Parasite Immunology

WILEY

Parasite Immunology

ORIGINAL ARTICLE OPEN ACCESS

Characteristics of Intestinal Flora in Patients With *Schistosoma japonicum* Infection Undergoing Splenectomy

Chen Zhou | Pengpeng Zhang | Yingzi Ming

Transplantation Center, Engineering and Technology Research Center for Transplantation Medicine of National Health Comission, The Third Xiangya Hospital, Central South University, Changsha, Hunan, China

Correspondence: Yingzi Ming (myz_china@aliyun.com)

Received: 17 August 2024 | **Revised:** 21 March 2025 | **Accepted:** 26 March 2025

Funding: This work was supported by the National Natural Science Foundation of China (81771722 to Y. Ming) and the Key Research and Development Plan of Hunan Province (2021SK2032 to Y. Ming).

Keywords: 16sRNA | gut microbiome | *S. japonicum* infection | splenectomy

ABSTRACT

Schistosomiasis japonica is a parasitic disease that seriously endangers human health. Patients with advanced *Schistosoma japonicum* infection often suffer from cirrhosis and portal hypertension. Splenectomy has been widely used in the treatment of these patients. Previous studies have confirmed that *S. japonicum* infection is closely related to the gut microbiota, but the impact of splenectomy on the gut microbiota of patients with advanced *S. japonicum* infection remains unclear. This study used 16sRNA sequencing technology to compare the differences in intestinal flora between patients with advanced *S. japonicum* infection who underwent splenectomy and non-surgical patients. We focused on the changes in the species composition, diversity and functions of the intestinal flora. Our study shows that dysbiosis of the gut microbiome occurred in patients with advanced *S. japonicum* infection, including changes in abundance and diversity and the disorder of biological function. The intestinal flora structure, diversity and function of patients who underwent splenectomy were significantly changed compared with those who did not undergo surgery.

1 | Introduction

Schistosomiasis is a tropical disease that is widely prevalent in poor and remote areas. The disease has caused a large number of disabilities, and more than 200,000 people die of schistosomiasis every year worldwide [1]. There are six kinds of schistosomiasis that parasitise humans, among which *Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma japonicum* (*S. japonicum*) are the main pathogenic species [2]. The prevalent schistosomiasis in China is *S. japonicum* [3]. Once the cercariae of *S. japonicum* infect humans, they migrate to the mesenteric vein and develop into adult *S. japonicum* [4]. The eggs laid by the adult *S. japonicum* are deposited in the liver and form egg granulomas, which lead to tissue damage and liver fibrosis [5]. The disease is divided into three infection stages: acute stage, chronic stage and advanced stage [6]. Patients with *S. japonicum* infection will reach the advanced stage if they do not receive timely and effective treatment during the acute and chronic stages [7]. Patients with advanced *S. japonicum* infection, who manifest portal hypertension, splenomegaly, hypersplenism and other complications, have a high mortality rate [8].

The pathogenesis, pathology, clinical manifestations and prognosis of portal hypertension caused by *S. japonicum* are different

Abbreviations: 16S rRNA, 16S ribosomal ribonucleic acid; ANOSIM, analysis of similarities; DNA, deoxyribonucleic acid; KEGG, Kyoto Encyclopedia of Genes and Genomes; LEfSe, linear discriminant analysis effect size; NMDS, non-metric multidimensional scaling; OUT, operational taxonomic unit; PCR, polymerase chain reaction; PCA, principal component analysis; PCoA, principal co-ordinates analysis; *S. japonicum*, *Schistosoma japonicum*.

from those of portal hypertension with other causes [9]. Portal hypertension can be divided into three types: pre-sinusoidal, sinusoidal and post-sinusoidal [10]. Portal hypertension caused by Schistosoma japonicum is pre-sinusoidal. Usually, the liver parenchyma is well preserved in these patients [11]. In addition, patients with splenomegaly caused by *S. japonicum* demonstrate beneficial effects after simple splenectomy [12]. Splenectomy for patients with advanced *S. japonicum* infection can not only correct hypersplenism, but can also reduce portal blood flow, thereby relieving portal hypertension [13]. Therefore, splenectomy has been widely used in the treatment of patients with advanced *S. japonicum* infection.

Intestinal microbes have been widely studied in various diseases [14]. Intestinal microbes help digest complex food components, boost immune system function and protect the gut from harmful bacteria and disease [15]. After a patient is infected with *S. japonicum*, the adult worms will deposit eggs in the intestinal wall, causing damage to the intestinal wall tissue, changing the permeability of the intestinal wall and changing the intestinal microbiota [16]. The gut-liver axis is one of the important links between the intestinal microbiota and the liver [17]. When the intestinal barrier is severely damaged, intestinal toxic substances flow from the superior and inferior mesenteric veins into the portal vein and liver [18]. *S. japonicum* infection is a chronic disease capable of causing intestinal and hepatic damage [19]. A previous study has revealed the relationship between intestinal microbiota and the progression of *S. japonicum* disease, with the discovery of a series of intestinal microbes characteristic of different stages of *S. japonicum* and has proposed a series of potential biomarkers for disease diagnosis and targets for therapeutic intervention [20]. Our recent study also demonstrated that alterations in the gut microbiota in different stages of *S. japonicum* infection play a potential role in the pathogenesis of the transition from chronic to advanced *S. japonicum* infection [6]. Therefore, intestinal microbiota has been confirmed to be closely related to *S. japonicum* infection.

