Differences in intestinal microbiota profiling after upper and lower gastrointestinal surgery

Xi-Hsuan Lin[a,b], Ueng-Cheng Yang[c], Jiing-Chyuan Luo[b,d,e,*], Tien-En Chang[b,d], Hung-Hsin Lin[b,f], Chi-Wei Huang[c], Jen-Jie Chiou[c], Wen-Liang Fang[b,g], Kuo-Hung Huang[b,g], Yi-Hsiang Huang[b,d], Ming-Chih Hou[b,d,*], Fa-Yauh Lee[b,d]

[a]Department of Medicine, National Yang Ming Chiao Tung University Hospital, Yilan, Taiwan, ROC; [b]Department of Medicine, National Yang Ming Chiao Tung University, School of Medicine, Taipei, Taiwan, ROC; [c]School of Medicine, Institute of Biomedical Informatics, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC; [d]Division of Gastroenterology and Hepatology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; [e]Department of Medicine, Keelung Hospital, Ministry of Health Welfare, Keelung, Taiwan, ROC; [f]Division of Colon and Rectal Surgery, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; [g]Division of General Surgery, Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan, ROC

Abstract

Background: We aimed to investigate the long-term effects of metabolic profiles and microbiota status in patients after upper gastrointestinal (GI) surgery and lower GI surgery and compared them with a control group.

Methods: In this cross-sectional study, we analyzed the occurrence of metabolic syndrome (MS) in 10 patients who underwent curative total gastrectomy with Roux-en-Y esophagojejunostomy (RYEJ) anastomosis, 11 patients who underwent curative partial colectomy with right hemicolectomy (RH), and 33 age- and sex-matched controls. Fecal samples were also analyzed by a next-generation sequencing method.

Results: Compared with the control group, the occurrence of MS was significantly lower among patients who underwent total gastrectomy with RYEJ than the controls over the long-term follow-up (>8 years; $p < 0.05$). Patients who received RH only had a trend of higher serum fasting glucose ($p = 0.10$). The diversity of the gut microbiota significantly decreased after RH in comparison with the control group and RYEJ group, respectively ($p < 0.05$). Principal component analysis revealed significant differences between the control, RYEJ, and RH groups ($p < 0.001$). At the genus level, the ratio of *Prevotella* to *Bacteroides* (P/B) was significantly higher in the RYEJ group than in the control group, whereas the P/B ratio was significantly lower in the RH group than in the control group ($p < 0.05$).

Conclusion: Early gastric cancer patients who received total gastrectomy with RYEJ had a lower occurrence of MS than the controls, while early colorectal cancer patients who received RH were associated with a higher serum fasting glucose than the controls during long-term follow-up. In parallel with the metabolic differences, the P/B ratio was also significantly altered in patients after upper and lower GI surgery.

Keywords: General surgery; Gastrointestinal microbiome; Metabolic syndrome

1. INTRODUCTION

The gastrointestinal (GI) tract plays an important role in metabolic control and nutritional homeostasis.[1] Bariatric/metabolic procedures that modify the structure and function of the proximal gut have widely been demonstrated to have a beneficial effect on obesity and type II diabetes mellitus (DM).[2,3] Furthermore, our previous study also demonstrated a lower occurrence of metabolic syndrome (MS) in early gastric cancer patients after subtotal gastrectomy during long-term follow-up periods.[4] Nevertheless, the research on the metabolic effects of the distal gut is very limited.

Some characteristics of insulin resistance were found in patients after total colectomy.[5,6] The gut microbiota was recently found to play important roles in the development of metabolic diseases.[7] The composition of the microbiome changes along the GI tract, with major populations in different locations performing various functions. As a result, upper and lower GI surgery may provide experimental tools to explore the metabolic physiology of the GI tract. Moreover, this approach could enhance our understanding of metabolic regulation and help identify novel therapeutic targets. Thus, we aimed to investigate the long-term effects of metabolic profiles and microbiota status in early gastric cancer patients after curative total gastrectomy and early colorectal cancer (CRC) patients after curative colectomy in comparison to a control group.

2. METHODS

2.1. Study population

In this cross-sectional study, we collected anthropometric, laboratory data, and stool specimens from patients in our outpatient

Address correspondence: Dr. Jiing-Chyuan Luo and Dr. Ming-Chih Hou, Division of Gastroenterology and Hepatology, Department of Medicine, Taipei Veterans General Hospital, 201, Section 2, Shi-Pai Road, Taipei 112, Taiwan, ROC. E-mail addresses: jcluo@vghtpe.gov.tw (J.-C. Luo); mitmit1994@gmail.com (M.-C. Hou).

Dr. Yi-Hsiang Haung, Dr. Ming-Chih Hou and Dr. Fa-Yauh Lee, editors at Journal of the Chinese Medical Association, have no roles in the peer review process of or decision to publish this article.

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2021) 84: 354-360.

Received November 8, 2020; accepted January 22, 2021.

doi: 10.1097/JCMA.0000000000000510.

clinics. The upper GI surgery group comprised patients who had early gastric cancer and had undergone curative total gastrectomy with Roux-en-Y esophagojejunostomy (RYEJ) anastomosis more than 7 years before the analysis at Taipei Veterans General Hospital between 2003 and 2007. Overall, the mean time interval between surgery and data collection was 8.75 years. Early gastric cancer is defined by the Japanese Research Society for Gastric Cancer as cancer in which the tumor cells invade only the mucosal and submucosal layers.[8] The lower GI surgery study group comprised patients who had undergone curative right hemicolectomy (RH) for primary nondisseminated CRC for more than 7 years before the analysis at Taipei Veterans General Hospital between 2003 and 2007. Their mean time interval between surgery and data collection was also 8.75 years.

