WJGP World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: http://www.f6publishing.com

World J Gastrointest Pathophysiol 2018 September 29; 9(2): 47-58

DOI: 10.4291/wjgp.v9.i2.47

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Basic Study

Gut microbiome profiling and colorectal cancer in African Americans and Caucasian Americans

Lulu Farhana, Fadi Antaki, Farhan Murshed, Hamidah Mahmud, Stephanie L Judd, Pratima Nangia-Makker, Edi Levi, Yingjie Yu, Adhip PN Majumdar

Lulu Farhana, Farhan Murshed, Hamidah Mahmud, Pratima Nangia-Makker, Yingjie Yu, Adhip PN Majumdar, Department of Internal Medicine, John D Dingell Veterans Affairs Medical Center, Detroit, MI 48201, United States

Lulu Farhana, Fadi Antaki, Pratima Nangia-Makker, Yingjie Yu, Adhip PN Majumdar, Department of Internal Medicine, Wayne State University School of Medicine, Detroit, MI 48201, United States

Fadi Antaki, Stephanie L Judd, Division of Gastroenterology, John D Dingell VA Medical Center, Detroit, MI 48201, United States

Pratima Nangia-Makker, Adhip PN Majumdar, Department of Medicine, Karmanos Cancer Institute, Detroit, MI 48201, United States

Edi Levi, Department of Pathology Service, John D Dingell VA Medical Center, Detroit, MI 48201, United States

Author contributions: Farhana L contributed to study design, performed the experiments, data analysis and interpretation and wrote the manuscript; Antaki F and Judd SL performed the colonoscopy; Murshed F performed experiments and reviewed the manuscript; Mahmud H laboratory supported and reviewed the manuscript; Nangia-Makker P, Levi E and Yu Y laboratories provided material support; Majumdar APN was the principal investigator who conceptualized the study, revised and approved the final version of the manuscript.

ORCID number: Lulu Farhana (0000-0003-1057-8845); Fadi Antaki (0000-0002-2273-9346); Farhan Murshed (0000-0003-0334-7846); Hamidah Mahmud (0000-0002-7834-8213): Stephanie L Judd (0000-0001-7296-5694); Pratima Nangia-Makker (0000-0002-1917-169X); Edi Levi (0000-0003-1809-2866); Yingjie Yu (0000-0001-7497-7832); Adhip PN Majumdar (0000-0002-6413-5102).

Supported by Department of Veteran Affairs, No. 1I101BX001927; National Institutes of Health, No. 1R21CA175916.

Institutional review board statement: The study followed a Full Board Review by the Wayne State University Institutional Review Board.

Informed consent statement: All routine colonoscopy, collection of colonic effluents and biopsy specimens from the patients were performed after obtaining informed consent and ethical permission.

Conflict-of-interest statement: To the best of our knowledge, no conflict of interest exists.

ARRIVE guidelines statement: The ARRIVE Guidelines had been adopted.

Manuscript source: Unsolicited Manuscript

Correspondence to: Adhip PN Majumdar, DSc, PhD, Professor, Department of Internal Medicine, John D Dingell Veterans Affairs Medical Center, 4646 John R, Detroit, MI 48201, United States. a.majumdar@wayne.edu
Telephone: +1-313-5764460
Fax: +1-313-5761112

Received: May 11, 2018
Peer-review started: May 11, 2018
First decision: July 10, 2018
Revised: August 8, 2018
Accepted: August 26, 2018
Article in press: August 26, 2018
Published online: September 29, 2018

Abstract

AIM
To determine whether and to what extent the gut microbiome is involved in regulating racial disparity in colorectal cancer (CRC).

METHODS
All patients were recruited and experiments were performed in accordance with the relevant guidelines and regulations by the Institutional Review Boards (IRB), committees of the John D. Dingell VAMC and Wayne State University guidelines. African American (AA) and Caucasian American (CA) patients were scheduled for an outpatient screening for colonoscopy, and no active malignancy volunteer patients were doubly consented, initially by the gastroenterologist and later by the study coordinator, for participation in the study. The gut microbial communities in colonic effluents from AAs and CAs were examined using 16sRNA profiling, and bacterial identifications were validated by performing SYBR-based Real Time PCR. For metagenomic analysis to characterize the microbial communities, multiple software/tools were used, including Metastats and R statistical software.

RESULTS
It is generally accepted that the incidence and mortality of CRC is higher in AAs than in CAs. However, the reason for this disparity is not well understood. We hypothesize that the gut microbiome plays a role in regulating this disparity. Indeed, we found significant differences in species richness and diversity between AAs and CAs. *Bacteroidetes* was more abundant in AAs than in CAs. In particular, the pro-inflammatory bacteria *Fusobacterium nucleatum* and *Enterobacter* species were significantly higher in AAs, whereas probiotic *Akkermansia muciniphila* and *Bifidobacterium* were higher in CAs. The polyphyletic *Clostridia* class showed a divergent pattern, with *Clostridium XI* elevated in AAs, and *Clostridium IV*, known for its beneficial function, higher in CAs. Lastly, the AA group had decreased microbial diversity overall in comparison to the CA group. In summary, there were significant differences in pro-inflammatory bacteria and microbial diversity between AA and CA, which may help explain the CRC disparity between groups.

CONCLUSION
Our current investigation, for the first time, demonstrates microbial dysbiosis between AAs and CAs, which could contribute to the racial disparity of CRC.

Key words: Human gut; Microbiome; Colorectal cancer; *Fusobacterium nucleatum*; African Americans; 16S RNA profiling; Metagenomics

Core tip: Several studies have demonstrated that the incidence of colorectal cancer (CRC) is higher in African Americans than Caucasian Americans. Reasons for this racial disparity are unknown. The current study, for the first time, demonstrated that dysbiosis in the gut microbiome plays a determinant role in the racial disparity of CRC. Determining the influence of the microbiota on the risk of developing CRC will have a major impact on health, since early-stage CRC hinges on the ability to detect early pathological changes. Subsequent translational studies could also be developed to alter microbiota with medications or diet, thus reducing the risk of developing CRC.

Farhana L, Antaki F, Murshed F, Mahmud H, Judd SL, Nangia-Makker P, Levi E, Yu Y, Majumdar APN. Gut microbiome profiling and colorectal cancer in African Americans and Caucasian Americans. *World J Gastrointest Pathophysiol* 2018; 9(2): 47-58 Available from: URL: http://www.wjgnet.com/2150-5330/full/v9/i2/47.htm DOI: http://dx.doi.org/10.4291/wjgp.v9.i2.47

INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of cancer death in the United States, and several studies have demonstrated that African Americans (AAs) have the highest rate of CRC in the United States[1-6]. AAs have the highest incidence and death rates for CRC than Caucasian Americans (CAs), Hispanics and Asian/Pacific Islanders[7]. CRC typically correlates with age, reflecting a multistep progression from normal epithelium to carcinoma. However, a significant number of AAs are diagnosed with CRC at a younger age compared to CAs[8-11]. Genomic alterations in oncogenes and tumor suppressor genes drive the epithelial cell transformation to carcinoma - including the Apc/Wnt-β-catenin signaling pathway and the tumor suppressor gene Apc[12-14]. Previous studies have shown that microRNA drivers upregulated in AAs lead to an increased proportion of cancer stem cells in human colonic epithelial cells[15].

The human microbiome is at the interface of intrinsic and environmental factors - and abnormalities in the gut microbiome have been noted in patients with CRC[16-19]. The colonic microbiota is mostly bacteria consisting of approximately 10^3 different microbial species[20]. Gut microbiota is essential in the maintenance of homeostasis, and it contributes to immune development, inhibits pathogen colonization, processes drug metabolites, metabolizes nutrients from the diet and also modulates their biological activities[10,21]. Dysregulation of gut microbiota and a concomitant state of chronic inflammation and persistent activation of the host immune system have been implicated in the initiation and development of CRC[22-24].

One primary role of gut bacteria is to participate in the biotransformation of products in the gut, which include bile acids secreted from the liver. The gut microbiota may alter cancer susceptibility and has anti-cancer effect through the production of microbial secretory

Table 1 General characteristics of African American and Caucasian American patients

Race	Gender	Age	Height (inches)	Weight (lbs)	Body mass index	Polyp	Adenoma
AA	Male	65.2	70.4	181	28.9	4.6	3.8
CA	Male	62.6	69.0	194	31.0	1.43	1.0

AA: African Americans; CA: Caucasian Americans.

metabolites, such as short chain fatty acids (SCFAs), secondary bile acids (SBA), and trimethylamine N-oxide (TAMO)[25-28]. SBAs, deoxycholic acid (DCA) and lithocholic acid (LCA), are noted in particular for their carcinogenic activity[29-33]. Murine models have demonstrated that DCA shifts the microbiota community to dysbiosis and promotes intestinal tumorigenesis in Apc$^{min/+}$ mice when DCA-treated fecal microbiota inoculated in one mouse is transferred to another[34].