Several studies have indicated that splenectomy could alter the gut microbiota in trauma [21] and liver cirrhosis patients [22]. However, whether splenectomy can affect the gut microbiota in patients with advanced *S. japonicum* infection has not yet been determined. Considering the close relationship between the gut microbiota and *S. japonicum* infection, we used 16s RNA sequencing to compare the diversity and structural changes in gut microbiota in healthy people, patients with advanced *S. japonicum* infection and patients with advanced *S. japonicum* infection who received splenectomy in order to investigate the effect of splenectomy on intestinal flora in patients with *S. japonicum* infection.

2 | Methods

2.1 | Study Design

This study was carried out from November 2021 to November 2022 and all participants were from Xiang Yue Hospital in Yue Yang, Hunan Province, China. Our study ultimately enrolled 13 healthy people (Healthy group), 8 patients with advanced *S. japonicum* infection (AS group) and 11 patients with advanced *S. japonicum* infection who underwent splenectomy (AS-SC group) according to strict inclusion and exclusion criteria. The healthy controls were recruited from people who underwent annual health checkups at the Health Checkup Center of Xiang Yue Hospital during the study period. In order to ensure that the intestinal flora of the control group subjects was not affected by other diseases, we focused on selecting people in good health who did not have any abnormal diseases found in the annual health checkups. At the same time, we required the control group subjects to have never been infected with *Schistosoma japonicum*. Then, we matched them according to age and gender to ensure that there was no statistical difference in age and gender composition between the control group and the other two study groups. The eligibility and exclusion criteria were shown in Table 1. The relevant statistical results were shown in Table 2.

The fresh faeces, general characteristics and clinical examination findings of these participants were collected. We used B-ultrasound technology to measure the main portal vein (MPV) diameter to evaluate the portal hypertension of the three groups of subjects. The MPV diameter in the H group was 1.01–1.28 cm, with an average of 1.10 cm. In the AS group, it was 1.51–1.9 cm, with an average of 1.65 cm. In the AS-SC group, it was 1.21–1.7 cm, with an average of 1.63 cm. Regardless of whether patients with *S. japonicum* underwent splenectomy, the MPV diameter was wider than that in the healthy group (t test, $p < 0.05$). However, no significant statistical difference was observed in the width of the portal vein trunk between the AS group and the AS-SC group (t test, $p > 0.05$). According to the National Standardised Diagnostic Criteria for *S. japonicum* infection (WS261-2006) of the Ministry of Health of China, patients with advanced *S. japonicum* infection should meet the following conditions (Table 1). Basic information on the study population per group is shown in Table 2.

2.2 | Treatment Histories for Schistosomiasis

Group H did not receive any treatment for schistosomiasis. The subjects of the AS group and the AS-SC group were all patients with advanced *S. japonicum* infection, and they were treated with the same drug regimen. The main treatment drug is praziquantel, which is administered at a standard dose of 60 mg/kg, divided into three doses per day, 0.5 h after a meal, for a total of 2 days. Patients also need to take liver protection drugs, mainly glutathione and silymarin. The medical treatment of patients in the AS-SC group after splenectomy was similar to that of patients without surgery. The dosage of praziquantel was 45–50 mg/kg given in three divided doses per day, 0.5 h after a meal, for a total of 2 days.

2.3 | Sample Collection

The faeces of patients in the AS-SC group were collected from those patients who had undergone splenectomy 3 or more years previously. All subjects received training from professional biomedical researchers. Fresh stool specimens were required to be collected using a sterile collection tube, and the specimens were quickly frozen in liquid nitrogen for 30 min and then transferred to a −80°C environment until subsequent experiments [23].

TABLE 1 | Inclusion and exclusion criteria of the study.

Group	Inclusion criteria	Exclusion criteria
H	1. No abnormal diseases found in the annual health checkups. 2. No history of Schistosoma japonicum infection. 3. 35–65 years old.	1. History of cancer. 2. History of chronic diseases such as hypertension and diabetes. 3. History of heart disease, hepatitis, or kidney disease. 4. Acute infection state. 5. Use of antibiotics within the past 6 months. 6. Use of proton pump inhibitors in the past 6 months.
AS	4. Living in an epidemic area or having a history of contact with epidemic water for many times. 5. There are clinical manifestations of portal hypertension or spleen hyperactivity. 6. Schistosoma eggs were found in faecal examination or serological examination was positive. 7. B-ultrasound showed hepatic fibrosis and splenomegaly. 8. 35–65 years old. 9. Positive serological test for schistosomiasis.	1. History of cancer. 2. Acute infection state. 3. Patients infected with hepatitis A, B, C, D and E viruses. 4. The patient infected with other parasitic diseases or infectious diseases. 5. Use of antibiotics within the past 6 months. 10. Use of proton pump inhibitors in the past 6 months.
AS-SC	1. Living in an epidemic area or having a history of contact with epidemic water for many times. 2. Schistosoma eggs were found in faecal examination or serological examination was positive. 3. B-ultrasound showed hepatic fibrosis. 4. History of splenectomy for portal hypertension and hypersplenism. 5. 35–65 years old. 6. 3 years or more after splenectomy. 7. Positive serological test for schistosomiasis.	1. History of cancer. 2. Acute infection state. 3. Patients infected with hepatitis A, B, C, D and E viruses. 4. The patient infected with other parasitic diseases or infectious diseases. 5. Use of antibiotics within the past 6 months. 6. Use of proton pump inhibitors in the past 6 months.

TABLE 2 | Basic information of study population per group.

