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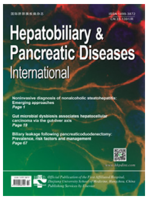

Original Article/Liver

Relationship between intestinal microbial dysbiosis and primary liver cancer

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ABSTRACT

Background: Intestinal microbial dysbiosis is involved in liver disease pathogenesis. However, its role in primary liver cancer (PLC), particularly in hepatocarcinogenesis remains unclear. The present study aimed to study the changes in intestinal flora at various stages of PLC and clarify the relationship between intestinal microbes and PLC.

Methods: Twenty-four patients with PLC (PLC group), 24 patients with liver cirrhosis (LC group), and 23 healthy control individuals (HC group) were enrolled from October 2016 to October 2017. Stool specimens of the participants were collected and the genomic DNA of fecal bacteria was isolated. High-throughput pyrosequencing of 16S rDNA was used to identify differences in gut bacterial diversity among HC, LC, and PLC groups. We also analyzed the relationship between clinical factors and intestinal microorganisms in LC and PLC groups.

Results: Diversity of Firmicutes tended to decrease from the HC to LC and PLC groups at the phylum level. Among species, *Enterobacter ludwigii* displayed an increasing trend in the PLC group, wherein the relative abundance of *Enterobacter ludwigii* in the PLC group was 100 times greater than that in the HC and LC groups. The ratio of Firmicutes/Bacteroidetes was significantly decreased with the disease progression. In addition, the linear discriminant analysis effect size method indicated that Clostridia were predominant in the gut microbiota of the HC group, whereas Enterococcaceae, Lactobacillales, Bacilli and Gammaproteobacteria may be used as diagnostic markers of PLC. Redundancy analysis showed a correlation between intestinal microbial diversity and clinical factors AST, ALT, and AFP. *Veillonella* showed a significant positive correlation with AFP in the PLC group, whereas *Subdoligranulum* showed a negative correlation with AFP.

Conclusions: This study indicates that dysbiosis of the gut microbiota might be involved in PLC development and progression.

Introduction

Primary liver cancer (PLC) is a serious threat to human health. It is estimated that there are 26,000 new cases each year worldwide and it is the third malignant tumor in China [1].

Gut microbes are a community of various bacteria colonizing the intestine. The number of bacteria harbored by the human body is estimated to be 100 times of human body cells [2]. Increasing evidence indicates that an altered human gut microbiome is associated with liver cirrhosis (LC) and its complications [3–5]. Many factors could affect the composition of intestinal microflora including antibiotics, alcohol consumption, diet, race, and geographical location [6].

The intestinal microbial community is currently considered an independent organ that regulates gut metabolism. The alteration of

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this community probably affects the prognosis of LC patients [7]. In recent years, modification of intestinal flora has been used as an adjuvant therapy to reduce bacterial shift and prevent liver cancer [8]. In the intestinal environment, microbial metabolites play a crucial role in liver disease pathogenesis [9]. A previous study has found that in adult mice, the hepatic immunity activated by establishment of intestinal microbiota could accelerate the clearance of HBV [10].

More than 700 species of bacteria have been found in the gut, of which approximately 35% have not been cultured [11]. The gut microbiota interacts with its host, thus contributing to diseases like inflammatory bowel disease [12], pancreatic diseases [13], obesity [14], and atherosclerosis [15]. Oral bacteria were found to be able to invade the gut of LC patients, causing the overgrowth of LC-associated small-intestinal bacteria [4]. However, the relationship between intestinal microbial dysbiosis and PLC has not been reported yet. Our study compared the differences in the intestinal microbial composition in patients with PLC, patients with LC, and healthy controls (HC) individuals.

Methods

Sample collection

A total of 71 individuals participated in this study. Twenty-four patients with primary liver cancer (PLC group) were admitted to the First Hospital of Lanzhou University from October 2016 to October 2017. The average age was 51.8 years old (range: 32–65 years). The patients in the PLC group were confirmed by pathological diagnosis, including patients with pathologically confirmed hepatocellular carcinoma (10 cases), cholangiocarcinoma (8 cases), and mixed type liver cancer (6 cases). Patients with LC (24 cases, LC group), average age 50.2 years old (range: 32–65 years), were also enrolled. All patients in the LC group were examined by ultrasound, CT and tumor markers to rule out PLC. The LC group included post-hepatitis cirrhosis (10 cases), alcoholic cirrhosis (6 cases), autoimmune cirrhosis (4 cases), and other disorders (4 cases). These diagnoses were based on the ESMO-ESDO Clinical Practice Guidelines [16]. Furthermore, 23 patients who underwent routine physical examination in our hospital were enrolled as healthy control (HC group), with an average age of 50.4 years old (range: 32–65 years). All the participating individuals provided written informed consent. The inclusion criteria of the HC group were as follows: (1) no serious digestive system disease; (2) no recent digestive system discomfort or disease; (3) no other diseases or infections within two months; (4) no recent medications; (5) not pregnant or lactating, nor menstruating.

Microbial DNA extraction and sequencing

Stool samples were obtained in the morning before breakfast using a sterile stool case. The microbial genomic DNA was isolated by QIAamp DNA micro kit (QIAGEN, Hilden, Germany) as reported previously [17–19]. Absorbance at 260 nm and 280 nm was measured using an ultraviolet spectrophotometer (DU1 640, Beckman Instruments, Inc., CA, USA) to evaluate the final amount and quality of DNA [20]. The 16S rRNA V3-V4 hypervariable region was sequenced on the Illumina HiSeq platform from Novogene (Beijing, China). The PCR conditions were as follows: denaturation at 98 °C for 1 min; 25 cycles of denaturation at 98 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 60 s; and extension at 72 °C for 10 min [21]. The PCR product was then mixed and purified using the Gene Extraction Kit (Thermo Scientific, Massachusetts, USA).