Thus, multifactorial reasons underlie the racial disparity of CRC. The current investigation was aimed at studying microbial dysbiosis in the gut between AAs and CAs. In this pilot study, we investigate the diversity and abundance of specific gut microbes in colonic effluents using 16S rRNA gene community profiles in AAs and CAs and their possible role in increased incidence of CRC in AAs.

MATERIALS AND METHODS

Study subjects and collection of samples

In the current pilot investigation, 52 AA and 46 CA patients were recruited. The study was approved by the Institutional Review Boards and Committees of the John D. Dingell-Veterans Affairs Medical Center (JDD-VAMC) and Wayne State University School of Medicine. Patients excluded from the study were those with active malignant disease, inflammatory bowel disease, recent infection and those recently treated with antibiotics. In addition, patients with psychiatric or addictive disorder, hemorrhagic diathesis or on warfarin were excluded. General characteristics of study participants are the same as described in our earlier publication[15]. None of the patients were taking probiotics as supplements. General characteristics of each group of patients are presented in Table 1.

Eligible study subjects were scheduled for an outpatient screening colonoscopy at the JDD-VAMC. All study subjects received standard colonoscopy purgative preparation. Briefly, patients were asked to stay on a clear liquid diet for 24 h and to take a preparation containing 15 mg Bisacodyl the morning prior to their colonoscopy. The patients were also instructed to split the dose (4 L) of poly-ethylene glycol solution (PEG) into a first half (2 L) the evening prior to colonoscopy, and to drink the second half (the remaining 2 L) 5 h prior to the procedure and to finish it 3 h prior to the procedure, regardless of appointment time (morning or afternoon). Colonic effluent was aspirated prior to the colonoscopy through the working channel of the endoscope, as reported earlier[15]. Additionally, 8 forceps biopsies were taken from macroscopically normal appearing colonic mucosa (< 10 cm anal verge), as described previously[15].

DNA extraction for 16S rRNA gene microbial community profiling

Genomic DNA was extracted from colonic effluents using QIAamp DNA Stool Mini Kit (QIAGEN) according to the manufacturer's instructions. Purified DNA used for analysis of 16S rRNA community profiling was performed by LC Sciences (Houston, Texas). The V3 and V4 regions of the 16S rRNA gene were amplified to generate approximately 469 bp amplicons, automating cluster generation and sequencing on the MiSeq system. For data analysis, the merge paired-end reads from DNA fragments were analyzed using next generation sequencing FLASH (Fast Length Adjustment SHort reads) software, and raw sequencing data quality control was checked using FastQC software. Operational taxonomic units (OTUs) clustering was based on 97% sequence similarity using CD-HIT software. Microbial strain identification software Quantitative Insights Into Microbial Ecology (QIIME) was used for alpha diversity and beta diversity, visualization of high throughput microbial community, and for principal coordinates analysis. Ribosomal Database Project (RDP) classifier, Greengenes, NCBI 16SMicrobial (TUIT tool) and GraPhlAn software were used for taxonomic classification and circular taxonomic phylogenetic trees.

Genomic DNA isolation from colonic effluents and validation

Bacterial genomic DNA was isolated from colonic effluents using QIAamp DNA Stool Mini Kit (QIAGEN) according to the manufacturer's instructions. For real time PCR, DNA (40-50 ng) and appropriate blank were used for RT-PCR analysis in triplicate using the 2 × Power-Up SYBR Green PCR Master Mix (Applied Biosystems) and the ABI Prism 7500 sequence detection system. PCR consisted of 40 cycles of 95 ℃ for 10 min and then 95 ℃ for 15 s, 60 ℃ for 60 s. The primer sequences were used to evaluate the presence of specific types of bacteria. Ct values were utilized to assess the relative concentration of specific DNA for each sample as described by the manufacturer. Each sample $^{\Delta\Delta}$Ct values was calculated by normalizing to the CT value of total bacteria (Eubacteria). 16S rDNA served as an internal control and each value represented the mean of three replicates. All oligonucleotide primers were synthesized by Integrated DNA technology Inc. (Coralville, IA, United States). The primer set for each

Table 2 List of primers for bacterial genes specific for family, genus and species

Bacterial gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Ref.
Clostridium cluster XI	TGACGGTACYYNRKGAGGAAGCC	CTACGGTTRAGCCGTAGCCTTT	[63]
Clostridium cluster XIVa	GCGGTRCGGCAAGTCTGA	CCTCCGACACTCTAGTMCGAC	[64]
Clostridium cluster IV	GCACAAGCAGTGGAGT	CTTCCTCCGTTTTGTCAA	[65]
Bifidobacterium genus	GATTCTGGCTCAGGATGAACGC	CTGATAGGACGCGACCCCAT	[66]
Lactobacillus spp.	AGCAGTAGGGAATCTTCCA	CACCGCTACACATGGAG	[66]
Enterobacter (Family)	CATTGACGTTACCCGCAGAAGAAGC	CTCTACGAGACTCAAGCTTGC	[66]
Fusobacterium (genus)	GGATTTATTGGGCGTAAAGC	GGCATTCCTACAAATATCTACGAA	[67]
Fusobacterium nucleatum	CAACCATTACTTTAACTCTACCATGTTCA	GTTGACTTTACAGAAGGAGATTATGTAAAAATC	[68]
Clostridium sordelli	CTGAGACACGTCCAAACTCTAC	CCTCCTCAAGTACCGTCATTATC	-
Total bacteria	CGTGCCAGCAGCCGCGG	TGGACTACCAGGGTATCTAATCCTG	

gene is listed in Table 2.

Real-Time PCR from biopsy

To determine the specific bacterial abundance between serrated and tubular adenomatous patients, total RNA was prepared from patient biopsy samples using TRIzol as recommended by the manufacturer and purified using the Rneasy Mini Kit (QIAGEN). For real time PCR, cDNA was prepared with the SuperScript Ⅲ First-Strand cDNA synthesis system for RT-PCR (Invitrogen) and analyzed in triplicate using the 2 × Power-Up SYBR Green PCR Master Mix (Applied Biosystems) and the ABI Prism 7500 sequence detection system. Primers and PCR were performed as described in the previous section.

For determination of RT-PCR expression of 7-α-dehydroxylase (BaiCD), primers were as follows, *baiCD* forward: 5′-GGWTTCAGCCCRCAGATGTTCTTTG-3′; reverse: 5′GAATTCCGGGTTCATGAACATTCTKCKAAG-3′[35].

Statistical analysis

For microbiota data statistical analysis, Metastats software was used for metagenomics sequencing data analyzed from two groups to characterize the microbial communities. CD-HT and R statistical software was used for BIOM-formatted OTU communities clustering and OTU statistics. For examining alpha diversity, QIIME software was used for graphics and statistical purposes. RDP classifier, QIIME, TUIT GraPhlAn, MetaPhlAn, R software/Too were used for taxonomic classification and statistics. For Real Time PCR data, the standard deviation of mean between two groups and t-test were performed to determine the significance level between two groups.

RESULTS

Phylogenetic analysis of microbial communities in colonic effluents

Microbiota composition of colonic effluent was compared by high throughput analysis of 16S small ribosomal subunit gene (16S rRNA) amplicon. Sequencing of the V3 + V4 region was used for OTU clustering based on 97% sequence similarity. We found unique OTUs in AAs (7234) and CAs (5252), with an overlap of 742 OTUs between the two groups (Figure 1A). We found higher species richness and species diversity in CAs using the number of OTUs in Shannon index (Figure 1B). Irrespective of high inter-individual variances, the Principal Coordinates Analysis (PCoA) showed AAs to possess an abundance of common microbiota, compared to dispersed CA counterparts, revealing more microbial homogeneity within AAs than CA patients (Figure 1C).

The microbiota composition of AAs and CAs showed significant differences in the *Bacteroidetes* and *Proteobacteria* phyla (Figure 2A and Table 3). Taxonomic phylum from 11 from AAs and 24 from CAs were identified (Figure 2A). *Bacteroidetes* was the most abundant bacterial phylum in AAs (70%), whereas *Firmicutes* occurrence of 36% was higher in CAs (Figure 2A). Phylogenetic analysis further identified 44 classes of microbiota, with CAs showing more diverse population than AAs (Figure 2B). *Bacteroidia* was significantly higher in AAs, while *Clostridia, Bacteria-unclassified, Gammaproteobacteria, Bacilli, Verrucomicrobiae, Acinobacteria, Fusobacteria* and *Alphaproteobacteria* were more abundant in CAs (Figure 2B). Again, the microbial species richness and diversity was higher in CAs compared to AAs (Figure 2B).

By 16S rRNA gene profiling, there were eight predominant families found between the two racial groups, *Bacteroidaceae*, *Ruminococcaceae*, *Lachnospiraceae*, *Rikenallaceae, Porphymonadaceae, streptococaceae, Acidaminocococaceae and Veillonellaceae* (Table 3). The most predominant genus in AAs and CAs was *Bacteroides*, comprising 56% and 29%, respectively. Genus abundance of other microbiota was less than 7% with some degree of variation, as observed for *Gemmiger, Allistipes, Parabacterroides, Faecalibacterium, Biophila, Ruminococcus2, Escherichia/Shigella (E/S), Streptococcus, Clostridium IV, Clostridium XIVa, Barnesiella, Akkermansia, Phascolartobacterium, Veillonella, Blautica, Roseburia, Haemophilus, Dialister* and *Fusobacterium* (Table 3).