Variables	H	AS	AS-SC	*p*
n	13	8	11	
Age(years)	55 ± 3.24	55 ± 3.28	56 ± 3.65	0.6038
Gender(male/female)	7/6	4/4	6/5	0.9787
Active smoker (%)	23.1	12.5	27.3	0.7371
Active drinker (%)	15.4	25	27.3	0.7582
Dietary type	Eastern diet	Eastern diet	Eastern diet	No
	100%	100%	100%	

Note: There were no statistically significant differences among three groups. Differences in age were determined using ANOVA, $F = 0.5134$, $R^2 = 0.03419$. Differences in gender, active smoker and drinker were determined using the Chi-square test, χ^2 of gender = 0.0430, df = 2, χ^2 of smoker = 0.6100, df = 2, χ^2 of drinker = 0.55371, df = 2.
Abbreviations: AS, advanced *S. japonicum* infection, AS-SC, advanced *S. japonicum* infection undergoing splenectomy; H, health people.

2.4 | 16sRNA Sequencing

2.4.1 | Extraction of Genome DNA

The cetyltrimethylammonium bromide method was used to extract total DNA. DNA Kit (TIANGEN DP712) was utilised, and 1.0% agarose gels were used to monitor DNA purity and concentration. DNA was diluted with sterile water to 1 ng/μL.

2.4.2 | Amplicon Generation

Here, 15 μL Phusion High-Fidelity PCR Master Mix (New England Biolabs) was applied to finish the polymerase chain reaction (PCR) reaction. The following primers were used in the experiment: 16S V3–V4: 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GGACTACHVGGGTATCTAAT-3′) [24]. All PCR reactions were carried out in 30 μL reactions with 15 μL of

Phusion High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s and elongation at 72°C for 60 s [6].

2.4.3 | PCR Product Quantification and Qualification

The 1× loading buffer (containing SYB green) and PCR products were mixed at a ratio of 1:1, and electrophoresis was carried out on a 2.0% agarose gel. Samples with bright main bands between 400 and 450 BP were selected for further experiments.

2.4.4 | PCR Product Mixing and Purification

AxyPrepDNAGel Extraction Kit (AXYGEN) was used to purify PCR products after they were mixed in equally dense ratios.

2.4.5 | Library Preparation and Sequencing

Sequencing libraries were generated using TruSeq DNA PCR-Free Sample Preparation Kit (Illumina) following the manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina NovaSeq6000 platform and 250 bp paired-end reads were generated [25].

2.4.6 | Sequencing Data Analysis

Paired-end reads from the original DNA fragments were merged using FLASH (http://ccb.jhu.edu/software/FLASH/). The UPARSE software package (http://drive5.com/uparse/) was used to perform sequence analyses. Alpha and beta diversity were analysed using in-house Perl scripts. Sequences with ≥97.0% similarity were assigned to the same OTUs [26]. We picked a representative sequence for each OTU and used the RDP classifier to annotate taxonomic information for each representative sequence. QIIME was used to describe alpha diversity [27]. LEfSe (http://huttenhower.sph.harvard.edu/lefse/) was used for the quantitative analysis [28]. ANOSIM was performed based on the Bray–Curtis dissimilarity distance matrices to identify differences in microbial communities among groups [29].

2.4.7 | Statistical Analysis

For the species composition analysis and alpha diversity comparison, a *t* test was used to compare the H and AS groups, and the H and AS-SC groups. For the beta diversity analysis, Wilcoxon test was used to compare the H and AS groups, and the H and AS-SC groups. For the function prediction, ANOVA analysis was used to compare the three groups. A *p* value less than 0.05 was considered statistically significant.

3 | Results

3.1 | Participant Characteristics

A total of 33 participants were ultimately enrolled in our study according to the inclusion and exclusion criteria, and were divided into three groups: Healthy group ($n=13$), AS group ($n=8$) and AS-SC group ($n=11$). Table 1 shows the general characteristics of all participants, and no significant difference was observed among groups.

3.2 | Species Composition Analysis

In order to explore the changes in gut microbiota in patients with advanced *S. japonicum* infection and the influence of splenectomy, we analysed the relative abundance of gut microbiota among three groups at the phylum and genus levels. At the phylum level, *Firmicutes, Bacteroidetes*, and *Proteobacteria* were the top three in abundance among the three groups. Compared with healthy people, the composition of the gut microbiota in patients with advanced *S. japonicum* infection changed significantly, and this was demonstrated by the decrease in relative abundance of *Firmicutes* (*t* test, $t_{(19)}=3.603$, $p=0.0015$) and the increase in relative abundance of *Proteobacteria* (*t* test, $t_{(19)}=3.361$, $p=0.0049$). However, splenectomy did not lead to changes in the composition of gut microbiota at the phylum level (Figure 1a). At the genus level, compared with the healthy group, the relative abundance of *Bacteroides* (*t* test, $t_{(19)}=2.372$, $p=0.0421$) in the AS group increased, while the relative abundance of *Faecalibacterium* (*t* test, $t_{(19)}=2.941$, $p=0.0072$) decreased. After splenectomy, we found that the relative abundance of *Bacteroides* decreased from 36.3% to 25.3%, while the relative abundance of *Faecalibacterium* and *Prevotella* 9 increased from 5.5% and 0.9% to 11.0% and 11.0%, respectively (Figure 1b). Tables S1 and S2 showed the relevant statistical results at the phylum and genus levels. Our results indicated that the intestinal flora composition of the AS group and AS-SC group were different.

