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Gut microbiota differences in Island Hispanic Puerto Ricans and mainland non-Hispanic whites during chemoradiation for rectal cancer: A pilot study

Velda J. González-Mercado[a,*], Jean Lim[a], Lawrence Berk[b], Mary Esele[c], Carmen S. Rodríguez[a], Gerardo Colón-Otero[d]

[a] College of Nursing, University of South Florida, Tampa, Florida
[b] Radiation Oncology, College of Medicine Radiology, University of South Florida, Tampa, Florida
[c] School of Nursing, South University, Tampa, Florida
[d] Division of Hematology-Oncology, Mayo Clinic Cancer Center, Mayo Clinic College of Medicine, Jacksonville, Florida

ABSTRACT

Purpose: To investigate whether there are differences in diversity, taxonomic composition, and predicted functional pathways of the gut microbiome between Island Hispanic Puerto Ricans (HPR) and mainland non-Hispanic whites (NHW) measured before and at the end of chemo-radiation (CRT) for Rectal Cancer.
Methods: Fifty-six stool samples of newly diagnosed rectal cancer patients (25 HPR and 31 NHW) were amplicon-sequenced during chemo-radiotherapy. 16S rRNA gene data was analyzed using QIIME2, phyloseq, and LEfSe.
Results: We observed similar within-sample alpha diversity for HPR and NHW participants during CRT. However, at the end of CRT, several taxa were present at significantly different abundances across both groups. Taxa enriched in the gut of HPR compared to NHW included *Muribaculaceae, Prevotella 2* and *7, Gemella, Bacillales Family XI, Catenibacterium, Sutterella, Pasteurellales*, and *Pasteurellaceae* genera, whereas over-represented taxa in NHW participants were *Turicibacter* and *Eubacteriaceae*. Significant differences in predicted HPR microbiota functions included pathways for synthesis of L-methionine and degradation of phenylethylamine and phenylacetate.

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* Correspondence to: Velda J. González-Mercado, College of Nursing, University of South Florida, 12901 Bruce B. Downs Blvd., MDC22, Tampa, FL 33612-4766.
E-mail address: veldag@usf.edu (V.J. González-Mercado).

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Conclusion: In this pilot study, taxonomic analyses and functional predictions of the gut microbiomes suggest greater inflammatory potential in gut microbial functions among HPR rectal cancer patients undergoing CRT compared to that of NHW participants.

ARTICLE INFO

Keywords: Gut microbiota differences; Island Hispanic Puerto Ricans; Mainland non-Hispanic whites; Chemo-radiation; Rectal cancer

Introduction

Island Hispanic Puerto Ricans (HPR) and mainland non-Hispanic whites (NHW) have different incidence, severity, and health outcomes of cancer treatment contributing to health disparities.[1] Although health disparities may be attributed to multiple known factors (eg, socioeconomic position, complex sociocultural and geographic factors, excess weight and physical inactivity),[2-4] recent evidence underlining gut microbiota alterations in colorectal cancer (CRC) suggest potential ethnic differences in gut microbiomes that may contribute to disparities in patients undergoing chemo-radiation (CRT) for rectal cancer (RC).[5,6] This pilot study is aligned with that area of interest. The objectives of this pilot study were to investigate whether there are differences in diversity, taxonomic composition, and predicted functional pathways of the gut microbiome between Island HPR and mainland NHW measured before and at the end of CRT for RC. Uncovering these potential differences may be a first step in identifying a source of health disparities in treatment outcomes for the optimization of clinical care, quality of life, and health outcomes of these patients.

Materials and methods

Study population

Newly diagnosed RC patients of at least 18 years of age or older scheduled to receive CRT were recruited for this study. Exclusion criteria included history of intestinal chronic inflammatory diseases or history of previous abdominal surgery, diagnosed psychiatric and/or sleep disorders, comorbidities associated with sleep disorders (eg sleep apnea), use of insomnia medications, antibiotics, prebiotics, probiotics, steroids, and/or immune-suppressants agents within 1 month prior to sample collection at each assessment time point. Data collection was conducted from September 2017 to April 2019. Ethics approval from both the Southeastern Academic Medical Center and the University of Puerto Rico Medical Science Campus were obtained prior to data collection. All participants included in the study provided written informed consent.