Bioinformatics analysis

Sequencing and analysis were carried out by Novogene. A sequencing library was constructed using Illumina's DNA Library Kit, which was evaluated on the Qubit 2.0 Fluorometer (Thermo Scientific). Finally, the library was sequenced on the Illumina MiSeq platform. Sequence analysis was performed using UPARSE software. Sequences with 97% similarity were assigned to the same service classification unit operational taxonomic unit (OTU). We selected the representative sequence of each OTU and used the Ribosomal Database Project (RDP) classifier to annotate the classification information for each representative sequence. The super index and Shannon index were calculated based on the OTU results. Spearman correlation coefficients (SCC) were calculated for each pair of core genera. Pairwise differential features of the three groups were plotted using the linear discriminant analysis (LDA) effect size (LEfSe) method [22], and the threshold logarithmic LDA score for discriminative features was 2.0. Redundancy analysis (RDA) and Spearman relationship analysis were conducted to better understand the relationship between gut microbial diversity and its clinical factors in the LC and PLC groups.

Statistical analysis

Continuous variables were summarized as means and standard deviations and compared with one-way ANOVA test. Discrete variables were expressed as numbers and percentages and compared with Chi-square test. All analyses were performed using SPSS software (Version 22.0, SPSS Inc., Chicago, IL, USA). A P value of <0.05 was considered statistically significant.

Results

Clinical characteristics of the three groups

The HC, LC, and PLC groups did not show significant differences in baseline characteristics, including weight and height (Table 1) after adjusting for smoking and drinking. However, the PLC group showed significantly higher levels of AST ($P < 0.001$), ALT ($P < 0.001$) and AFP ($P < 0.001$) than the HC and LC groups.

Changes in gut microbiota in PLC patients

The 16S rDNA sequencing results showed a decreasing tendency of bacterial diversity in gut microbiota from the HC group to the advanced LC and PLC groups. After quality trimming, 6 423 779 high-quality reads were obtained. These results are summarized in Table 2.

We finally detected 1576 OTUs from all the samples, representing all phenotypes present among gut microbes. Good's coverage was high, indicating that the sequencing depth was sufficient to investigate the gut microbiota of all samples. The Shannon and Chao indices of alpha diversity between the HC, LC and PLC groups have no significant differences ($P > 0.05$) (Fig. 1A and B). The results of the OTU analysis showed that the rank-abundance curve of bacterial communities in the HC, LC, and PLC patients presented a similar pattern. In addition, a Venn diagram representing three sets of overlapping OTU data showed that the total number of OTUs in all samples is only 655 (Fig. 1C).

Gut microbiota composition associated with PLC

The majority of sequences were arranged in six bacterial phyla (i.e., Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia), whereas sequences representative of Acidobacteria, Euryarchaeota, Gemmatimonadetes,

Table 1
Clinical characteristics.

Characteristics	HC group ($n = 23$)	LC group ($n = 24$)	PLC group ($n = 24$)	*P* value*
Age (yr)	50.4 ± 10.6	50.2 ± 11.9	51.8 ± 10.9	0.908
Sex (male/female)	18/5	17/7	18/6	0.549
Current smoker (%)	11 (48%)	12 (50%)	14 (58%)	0.093
Alcohol drinker (%)	8 (35%)	10 (42%)	12 (50%)	0.072
BMI (kg/m^2)	24.5 ± 0.4	24.1 ± 0.7	24.3 ± 0.9	0.901
ALT (U/L)	8.4 ± 2.7	40.8 ± 18.3	90.4 ± 10.1	**<0.001**
AST (U/L)	11.9 ± 3.5	61.4 ± 21.6	137.9 ± 23.6	**<0.001**
AFP (IU/mL)	4.7 ± 1.3	67.8 ± 13.5	1023.3 ± 42.9	**<0.001**

[*] Adjusting for smoking and drinking. ALT: alanine aminotransferase; AST: aspartate aminotransferase; AFP: alpha fetoprotein.

Table 2
Diversity estimation of 16S rRNA from the pyrosequencing analysis.

Group	No. of reads	No. of OTUs[a]	Good's[b]	Richness estimator	Diversity index
				Chao	Shannon
HC	4 254 750**	558	0.9996	362	5.116
LC	2 169 029	526	0.9998	398	5.114
PLC	2 163 919	492	0.9998	404	4.815

[a] The operational taxonomic units (OTUs) were defined at the 97% similarity level.

[b] The coverage percentage (Good's), richness estimators Chao and diversity indices Shannon were calculated using Good's method and the mothur program, respectively. HC: healthy control; LC: liver cirrhosis; PLC: primary liver cancer.

[**] indicates a significant *P* value <0.01.

Tenericutes, and Synergistetes were also identified but were at lower proportions.

The most abundant phylum was Firmicutes, followed by Bacteroidetes, and Proteobacteria (Fig. 2A). Firmicutes were found to be significantly decreased in the PLC group. The HC, LC, and PLC groups were also significantly different with respect to the relative abundance of family, genus, and species of their microbiota (Fig. 2B–D). The most abundant family in the total samples belonged to Prevotellaceae and Bacteroidaceae. In particular, Bacteroidaceae was significantly increased in LC patients compared with HC and PLC groups, and was decreased in PLC patients compared with LC patients. Percentage of Prevotellaceae in LC and PLC patients was significantly lower compared with HC (Fig. 2B). At the genus level, *Enterobacter* and *Escherichia-Shigella* were most significantly represented in the PLC group than in the HC and LC groups (Fig. 2C). Finally, among species, *Escherichia coli* displayed an increasing trend from HC to PLC patients. In addition, we found a very interesting result that the relative abundance of *Enterobacter ludwigii* was the highest in the PLC group and was 100 times greater than that in the HC and LC groups, whereas *Lactococcus* was decreased in the PLC group than that in the HC group (Fig. 2D).