3.3 | Alpha and Beta Diversity of Intestinal Microbiota

First, we conducted an ANOSIM analysis and found that the differences among the three groups were greater than the differences within the groups, which suggested that this study was meaningful (Anosim, $R=0.274$, $p=0.001$) (Figure 2a). Species accumulation curves were used to evaluate whether the sample size of our study was sufficient. As the sample size increased, the position of the box plot tended to flatten, indicating that a further increase in sample size would not significantly increase the number of species discovered, which indicated that the sample size was sufficient for data analysis (Figure 2b).

The alpha diversity is the diversity within a given community. Four indices were used to evaluate alpha diversity in our data: ACE (Figure 3a), Chao1 (Figure 3b), observed species (Figure 3c) and PD whole tree (Figure 3d). Our results showed that there were differences regarding ACE (*t* test, $t_{(19)}=3.011$, $p=0.0088$), Chao1 (*t* test, $t_{(19)}=2.698$, $p=0.0113$), observed species (*t* test, $t_{(19)}=1.116$,

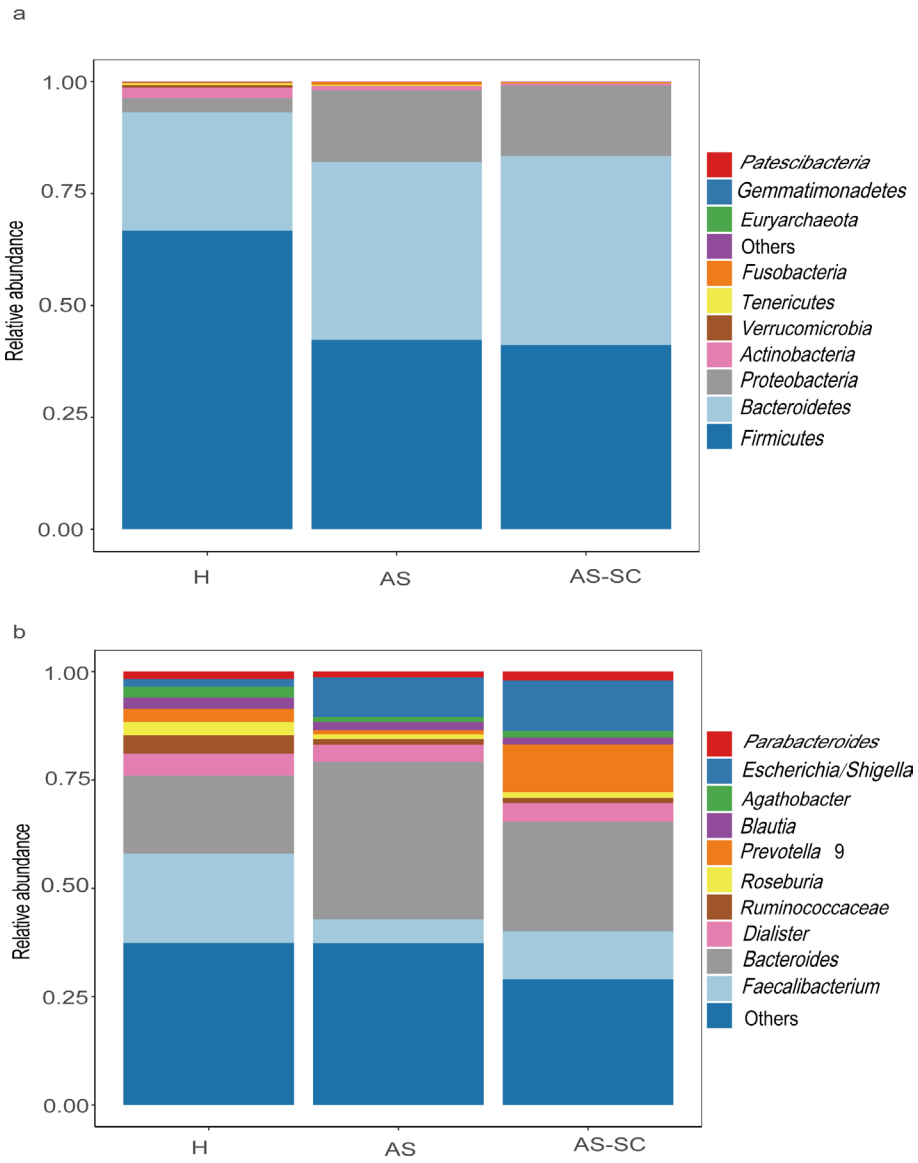

FIGURE 1 | Community of gut microbiota in healthy people, patients with advanced *S. japonicum* infection and patients with advanced *S. japonicum* infection undergoing splenectomy. (a) Phylum level. (b) Genus level. AS, advanced *S. japonicum* infection; AS-SC, advanced *S. japonicum* infection undergoing splenectomy; H, healthy people.

$p=0.0202$) and PD whole tree (Wilcoxon, $Z=-1.88$, $p=0.0302$) between the healthy group and the AS group. Compared with the healthy group, the alpha diversity of the gut microbiota in patients of the AS group was significantly lower (t test, $p<0.05$). However, according to the results of Chao1 (t test, $p=0.0808$), Observed species (t test, $p=0.0638$) and PD whole tree (t test, $p=0.0629$) between the healthy group and the AS-SC group, there was no statistical difference between the alpha diversity of intestinal flora in patients of the AS-SC group compared with that of the healthy group, indicating that splenectomy may have the ability to improve intestinal microbiota diversity in patients with advanced *Schistosoma japonicum* infection.