16S rRNA gene sequencing

After obtaining informed consent, participants completed demographics and clinical information (ie age, weight, height). Participants collected approximately 5 g of stool using a sterile plastic container at 2 time-points: before, and at the end (after 24-28 treatments) of CRT. DNA was extracted from stool samples using the Power Soil DNA Isolation kit, (MoBio, Carlsbad, CA). V3-V4 regions of the 16S rRNA gene were amplified and sequenced on the MiSeq 2×300 bp platform (Illumina, San Diego, CA) following existing protocols.

Table
Clinical characteristics of sample (n = 56).

Variables	All participants (n = 56)	NHW (n = 31)	HW (n = 25)	
Gender				
M	31 (55%)	14 (45%)	17 (68%)	
F	25 (45%)	17 (55%)	8 (32%)	
Occupation				
Working	54%	50%	58%	
Retired	17%	17%	17%	
Handicapped	29%	33%	25%	
Chemotherapy				
5FU	54%	56%	58%	
Xeloda	46%	44%	42%	
	Mean (SD)	Mean (SD)	Mean (SD)	*P* value
Age	60.5 (13.1)	59.1 (12.4)	62.4 (14.1)	0.34
Education	13.3 (3.0)	13.8 (2.6)	12.5 (3.6)	0.56
# Treatment	28.7 (3.7)	29.0 (3.5)	27.8 (4.1)	0.43
BMI	27.1 (5.1)	27.6 (4.6)	26.0 (6.3)	0.55

BMI, body mass index; HW, Hispanic whites; NHW, non hispanic white.

Data analysis

Demultiplexed reads were quality-checked and trimmed at a Q = 25 cutoff using the Trim Galore! v0.4.4, (https://github.com/FelixKrueger/TrimGalore) wrapper package. Trimmed reads were imported into QIIME2-2019.1 [7] and denoised with DADA2[8] without further trimming. For taxonomic assignment, a naïve Bayes classifier was trained on reference sequences from the SILVA v132 database[9] matching the sequencing primer pair. The resulting feature table was rarefied to 4226 sequences per sample (smallest 4-digit number), after eliminating 1 sample. Alpha and beta diversity metrics were calculated from the rarefied table using QIIME2 (alpha diversity) and the phyloseq R package (beta diversity).[10] Metagenomic inference (Enzyme Commission and MetaCyc pathways) was performed on the rarefied table using QIIME2's q2-picrust2[11] plugin. Linear discriminant analysis Effect Size (LEfSe)[12] was performed on subsets (before and end-CRT) of the rarefied feature table using default parameters with per-sample normalization to a sum of 1 million.

Results

Participant characteristics

The sample consisted of 25 islander HPR and 31 mainlander NHW participants. The HPR participants were accrued from an ambulatory Radiation therapy (RT) facility located in San Juan, Puerto Rico, while the NHW participants were accrued from 2 RT facilities located in the Tampa Bay, Florida area. Demographics and clinical characteristics are presented in Table. There were no significant differences between HPR and NHW participants in age, body mass index, years of education, and chemotherapy treatment (infusion of 5-FU vs those on oral capecitabine) (Table).

Composition of the gut microbiota

Shannon Diversity and number of observed operational taxonomic units (OTUs) indicated similar alpha diversity for HPR and NHW participants at each time of assessment (Fig. 1A). Beta diversity analyses did not reveal consistent statistically significant groupings based on ethnic-