Patients with the following conditions were excluded: (1) age <20 years;, (2) other underlying malignancies; (3) pre- and postoperative chemotherapy or chemoradiotherapy for gastric cancer or CRC; 4) recurrent or uncured gastric cancer or CRC after curative surgery; (5) moderate to severe cardiovascular, pulmonary, hepatic, or renal disease; (6) DM or MS before receiving gastric or colonic surgery or 8.75 years before enrolling in the control group; and (7) patients who had received proton pump inhibitors, histamine-2 receptor antagonists, nonsteroidal anti-inflammatory drugs, antibiotics, or probiotics within 1 month of sample collection.

Subjects matched in terms of age, gender, and time of follow-up without GI tract surgery were also enrolled as the control group. The same exclusion criteria as in the study groups were applied. Comprehensive oral explanations were carried out, and signed informed consent was obtained from all study subjects. Anthropometric measurements were obtained by experienced nursing staff (body mass index [BMI], waist circumference, and blood pressure). This study complies with the standards of the Declaration of Helsinki and current ethical guidelines and has been approved by the hospital's institutional review board (2016-07-008B).

2.2. Stool bacterial genomic DNA extraction and polymerase chain reaction amplification

Fresh stool samples were collected, and bacterial genomic microbial DNA was extracted using a QIAamp DNA Stool Mini Kit (Qiagene, MD, USA) according to the manufacturer's protocols.[4] Briefly, tissue samples (180-220 mg) yielded 5 to 100 μg of genomic DNA for direct use in 16S rRNA gene sequencing. The amount and quality of isolated genomic DNA were determined with a NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE, USA). Genomic DNA was stored at –80°C before 16S rRNA sequencing.

A 1-μL sample of DNA (10 pg-500 ng) was used as a template in a polymerase chain reaction (PCR) reaction for bacteria 16S rRNA variable region V3 to V4. The primer set for the reaction was chosen with 341F_V3_illumina (5′-CCTACGGGNGGCWGCAG-3′) and 805R_V4_illumina (5′-GACTACHVGGGTATCTAATCC -3′).[4] The PCR consisted of an initial denaturation at 94°C for 2 minutes, 30 cycles of 92°C for 20 seconds, 55°C for 30 seconds, and 68°C for 1 minute for amplification, followed by 68°C for 1 minute to finish the replication on all templates and storage at 4°C. Dual-indexes (barcodes) were used for each sample before sequencing, and NGS was performed Illumina MiSeq Desktop Sequencer according to the standard protocol (Illumina Inc., San Diego, CA, USA).

2.3. Sequence processing and statistical analysis

Raw sequencing fastqc files were quality filtered and analyzed using QIIME2 version 2018.06.[9] We used the DADA2 software package wrapped in QIIME2 for dereplicating, denoising, and chimera removal to generate the Amplicon Sequencing Variant (ASV) group.[10] Alpha and beta-diversity analyses were performed using the built-in core analysis pipeline of QIIME2, including the Chao 1 richness estimator, Shannon diversity index (SI), Faith's Phylogenetic Diversity (PD), and UniFrac distance metrics.

The alpha diversity of the groups of patients was compared using the Kruskal-Wallis test. A permutational multivariate analysis of variance test was used to analyze the statistical differences in beta diversity with QIIME2. Principal coordinate analyses were performed based on the unweighted UniFrac distance in QIIME2, and p values <0.05 were considered significant.

To identify the taxonomy compositions, the ASVs were aligned and assigned to each taxonomic level using VSEARCH against Greengenes 13.5 using 99% similarity.[11] To find which taxa were most likely to explain the differences between clinical groupings, the samples' microbiota profiles generated in QIIME2 were reformatted for input into LDA effect size (LEfSe) via the Huttenhower Lab Galaxy Server.[12] This algorithm performs nonparametric statistical testing to determine whether individual taxa differ between clinical groups and ranks differentially abundant taxa by their linear discriminant analysis (LDA) log-score.[13] Bar plots were used to illustrate differentially abundant taxa that were statistically significant using an alpha of 0.05 and exceeded an LDA log-score of at least ±3

All statistical analyses other than the gut microbiota profile analyses were performed using the software SPSS (version 17.0; SPSS Inc., Chicago, IL, USA). Demographic data are expressed as the frequency (percentage) or as the mean ± standard deviation. Continuous variables were compared using a Student's t-test, while categorical data were compared using a chi-square test and Yates' correction or Fisher's exact test as appropriate. A two-sided $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Metabolic status in patients post upper and lower GI surgery

From January 2003 to December 2007, there were 674 patients with gastric cancer who visited the outpatient clinics or were hospitalized our general surgery ward. At the time of enrollment for this study, there were 473 patients who had passed away, 25 patients who were lost follow-up, 13 patients with other malignancy, 8 patients with recurrent gastric cancer, 26 patients with DM or MS, and 18 patients with incomplete clinical or laboratory data when gastric cancer was diagnosed. Furthermore, the excluded patients comprised 27 patients with underlying severe comorbidity, 6 patients who had taken medications mentioned in the exclusion criteria, 20 patients who refused to participate, 16 patients who received subtotal gastrectomy with B-I, 19 patients who received subtotal gastrectomy with B-II, and 13 patients received subtotal gastrectomy with Roux-en-Y anastomosis, only 10 patients with total gastrectomy with RYEJ enrolled for this study.

There were 777 patients with CRC who visited the outpatient clinics or were hospitalized in our colorectal surgery ward from January 2003 to December 2007. At the time of enrollment for this study, there were 513 patients who had passed away, 43 patients who were lost to follow-up, 12 patients with another malignancy, 21 patients with recurrent CRC, 30 patients with DM or MS, and 22 patients with incomplete clinical or laboratory data when CRC was diagnosed. The excluded patients comprised 37 patients with underlying severe comorbidity, 13 patients taking indicated medications, 46 patients who refused to participate, 10 patients who received left hemicolectomy, and 19 patients who received low anterior resection. We enrolled the remaining 11 patients who received RH in this study.