We further analysed the beta diversity based on Weighted Unifrac distances analysis to assess the structure of the intestinal microbiota community among three groups. As shown in Figure 4a, the beta diversity of intestinal flora of patients in the AS group was higher than that of the healthy group (Wilcoxon, $Z=-2.44$, $p=0.0063$), but there were no statistical differences between the healthy and AS-SC group (Wilcoxon, $Z=0.48$, $p=0.6860$). In order to make the results more visible, we then carried out the PCoA analysis (Figure 4b) and an NMDS analysis (Figure 4c), both of which indicated that the distribution of intestinal flora of patients in the AS group was separate from that of the healthy group and the distribution of gut microbiota of patients in the AS-SC group was closer to that of the healthy group. Therefore, our results indicate that patients with advanced *S. japonicum* infection had significant differences in the alpha and beta diversity of their intestinal microbiota compared with the healthy group. However, the diversity of gut microbiota in patients who underwent splenectomy was more similar to that of healthy people.

3.4 | The Functional Prediction of Different Microbiota

To explore the function of different microbiota among the three groups, we conducted a functional analysis for them

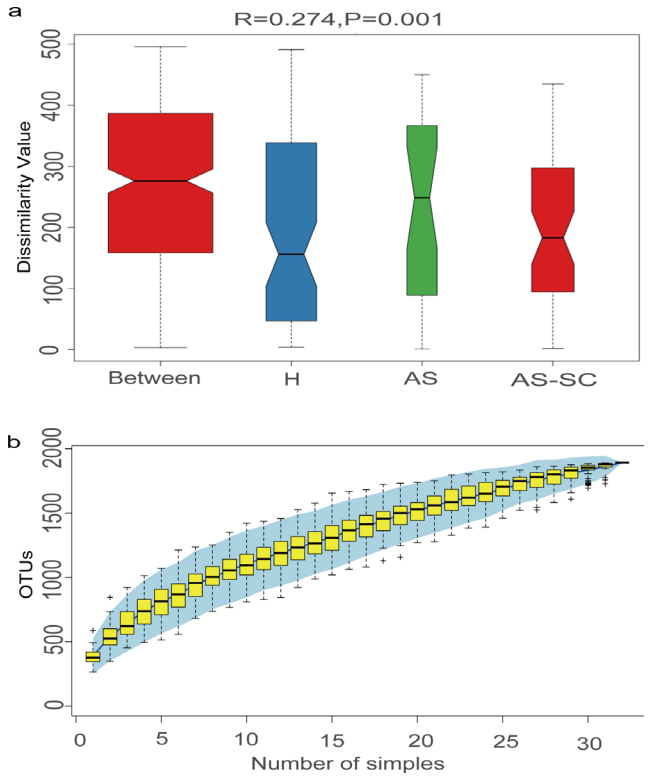

FIGURE 2 | Quality control. (a) Anosim analysis ($R = 0.274$; $p = 0.011$). (b) Species accumulation curves. ANOSIM, analysis of similarities; AS, advanced *S. japonicum* infection; AS-SC, advanced *S. japonicum* infection undergoing splenectomy; H, healthy people.

based on the KEGG database. First, we compared the differences in biological functions of the different microbiota of patients among the three groups using PCA analysis (ANOVA, $F = 6.86$, $p = 0.0036$). The results showed that compared with the healthy group, the biological functions of the different microbiota of patients in the AS group changed significantly, but the biological functions of the different microbiota of patients in the AS-SC group were more similar to those of the healthy group (Figure 5a). Subsequently, an LEfSe analysis was carried out to find the most significantly changed function among the three groups. Our results showed that the biological functions of intestinal microbiota of patients in the healthy groups were mainly related to amino acid metabolism, transcription and environmental adaptation. The biological functions of intestinal microbiota of patients in the AS group were glycon biosynthesis metabolism, cellular processes and signalling and metabolism, and so on. After splenectomy, the biological functions of intestinal microbiota of patients were mainly reflected in digestive system changes and infectious diseases (Figure 5b) To further explore which digestive and infectious diseases AS-SC patients were more susceptible to, we further performed analysis and found that patients in the AS-SC group were more susceptible to Shigellosis, and their carbohydrate, vitamin, cofactor and riboflavin metabolic pathways were significantly changed (Figure S1). In order to show the changes in intestinal flora function among the three groups more intuitively, a heat map was performed (Figure 6).

To sum up, compared with the healthy group, the biological function of gut microbiota of patients with *S. japonicum* infection changed significantly, especially regarding glycon biosynthesis metabolism, cellular processes and signalling and metabolism. Among the important physiological functions of the liver are glucose and lipid metabolism. Our results showed significant changes in the carbohydrate metabolic function in patients with advanced schistosomiasis, which may suggest that the carbohydrate metabolic function is disrupted in these patients. Previous studies have reached similar conclusions [30]. Changes in the cell cycle indicate that liver parenchymal cells in patients with advanced schistosomiasis may be seriously damaged or even die. Cell damage and death enhance liver regeneration. Some liver cells in the quiescent phase re-enter the cell cycle and begin to proliferate. However, this dysbiosis in the biological functions of the intestinal flora was alleviated in splenectomy patients.

4 | Discussion

As a chronic and progressive disease, *S. japonicum* infection has seriously endangered the health of patients in China [31]. Patients with advanced *S. japonicum* infection, who manifest portal hypertension, splenomegaly, hypersplenism and other complications, have a high mortality rate [32]. As a valuable treatment, splenectomy has played an important role in the treatment of patients with advanced *S. japonicum* infection. However, the potential mechanism at work remains unknown [33]. Our study was the first to explore the relationship between splenectomy and gut microbiota and showed that the intestinal flora of patients with advanced *S. japonicum* infection was altered compared with that of healthy people. The composition, diversity and function of the intestinal flora of patients with advanced *S. japonicum* infection who underwent splenectomy were also different from those who did not undergo surgery.