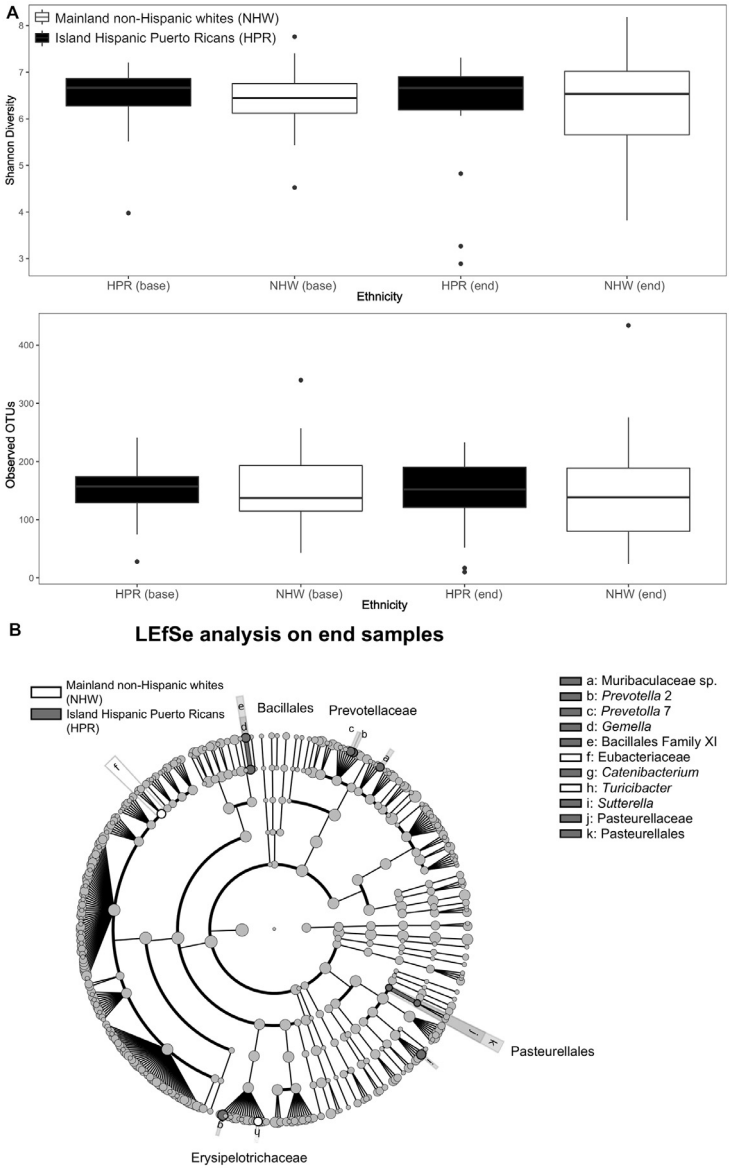

Fig. 1. (A) Alpha diversity measures within HPR and NHW, split between CRT time points (before, and at the end of CRT for RC) and (B) LEfSe analyses of taxon abundances between HPR and NHW samples collected at end of CRT.

ity and treatment time points (Fig S1). At the end of CRT, however, statistical comparisons using the LEfSe algorithm showed fine-scale differences in bacterial taxon abundances between both groups (Fig. 1B). Bacterial genera enriched in HPR compared to NHW participants included *Muribaculaceae, Prevotella 2* and *7, Gemella, Bacillales Family XI, Catenibacterium, Sutterella, Pasteurellales,* and *Pasteurellaceae*. At the end of CRT, 2 taxa classified to *Turicibacter* and *Eubacteriaceae* were over-represented in the NHW compared to the HPR group. Further, LEfSe comparison showed marked differences of functional gene pathways overrepresented in gut microbiota of HPR and NHW participants at the end of CRT (Fig. 2).

