

Name of Journal: World Journal of Gastrointestinal Pathophysiology

Manuscript NO: 39764

Title: Human gut microbiome profiling and colorectal cancer in African Americans and Caucasian Americans

We wish to thank the reviewers for critically reviewing the manuscript and providing us with thoughtful comments, which have greatly improved the quality of the manuscript.

Reviewer's code: 03713770

Comment 1: General comments; this manuscript reveals the microbiome profiling of human gut in African and Caucasian Americans. The study design, methods and outcomes are well described. The results are presented clearly and concisely accompanied by appropriate table and figures. In the literature review, recent researches are listed to this topic.

Answer: We are extremely pleased to know that the reviewer found the study design, methods and other section are well presented.

Comment 2: Correct the title (American/Americans)

Answer: Per your request, the title has been corrected by adding "Americans"

Comment 3: Abstract: meliorate the abstract to improve the scientific strength

Answer: As suggested, we have modified the abstract to improve its scientific strength. That multiple software was used to analyze metagenomics has been stated.

Comment 4: Material & Methods: Include the primer sequences in separate table not in the text.

Answer: As per reviewer's request, we have incorporated a new table (Table 1) that lists the primers and their sequences.

Comment 5: In paragraph of statistical analysis the author stated that data were presented as standard error of mean but the data were presented as mean \pm standard deviation in the figure legends.

Answer: This was an oversight, which we have corrected. Standard error has been changed to standard deviation.

Reviewer's code: 00253974

Comment-1: Novelty of the study: Differences in microbiome in different human races have been investigated in numerous studies. (1) Moreover, the correlation between dysbiosis and the development of CRC has been established recently. (2-5). Exclusion of dietary effects on the microbiome: the dietary habits of the study population were ignored. Diet has been proven to have a massive effect on the gut microbiome.

Answer: We agree with the reviewer that differences in microbiome in different races have been investigated. However, our primary objective was to determine whether changes in gut microbiome could partly be attributed to increased incidence in colorectal cancer seen among African Americans. In view of this, we performed a detailed profiling of gut microbiome in African Americans and Caucasian Americans and found substantial differences including changes in inflammatory bacteria.

Comment-2: Small sample size: a total of 98 patients is quite few for profiling the highly complexed human gut microbiome in different races.

Answer: This is a pilot study which shows substantial differences in gut microbiome between African Americans and Caucasian Americans. This provides the impetus to carry out additional investigation with large number of patients.

Comment: 3. Microbiome profiling is actually an interesting tool in modern research. However, this study needs some work to get 1st results validated.

Answer: As stated above, this is a pilot study. However, marked changes observed from the profiling data, have been validated by RT-PCR.

Reviewer's code: 03567380

Comment-1: The authors state that the general characteristics of the study participants are described in reference 15. However, that study used a different number of patients per group than the current study. Please provide all important patient parameters in a table or expand the methods to include these details.

Answer: Per your request, general characteristics of each group have been stated in the Material & Method section.

Comment-2. Were any of these patients on probiotics or taking supplements that could influence the gut microbiome? If this was not an exclusion criteria, this should be listed as a weakness in this study.

Answer: *None of the patients were taking probiotics. This is now stated in the Material & Method section.*

Comment-3: Figure 5 should be expanded to investigate these measures between AA and CAs rather than combining both AA and CA into a single group.

Answer: *We agree that the observation presented in Fig 5 should be expanded. However, our objective at this time was to determine whether there is any change in the relative abundance in and differential abundance of Fuso bacterium Spp. between serrated and tubular adenomatous patients. In view of this, we produced combined our results. Undoubtedly, additional studies need to be carried out to investigate changes in gut microbiome in tubular adenomas and serrated polyps in different racial groups, particularly in AA and CA.*

Comment-4. The data with 7alpha dehydroxylase is very interesting but would be strengthened by assessing specific bile acid concentrations in the stool.

Answer: *Indeed, the changes observed in 7-a dehydroxylase between AAs and CAs are very interesting. Bile concentrations need to be examined and is a subject of future investigation.*

Comment-5. There have been differences identified in the microbiome and metabolism between AA and CA in other studies (PMID: 25759547). The authors need to expand their discussion to detail this study and how this may relate to their findings.

Answer: *Per reviewer's request, we have discussed differential microbial profiling between AA and CA (PMID: 25759547) and have cited the reference. These observations were discussed in the "Discussion" section (second paragraph).*

Comment-6. The sentence, "In contrast, Unclassified-Bacteria and Unclassified-Unclassified micro-organisms were only present in CAs." is repeated in the discussion twice.

Answer: *We have removed the repeated sentence in discussion.*

Reviewer's code: 02441737

Comment-1. Abstract: It would be recommendable for the authors to present some general characteristics of the patients such as age, sex, and data of the socioeconomic level of the CAs and AAs patients. Background

Answer: Because of space constraint, we did not present the general characteristics in the abstract. However, they were presented in Method & Material section.

Comment- 2: The introduction is adequate and allows a proper understanding of the problem of study. It would be of interest for the authors to present more studies on the characteristics of the microbiota in different ethnic and racial groups; in addition to commenting more data on the general characteristics of the populations studies such as: the age, the sex, and the characteristics of the socioeconomic level.

Answer: We understand the reviewer's points of view. But, this is a pilot study intended to examine the changes in gut microbiome in AAs and CAs. In view of this, we felt that discussion of previous observations of changes in microbiome in different racial and ethnic groups was not warranted.

Comment-3: Methods. Although the authors have already published an article describing the general characteristics of patients CAs and AAs, it is important that they mention more details of the characteristics of these patients; especially the external variables that are known to influence the characteristics of the microbiota.

Answer: Patients characteristics have been presented in our previous manuscript (see ref 15). However, general characteristics were presented in Table 1.

Comment: 5: Discussion. It is important that the authors make an effort to explain the internal and external reasons to the host, for which there are differences in the distribution of the different species of bacteria in the microbiota of the CAs and AAs groups.

Answer: As stated in the Methods and Materials section, we recruited patients who came to the clinic for routine colonoscopy, but met the exclusion criteria. At this time, we did not collect their dietary history, socio-economic status or life style. In view of this, we are unable to provide the reasons for the observed microbial differences between the two racial groups.

Comment-6: Illustrations and tables. It is recommended to place a more descriptive title in all the figures. Biostatistics. It is recommended that the statistical procedure followed for the analysis of the data be described in more detail, for example in Table 1. It would be recommended to present the results of the statistical test carried out.

Answer: Per reviewer's request, legends to Figure 1, 2 and 3 have been expanded to include more information. Bio-statistical part of the write-up has also been expended.

Comment-7: References. If possible, it is recommendable to reduce the number of references.

Answer: *We felt that the references cited are needed to discuss the manuscript.*