Gut microbiome signature associated with mycophenolate mofetil related diarrhea in kidney transplant recipients.

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INTRODUCTION

- Mycophenolate mofetil (MMF) is used in >90% of kidney transplant recipients (KTRs) and associated with diarrhea.
- Recipients developing diarrhea are generally managed with lower MMF daily doses or longer dosing intervals, which are associated with reduced adherence to immunosuppression and poorer outcomes.
- 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 esp by beta-glucuronidase (BGUS) producers.

HYPOTHESIS

- We hypothesized that KTRs who experienced severe diarrhea after transplantation would have a distinct microbiome signature, likely associated with the presence of beta-glucuronidase producing bacteria.

METHODS

- The Microbiome and Immunosuppression in Kidney Transplantation (MISSION) study assessed diarrhea posttx twice weekly for 6 months, using a HIPAA compliant text-based survey (Mosio, Inc , Seattle, WA). Diarrhea events were defined using the V 5.0 definition of the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE).
- Diarrhea events of CTCAE grade 2 (increase of 4-6 stools per day compared to the previous week) were the primary outcome.
- A baseline stool sample and 24-hour food recall was collected one-week post-transplant in each participant using the Nutrition Data System for Research (NDSR).
- 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.

RESULTS

Table 1. Characteristics of study participants

Demographic Information (n=57)		
	Reported Diarrhea grade 2+ during follow up (n=24)	Did not report severe diarrhea during follow up (n=33)
Age at transplant, mean (sd)	51.16 (14.05)	54.51 (14.56)
Weight at baseline in kg, mean(sd)	83.34 (16.54)	86.14 (16.67)
White, n(%)	19 (79.2)	20 (60.1)
Male, n(%)	14 (58.3)	24 (72.7)
Clinical Information (n=57)		
Time to first severe diarrhea event in days, median(range)	9 (2 - 152)	NA
Dietary Information (n=41)		
	Reported Diarrhea grade 2+ during follow up (n=19)	Did not report severe diarrhea during follow up (n=22)
Average daily energy intake in kcal, mean(sd)	1925.7 (736.2)	1843.1 (651.2)
Daily total fat consumption in grams, mean(sd)	86.83 (41.2)	70.9 (32.4)
Daily total carbohydrate consumption in grams, mean(sd)	204.2 (80.9)	225.2 (90.3)
Daily total protein consumption in grams, mean(sd)	90.6 (33.2)	82.3 (38.9)
Daily total fiber consumption in grams, mean(sd)	18.6 (8.9)	16.0 (9.5)
HEI score, mean(sd)	53.4 (12.7)	50.6 (15.5)

*41 out of the 57 participants provided a 24-hour dietary recall at baseline.

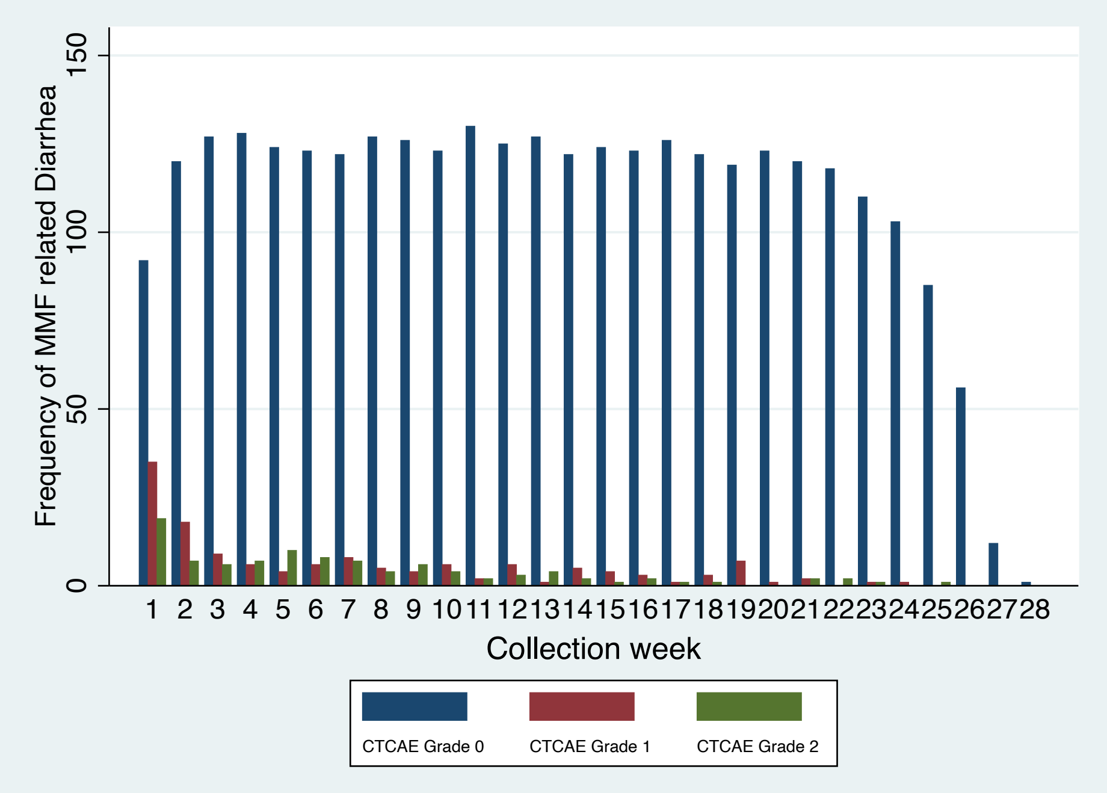

Figure 1: Frequency of diarrhea during the first 6 months of follow up among KTRs taking MMF. During follow up, 138 (4.1%) responses reported CTCAE grade 1 diarrhea and 100 (3%) reported CTCAE grade 2. Twenty-four (42%) participants experienced CTCAE grade 2 diarrhea.