The Firmicutes/Bacteroidetes ratios in the HC, LC, and PLC samples were significantly different (Fig. 3A), with lower ratios in the PLC group compared with the HC group ($P < 0.05$). The heatmap in Fig. 3B shows the correlation between samples and abundance of the phyla represented in the microbiota samples.

The composition of the gut microbiota in the three groups was compared using the LEfSe method. Fig. 4A shows the structure of the intestinal microbiota and the major bacteria in the three groups. Changes in the composition of PLC gut microbiota were also explored using the Mann–Whitney *U* test at different taxon levels. LEfSe indicated that Clostridia were the predominant contributors to the gut microbiota of HC, whereas Enterococcaceae, Lactobacillales, Bacilli, and Gammaproteobacteria were mostly contributing to the dysbiosis of gut microbiota in the PLC group (Fig. 4A). Overall, Fig. 4 shows the taxon level differences represented in the gut bacterial communities of the HC, LC, and PLC groups. These phylotypes could be used as microbiological markers at phylogenetic levels to discriminate HC, and patients with LC and PLC. Specifically, at the phylum level, only Porphyromonadaceae members were found at a significantly higher proportion in PLC patients.

The gut microbial composition of the PLC group was correlated with AFP, AST, and ALT (Fig. 5A). *Veillonella* had a significant positive correlation with AFP, whereas *Subdoligranulum* had a highly significant negative correlation with AFP in the PLC group (Fig. 5B).

Discussion

To study gut microbiota in PLC, we first studied the changes in the characteristics of intestinal flora at various stages of PLC, and found differential intestinal microbes and clarified the relationship between intestinal microbes and PLC, including 24 PLC patients, 24 LC patients, and 23 healthy individuals. We observed that *Enterobacter ludwigii* was highly represented in PLC patients. An earlier study has reported that *Enterobacter ludwigii* could grow well in a solution containing high concentrations of cadmium and had a good Cd^{2+} removal rate [23]. Results of our previous study indicated that cadmium stress might play an important role in PLC development [24]. We thus speculated that the high abundance of *Enterobacter ludwigii* in PLC samples is related to the accumulation of excessive cadmium in PLC patients, which needs further investigation.

Increasing evidence has shown that alteration in the human gut microbiome is associated with liver disease [4,5] and its complications [3]. However, the cause has not been clarified yet. The liver interacts directly with gut through hepatic portal and bile secretion systems [25–28]. Thus, changes in the gut microbiota are important for liver diseases.

The changes in the oral microbiota of patients with chronic hepatitis B (CHB) and LC are useful to determine the causative bacteria [29]. However, changes occurring in the gut microbiota of patients with LC and PLC remain partially unclear. In this study, we analyzed the differences in the gut microbiota of PLC, LC, and HC groups. Overall, our results indicated a decreased bacterial