Several previous studies have confirmed that the gut microbiota of patients with advanced *S. japonicum* infection changes significantly [34, 35]. Our study suggests that the abundance of *Bacteroides* in patients with advanced *S. japonicum* infection increased, which was consistent with our team's previous study [6]. As a common bacterium in the human intestinal tract, *Bacteroides* can metabolise carbohydrates to provide energy for human body [36]. However, it is also an important bacterium that causes a variety of infections [37]. In a study about colon cancer in patients with advanced *S. japonicum* infection, the researchers believed that the abundance of *Bacteroidetes* was closely related to tumorigenesis [38]. In our study, splenectomy treatment reduced the abundance of intestinal *Bacteroides*. Whether this suggests that splenectomy may reduce the risk of infection and tumorigenesis deserves further study in the future. In addition to *Bacteroidetes*, compared with healthy group, the abundance of intestinal *Faecalibacterium* in patients with advanced *S. japonicum* infection decreased. *Faecalibacterium* is considered to be the main source of butyrate in the gut [39]. Butyrate is important for maintaining intestinal homeostasis, which is not only the main energy source for colon cells [40], but also has a strong anti-inflammatory effect [41]. The growth of *Faecalibacterium* is extremely sensitive to concentration changes in bile salts [42]. In our study, the decreased abundance of *Faecalibacterium* in patients with