To investigate the long-term effects of upper and lower GI surgery on the metabolic profile and gut microbiota, we analyzed

Table

Anthropometric and laboratory data between patients with total gastrectomy with RYEJ, RH, and controls

	Control subjects n = 32	Patients with RYEJ n = 10	Patients with RH n = 11
Age y/o	64.3 ± 9.3	66.4 ± 8.2	66.1 ± 7.3
Sex (M:F)	11:21	7:3	5:6
Body mass index	24.3 ± 3.7	20.8 ± 1.8*	23.7 ± 3.3
Waist, cm	86.0 ± 10.2	78.9 ± 10.1	86.0 ± 8.8
Systolic BP, mm Hg	122 ± 17	123 ± 22	124 ± 19
Diastolic BP, mm Hg	77 ± 11	76 ± 13	78 ± 12
HDL-cholesterol, mg/dL	53 ± 13	52 ± 7	57 ± 15
Total cholesterol, mg/dL	197 ± 27	169 ± 27*	189 ± 22
Triglyceride, mg/dL	133 ± 78	78 ± 28*	100 ± 42
Serum glucose, mg/dL	96 ± 17	97 ± 13	106 ± 22
HbA1c	5.8 ± 0.7	5.8 ± 0.8	6.0 ± 0.5
Metabolic syndrome (+/–)	13:19	0: 10*	5: 6

BP = blood pressure; HbA1c = hemoglobin A1c; HDL = high density lipoprotein; RH = right hemicolectomy; RYEJ = Roux-en-Y esophagojejunostomy.
*$p < 0.05$ when compared with the control group.

anthropometric, laboratory, and fecal microbiome data from 10 patients who had undergone total gastrectomy with RYEJ, 11 patients with RH, and 32 controls who were matched in terms of age, sex, and follow-up time. Compared with the control group, patients who had undergone RYEJ had lower BMI, total cholesterol levels, serum TGs, and MS occurrence ($p < 0.05$) (Table). Compared with the control group, patients who received RH only had a trend of higher serum fasting glucose and higher hemoglobin A1c (HbA1c) ($p < 0.10$) (Table).

3.2. Statistical summaries of sequencing results

After 16S rRNA gene sequencing and quality filtering, 2.6 million reads from a total of 3.4 million pair-end reads were obtained, which corresponded to a mean of 65 ± 19 thousand reads per sample.

3.3. Richness and diversity of gut microbiota

The gut microbiota richness was estimated using Chao 1 (Fig. 1A). Compared with the control group, both the RYEJ and RH groups showed no difference in bacterial richness at the genera level (Chao 1, $p = 0.40$). SI and Faith's PD were used to evaluate the ecological diversity of microbiota from each sample (Fig. 1B, C). Compared with the control group, both the RYEJ and RH groups showed no difference in SI at the genera level (SI, $p = 0.39$). However, compared with the control group, the RH group showed a lower Faith's PD index ($p = 0.001$), whereas the RYEJ group showed no difference in Faith's PD at the genera level (Faith's PD, $p = 0.39$)

3.4. Long-term effects on gut microbiota composition

We visualized the changes in overall gut microbial genera composition using a principal component analysis (PCA) of the log-transformed relative abundances (Fig. 2). The results showed a clear separation between the control group and the RYEJ and RH groups. Compared with the control group, PCA revealed significant differences in bacterial genera abundance in the RYEJ group ($p < 0.001$, Monte-Carlo simulation; Fig. 2) and RH group ($p < 0.001$, Monte-Carlo simulation; Fig. 2).