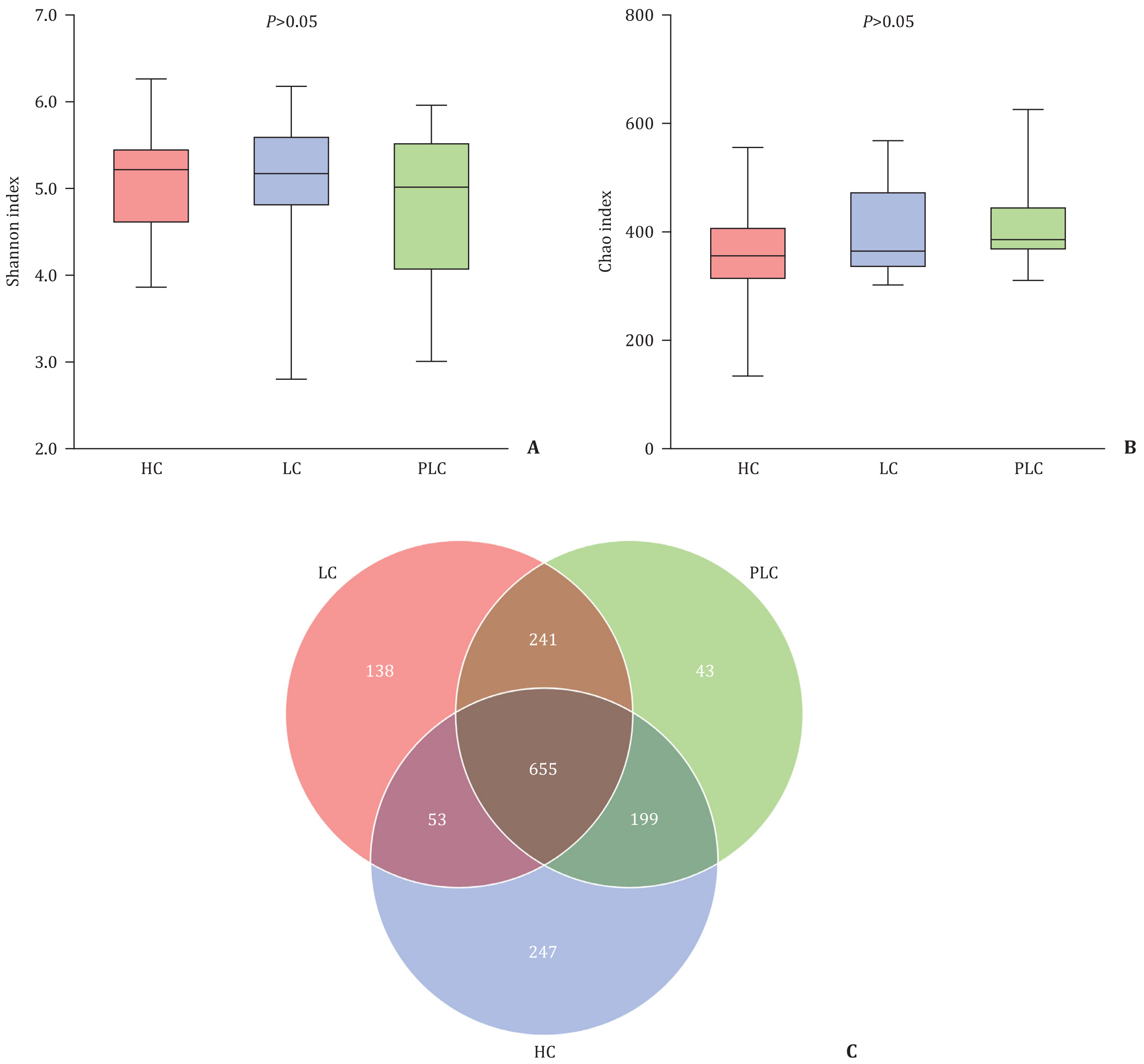

Fig. 1. Gut microbiota structures in the HC, LC, and PLC groups. Shannon (**A**) and Chao (**B**) indices were used to estimate the level of gut microbial diversity. Venn diagram illustrating the overlap of OTUs identified in the gut microbiota from the three groups (**C**). HC: healthy control; LC: liver cirrhosis; PLC: primary liver cancer; OUT: operational taxonomic units.

diversity in the gut microbiota associated with liver disease, especially PLC. At the phylum level, diversity of Firmicutes decreased from HC group to the advanced LC and PLC groups. This finding supported previous studies that the diversity of Firmicutes in patients with colorectal cancer was decreased compared to that of HC group. The study indicated that colon cancer changed the characteristics of intestinal microbes, and the ratio of beneficial microorganisms decreased significantly [30]. Alterations in the natural balance of the microbiota could lead to pathogenesis.

A previous study found that, in healthy people, the gut microbiota is distinct from that in other organs of the human body [31]. However, the bacterial diversity and make-up were dramatically different among different samples such as conventional adenoma, sessile serrated adenoma, and colorectal cancer [30]. Indeed, the ratio of Firmicutes/Bacteroidetes has been already used as an effective indicator for the status of human gut microbes [32]. Our study revealed that the Firmicutes/Bacteroidetes ratio was significant decreased in the LC and PLC groups compared to that in the HC group. Similar to our results, Zhang et al. demonstrated that the ratio of Firmicutes/Bacteroidetes was reduced in the residents of Baiyin (a Chinese city) compared to that in the city of Liujiaxia. Baiyin is a city with heavy-metal pollution and their results indicated that the proportion of Bacteroidetes was significantly increased under heavy metal exposure, whereas the proportion of Firmicutes decreased [33].

The previously reported profile of gut microbiota associated with PLC in cirrhotic patients was shown to be characterized by increased fecal counts of *E. coli*. The study compared the patients with PLC and those without PLC who underwent liver transplantation. However, only the fecal counts were used for the