At the species levels, the relative abundance of *B. caccae* and *B. massiliensis, P. distasonis, P. unclassified, Biophila unclassified* and *Clostridium XI unclassified* were noted to be significantly higher in AAs compared to CAs (Table 3). In CA, the abundance of *G. formicilis, Clostridium IV, B. intestinihominis, E/S. unclassified, H. parainfluenza, A. muciniphila, D. invisus, S. faecium* and *F. unclassified* abundance was higher than AA patients.

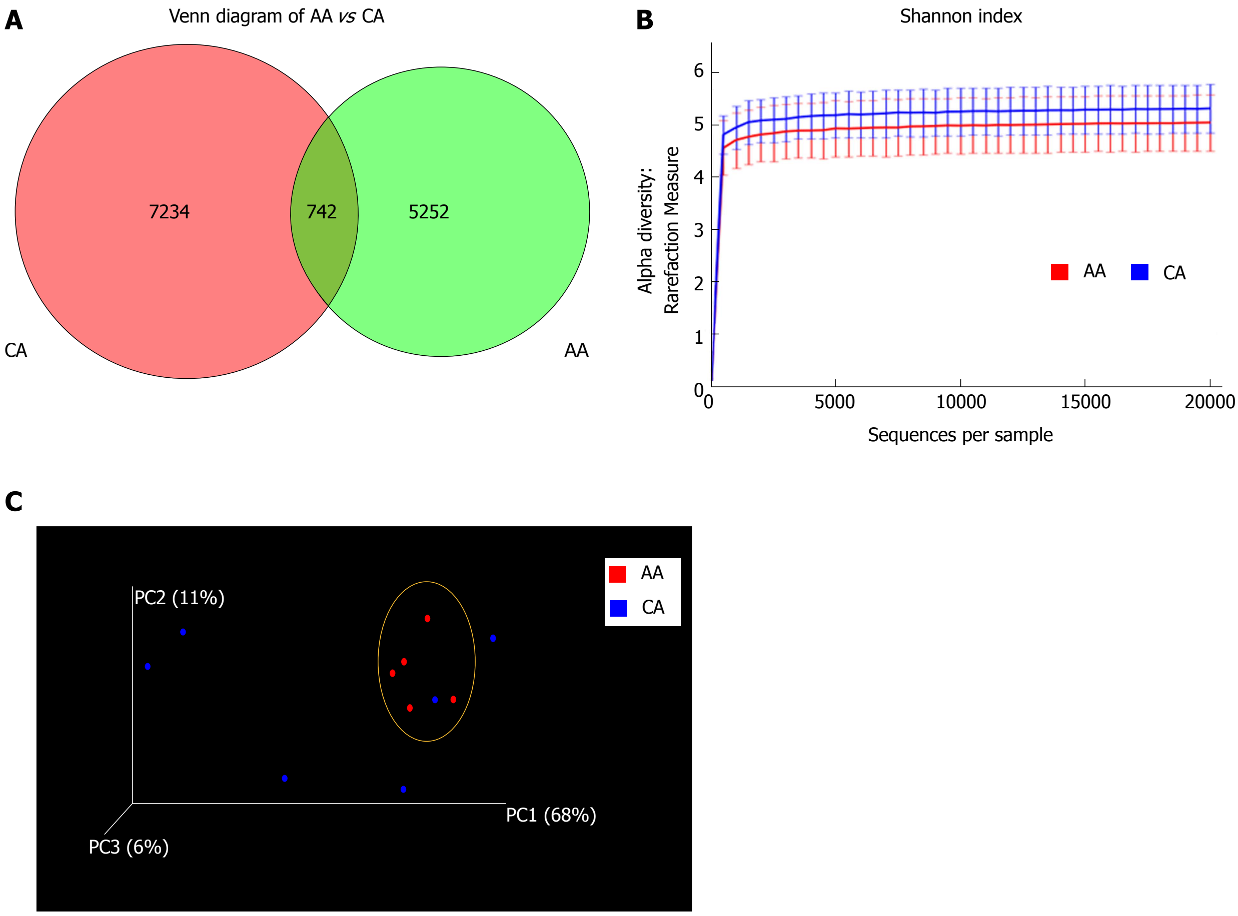

Figure 1 Microbial diversity in colonic effluent from African Americans and Caucasian Americans. A: Venn diagram showing the unique Operational taxonomic units (OTUs) in different subsets of African American (AA) and Caucasian Americans (CA) including overlap community; B: Rarefaction curves showing the species richness from the average number of OTUs for AA and CA (alpha diversity); C: Principal coordinates analysis for beta diversity showing the very dissimilar individual in the CA group while closely similar to the group indicated by the yellow circle. CD-HIT and R software were used for BIOM format OTU clustering and OTU statistics. For measurement of alpha diversity of observed species, QIIME software and Shannon index were used and the emperor tool generated the PCoA plot.

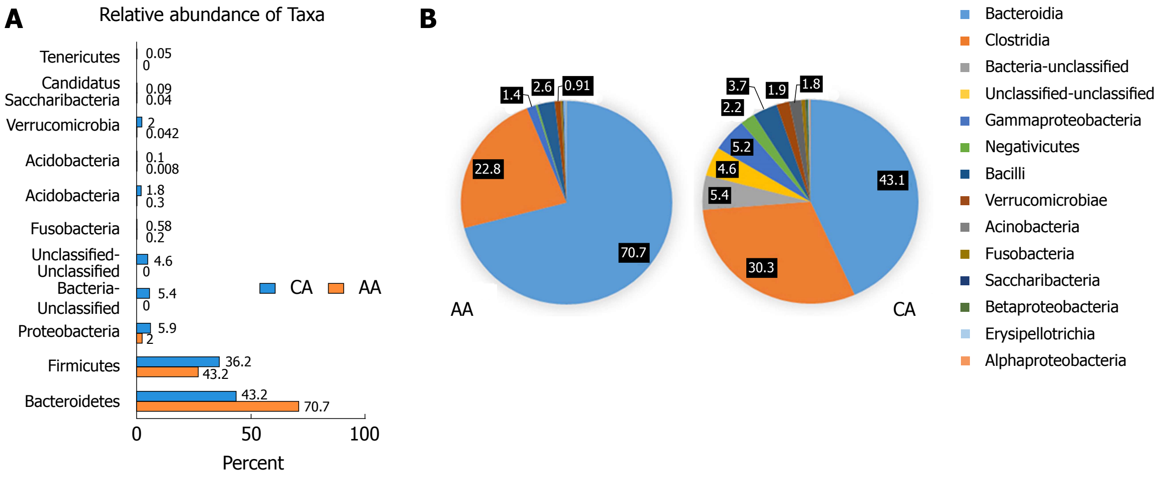

Figure 2 Microbial composition of each group at the phylum and class levels. A: Bar chart shows the relative abundance of phylum; B: Pie chart show the proportion of predominant different classes of microbial community in African Americans and Caucasian Americans. In order to get more comprehensive and accurate taxonomies, multiple databases, Ribosomal Database Project classifier, Greengenes and NCBI 16S Microbial were used for analysis of plot bars and pie charts.

However, with respect to *F. prausnitzii, Ruminococcus2 unclassified, A. putredinis,* and *P. clara*, the relative abundance was found to be similar in both groups (Table 3). On the other hand, the *Fusobacterium* genus was higher in CAs compared to AAs (Figure 3A), and the *Fusobacterium* species level was identified as unclassified by microbial 16sRNA gene profiling.