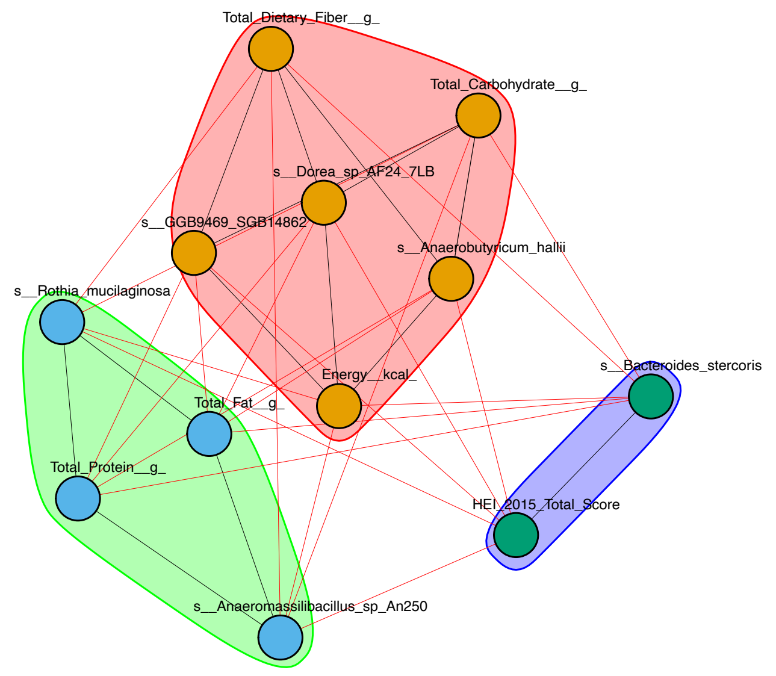

Figure 2: Correlation network between the relative abundance of gut microbiota and dietary indices derived from a 24h food recall at baseline. The color nodes (orange vs blue vs green vs yellow) indicate group membership to a specific cluster Louvain modularity maximization. A black line indicates a correlation with another node within the cluster, whereas a red line indicates a correlation with a node outside of the cluster.

Table 2. Association between bacterial taxa and severe diarrhea (CTCAE grade 2+) during follow-up.

Univariate a-priori analysis of BGUS producing bacteria (n=57)		
Bacterial taxa (species level)	organism prevalence	p-value†
*Bacteroides thetaiotaomicron**	36.80%	0.02
*Clostridium butyricum**	57.80%	0.01
Unadjusted analysis (n=57)		
Bacterial taxa (species level)	organism prevalence	p-value
Anaeromassilibacillus sp An250	15.78%	0.01
Bacteroides stercoris	14.03%	0.02
GGB3537_SGB4727 (Lachnospiraceae)	12.28%	0.03
Dorea sp AF24 7LB	12.28%	0.03
Eubacterium ramulus	10.52%	0.04
GGB9469_SGB14862 (Synergistaceae)	14.03%	0.05
Coprococcus catus	10.52%	0.05
Analysis adjusted for diet at the time of stool collection (n=41)		
Bacterial taxa (species level)	organism prevalence	p-value
Bacteroides_stercoris	19.50%	0.01
Anaerobutyricum_hallii	12.20%	0.02
Anaeromassilibacillus sp An250	17.10%	0.03
Dorea sp AF24 7LB	12.20%	0.03
Rothia_mucilaginosa	12.20%	0.03
GGB9469_SGB14862 (Synergistaceae)	14.60%	0.04

*Beta-glucuronidase producing organisms

†The p-value for the association in the univariate a-priori model was generated using zero inflated Poisson regression. The unadjusted and adjusted microbiome models were analyzed with MaAsLin2, using the first two principal components from the 6 dietary variables shown in table 1 for the adjusted analysis.

- We collected 3298 text-based surveys from 57 participants in the first 6 months post transplant (FIG.1).
- We implemented the Louvain Modularity Maximization (LMM) algorithm for community detection, focusing on the correlation between dietary indices and the relative abundance of gut bacteria present in more than 10% of the samples (Threshold R=0.3, FIG 2).
- Our microbiome association analysis identified several taxa which are possibly associated with the occurrence of severe diarrhea in this cohort of KTRs. However, the findings were not statistically significant after multiple hypothesis testing correction.

CONCLUSIONS

- Our preliminary findings suggest that the relative abundance of some specific taxa is associated with severe diarrhea occurrence in KTRs.
- Interestingly, the *Anaeromassilibacillus* and *Rothia* taxa have previously been associated with diet in other cohorts, but not diarrhea.
- Larger studies are needed to understand the interplay between diarrhea, the gut microbiome and dietary changes posttx.

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