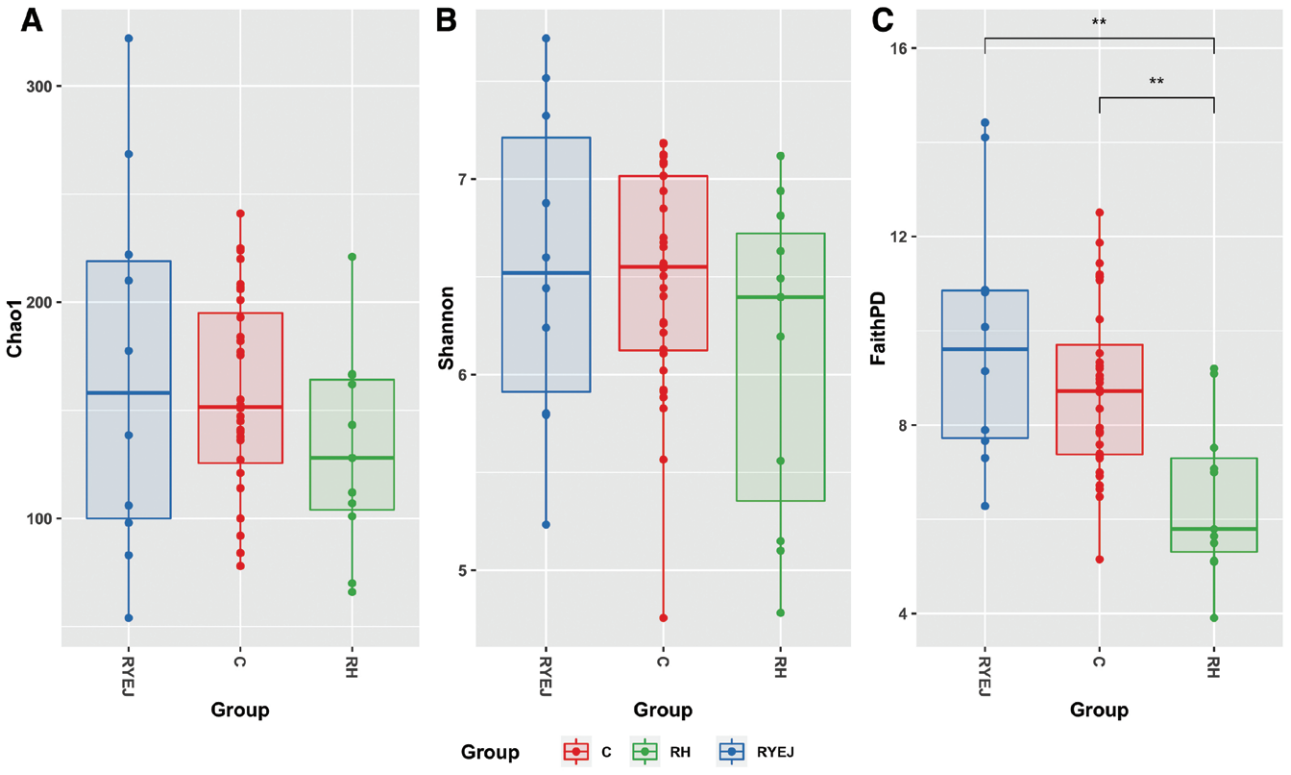

Fig. 1 Richness and diversity of gut microbiota in the total gastrectomy with Roux-en-Y esophagojejunostomy (RYEJ) anastomosis, right hemicolectomy (RH), and control groups. A, No significant difference among RYEJ, RH, and control group in Chao1. B, No significant difference among RYEJ, RH, and control group in Shannon diversity index. C, The RH group with lower bacterial diversity, as estimated by the Faith's Phylogenetic Diversity (Faith's PD) when compared with the control group and RYEJ group, respectively ($p < 0.05$). The boxes (containing 50% of all values) show the median (horizontal line across the middle of the box) and the interquartile range, whereas the spots represent the 10th and the 90th percentiles.

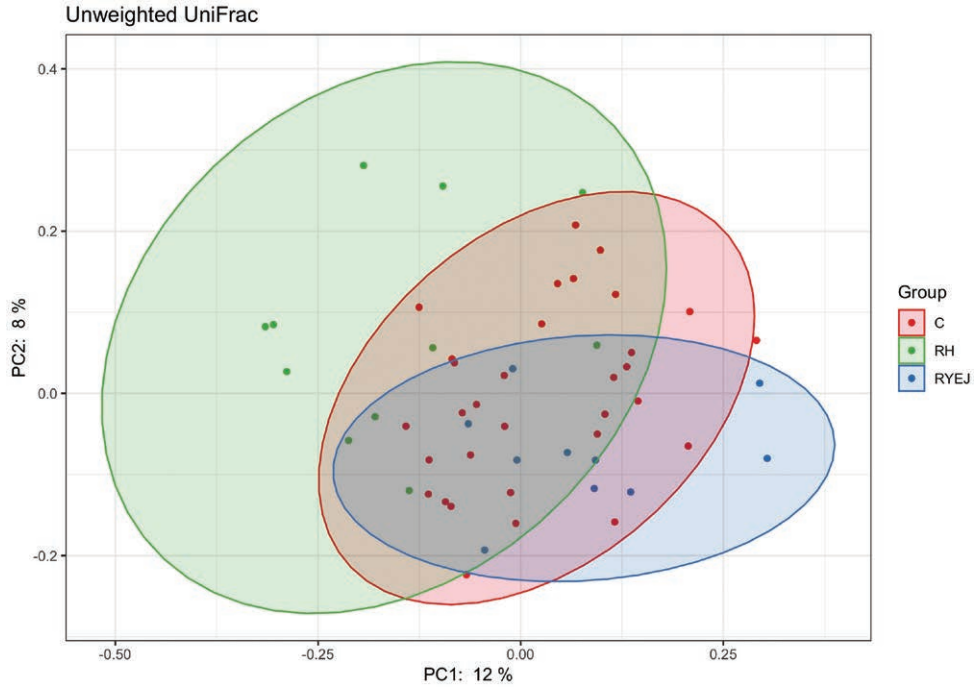

Fig. 2 Principal component (PC) analysis of bacterial genera abundance. PC analysis score plot based on unweighted UniFrac metrics showed a clear separation between control group, and those after total gastrectomy with Roux-en-Y esophagojejunostomy (RYEJ) anastomosis or right hemicolectomy (RH). Compared with the control (C) group, RYEJ group and RH group revealed significant differences in bacterial genera abundance, respectively (p all <0.001, Monte-Carlo simulation).

The four major phyla in the microbiota of the RYEJ, RH, and control groups were Bacteroidetes, Firmicutes, Proteobacteria, and Fusobacteria (Fig. 3A). RYEJ patients had a higher proportion of Proteobacteria than the controls (17.8% vs 10.1%, $p < 0.05$). RH patients had a lower proportion of Firmicutes (23.3% vs 39.4%, $p < 0.05$) and a higher proportion of Fusobacteria than the controls (7% vs 5%, $p < 0.05$).