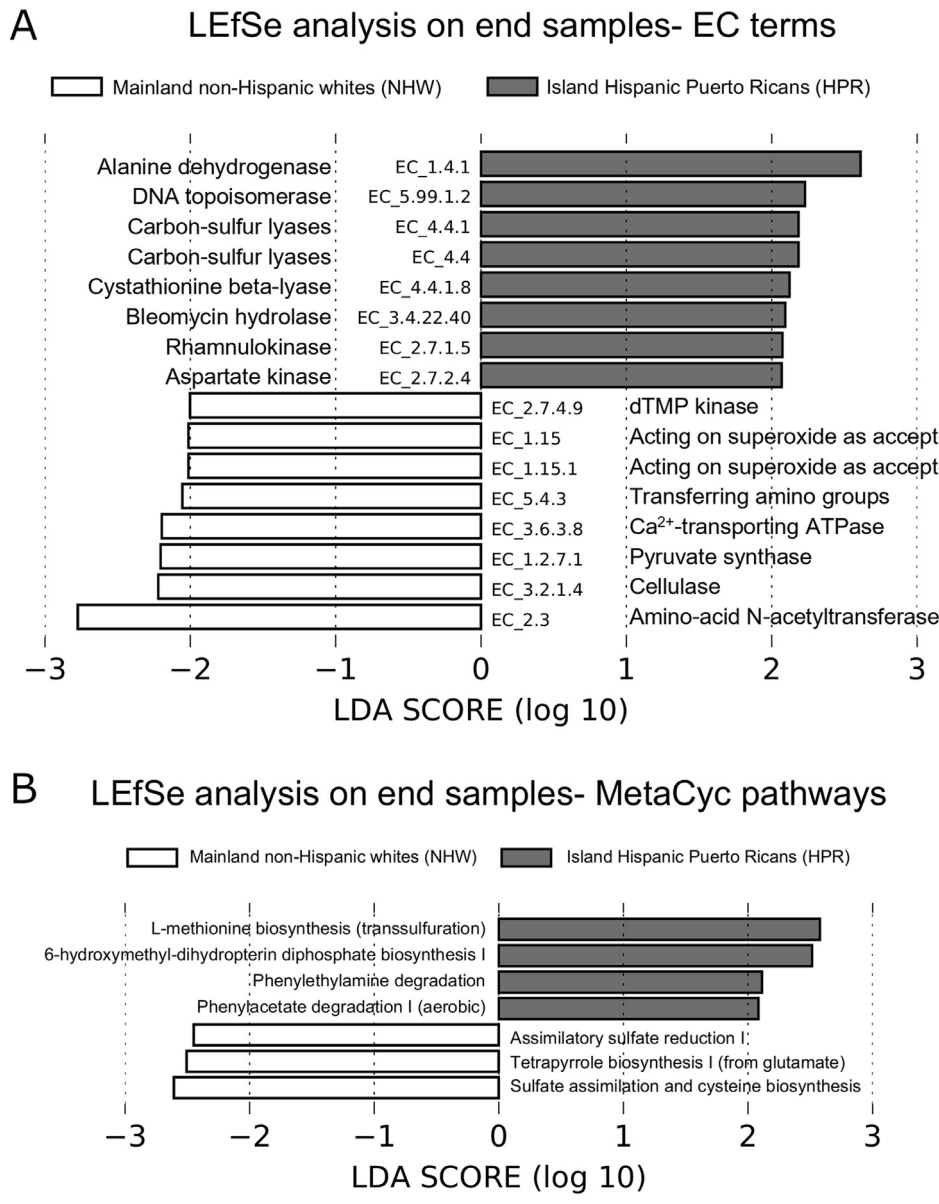

Fig. 2. LEfSe analyses of over-represented (A) EC terms and (B) MetaCyc pathways between HPR and NHW samples collected at the end of CRT. EC, Enzyme Commission.

Discussion

Emerging evidence suggest that diversity of the gut microbiome in different ethnic groups[13,14] may contribute to disparities in treatment outcomes and/or influence the susceptibility to chronic disease in the intestinal tract (eg, Irritable bowel syndrome (IBS)).[15] We observed no differences in alpha or beta diversity in our cohort of HPR and NHW, suggesting homogene-

ity across participants. Similar findings have been reported comparing healthy African American (n = 47) and NHW women (n = 33).[5]