To further compare the microbial population between

Table 3 Abundance of microbiota in colonic effluents from African Americans and Caucasian Americans (green, black, blue and red color represent phylum, family, genus and species, respectively)

Phylum	Family	Genus	Species	AA (%)	CA (%)
Bacteroidetes				70.74	43.17
	Bacteroidaceae			56.75	29.94
		Bacteroides		56.8	29.9
			Unclassified	30.3	17.8
			Caccae	13.6	5.5
			Massiliensis	6.7	1.7
			Uniformis	2.8	1.5
			Fragilis	1	2
	Rikenellaceae			6.44	5.47
		Allistipes	Putredinis	6.4 (3.9)	5.4 (3.0)
	Porphymonadaceae			6.4	6.95
		Parabacteroides	Distasonis	4.9 (3.4)	3.2 (1.0)
		Barnesiella	Intestinihominis	0.16 (0.16)	2.7 (2.6)
	Prevotellaceae			1.9	1.1
		Paraprevotella	Clara	1.0 (0.9)	0.6 (0.6)
Firmicutes				26.74	36.25
	Ruminococaceae			11.35	15.7
		Faecalibacterium	Prausinitzii	4.5 (4.5)	3.1 (3.1)
		Unclassified	Unclassified	2.5 (2.5)	2.9 (3.0)
		Gemmiger	Formicilis	1.9 (1.6)	6.1 (6.0)
		Clostridium IV	Unclassified	0.3 (0.3)	1.5 (1.5)
	Lachnospiraceae			11.19	13.7
		Ruminococcus 2	Unclassified	3.4 (3.4)	4.7 (4.7)
		Clostridium XIVa	Unclassified	2.05 (2.0)	1.03 (1.0)
		Unclassified	Unclassified	1.6 (1.7)	2.4 (2.4)
		Blautica	Producta	0.22 (0.06)	1.2 (0.0)
		Dorea	Unclassified	1.8 (0.8)	1.3 (1.0)
		Roseburia	Unclassified	0.5 (0.3)	1.3 (0.9)
	Steptococaccae	Steptococcus	Faecium	0.9 (0.9) (0.8)	3.2 (3.2) (3.2)
	Acidaminococcaceae	Phascolarctobacterium	Unclassified	1.8 (1.9) (1.4)	0.9 (0.9) (1.3)
	Peptostreptococcaceae	Clostridium XI	Unclassified	0.02 (0.02) (0.02)	0.01 (0.008) (0.008)
	Veillonellaceae	Veillonella	Atypica	0.81 (0.03) (0.03)	1.27 (0.02) (0.02)
			Unclassified	0.6 (0.6)	0.5 (0.5)
		Dialister	Invisus	0.002 (0.0)	0.68 (0.65)
	Lactobacillaceae	Lactobacillus	Sanfrancisecnsis	0.035 (0.02) (0.004)	0.01 (0.01) (0.001)
Proteobacteria				2.02	5.9
	Desulfovibrionaceae	Biophila	Unclassified	0.16 (0.12) (0.12)	0.07 (0.07) (0.01)
	Sutterrellaceae	Parasuttrella	Excrementihomis	0.50 (0.76) (0.4)	0.24 (1.9) (0.1)
	Pasteurellaceae	Haemophilus	Parainfluenza	0.57 (0.9) (0.4)	1.98 (1.9) (1.9)
	Enterobacteiaceae	Escherichia/Shigella	Unclassified	0.92 (0.8) (0.9)	3.14 (3.1) (3.1)
		Klebsiella	Unclassified	(0.09) (0.02)	0.003 (0.01)
Fusobacteria				0.18	0.58
	Fusobactereaceae	Fusobacterium	Unclassified	0.18 (0.15) (0.15)	0.58 (0.6) (0.6)
Verrucomicrobia				0.04	1.9
	Verrucomicrobiaceae	Akkermansia	Muciniphila	0.04 (0.04)	1.9 (1.9)
Bacteria-unclassified	Unclassified	Unclassified	Unclassified	0	5.4 (5.4) (5.4)
Unclassified-unclassified	Unclassified	Unclassified	Unclassified	0	4.6 (4.6) (4.6)

AA: African Americans; CA: Caucasian Americans.

AAs and CAs, we examined the abundance of specific bacterial populations using species-specific primers. The occurrence of the *Enterobacter* genus was found to be considerably higher in AAs than CAs (Figure 3B), while the *Enterobacteriaceae* family showed the opposite pattern (Table 3). Taxonomic analysis showed CAs to contain *Citrobacter, Klebsiela Escherichia coli, Enterobacter and Shigella sp.* (Table 3). In contrast, the relative abundance of the probiotic bacteria genuses *Bifidobacterium* and *Akkermansia muciniphila* was considerably lower in AAs compared to CAs (Figures 3C and D). These observations demonstrate that the population of pro-inflammatory bacteria is higher in the gut of AAs than CAs.

Occurrence of Fusobacterium nucleatum and Clostridium genus

The relative abundance of *Fusobacterium nucleatum* has been associated with the development and progression of CRC[23,36,37]. We found that the relative abundance of *F. nucleatum* in colonic effluents was significantly higher in AAs than CAs (Figure 3E), indicating a greater risk for the development of CRC in AAs. On the other hand, serrated polyps, which supposedly possess a greater risk of developing CRC, did not exhibit an increased abundancy of *F. nucleatum*. In fact, we found the relative

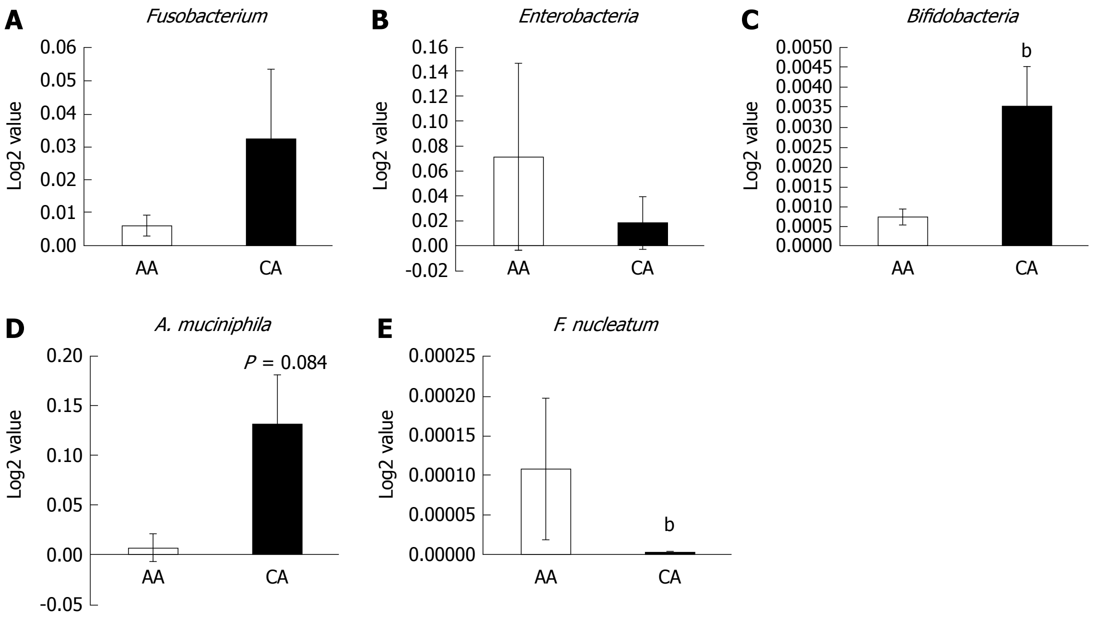

Figure 3 Abundance of inflammatory and probiotic bacteria in colonic effluent from two racial groups using RT-PCR. A: Genus, *Fusobacterium* occurrence higher in Caucasian American (CA); B: The relative abundance of pro-inflammatory *Enterobacter* occurrence is higher in African Americans (AAs); C and D: Probiotic *Bifidobacteria* and *A. muciniphila* is higher in CAs; E: Relative abundance of *F. nucleatum* is higher in AAs than CAs. Data represent mean ± SD of all samples from each group ($^bP < 0.001$).

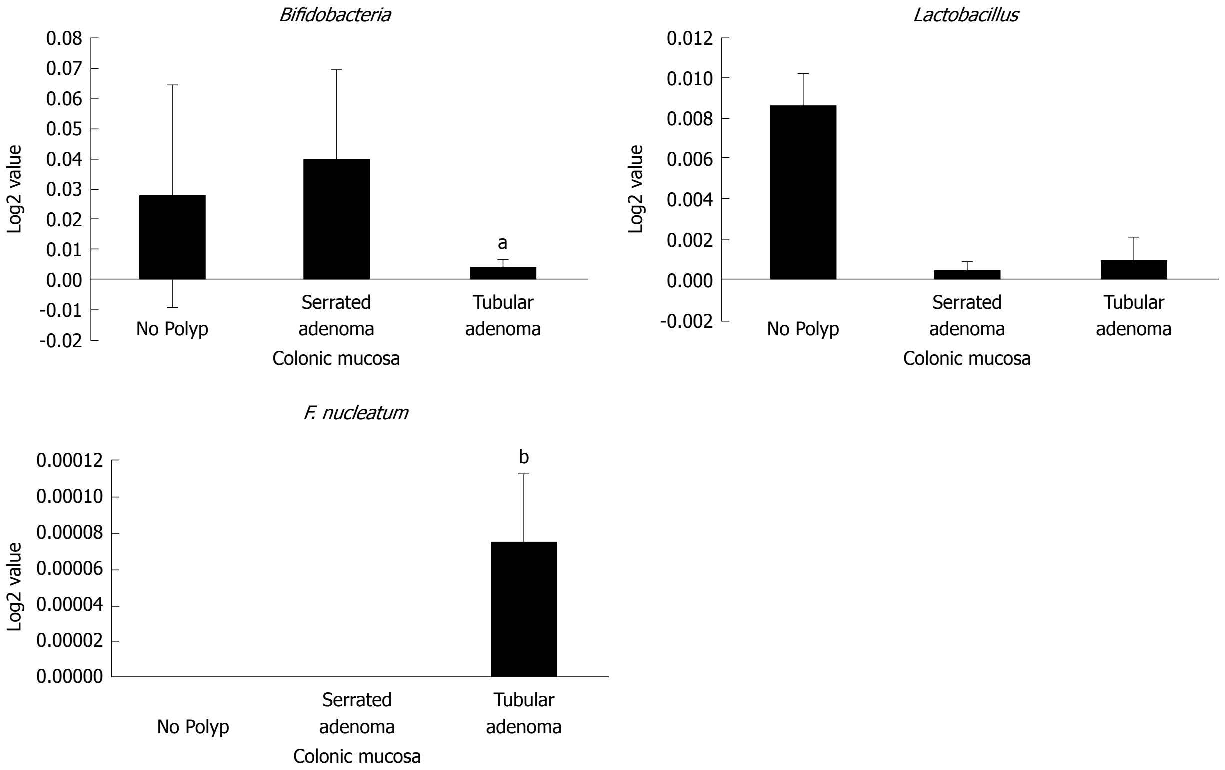

Figure 4 Abundance of probiotic and pro-carcinogenic bacteria in serrated and tubular adenomatous in colonic mucosa. African American and Caucasian American patients were combined. Data represent mean ± SD of all samples from each group ($^aP < 0.05$, $^bP < 0.001$).

