Association between fecal microRNAs and beta-glucuronidase activity in kidney transplant recipients..

G. Onyeaghala[1,2], S. Elmer[1], M. H. Khan[6], P. Yang[1], M. Wagner[6], L. Teigen[3], M. Al-Kofahi[5], B. Wu[4], W. Guan[4], C. Staley[6], S. Riad[2], A. Matas[6], R. Remmel[5], W. Oetting[5], C. Dorr[1,2], PA. Jacobson[5], A. Israni[1,2,7]

[1]Hennepin Healthcare Research Institute [2]Nephrology Division, Hennepin Healthcare, Department of Medicine, University of Minnesota [3]Division of Gastroenterology, Hepatology and Nutrition, University of Minnesota [4]Division of Biostatistics, University of Minnesota [5]Department of Experimental and Clinical Pharmacology (ECP), University of Minnesota [6]Department of Surgery, University of Minnesota [7]School of Public Health, University of Minnesota

INTRODUCTION

- Mycophenolate mofetil (MMF) is used in >90% of kidney transplant recipients.
  - Its inactive metabolite, MPAG, is de-glucuronidated by bacterial beta-glucuronidase in the gut and the active metabolite MPA is reabsorbed back into the blood in a process know as enterohepatic recirculation (EHR).
  - Enterohepatic recirculation increases blood concentrations, enhances immunosuppression and possibly toxicity in kidney transplant recipients.
- Host microRNA (miRNA) can influence the microbiome, leading to changes in beta-glucuronidase levels.

HYPOTHESIS

- We hypothesized that host miRNA levels would be associated with beta-glucuronidase levels in kidney transplant recipients.

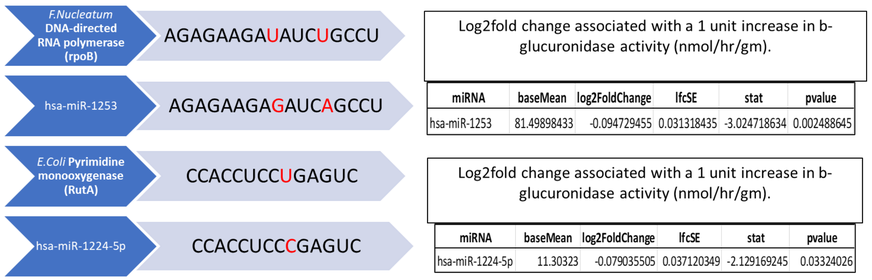
METHODS

- Stool samples from 30 participants were collected from the Microbiome and Immunosuppression in Kidney Transplantation (MISSION) prospective study within 60 days post-transplant.
- Fecal miRNA was profiled using the NanoString nCounter human v3 miRNA codeset.
- Beta-glucuronidase activity levels were measured using the Abcam ab234625 beta-Glucuronidase Activity Assay Kit.
- After QC and data normalization, we examined the differential expression of 798 miRNA probes with beta-glucuronidase activity levels.
- In a secondary analysis, we conducted focused analyses of 17 a priori, microbiome associated fecal miRNAs and their association with beta-glucuronidase activity.

RESULTS

Figure 1: Distribution of logfold change in miRNA expression among the top 25 miRNAs (ranked by pvalue)

Figure 2: Alignment of microbiota-associated host miRNA-1253 and miRNA-12245p with *F.nucleatum* and *E.coli* targets.



Log2fold change associated with a 1 unit increase in b-glucuronidase activity (nmol/hr/gm).

miRNA	baseMean	log2FoldChange	lfcSE	stat	pvalue
hsa-miR-1253	81.49898433	-0.094729455	0.031318435	-3.024718634	0.002488645

Log2fold change associated with a 1 unit increase in b-glucuronidase activity (nmol/hr/gm).

miRNA	baseMean	log2FoldChange	lfcSE	stat	pvalue
hsa-miR-1224-5p	11.30323	-0.079035505	0.037120349	-2.129169245	0.03324026

*miRbase alignment data was adapted from Liu et al. (PMID:26764595)

- For data analysis, we performed global differential expression analysis treating beta-glucuronidase as a continuous variable using the DESeq2 package (version 3.15).
  - Detectable mean miRNA expression levels were defined as more than a one count per million.
- At the Bonferroni corrected 0.05 significance level, we found 20 fecal miRNAs associated with beta-glucuronidase activity in this cohort, with miR-2116-5p, miR-4888 and miR-600 being the most abundant **(Figure 1)**.
- In our secondary, focused analyses, we found that miR-1253, miR-1224-5p, miR-194-5p and miR-200a-3p were associated with beta-glucuronidase activity (p-value < 0.05).
- miR-1224-5p was shown to align with *Escherichia. coli* DNA, while miR-1253 was shown to align with *Fusobacterium. nucleatum* DNA **(Figure 2)**.
  - Both organisms are known producers of beta-glucuronidase enzymes.

CONCLUSIONS

- Our preliminary findings show that fecal miRNAs are associated with beta-glucuronidase.
- Further mechanistic studies are needed to validate these findings.
- Given sequence homology, it is feasible that fecal miRNAs may enter bacteria, such as *E. coli* and *F. nucleatum* and potentially regulate bacterial growth or gene transcripts such as beta-glucuronidase.

Acknowledgements: This study was supported in part by NIH/NIAID grant **5R01AI140303** and Hennepin Healthcare Research Institute's Post-doctoral Support Career Development Award