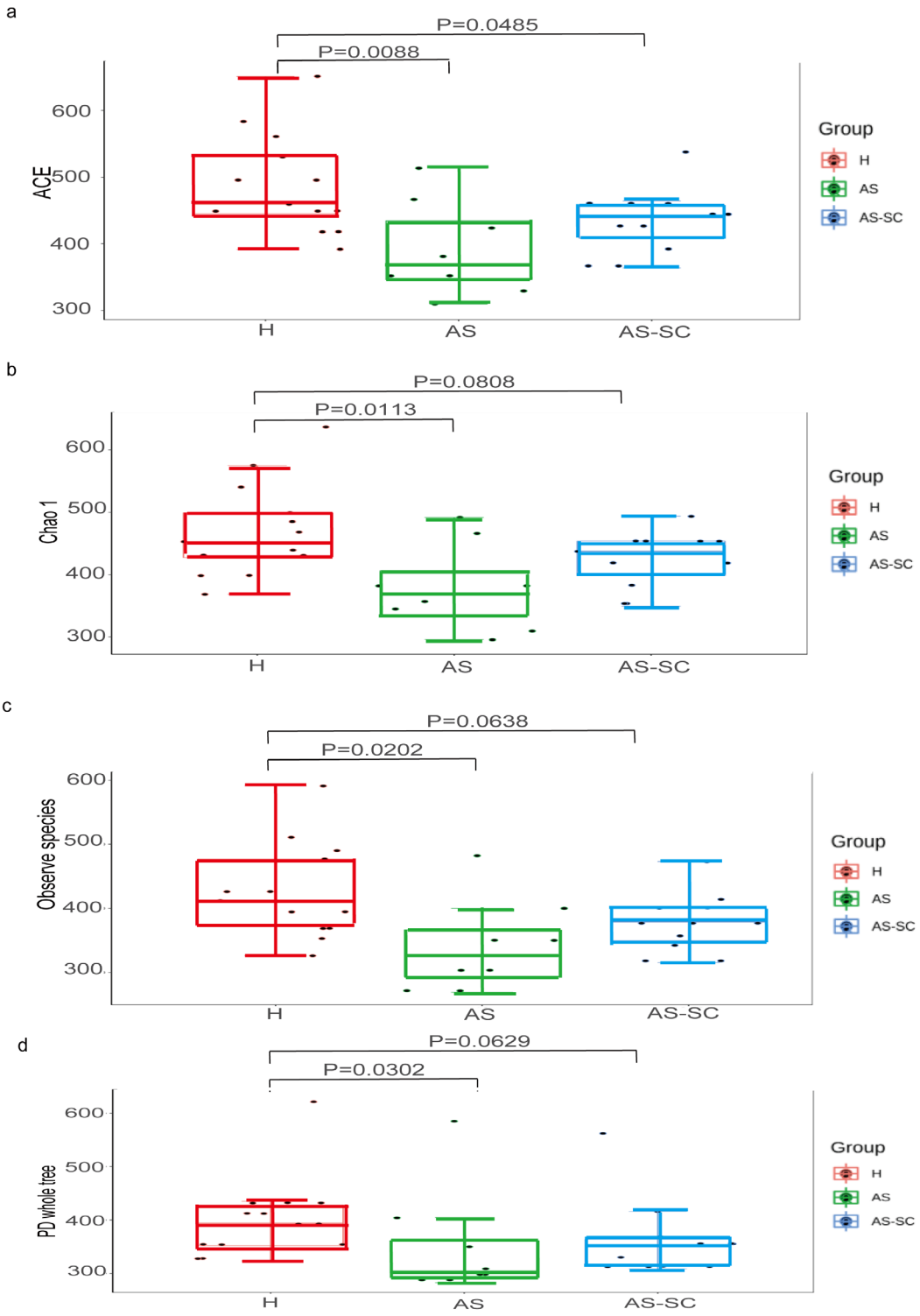

FIGURE 3 | Alpha diversity analysis of intestinal flora in healthy people, patients with advanced *S. japonicum* infection and patients with advanced *S. japonicum* infection undergoing splenectomy. (a) ACE index (H vs. AS, *t* test, $t_{(19)} = 3.011$, $p = 0.0088$, H vs. AS-SC, *t* test,$p = 0.0485$); (b) Chao 1 index (H vs. AS, *t* test, $t_{(19)} = 2.698$, $p = 0.0113$, H vs. AS-SC, *t* test, $p = 0.0808$); (c) Observed species index (H vs. AS, *t* test, $t_{(19)} = 1.116$, $p = 0.0202$, H vs. AS-SC, *t* test, $p = 0.0638$); (d) PD whole tree index (H vs. AS, Wilcoxon, $Z = -1.88$, $p = 0.0302$, H vs. AS-SC, $p = 0.0629$). AS, advanced *S. japonicum* infection; AS-SC, advanced *S. japonicum* infection undergoing splenectomy; H, healthy people.

advanced *S. japonicum* infection suggested that the bile concentration could change significantly. This provided a good inspiration for further exploring the related changes in bile and metabolism in patients with advanced *S. japonicum* infection. In addition, splenectomy could effectively reverse the reduction in the abundance of intestinal *Faecalibacterium* in patients with advanced *S. japonicum* infection, which suggested the advantage of splenectomy treatment. The abundance of *Prevotella* 9 increased after splenectomy. According to the literature review, the reduction of abundance of intestinal

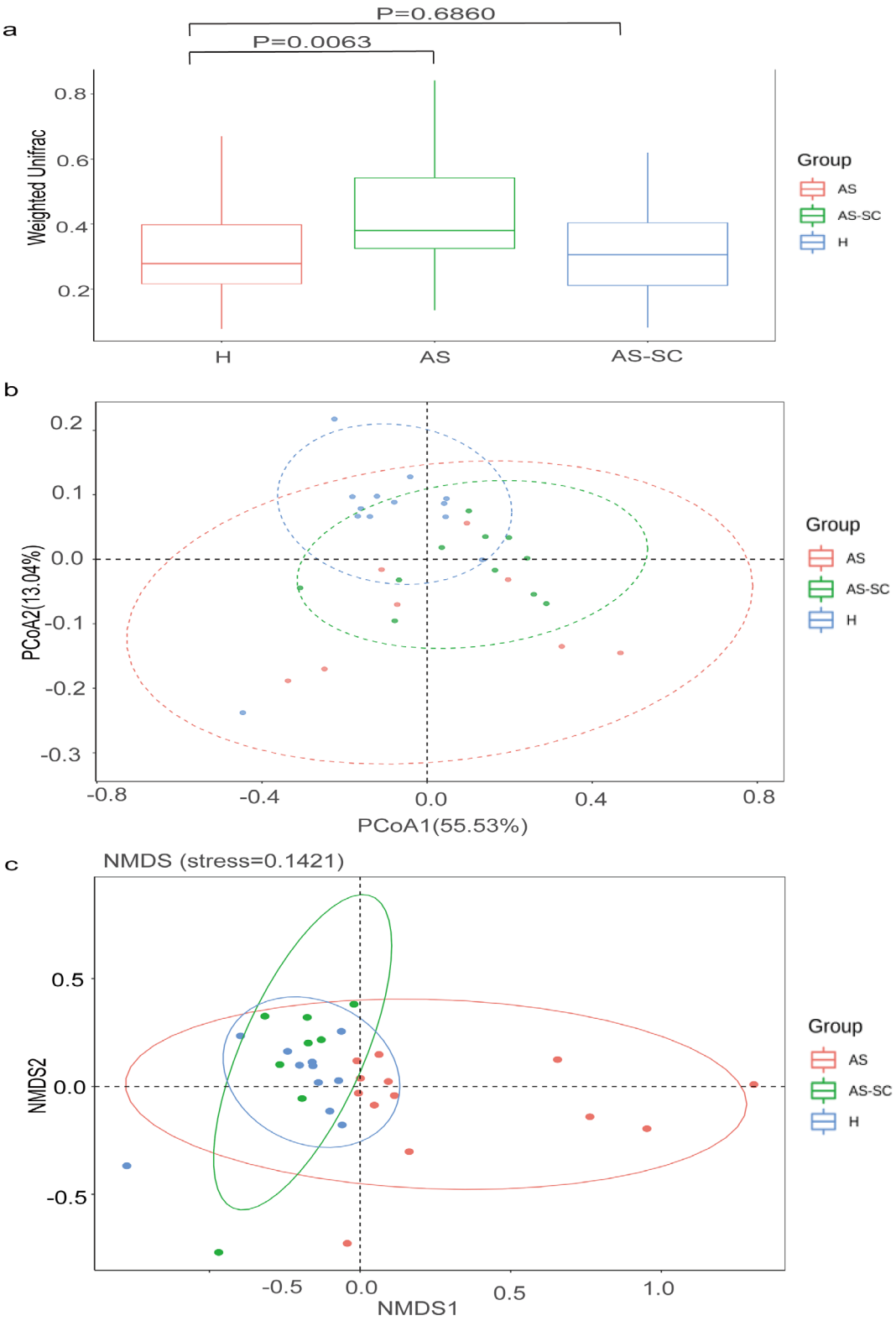

FIGURE 4 | Beta diversity of gut microbiota in healthy people, patients with advanced *S. japonicum* infection, and patients with advanced *S. japonicum* infection undergoing splenectomy. (a) Result based on weighted UniFrac (Wilcoxon, H vs. AS, $Z = -2.44$, $p = 0.0063$; H vs. AS-SC, $Z = 0.48$, $p = 0.6860$); (b) PCoA and (c) NMDS analysis. AS, advanced *S. japonicum* infection; AS-SC, advanced *S. japonicum* infection undergoing splenectomy; H, healthy people; NMDS, non-metric multidimensional scaling; PcoA, principal co-ordinates analysis.