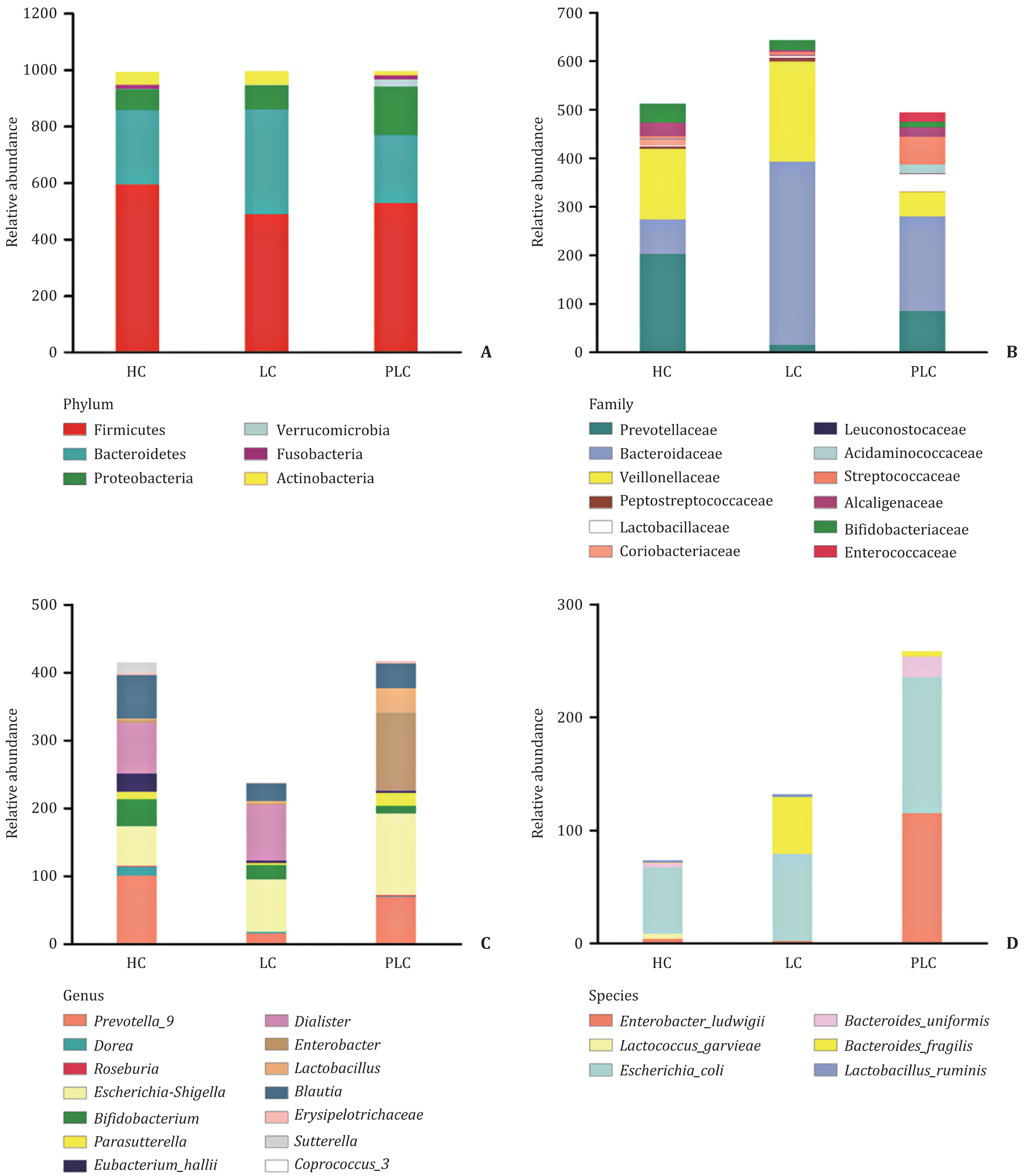

Fig. 2. The relative abundance of the gut microbiota phyla (**A**), family (**B**), genera (**C**), and species (**D**) in the HC, LC, and PLC patients. HC: healthy control; LC: liver cirrhosis; PLC: primary liver cancer.

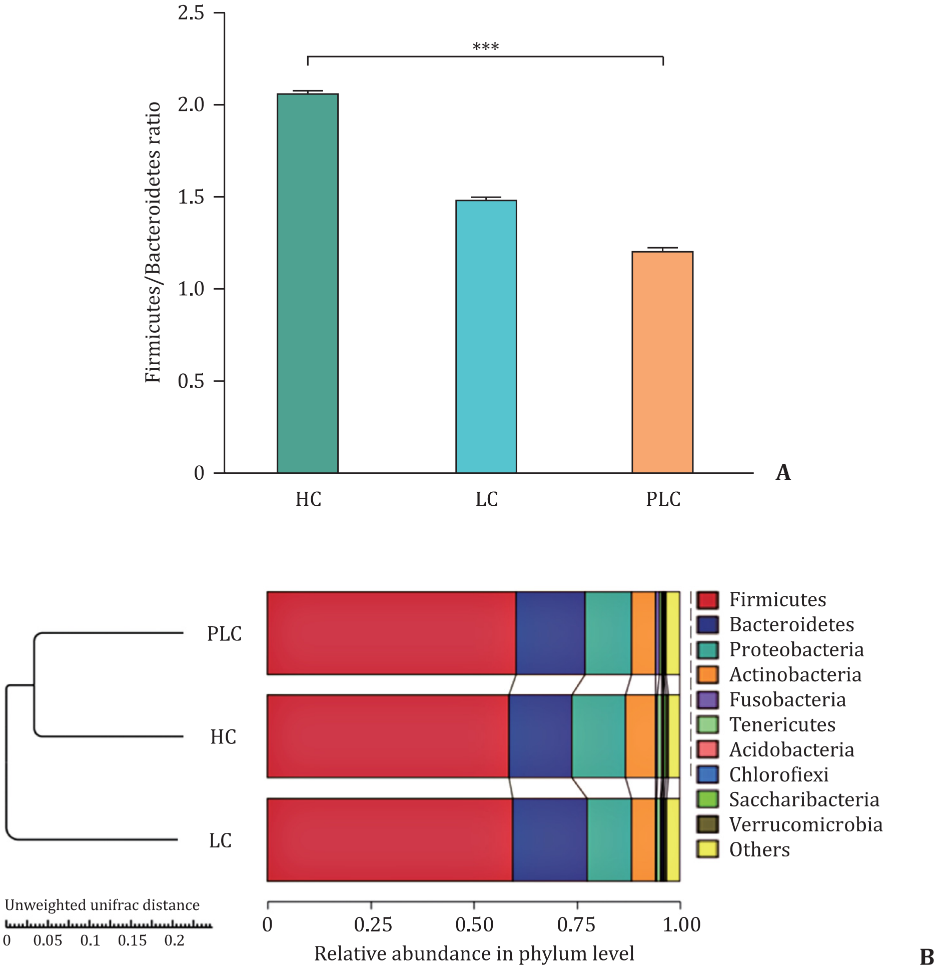

Fig. 3. Gut microbiota changes in the HC, LC, and PLC groups. **A:** Firmicutes/Bacteroidetes ratio in the three groups. **B:** The heatmap indicates changes at the phylum level in the HC, LC, and PLC groups. HC: healthy control; LC: liver cirrhosis; PLC: primary liver cancer. *** indicates a significant *P* value <0.001.

comparison [34]. In the present study, we obtained detailed knowledge about the composition of the gut microbiota in PLC patients using high-throughput pyrosequencing analysis. With 6 423 779 high-quality reads, numerous members from rare taxa that were present at relatively low abundance were detected. The lower level of bacterial diversity found in LC and PLC patients may indicate that the process of PLC development disturbs the gut microbiota homeostasis. Decreased bacterial diversity in gut eventually leads to dysbiosis of the gut microbiota.