The dominant class represented ≥0.2% of the obtained gut microbiota sequences (Fig. 3B). Compared with the control group, the class Gammaproteobacteria (belonging to Proteobacteria) was significantly higher in the RYEJ group (15.2% vs 7%, $p < 0.05$). The bacterial class Fusobacteria (Fusobacteria) was significantly higher in the RH group than in the control group (7% vs 5%, $p < 0.05$). Moreover, compared with the control group, the

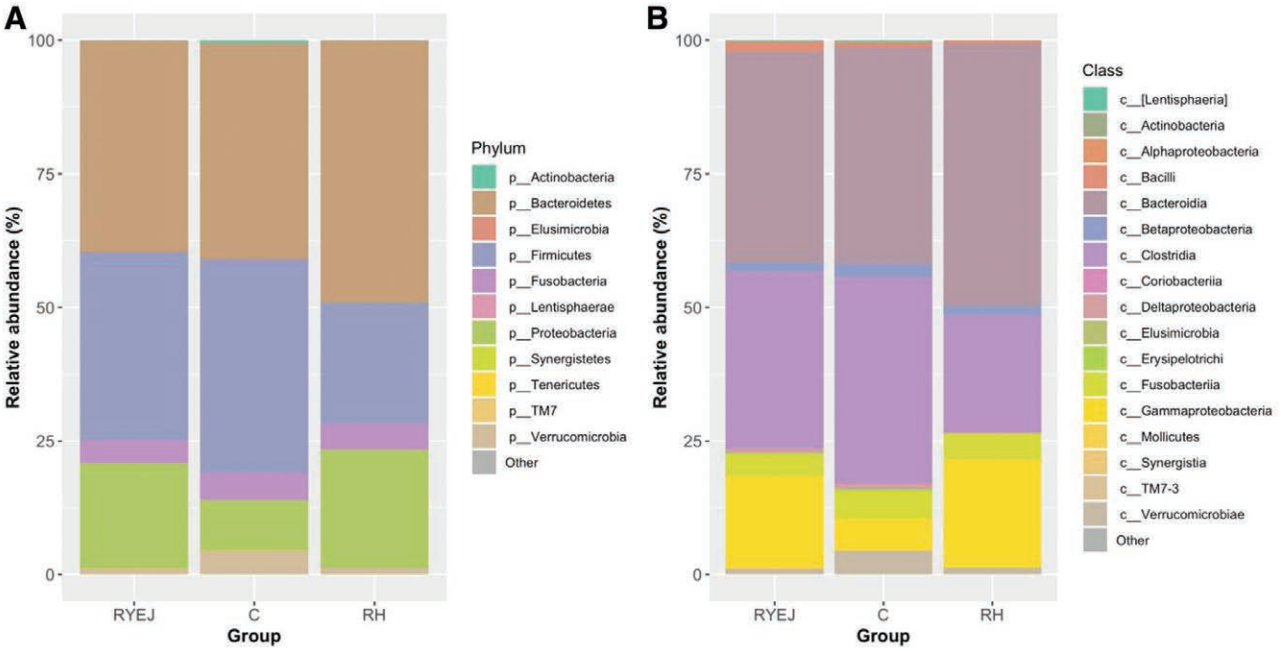

Fig. 3 Relative abundances of phylum and classes across three groups. A, Relative abundances of phylum across total gastrectomy with Roux-en-Y esophagojejunostomy (RYEJ) anastomosis, right hemicolectomy (RH), and control (C) groups. B, Relative abundances of classes across RYEJ, RH, and C groups.