abundance of *F. nucleatum* to be higher in tubular adenoma than serrated adenoma (B-Raf proto oncogene, serine/threonine kinase (BRAF) mutation), whereas the probiotic *Lactobacillus* was lower in both serrated and tubular adenomas than those without adenoma (Figure 4). Likewise, the relative abundance of *Bifidobacteria* was found to be lower in tubular adenoma than those without adenoma (Figure 4).

Using a clostridium cluster analysis and RT-PCR, we found that the relative abundance of *Clostridium IV* was higher in CAs (Figure 5A), while *Clostridium XI* was significantly higher in AAs (Figure 5B). *Clostridium IV*

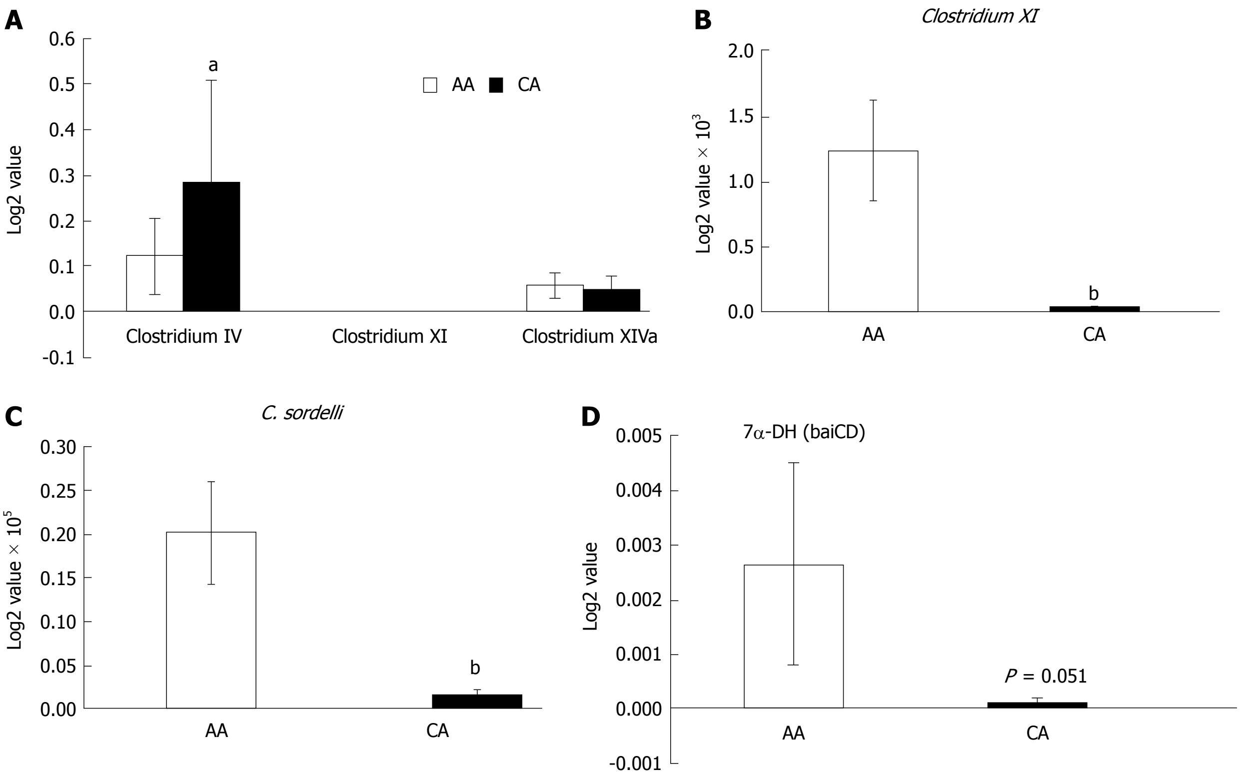

Figure 5 Expression of secondary bile acids transforming enzyme 7-α-DH in the colonic effluent from African Americans and Caucasian Americans using RT-PCR. A: Distribution of different *Clostridium* cluster between African Americans (AA) and Caucasian Americans (CA); B: *Clostridium XI* expression was higher in AAs; C: Secondary bile acids transforming bacteria *Clostridium sordelli* was higher in AAs than CAs; D: Increased 7α-DH expression in AAs. Data represent mean ± SD of all samples from each group ($^{a}P < 0.05$, $^{b}P < 0.01$).

is known to be mediate anti-inflammatory effects[38-40]. *Clostridium sordelli* in the *Clostridium XI* cluster group is known to transform SBA[41]. AA patients also showed higher concentrations of *C. sordelli,* compared to CAs (Figure 5C). A few species of gut anaerobes in the *Clostridium* genus promote the biotransformation of primary to SBA. Given the role of SBA (DCA and LCA) in promoting CRC, the expression of 7-α-dehydroxylase, an enzyme that participates in de-conjugation of primary bile acids, was examined. We found the expression of 7-α-dehydroxylase (baiCD) in colonic effluent from AA patients to be markedly higher than CA subjects (Figure 5D).

DISCUSSION

Our pilot study comparing AA and CA gut microbiota from colonic effluents reveal three major differences between the groups: the AA gut microbiota was less diverse overall, AAs had more pro-inflammatory gut bacteria, and AAs had fewer anti-inflammatory gut bacteria. The phylogenetic tree of microbiota between AAs and CAs reveal an abundance of taxon in CA *vs* AA (Figure 6). Analyzing PCoA of patients, we noted trends found previously in comparing normal colon to adenomas and CRC[36,42,43]. Patients with colonic adenoma typically demonstrate reduced species richness and diversity compared to those without adenomas[44,45]. Furthermore, a fecal microbiota shift occurs in patients with adenomas[46], and they exhibit increased diversity in mucosa than those without adenoma[47].

The human gut microbiota composition is generally represented by three primary phyla: *Firmicutes* (30%-50%), *Bacteroidetes* (20%-40%), and *Actinobacteria* (1%-10%)[22]. The two predominant bacterial phyla, *Bacteroidetes*, and *Firmicutes,* which contribute to 95% of the total GI ecosystem, are associated with adenomas and CRC[48]. The abundance of phylum *Bacteroidetes (P. distasonis, Alistipes spp.)* in the gut may increase the rate of tumorigenesis[49]. Our current study demonstrates an abundance of *Bacteroidetes* (70%) and reduction in *Firmicute* and *Actinobacteria* in AAs, whereas the microbial balance between predominant groups was unchanged in CAs, as has been demonstrated previously[22]. These observations are similar to what Hester *et al*[50] noted in their investigation in that the *Firmicutes/Bacteroidetes* ratio is higher in AAs compared to CAs. There is also a relative abundance of *B. massiliensis* in AAs compared to CAs. CRC has also been shown to be associated *F. nucleatum* and pro-inflammatory bacteria, *Enterobacter* and *Clostridium XI*-species. *F. nucleatum* strains have been shown to promote carcinogenesis and invasion of host cells and potentiate tumorigenesis in mouse model of colon cancer[23,37]. CRC patients demonstrate a higher abundance of *F. nucleatum* and *Clostridium difficile,* a member of *Clostridium XI*[51,52]. Others have demonstrated that the relative increase in *Clostridium*

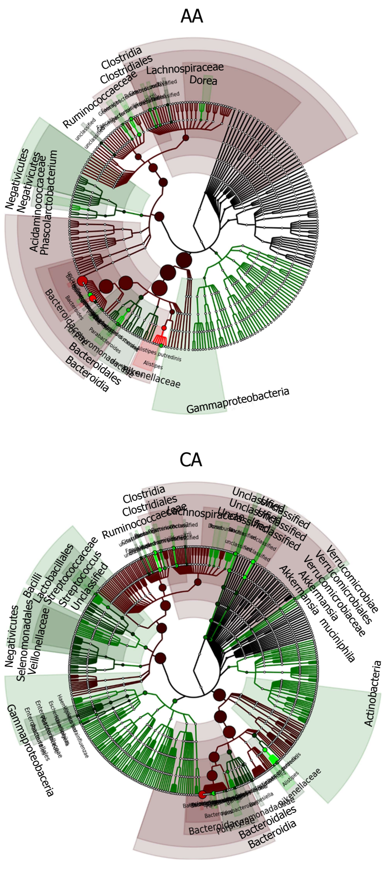

Figure 6 Phylogenetic tree showing the differences and abundance of taxa in African Americans and Caucasian Americans colonic effluents. The taxon size and color indicate the relative abundance of family. GraPhlAn software was used for taxonomic classification and circular taxonomic phylogenetic trees and Ribosomal Database Project classifier, Quantitative Insights into Microbial Ecology, R software/Tool were used for taxonomic data analysis.

cluster XI and *Enterobacteriaceae* are associated with intestinal dysbiosis[53].