Finally, according to the LEfSe method, composition of gut microbiota in HC, LC, and PLC groups was compared. The results indicated that Clostridia were the predominant contributors to the HC gut microbiota, whereas Enterococcaceae, Lactobacillales, Bacilli, and Gammaproteobacteria were the key factors contributing to dysbiosis of the gut microbiota in the PLC group. The data fit well with the results of a previous study [31] which showed that *Lactococcus, Acinetobacter, Bacilli/Bacillus* and *Pseudomonas* were predominant contributors to the distribution of adjacent normal mucosal tissue associated microbiota, whereas *Fusobacterium* was the key contributor to dysbiosis of the microbiota in tumor tissues.

Clinical factors have great influence on the composition of human gut microbiota, and our study clearly indicates that clinical factors overtly influenced their gut microbiota composition. Compared to the results of a previous study, we found that *Veillonella* was positively correlated with AFP. *Veillonella* is prone to intestinal infections and is significantly elevated in children with type 1 diabetes [35]. AFP is the gold standard for the serodiagnosis of liver cancer. In contrast, *Subdoligranulum* showed a highly significant negative correlation with AFP. *Subdoligranulum* species contain known short-chain fatty acid (SCFA)-producing lineages [36–38] such as butyrate producers. SCFAs and butyrate are major anions in gut, where they are rapidly absorbed. A large amount of acetate enters the systemic circulation, and propionate is mainly consumed by the liver for glycogen production. Evidence indicated that these small molecules can positively affect the barrier function of gut and exhibit anti-inflammatory effects [39].

In conclusion, our study demonstrated that PLC patients were characterized by a decreased gut bacterial diversity. Changes in the Firmicutes/Bacteroidetes ratio indicated that dysbiosis of the gut microbiota occurred in both patients with LC and with PLC. Consequently, adjustments in the gut microbiota of PLC patients may disrupt the gut defenses, resulting in gut microbial imbalances. The gut microbial composition of patients with LC and with PLC was closely related to clinical factors. These changes in the gut microbiota suggest that dysbiosis might be involved in the development of PLC. We found differences in the intestinal microbiota among the three groups studied. Further, we clarified the relationship between intestinal microbes and PLC; especially, *Enterobacter ludwigii* may play an important role in the development of PLC. Further investigations focusing on the restoration of gut microbiota are needed for providing new insights into PLC treatment.

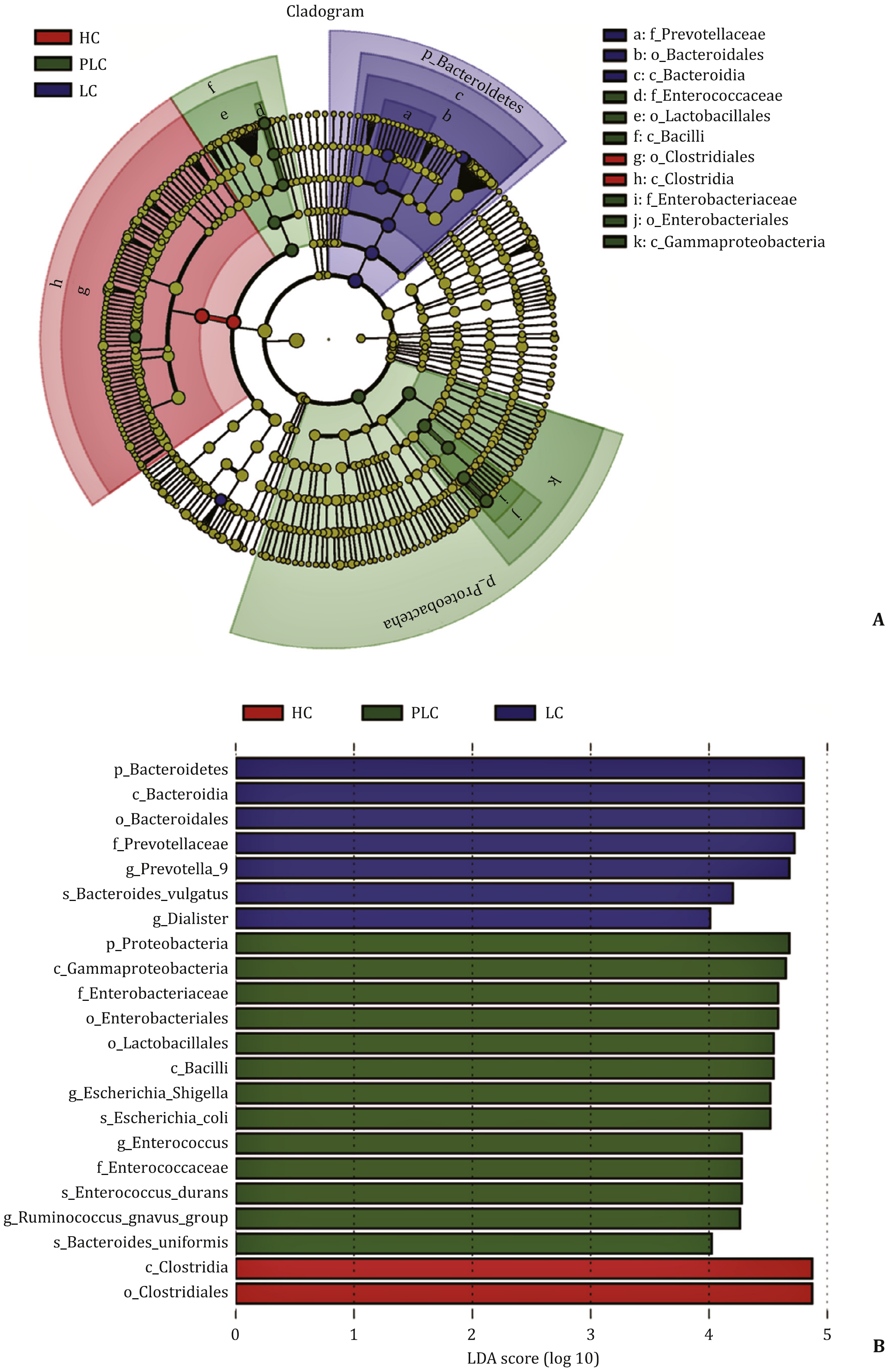

Fig. 4. A: Cladogram for taxonomic classification of gut microbiota. Red, HC-enriched taxa; green, PLC-enriched taxa; blue, LC-enriched taxa. **B:** Key microbiota contributing to the composition of gut microbiota in HC, LC, and PLC samples. HC: healthy control; LC: liver cirrhosis; PLC: primary liver cancer; LDA: linear discriminant analysis.