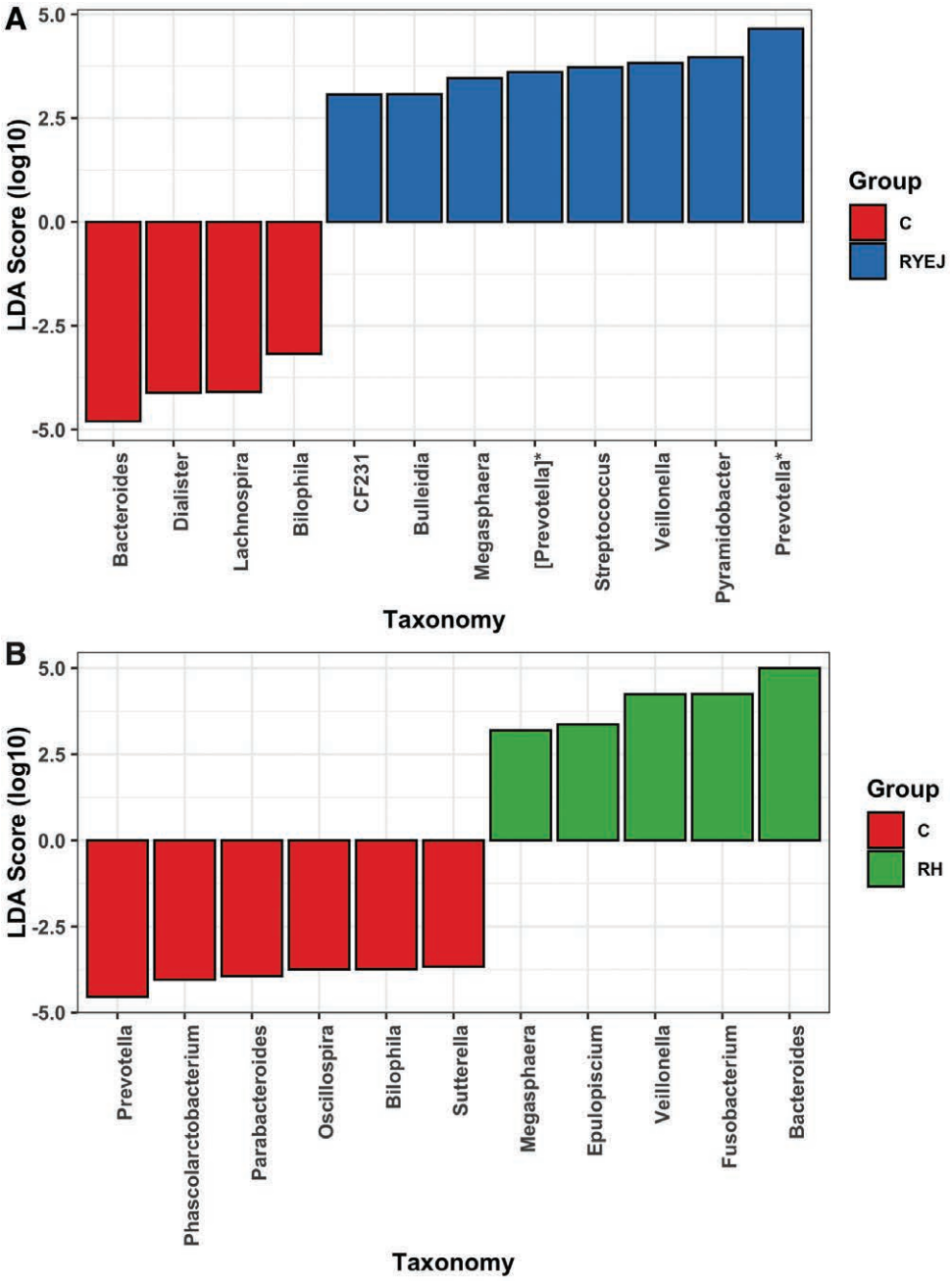

Fig. 4 Known genera abundance reported by LDA effect size (LEfSe) in the bacterial community. A, Known genera reported by LEfSe in the bacterial community, comparison between total gastrectomy with Roux-en-Y esophagojejunostomy (RYEJ) anastomosis and control (C) groups. B, Known genera reported by LEfSe in the bacterial community, comparison between right hemicolectomy (RH) and C.

bacterial class Clostridia (Firmicutes) was significantly lower in the RH group (23% vs 38%, $p < 0.05$). The bacterial class Deltaproteobacteria (Proteobacteria) was significantly lower in the RH group than in the control group (0.2% vs 0.8%, $p < 0.05$).

The LEfSe analysis identified a total of 11 known genera, which were differentially abundant between the RYEJ and control groups (Fig. 4A). Among the seven differentially abundant genera in the RYEJ group, *Prevotella* and *Pyramidobacter* represented the top two genera. There were 11 known genera in total, which were differentially abundant between the RH and control groups (Fig. 4B). Among the five genera known to be more abundant in the RH group, *Bacteroides* and *Fusobacterium* represented the top two genera. Interestingly, the ratio of *Prevotella* to *Bacteroides* (P/B) was significantly higher in the RYEJ group than in the control group ($p < 0.05$) (Fig. 5). On the other hand, the P/B ratio was significantly lower in the RH group than in the control group ($p < 0.05$) (Fig. 5).

4. DISCUSSION

To the best of our knowledge, no previous studies have explored the long-term metabolic changes (at least 8.75 years on average) in patients after upper and lower GI tract surgery. In our study, early gastric cancer patients who had undergone total gastrectomy with RYEJ were found to have benefits on metabolic effects, including lower BMI, lower serum total cholesterol, and TGs when

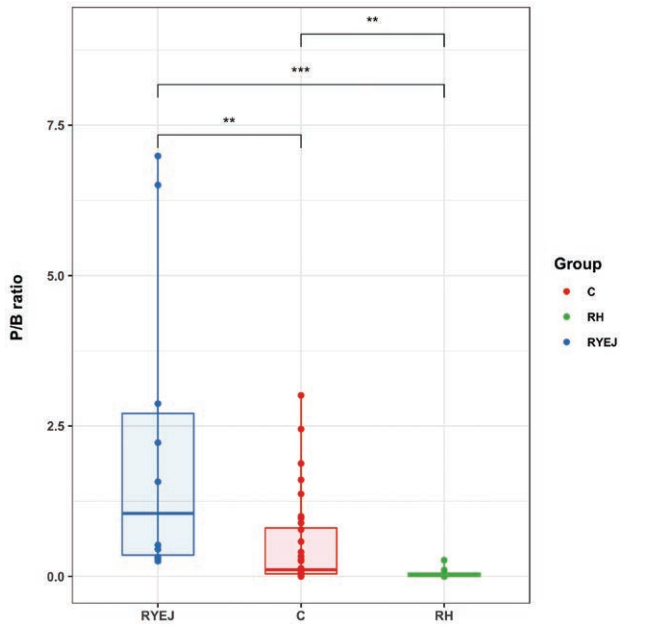

Fig. 5 *Prevotella* to *Bacteroides* (P/B) ratio in the Roux-en-Y esophagojejunostomy (RYEJ) anastomosis, right hemicolectomy (RH), and control (C) groups. At the genus level, P/B ratio showed significantly higher in the total gastrectomy with the RYEJ group and significantly lower in the RH group when compared with the C group. The boxes (containing 50% of all values) show the median (horizontal line across the middle of the box) and the interquartile range, whereas the spots represent the 10th and the 90th percentiles.

compared with the controls. In addition, RYEJ patients had significantly lower MS occurrence when compared with the controls.

The results were consistent with a recent systemic review and meta-analysis conducted by Sheng et al,[2] which revealed evidence that bariatric surgery provides benefits for the long-term (≥5 years) outcomes of diabetes remission, microvascular events, and macrovascular events among type 2 DM patients compared with no surgical treatment. Although MS occurrence was not significantly higher in CRC patients after partial colectomy with RH, we found metabolic changes including higher fasting serum glucose and higher serum HbA1c. The results support the evidence that *concomitant hyperglycemia and* hyperinsulinemia (the *key feature* of MS) are the most consistent finding following total colectomy.[5,6,14] Moreover, in agreement with a previous study, our results also imply that the proximal colon may *play an* important role in the regulation of glucose metabolism.[15]

This is the first study in which 16S rRNA amplicon deep sequencing was applied to investigate the effect of microbiota status in patients after upper and lower GI surgery. In parallel with the metabolic changes, including higher serum fasting glucose and higher HbA1c, the gut microbial diversity was significantly lower in the RH group. Furthermore, in parallel with the metabolic profile improvements, gut microbial diversity was significantly higher in the RYEJ group than in the RH group.