At the end of CRT, we did, however, observe ethnic differences in bacterial communities down to the genera level. For example, certain strains of *Catenibacterium* enriched in HPR relative to NHW participants have been linked to the high-sugar, high-fat "Western" diet.[15,16] Western diet-associated dysbiosis affects host gastrointestinal tract metabolism and immune homeostasis.[15,17] Contrarily, other *Catenibacterium* species produce short chain fatty acids, such as butyric acids, from glucose fermentation.[18,19] Butyric acid may serve beneficial roles in colonic anti-inflammation and metabolic health parameters.[20] Another genus enriched in HPR relative to NHW participants, *Sutterella*, may be related to worse outcomes of cancer treatment such as chemoresistance among CRC patients.[21] Similarly, other gram-negative genera enriched in the HPR compared to NHW group, such as *Prevotella* and *Pasteurella*, are associated with worse outcomes, including chemotherapy-induced oral mucositis, respiratory tract infection, or even sepsis among cancer patients.[22,23] From the ethnic perspective, higher abundances of *Prevotella* were found in stool samples from a healthy Hispanic Cohort compared to Human Microbiome Project,[24] although *Prevotella* can also be related to cancer. Further, *Gemella* and *Prevotella* are among intestinal microbes previously associated with CRC.[25] The contribution of specific bacterial genera to negative outcomes in HPR participants is an area of future research.

Conversely, *Turicibacter* enriched in the NHW compared to HPR group is correlated with the anti-inflammatory compound butyric acid.[20] This agrees with our previous report that gut *Turicibacter* abundances were associated with lower sleep disturbance scores among RC participants during CRT.[26] *Eubacteriaceae*, which is also enriched in NHW compared to HPR participants, participates in the production of medium-chain fatty acids.[27] Medium-chain fatty acids exert beneficial effects on the intestinal health, including energy production, integrity support of the intestinal tissue, and immune modulation.[28]

Exploratory functional analyses suggest that after CRT, the gut of HPR participants is enriched in L-methionine biosynthesis (via transsulfuration) and carbon-sulfur lyase pathways, implying the presence of hydrogen sulfide in the gut,[29,30] although these metabolites were not directly measured. Hydrogen sulfide can be detrimental to the intestinal epithelia cells via inhibition of mitochondrial cytochrome C oxidase, activation of proinflammatory T helper 17 cell, and inhibition of butyrate catabolism in the colonocytes.[31] Hydrogen sulfide can also protect gut bacteria from reactive oxygen species.[31] At the end of CRT, HPR participants also showed over-representation of pathways that degrade phenylethylamine and phenylacetate, suggesting the presence/availability of these metabolites in the gut. Increased phenylethylamine levels have been positively associated with inflammatory conditions such as Crohn's and inflammatory bowel disease,[32] while elevated phenylacetate concentrations has been linked to CRC.[33]

The enrichment of cysteine and tetrapyrrole biosynthesis pathways suggest some recovery of gut microbiota functions in NHW vs HPR in this cohort. Cysteine is used for protein and glutathione synthesis, and glutathione and other cysteine derivatives are important for protection against oxidative stress toxicity,[34] amelioration of intestinal inflammation,[35] and survival in RC patients who received RT.[36] The tetrapyrrole biosynthetic pathway encompasses the synthesis of porphyrins (such as heme, chlorophyll, and coproporphyrin III) and corrinoids (such as cobalamin).[37] While cobalamin is nutritionally beneficial and some porphyrins are anti-inflammatory,[38] elevated endogenous porphyrins have been associated with CRC.[39]

Limitations

Limitations of this pilot study include the moderate sample size and the use of fecal samples, which may not fully represent the structure of the mucosal microbiota. However, tissue-based studies can be invasive, riskier, and more expensive compared to fecal samples which are more commonly used to study microbial communities.[40] Another limitation of our 16S rRNA gene-based analyses is the inability to classify beyond the genus level, inaccuracy in resolving functional potential among taxa sharing high 16S rRNA gene identity, or inability to confirm

functional activity. Clinical data analyses are also limited by the exclusion of variables (eg diet) that were assessed but not yet analyzed, and data that was not available to us (tumor characteristics [eg, tumor target volume, rectal dose].

Conclusions

Our study suggests that, compared to NHW, HPR may have a greater abundance of CRC- and pro-inflammatory-associated bacterial taxa, which could potentially be related to poorer health outcomes of cancer treatment and contribute to health disparities. A large-scale, multicenter study is needed to validate these findings and confirm associations between gut microbiomes, metabolites, geography, diet, and/or lifestyle and comorbidities, including fatigue among RC survivors, of different ethnic groups.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.currproblcancer.2020.100551.

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