Previous studies have suggested that a decrease in commensal microbiota in AAs may contribute to the tumorigenic microenvironment and that dysbiosis of gut microbiota may be partially responsible for promoting CRC and colitis-associated CRC[22,42,54]. In line with these observations, we found commensal bacteria *B. fragilis* to be slightly higher in CAs than AAs. *Bacteroides fragilis* is an immunomodulatory bacteria, which stimulates anti-inflammatory cytokine IL10 by Foxp3+ regulatory CD+ T (Treg) cells and suppresses mucosal inflammation[55,56]. In contrast, *Unclassified-Bacteria* and *Unclassified-Unclassified* micro-organisms were only present in CAs *A. municiphila,* a member of *Verrumicomicrobia,* is an intestinal symbiont and is known to induce an anti-inflammatory effect and enhance immune function[57]. Depletion of *A. muciniphila* is associated with a variety of diseases, including diabetes[58,59]. We found the relative abundance of *A. muciniphila* to be lower in AAs than their CA counterparts. Collectively, AAs have fewer bacterial populations that are known to suppress inflammation, improve mucus barrier function, and diminish permeability[60].

Human genetic variants can modulate the effects of the microbiome composition, and both are associated with many human complex diseases[61]. Microbiota changes with diet and stimulatory agents and can modulate disease development and progression[61,62]. Microbial dysbiosis in AAs may serve as a point for prevention and ultimately treatment of CRC. Identifying a microbial signature associated with CRC is complicated by many factors. This study was limited by focusing on a specific population with limited sample size and did not investigate dietary differences. However, there were significant differences between the colonic effluent microbiota of the AA and CA study groups - with less diversity of bacteria, greater abundance of pro-inflammatory bacteria, and reduced anti-inflammatory bacteria. Mechanisms for tumorigenesis may include bacteria that promote SBA transformation, as suggested by higher 7-α-dehydroxylase in the AA *vs* CA group. Further study is needed to evaluate the role of decreased diversity and structural imbalance in the colon microbial communities and the development of CRC.

ARTICLE HIGHLIGHTS

Research background

The incidence of colorectal cancer (CRC), the third most common malignancy, is not only higher among African Americans (AAs), but is also associated with higher mortality. In addition, AAs tend to be diagnosed with CRC at a younger age than Caucasian Americans (CAs) and exhibit worse prognoses than their CA counterparts. Despite this grim outlook, neither the extrinsic/intrinsic factor(s) nor the underlying molecular and/or biochemical mechanisms are fully understood. We hypothesize that imbalance in the gut microbiome between AAs and CAs results in alterations of metabolites, which changes symbiotic relationships and enhance gastrointestinal diseases, including CRC. A number of bacteria are known to promote carcinogenesis in the colon by altering gut microbial composition, which may play a major role in colorectal carcinogenesis.

Research motivation

CRC is the third leading malignancy world-wide, affecting both males and females equally. It represents one of the most common cancers in the United States and is estimated to be the second and third leading cause of cancer-related deaths in men and women, respectively, in the United States. Several studies have also demonstrated that AAs have the highest rate of CRC than any other racial group in the USA, and also AA men are even more likely to die from CRC than AA women. With these grim statistics, it is important to gain a better understanding of the underlying mechanism(s), particularly the role of gut microbiota, in regulating racial disparity in colorectal carcinogenesis.

Research objectives

The current investigation was aimed at studying microbial dysbiosis in the gut between AAs and CAs. The primary endpoint of this investigation was to determine whether the increased incidence of CRC in AAs could be attributed to alterations in gut microbiota. In this pilot study, we investigated the diversity and abundance of specific gut microbial communities in colonic effluents using 16S rRNA gene profiling in AAs and CAs and their possible role in the increased incidence of CRC in AAs.

Research methods

Male and female AA and CA patients, aged between 40 and 80 years, undergoing routine colonoscopy at the John D. Dingell VA Medical Center in Detroit were asked to participate. To determine the microbial diversity and the microbial richness in AAs and CAs, colonic effluent from each patient was used for DNA extraction and 16s RNA gene-based microbial community profiling which was performed and analyzed by LC Sciences (Houston, Texas, United

States). The composition of OTU and alpha diversity was measured by Venn diagram and Rarefaction measurement method. The relative abundance of phylum and classes was depicted by bar and pie chart. The phylogenic tree was plotted for AA and CA to determine the relative abundance of family in microbial community. Several inflammatory and probiotic bacterial marker candidates such as *Enterobacteria, Bifidobacteria, Lactobacillus, Fusobacterium* and/or *Clostridium genus* and species-specific bacteria were identified by real-time qPCR using specific primers designed on the basis of conserved and variable region in bacterial 16S rRNA genes according to our standard protocol. Statistical analysis was performed for each experiment accordingly.

Research results

The relative abundance of *Fusobacterium nucleatum,* which has been associated with the development and progression of CRC, was found to be significantly higher in AAs than CAs, indicating a greater risk for the development of CRC in AAs. *Clostridium IV,* a known mediator of anti-inflammatory effects, was found to be higher in CAs than AAs.

Research conclusion

The human colon harbors a complex microbial flora. Bacterial density in the human colon is among the highest found in nature, approaching 10^{12} bacteria/gm wet weight of feces. These bacteria are in a symbiotic relationship with the intestine, utilizing undigested nutrients as substrates and in return, produce various vitamins, amino acids, transform bile salts and assist in the maintenance of the intestinal barrier, and the appropriate immune response against pathogens. This homeostasis is altered in a state of dysbiosis, which is overgrowth of pathogenic bacteria that are normally inhibited by commensal bacteria. Our current investigation, for the first time, demonstrates microbial dysbiosis between AAs and CAs. This imbalance, we believe, is partially responsible for the racial disparity in CRC observed between AAs and CAs.

Research perspective

Although numerous studies have demonstrated that the incidence of CRC is higher in AAs than CAs, the reasons for this racial disparity are not fully understood. Data generated from this investigation reveal a role for the gut microbiome in racial disparity. The precise mechanisms by which changes in gut microbiota would lead to an increase CRC in AAs remain unexplored. However, it is tempting to speculate that this dysbiosis or overgrowth of certain bacteria in the gut of AAs resulting in alterations in microbial metabolites, specifically deoxycholic acid and lithocholic acid, which are known for their co-carcinogenic activity, could induce the process(es) of carcinogenesis in the colon of AAs. However, the levels of microbial metabolites, including bile acids in AAs and CAs with and without adenomas have not determined. Moreover, no information is available whether the observed dysbiosis in AAs is due to changes in diet and/or lifestyle. Undoubtedly, further investigations are needed to gain a better and fuller understanding of the intrinsic and extrinsic factors that are critically involved in regulating racial disparity in CRC.

REFERENCES

1 **Dimou A**, Syrigos KN, Saif MW. Disparities in colorectal cancer in African-Americans vs Whites: before and after diagnosis. *World J Gastroenterol* 2009; **15**: 3734-3743 [PMID: 19673013 DOI: 10.3748/wjg.15.3734]

2 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

3 **Lieberman DA**, Holub JL, Moravec MD, Eisen GM, Peters D, Morris CD. Prevalence of colon polyps detected by colonoscopy screening in asymptomatic black and white patients. *JAMA* 2008; **300**: 1417-1422 [PMID: 18812532 DOI: 10.1001/jama.300.12.1417]

4 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.20138]

5 **Wallace K**, Hill EG, Lewin DN, Williamson G, Oppenheimer S, Ford ME, Wargovich MJ, Berger FG, Bolick SW, Thomas MB, Alberg AJ. Racial disparities in advanced-stage colorectal cancer survival. *Cancer Causes Control* 2013; **24**: 463-471 [PMID: 23296454 DOI: 10.1007/s10552-012-0133-5]

6 **Wallace K**, Sterba KR, Gore E, Lewin DN, Ford ME, Thomas MB, Alberg AJ. Prognostic factors in relation to racial disparity in advanced colorectal cancer survival. *Clin Colorectal Cancer* 2013; **12**: 287-293 [PMID: 24188687 DOI: 10.1016/j.clcc.2013.08.001]

7 **Ashktorab H**, Kupfer SS, Brim H, Carethers JM. Racial Disparity in Gastrointestinal Cancer Risk. *Gastroenterology* 2017; **153**: 910-923 [PMID: 28807841 DOI: 10.1053/j.gastro.2017.08.018]