The relative abundances of the phylum Proteobacteria and the class Gammaproteobacteria were significantly higher in the RYEJ group. The changes in gut microbiota compositions were consistent with the changes induced by bariatric bypass, such as Roux-en-Y gastric bypass (RYGB).[16,17] Despite the different indications and gastric surgical modalities for different underlying diseases, our results support the concept that local and global metabolic effects arise after a shift toward Gammaproteobacteria as a major component of the microbiota after RYGB.[18]

The relative abundances of the phylum Firmicutes and the class Clostridia were significantly lower in the RH group. This result is consistent with Larsen's study, which showed that the proportions of Firmicutes and Clostridia were significantly reduced in the type II diabetic group compared with the control group.[19] Fusobacteria are aerobic bacteria distributed in the oral microbiota and can cause periodontal disease.[20]

Minty et al[21] showed that dysbiosis of oral microbiota components, like the genera *Fusobacterium*, *Porphyromonas*, or *Prevotella*, was associated with metabolic disorders. In the present article, the phylum Fusobacteria, class Fusobacteria, and genus *Fusobacterium* were significantly higher in subjects with RH. Thus, we speculated that the Fusobacteria may play an important role in metabolic control. This is consistent with Sakalauskiene's study, which showed that the presence of *Fusobacterium nucleatum* was more frequent in a diabetic group than in a healthy group.[22]

At the genus level, the relative abundances of *Streptococcus* and *Veillonella* were significantly higher in the RYEJ group. In agreement with a previous study, the possible mechanism might be the increase of pH after total gastrectomy, which could make the gastric barrier less stringent for oral microbiota such as *Streptococcus* spp. and a few *Veillonella* spp.[23] The LEfSe identified *Prevotella* as the most abundant genus in the RYEJ group and *Bacteroides* as the most abundant one in the RH group. Interestingly, the P/B ratio was significantly higher in the RYEJ group and significantly lower in the RH group than in the control group. The P/B ratio is an important biomarker associated with dietary weight loss.[24]

It is known that the intestinal microbial communities are resilient to change through dietary interventions[25–27] unless there are extreme changes such as anatomical rearrangements.[28] Thus, we speculated that the P/B ratio might be altered after upper and lower GI surgery and further resulted in the subsequent metabolic changes in our study. Kovatcheva-Datchary et al[29] also found that the improvements in post-prandial blood glucose and insulin after dietary fiber intake were positively associated with the abundance of *Prevotella*. Therefore, future diet-based intervention studies could be done with the aim of changing gut microbiota characteristics such as the P/B ratio to gain health benefits in glucose control after RH.

This study has several limitations. First, the sample size was limited. Second, in the cross-sectional study, we did not have metabolic and microbiota data from before and after upper GI/LGI surgery for comparison. As a result, we cannot clearly distinguish the status of metabolic and microbiota profiles caused by upper GI/LGI surgery. However, our team is currently conducting a prospective longitudinal study with data from before and after upper GI/LGI surgery associated with gut microbiota, genomic, proteomic, and metabolomic data. Third, we did not have data about gut hormones such as glucagon-like peptide 1,[30] metatranscriptomic data, and metabolomic short chain fatty acid measurements,[31] which may play important roles in the changes of metabolic profiles in patients who have undergone upper GI/LGI surgery. Fourth, the gut microbial community would be sensitive to environmental factors including lifestyles and diet. Although our study subjects were matched in terms of age, gender, and follow-up time, some phenotype differences were still unobservable. Fifth, we did not correlate the composition of the gut microbiota with the clinical parameters after upper GI/LGI surgery. As a result, it is still infeasible at this moment to draw any conclusions about the causal relationships of gut microbiota and the metabolic profiles after upper and lower GI surgery. Finally, patients with recurrent gastric cancer and CRC usually receive further therapy, such as chemotherapy, which can change the gut microbiota. The aim of our study was to compare long-term effects of metabolic profiles and microbiota status after

upper GI surgery and lower GI surgery to controls rather than patients with or without tumor recurrence or between stable and deteriorated metabolic profiles.

In conclusion, early gastric cancer patients had a lower occurrence of MS after total gastrectomy with RYEJ than the controls during long-term follow-up. Early CRC patients who underwent RH were associated with higher serum fasting glucose than the controls during long-term follow-up. In parallel with the metabolic differences, gut microbial diversity also significantly decreased after partial colectomy with RH. Future direct experimental studies (e.g., animal model studies) or fecal microbiota transplantation studies are needed to show the causal effect of microbiota on the metabolic regulation and expand our knowledge on the different roles of proximal and distal gut microbiota in metabolic control.

ACKNOWLEDGMENTS

This study was funded in part by the grants from Taipei Veterans General Hospital (VN 108-04, V108D41-001-MY3-2) and Ministry of Science and Technology of Taiwan (MOST 108-2314-B-010-050, MOST 109-2314-B-075-034) to JCL. But they did not involve in the conduct of the research, study design, data collection, analysis and interpretation of data, writing the manuscript, and in the decision to submit the article for publication. The authors express their gratitude to Mrs. Pui-Ching Lee (Department of Medicine, Taipei Veterans General Hospital) for her help in figure editing.