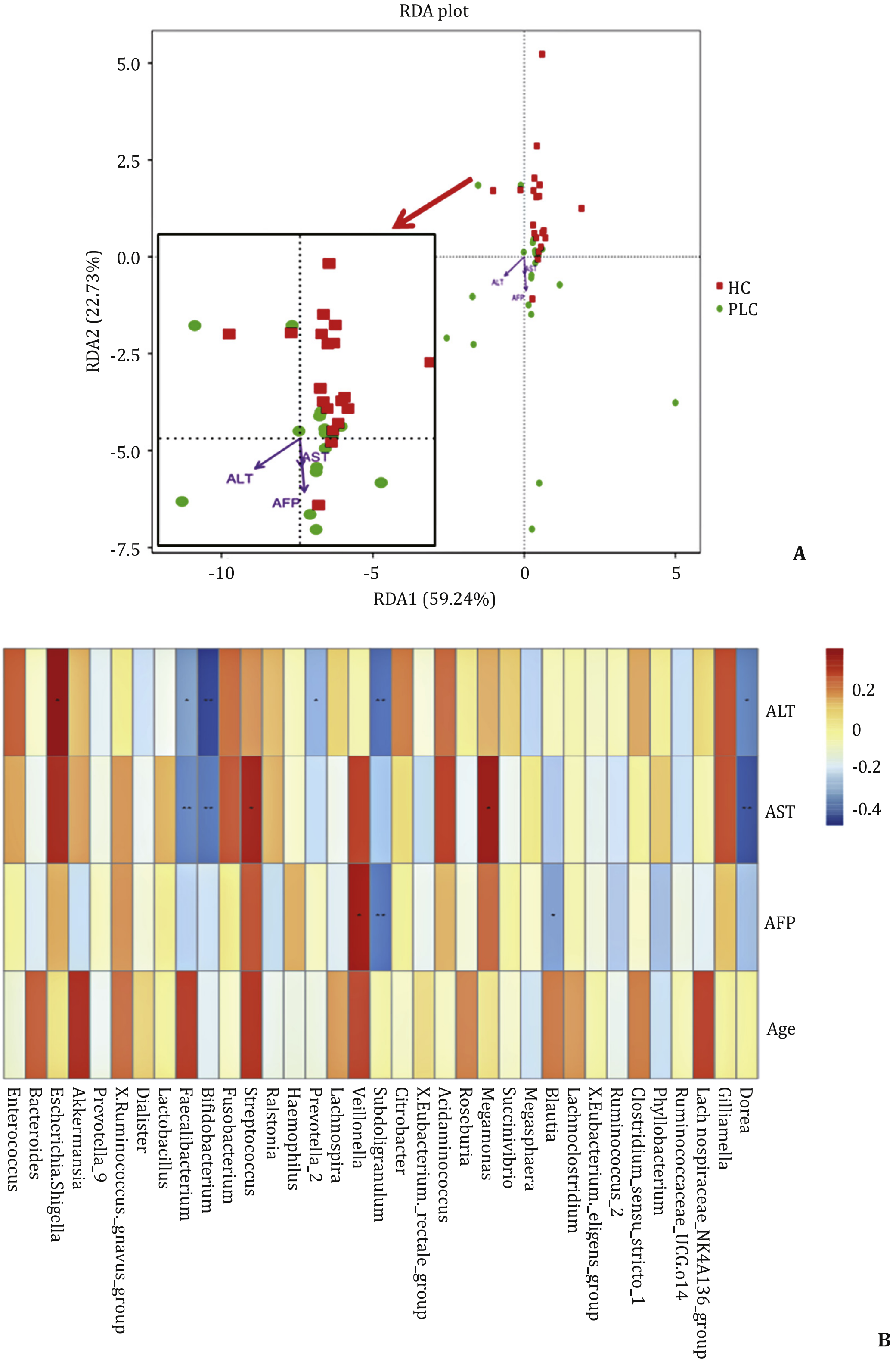

Fig. 5. **A:** Redundancy analysis (RDA) showing the correlation between gut microbial compositions of HC and PLC patients and clinical factors at genus level; **B:** The Spearman correlation analysis to study the relationship between clinical factors and species richness, the vertical is the clinical factor information, the horizontal is the alpha diversity index information, the corresponding value in the middle heatmap is the Spearman correlation coefficient r. * indicates a significant *P* value <0.05. ** indicates a significant *P* value <0.01.

Contributors

ZL and LGX conceived the study and wrote the article. CT coordinated the research. WYN collected the data and carried out the analysis. RCH and LX assisted in collecting specimens, read and corrected the manuscript. All authors read and approved the final manuscript. LGX is the guarantor.

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Ethical approval

The study was approved by the Ethnical Committee of the First Hospital of Lanzhou University, China (LLYYLL-2017-18).