8 **Amri R**, Bordeianou LG, Berger DL. The conundrum of the young colon cancer patient. *Surgery* 2015; **158**: 1696-1703 [PMID: 26298030 DOI: 10.1016/j.surg.2015.07.018]

9 **Ashktorab H**, Vilmenay K, Brim H, Laiyemo AO, Kibreab A, Nouraie M. Colorectal Cancer in Young African Americans: Is It Time to Revisit Guidelines and Prevention? *Dig Dis Sci* 2016; **61**: 3026-3030 [PMID: 27278956 DOI: 10.1007/s10620-016-4207-1]

10 **Carethers JM**. Screening for colorectal cancer in African Americans: determinants and rationale for an earlier age to commence screening. *Dig Dis Sci* 2015; **60**: 711-721 [PMID: 25540085 DOI: 10.1007/s10620-014-3443-5]

11 **Laryea JA**, Siegel E, Klimberg S. Racial disparity in colorectal cancer: the role of equal treatment. *Dis Colon Rectum* 2014; **57**: 295-302 [PMID: 24509450 DOI: 10.1097/DCR.0000000000000056]

12 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767 [PMID: 2188735 DOI: 10.1016/0092-8674(90)90186-I]

13 **Vogelstein B**, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. *Science* 2013; **339**: 1546-1558 [PMID: 23539594 DOI: 10.1126/science.1235122]

14 **Todaro M**, Iovino F, Eterno V, Cammareri P, Gambara G, Espina V, Gulotta G, Dieli F, Giordano S, De Maria R, Stassi G. Tumorigenic and metastatic activity of human thyroid cancer stem cells. *Cancer Res* 2010; **70**: 8874-8885 [PMID: 20959469 DOI: 10.1158/0008-5472.CAN-10-1994]

15 **Farhana L**, Antaki F, Anees MR, Nangia-Makker P, Judd S, Hadden T, Levi E, Murshed F, Yu Y, Van Buren E, Ahmed K, Dyson G, Majumdar AP. Role of cancer stem cells in racial disparity in colorectal cancer. *Cancer Med* 2016; **5**: 1268-1278 [PMID: 26990997 DOI: 10.1002/cam4.690]

16 **Alexander DD**, Cushing CA, Lowe KA, Sceurman B, Roberts MA. Meta-analysis of animal fat or animal protein intake and colorectal cancer. *Am J Clin Nutr* 2009; **89**: 1402-1409 [PMID: 19261724 DOI: 10.3945/ajcn.2008.26838]

17 **Carethers JM**. Racial and ethnic factors in the genetic pathogenesis of colorectal cancer. *J Assoc Acad Minor Phys* 1999; **10**: 59-67 [PMID: 10826011]

18 **Huxley RR**, Ansary-Moghaddam A, Clifton P, Czernichow S, Parr CL, Woodward M. The impact of dietary and lifestyle risk factors on risk of colorectal cancer: a quantitative overview of the epidemiological evidence. *Int J Cancer* 2009; **125**: 171-180 [PMID: 19350627 DOI: 10.1002/ijc.24343]

19 **Sandler RS**. Aspirin and other nonsteroidal anti-inflammatory agents in the prevention of colorectal cancer. *Important Adv Oncol* 1996: 123-137 [PMID: 8791132]

20 **Dethlefsen L**, Eckburg PB, Bik EM, Relman DA. Assembly of the human intestinal microbiota. *Trends Ecol Evol* 2006; **21**: 517-523 [PMID: 16820245 DOI: 10.1016/j.tree.2006.06.013]

21 **Abreu MT**, Peek RM Jr. Gastrointestinal malignancy and the microbiome. *Gastroenterology* 2014; **146**: 1534-1546.e3 [PMID: 24406471 DOI: 10.1053/j.gastro.2014.01.001]

22 **Gagnière J**, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E, Bringer MA, Pezet D, Bonnet M. Gut microbiota imbalance and colorectal cancer. *World J Gastroenterol* 2016; **22**: 501-518 [PMID: 26811603 DOI: 10.3748/wjg.v22.i2.501]

23 **Kostic AD**, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, Clancy TE, Chung DC, Lochhead P, Hold GL, El-Omar EM, Brenner D, Fuchs CS, Meyerson M, Garrett WS. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 2013; **14**: 207-215 [PMID: 23954159 DOI: 10.1016/j.chom.2013.07.007]

24 **Tözün N**, Vardareli E. Gut Microbiome and Gastrointestinal Cancer: Les liaisons Dangereuses. *J Clin Gastroenterol* 2016; **2015**: S191-S196 [PMID: 27741173 DOI: 10.1097/MCG.0000000000000714]

25 **Boutagy NE**, Neilson AP, Osterberg KL, Smithson AT, Englund TR,

Davy BM, Hulver MW, Davy KP. Short-term high-fat diet increases postprandial trimethylamine-N-oxide in humans. *Nutr Res* 2015; **35**: 858-864 [PMID: 26265295 DOI: 10.1016/j.nutres.2015.07.002]

26 **Fleming SE**, Fitch MD, Chansler MW. High-fiber diets: influence on characteristics of cecal digesta including short-chain fatty acid concentrations and pH. *Am J Clin Nutr* 1989; **50**: 93-99 [PMID: 2546417 DOI: 10.1093/ajcn/50.1.93]

27 **Northfield TC**, McColl I. Postprandial concentrations of free and conjugated bile acids down the length of the normal human small intestine. *Gut* 1973; **14**: 513-518 [PMID: 4729918 DOI: 10.1136/gut.14.7.513]

28 **Zitvogel L**, Pietrocola F, Kroemer G. Nutrition, inflammation and cancer. *Nat Immunol* 2017; **18**: 843-850 [PMID: 28722707 DOI: 10.1038/ni.3754]

29 **Ajouz H**, Mukherji D, Shamseddine A. Secondary bile acids: an underrecognized cause of colon cancer. *World J Surg Oncol* 2014; **12**: 164 [PMID: 24884764 DOI: 10.1186/1477-7819-12-164]

30 **Narisawa T**, Magadia NE, Weisburger JH, Wynder EL. Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation of N-methyl-N'-nitro-N-nitrosoguanidine in rats. *J Natl Cancer Inst* 1974; **53**: 1093-1097 [PMID: 4427390 DOI: 10.1093/jnci/53.4.1093]

31 **Ou J**, DeLany JP, Zhang M, Sharma S, O'Keefe SJ. Association between low colonic short-chain fatty acids and high bile acids in high colon cancer risk populations. *Nutr Cancer* 2012; **64**: 34-40 [PMID: 22136517 DOI: 10.1080/01635581.2012.630164]

32 **Payne CM**, Crowley-Skillicorn C, Bernstein C, Holubec H, Moyer MP, Bernstein H. Hydrophobic bile acid-induced micronuclei formation, mitotic perturbations, and decreases in spindle checkpoint proteins: relevance to genomic instability in colon carcinogenesis. *Nutr Cancer* 2010; **62**: 825-840 [PMID: 20661832 DOI: 10.1080/01635581003695756]

33 **Reddy BS**, Martin CW, Wynder EL. Fecal bile acids and cholesterol metabolites of patients with ulcerative colitis, a high-risk group for development of colon cancer. *Cancer Res* 1977; **37**: 1697-1701 [PMID: 322859]

34 **Cao H**, Xu M, Dong W, Deng B, Wang S, Zhang Y, Wang S, Luo S, Wang W, Qi Y, Gao J, Cao X, Yan F, Wang B. Secondary bile acid-induced dysbiosis promotes intestinal carcinogenesis. *Int J Cancer* 2017; **140**: 2545-2556 [PMID: 28187526 DOI: 10.1002/ijc.30643]

35 **Wells JE**, Williams KB, Whitehead TR, Heuman DM, Hylemon PB. Development and application of a polymerase chain reaction assay for the detection and enumeration of bile acid 7alpha-dehydroxylating bacteria in human feces. *Clin Chim Acta* 2003; **331**: 127-134 [PMID: 12691873 DOI: 10.1016/S0009-8981(03)00115-3]

36 **Mira-Pascual L**, Cabrera-Rubio R, Ocon S, Costales P, Parra A, Suarez A, Moris F, Rodrigo L, Mira A, Collado MC. Microbial mucosal colonic shifts associated with the development of colorectal cancer reveal the presence of different bacterial and archaeal biomarkers. *J Gastroenterol* 2015; **50**: 167-179 [PMID: 24811328 DOI: 10.1007/s00535-014-0963-x]

37 **Rubinstein MR**, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. *Cell Host Microbe* 2013; **14**: 195-206 [PMID: 23954158 DOI: 10.1016/j.chom.2013.07.012]

38 **Arpaia N**, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffer PJ, Rudensky AY. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013; **504**: 451-455 [PMID: 24226773 DOI: 10.1038/nature12726]

39 **Furusawa Y**, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013; **504**: 446-450 [PMID: 24226770 DOI: 10.1038/nature12721]