REFERENCES

1. Fournel A, Marlin A, Abot A, Pasquio C, Cirillo C, Cani PD, et al. Glucosensing in the gastrointestinal tract: impact on glucose metabolism. *Am J Physiol Gastrointest Liver Physiol* 2016;**310**:G645–58.
2. Sheng B, Truong K, Spitler H, Zhang L, Tong X, Chen L. The long-term effects of bariatric surgery on type 2 diabetes remission, microvascular and macrovascular complications, and mortality: a systematic review and meta-analysis. *Obes Surg* 2017;**27**:2724–32.
3. Tremaroli V, Karlsson F, Werling M, Ståhlman M, Kovatcheva-Datchary P, Olbers T, et al. Roux-en-Y gastric bypass and vertical banded gastroplasty induce long-term changes on the human gut microbiome contributing to fat mass regulation. *Cell Metab* 2015;**22**:228–38.
4. Lin XH, Huang KH, Chuang WH, Luo JC, Lin CC, Ting PH, et al. The long term effect of metabolic profile and microbiota status in early gastric cancer patients after subtotal gastrectomy. *PLoS One* 2018;**13**:e0206930.
5. Palnaes Hansen C, Andreasen JJ, Holst JJ. The release of gastric inhibitory peptide, glucagon-like peptide-I, and insulin after oral glucose test in colectomized subjects. *Scand J Gastroenterol* 1997;**32**:473–7.
6. Robertson MD, Bickerton AS, Dennis AL, Vidal H, Jewell DP, Frayn KN. Enhanced metabolic cycling in subjects after colonic resection for ulcerative colitis. *J Clin Endocrinol Metab* 2005;**90**:2747–54.
7. Ussar S, Griffin NW, Bezy O, Fujisaka S, Vienberg S, Softic S, et al. Interactions between gut microbiota, host genetics and diet modulate the predisposition to obesity and metabolic syndrome. *Cell Metab* 2015;**22**:516–30.
8. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma - 2nd. english edition. *Gastric Cancer* 1998;**1**:10–24.
9. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010;**7**:335–6.
10. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from illumina amplicon data. *Nat Methods* 2016;**13**:581–3.
11. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 2016;**4**:e2584.
12. Goecks J, Nekrutenko A, Taylor J; Galaxy Team. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol* 2010;**11**:R86.
13. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;**12**:R60.
14. Medhus AW, Bjørnland K, Emblem R, Haug E, Husebye E. Are patients with Hirschsprung's disease prediabetic? *Metabolism* 2001;**50**:1389–90.
15. Lin XH, Jiang JK, Luo JC, Lin CC, Ting PH, Yang UC, et al. The long term microbiota and metabolic status in patients with colorectal cancer after curative colon surgery. *PLoS One* 2019;**14**:e0218436.
16. Furet JP, Kong LC, Tap J, Poitou C, Basdevant A, Bouillot JL, et al. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes* 2010;**59**:3049–57.
17. Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, et al. Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci U S A* 2009;**106**:2365–70.
18. Li JV, Ashrafian H, Bueter M, Kinross J, Sands C, le Roux CW, et al. Metabolic surgery profoundly influences gut microbial-host metabolic cross-talk. *Gut* 2011;**60**:1214–23.
19. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 2010;**5**:e9085.
20. Matsha TE, Prince Y, Davids S, Chikte U, Erasmus RT, Kengne AP, et al. Oral microbiome signatures in diabetes mellitus and periodontal disease. *J Dent Res* 2020;**99**:658–65.
21. Minty M, Canceil T, Serino M, Burcelin R, Tercé F, Blasco-Baque V. Oral microbiota-induced periodontitis: a new risk factor of metabolic diseases. *Rev Endocr Metab Disord* 2019;**20**:449–59.
22. Sakalauskiene J, Kubilius R, Gleiznys A, Vitkauskiene A, Ivanauskiene E, Šaferis V. Relationship of clinical and microbiological variables in patients with type 1 diabetes mellitus and periodontitis. *Med Sci Monit* 2014;**20**:1871–7.
23. Mashima I, Nakazawa F. The interaction between Streptococcus spp. and Veillonella tobetsuensis in the early stages of oral biofilm formation. *J Bacteriol* 2015;**197**:2104–11.
24. Hjorth MF, Blædel T, Bendtsen LQ, Lorenzen JK, Holm JB, Kiilerich P, et al. Prevotella-to-Bacteroides ratio predicts body weight and fat loss success on 24-week diets varying in macronutrient composition and dietary fiber: results from a post-hoc analysis. *Int J Obes (Lond)* 2019;**43**:149–57.
25. Lim MY, Rho M, Song YM, Lee K, Sung J, Ko G. Stability of gut enterotypes in Korean monozygotic twins and their association with biomarkers and diet. *Sci Rep* 2014;**4**:7348.
26. Roager HM, Licht TR, Poulsen SK, Larsen TM, Bahl MI. Microbial enterotypes, inferred by the Prevotella-to-Bacteroides ratio, remained stable during a 6-month randomized controlled diet intervention with the new Nordic diet. *Appl Environ Microbiol* 2014;**80**:1142–9.
27. Hjorth MF, Roager HM, Larsen TM, Poulsen SK, Licht TR, Bahl MI, et al. Pre-treatment microbial Prevotella-to-Bacteroides ratio, determines body fat loss success during a 6-month randomized controlled diet intervention. *Int J Obes (Lond)* 2018;**42**:580–3.
28. Guyton K, Alverdy JC. The gut microbiota and gastrointestinal surgery. *Nat Rev Gastroenterol Hepatol* 2017;**14**:43–54.
29. Kovatcheva-Datchary P, Nilsson A, Akrami R, Lee YS, De Vadder F, Arora T, et al. Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of Prevotella. *Cell Metab* 2015;**22**:971–82.
30. Robertson MD, Livesey G, Hampton SM, Mathers JC. Evidence for altered control of glucose disposal after total colectomy. *Br J Nutr* 2000;**84**:813–9.
31. Scheppach W, Pomare EW, Elia M, Cummings JH. The contribution of the large intestine to blood acetate in man. *Clin Sci (Lond)* 1991;**80**:177–82.