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.
[2] DuPont AW, DuPont HL. The intestinal microbiota and chronic disorders of the gut. Nat Rev Gastroenterol Hepatol 2011;8:523–531.
[3] Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. J Hepatol 2014;60:940–947.
[4] Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, et al. Alterations of the human gut microbiome in liver cirrhosis. Nature 2014;513:59–64.
[5] Chen Y, Yang F, Lu H, Wang B, Chen Y, Lei D, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. Hepatology 2011;54:562–572.
[6] Stam R, Akkermans LM, Wiegant VM. Trauma and the gut: interactions between stressful experience and intestinal function. Gut 1997;40:704–709.
[7] Wei X, Yan X, Zou D, Yang Z, Wang X, Liu W, et al. Abnormal fecal microbiota community and functions in patients with hepatitis B liver cirrhosis as revealed by a metagenomic approach. BMC Gastroenterol 2013;13:175.
[8] Tao X, Wang N, Qin W. Gut Microbiota and Hepatocellular Carcinoma. Gastrointest Tumors 2015;2:33–40.
[9] Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. Gastroenterology 2014;146:1513–1524.
[10] Chou HH, Chien WH, Wu LL, Cheng CH, Chung CH, Horng JH, et al. Age-related immune clearance of hepatitis B virus infection requires the establishment of gut microbiota. Proc Natl Acad Sci U S A 2015;112:2175–2180.
[11] Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 2005;43:5721–5732.
[12] Bajer L, Kverka M, Kostovcik M, Macinga P, Dvorak J, Stehlikova Z, et al. Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis. World J Gastroenterol 2017;23:4548–4558.
[13] Ren Z, Jiang J, Xie H, Li A, Lu H, Xu S, et al. Gut microbial profile analysis by MiSeq sequencing of pancreatic carcinoma patients in China. Oncotarget 2017;8:95176–95191.
[14] Li J, Riaz Rajoka MS, Shao D, Jiang C, Jin M, Huang Q, et al. Strategies to increase the efficacy of using gut microbiota for the modulation of obesity. Obes Rev 2017;18:1260–1271.
[15] Getz GS, Reardon CA. Natural killer T cells in atherosclerosis. Nat Rev Cardiol 2017;14:304–314.
[16] Verslype C, Rosmorduc O, Rougier PESMO Guidelines Working Group. Hepatocellular carcinoma: ESMO-ESDO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012;23:vii41–vii48.
[17] Li Y, Zhang Q, Zhang F, Liu R, Liu H, Chen F. Analysis of the microbiota of black stain in the primary dentition. PLoS One 2015;10:e0137030.
[18] Zhang X, Tian X, Ma L, Feng B, Liu Q, Yuan L, et al. Biodiversity of the symbiotic bacteria associated with toxic marine dinoflagellate alexandrium tamarense. J Biosci Med 2015;3:23–28.
[19] 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;7:335–336.
[20] Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 2009;75:7537–7541.
[21] Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, et al. The ribosomal database project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 2009;37:D141–D145.
[22] Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol 2011;12:R60.
[23] Xu YY, Zhang JX, Zhu XL, Fan DD, Dong LP, Yao Q, et al. Analysis of treatment of cadmium wastewater using an Enterobacter ludwigii. Chem Eng (China) 2015;43:1–5.
[24] Liu T, He W, Yan C, Qi Y, Zhang Y. Roles of reactive oxygen species and mitochondria in cadmium-induced injury of liver cells. Toxicol Ind Health 2011;27:249–256.
[25] Cesaro C, Tiso A, Del Prete A, Cariello R, Tuccillo C, Cotticelli G, et al. Gut microbiota and probiotics in chronic liver diseases. Dig Liver Dis 2011;43:431–438.
[26] Wiest R, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. Hepatology 2005;41:422–433.
[27] Nolan JP. The role of intestinal endotoxin in liver injury: a long and evolving history. Hepatology 2010;52:1829–1835.
[28] Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, et al. Metagenomic analysis of the human distal gut microbiome. Science 2006;312:1355–1359.
[29] Ling Z, Liu X, Cheng Y, Jiang X, Jiang H, Wang Y, et al. Decreased diversity of the oral microbiota of patients with hepatitis b virus-induced chronic liver disease: a pilot project. Sci Rep 2015;5:17098.
[30] Yoon H, Kim N, Park JH, Kim YS, Lee J, Kim HW, et al. Comparisons of gut microbiota among healthy control, patients with conventional adenoma, sessile Serrated adenoma, and colorectal cancer. J Cancer Prev 2017;22:108–114.
[31] Ling Z, Liu X, Luo Y, Yuan L, Nelson KE, Wang Y, et al. Pyrosequencing analysis of the human microbiota of healthy Chinese undergraduates. BMC Genom 2013;14:390.
[32] Mariat D, Firmesse O, Levenez F, Guimarães V, Sokol H, Doré J, et al. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. BMC Microbiol 2009;9:123.
[33] Zhang W, Guo R, Yang Y, Ding J, Zhang Y. Long-term effect of heavy-metal pollution on diversity of gastrointestinal microbial community of Bufo raddei. Toxicol Lett 2016;258:192–197.
[34] Grąt M, Wronka KM, Krasnodębski M, Masior Ł, Lewandowski Z, Kosińska I, et al. Profile of gut microbiota associated with the presence of hepatocellular cancer in patients with liver cirrhosis. Transplant Proc 2016;48:1687–1691.
[35] Murri M, Leiva I, Gomez-Zumaquero JM, Tinahones FJ, Cardona F, Soriguer F, et al. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. BMC Med 2013;11:46.
[36] Holmstrøm K, Collins MD, Møller T, Falsen E, Lawson PA. Subdoligranulum variabile gen. nov., sp. nov. from human feces. Anaerobe 2004;10:197–203.
[37] Zhu L, Baker RD, Baker SS. Gut microbiome and nonalcoholic fatty liver diseases. Pediatr Res 2015;77:245–251.
[38] Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J Nutr 2009;139:1619–1625.
[39] Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature 2009;461:1282–1286.