{5\text{-}12\,hr}$ / $AUC_{0\text{-}12\,hr}$	-0.12	0.55
GALACTUROCAT-PWY	95.70%	MPA $AUC_{5\text{-}12\,hr}$ / $AUC_{0\text{-}12\,hr}$	-0.12	0.61
GLUCUROCAT-PWY	95.70%	MPAG $AUC_{0\text{-}12\,hr}$	-0.06	0.77
GALACTUROCAT-PWY	95.70%	MPAG $AUC_{0\text{-}12\,hr}$	0.14	0.55
GLUCUROCAT-PWY	95.70%	MPAG $AUC_{0\text{-}12\,hr}$ / MPA $AUC_{0\text{-}12\,hr}$	-0.07	0.71
GALACTUROCAT-PWY	95.70%	MPAG $AUC_{0\text{-}12\,hr}$ / MPA $AUC_{0\text{-}12\,hr}$	0.08	0.75

Table 2: Association of βGUS related pathways and MPA %EHR, MPAG $AUC_{0\text{-}12}$ and MPAG $AUC_{0\text{-}12}$/MPA $AUC_{0\text{-}12}$.

Conclusions

- The bacterial β-glucuronidase (βGUS) transcriptomic pathway, specifically GLUCUROCAT-PWY, was found to be associated with EHR due to MPA exposure in kidney transplant recipients during the early post-transplant period.
- This association suggests a potential role of microbial βGUS activity in modulating MPA metabolism and its clinical outcomes.
- To precisely identify the βGUS-expressing microbial enzymes contributing to EHR, integrated multi-omics approaches (e.g., metagenomics, metatranscriptomics, metabolomics) will be required.
- These integrated approaches will provide insights into the mechanistic role of βGUS enzymes in post-transplant complications and may inform targeted interventions to mitigate EHR risk.

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

Student name: Duy Vo