40 **Smith PM**, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, Glickman JN, Garrett WS. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 2013; **341**: 569-573 [PMID: 23828891 DOI: 10.1126/science.1241165]

41 **Ridlon JM**, Kang DJ, Hylemon PB, Bajaj JS. Bile acids and the gut microbiome. *Curr Opin Gastroenterol* 2014; **30**: 332-338 [PMID: 24625896 DOI: 10.1097/MOG.0000000000000057]

42 **Nakatsu G**, Li X, Zhou H, Sheng J, Wong SH, Wu WK, Ng SC, Tsoi H, Dong Y, Zhang N, He Y, Kang Q, Cao L, Wang K, Zhang J, Liang Q, Yu J, Sung JJ. Gut mucosal microbiome across stages of colorectal carcinogenesis. *Nat Commun* 2015; **6**: 8727 [PMID: 26515465 DOI: 10.1038/ncomms9727]

43 **Sanapareddy N**, Legge RM, Jovov B, McCoy A, Burcal L, Araujo-Perez F, Randall TA, Galanko J, Benson A, Sandler RS, Rawls JF, Abdo Z, Fodor AA, Keku TO. Increased rectal microbial richness is associated with the presence of colorectal adenomas in humans. *ISME J* 2012; **6**: 1858-1868 [PMID: 22622349 DOI: 10.1038/ismej.2012.43]

44 **Ahn J**, Sinha R, Pei Z, Dominianni C, Wu J, Shi J, Goedert JJ, Hayes RB, Yang L. Human gut microbiome and risk for colorectal cancer. *J Natl Cancer Inst* 2013; **105**: 1907-1911 [PMID: 24316595 DOI: 10.1093/jnci/djt300]

45 **Peters BA**, Dominianni C, Shapiro JA, Church TR, Wu J, Miller G, Yuen E, Freiman H, Lustbader I, Salik J, Friedlander C, Hayes RB, Ahn J. The gut microbiota in conventional and serrated precursors of colorectal cancer. *Microbiome* 2016; **4**: 69 [PMID: 28038683 DOI: 10.1186/s40168-016-0218-6]

46 **Hale VL**, Chen J, Johnson S, Harrington SC, Yab TC, Smyrk TC, Nelson H, Boardman LA, Druliner BR, Levin TR, Rex DK, Ahnen DJ, Lance P, Ahlquist DA, Chia N. Shifts in the Fecal Microbiota Associated with Adenomatous Polyps. *Cancer Epidemiol Biomarkers Prev* 2017; **26**: 85-94 [PMID: 27672054 DOI: 10.1158/1055-9965.EPI-16-0337]

47 **Shen XJ**, Rawls JF, Randall T, Burcal L, Mpande CN, Jenkins N, Jovov B, Abdo Z, Sandler RS, Keku TO. Molecular characterization of mucosal adherent bacteria and associations with colorectal adenomas. *Gut Microbes* 2010; **1**: 138-147 [PMID: 20740058 DOI: 10.4161/gmic.1.3.12360]

48 **Allen-Vercoe E**, Jobin C. Fusobacterium and Enterobacteriaceae: important players for CRC? *Immunol Lett* 2014; **162**: 54-61 [PMID: 24972311 DOI: 10.1016/j.imlet.2014.05.014]

49 **Baxter NT**, Zackular JP, Chen GY, Schloss PD. Structure of the gut microbiome following colonization with human feces determines colonic tumor burden. *Microbiome* 2014; **2**: 20 [PMID: 24967088 DOI: 10.1186/2049-2618-2-20]

50 **Hester CM**, Jala VR, Langille MG, Umar S, Greiner KA, Haribabu B. Fecal microbes, short chain fatty acids, and colorectal cancer across racial/ethnic groups. *World J Gastroenterol* 2015; **21**: 2759-2769 [PMID: 25759547 DOI: 10.3748/wjg.v21.i9.2759]

51 **Franks AH**, Harmsen HJ, Raangs GC, Jansen GJ, Schut F, Welling GW. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl Environ Microbiol* 1998; **64**: 3336-3345 [PMID: 9726880]

52 **Fukugaiti MH**, Ignacio A, Fernandes MR, Ribeiro Júnior U, Nakano V, Avila-Campos MJ. High occurrence of Fusobacterium nucleatum and Clostridium difficile in the intestinal microbiota of colorectal carcinoma patients. *Braz J Microbiol* 2015; **46**: 1135-1140 [PMID: 26691472 DOI: 10.1590/S1517-838246420140665]

53 **Marteau P**, Chaput U. Bacteria as trigger for chronic gastrointestinal disorders. *Dig Dis* 2011; **29**: 166-171 [PMID: 21734380 DOI: 10.1159/000323879]

54 **Sobhani I**, Amiot A, Le Baleur Y, Levy M, Auriault ML, Van Nhieu JT, Delchier JC. Microbial dysbiosis and colon carcinogenesis: could colon cancer be considered a bacteria-related disease? *Therap Adv Gastroenterol* 2013; **6**: 215-229 [PMID: 23634186 DOI: 10.1177/1756283X12473674]

55 **Donaldson GP**, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol* 2016; **14**: 20-32 [PMID: 26499895 DOI: 10.1038/nrmicro3552]

56 **Zitvogel L**, Daillère R, Roberti MP, Routy B, Kroemer G. Anticancer effects of the microbiome and its products. *Nat Rev Microbiol* 2017; **15**: 465-478 [PMID: 28529325 DOI: 10.1038/nrmicro.2017.44]

57 **de Vos WM**. Microbe Profile: Akkermansia muciniphila: a conserved intestinal symbiont that acts as the gatekeeper of our mucosa. *Microbiology* 2017; **163**: 646-648 [PMID: 28530168 DOI: 10.1099/mic.0.000444]

58 **Belzer C**, de Vos WM. Microbes inside--from diversity to function: the case of Akkermansia. *ISME J* 2012; **6**: 1449-1458 [PMID: 22437156 DOI: 10.1038/ismej.2012.6]

59 **Plovier H**, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, Chilloux J, Ottman N, Duparc T, Lichtenstein L, Myridakis A, Delzenne NM, Klievink J, Bhattacharjee A, van der Ark KC, Aalvink S, Martinez LO, Dumas ME, Maiter D, Loumaye A, Hermans MP, Thissen JP, Belzer C, de Vos WM, Cani PD. A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med* 2017; **23**: 107-113 [PMID: 27892954 DOI: 10.1038/nm.4236]

60 **Plotnikoff GA**. Three measurable and modifiable enteric microbial biotransformations relevant to cancer prevention and treatment. *Glob Adv Health Med* 2014; **3**: 33-43 [PMID: 24891992 DOI: 10.7453/gahmj.2014.021]

61 **Luca F**, Kupfer SS, Knights D, Khoruts A, Blekhman R. Functional Genomics of Host-Microbiome Interactions in Humans. *Trends Genet* 2018; **34**: 30-40 [PMID: 29107345 DOI: 10.1016/j.tig.2017.10.001]

62 **Ou J**, Carbonero F, Zoetendal EG, DeLany JP, Wang M, Newton K, Gaskins HR, O'Keefe SJ. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *Am J Clin Nutr* 2013; **98**: 111-120 [PMID: 23719549 DOI: 10.3945/ajcn.112.056689]

63 **Yoshimoto S**, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, Iwakura Y, Oshima K, Morita H, Hattori M, Honda K, Ishikawa Y, Hara E, Ohtani N. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013; **499**: 97-101 [PMID: 23803760 DOI: 10.1038/nature12347]

64 **Matsuki T**, Watanabe K, Fujimoto J, Miyamoto Y, Takada T, Matsumoto K, Oyaizu H, Tanaka R. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. *Appl Environ Microbiol* 2002; **68**: 5445-5451 [PMID: 12406736 DOI: 10.1128/AEM.68.11.5445-5451.2002]

65 **Matsuki T**, Watanabe K, Fujimoto J, Takada T, Tanaka R. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. *Appl Environ Microbiol* 2004; **70**: 7220-7228 [PMID: 15574920 DOI: 10.1128/AEM.70.12.7220-7228.2004]

66 **Collado MC**, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am J Clin Nutr* 2008; **88**: 894-899 [PMID: 18842773 DOI: 10.1093/ajcn/88.4.894]

67 **Kostic AD**, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, Ojesina AI, Jung J, Bass AJ, Tabernero J, Baselga J, Liu C, Shivdasani RA, Ogino S, Birren BW, Huttenhower C, Garrett WS, Meyerson M. Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. *Genome Res* 2012; **22**: 292-298 [PMID: 22009990 DOI: 10.1101/gr.126573.111]

68 **Castellarin M**, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, Barnes R, Watson P, Allen-Vercoe E, Moore RA, Holt RA. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. *Genome Res* 2012; **22**: 299-306 [PMID: 22009989 DOI: 10.1101/gr.126516.111]

P- Reviewer: Huerta-Franco M, Mann O, Maric I, McMillin MA
S- Editor: Cui LJ **L- Editor**: Filipodia **E- Editor**: Yin SY

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com