Prevotella 9 has been observed in various diseases, such as depression [43] and IgA nephropathy [44]. *Prevotella* is involved in mood-related tryptophan and glutamate synthesis, thereby affecting patients with depression [43]. There is a significant difference in the amount of *Prevotella* in healthy controls and patients with IgA nephropathy, so it is considered to have the potential to be an effective biomarker for IgA nephropathy [45]. In summary, splenectomy changes the abundance of

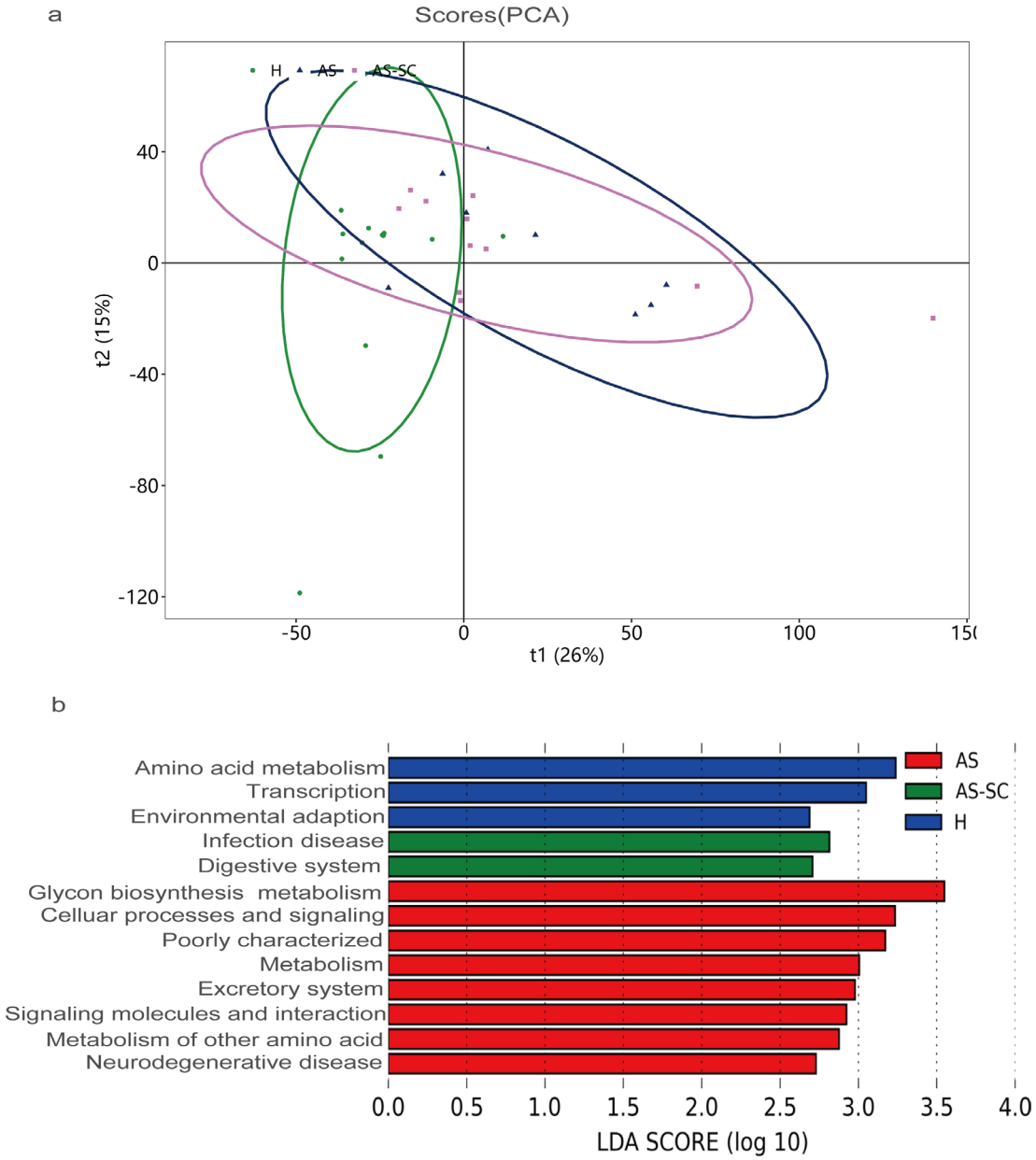

FIGURE 5 | Prediction of intestinal flora function in healthy people, patients with advanced *S. japonicum* infection, and patients with advanced *S. japonicum* infection undergoing splenectomy. (a) PCA analysis of three groups of patients based on KEGG database (ANOVA, $F = 6.86$, $p = 0.0036$). (b) Histogram showing the difference in the abundance of gut bacterial function between the three groups of patients based on KEGG database. AS, advanced *S. japonicum* infection; AS-SC, advanced *S. japonicum* infection undergoing splenectomy; H, healthy people; KEGG: Kyoto Encyclopedia of Genes and Genomes.

some key intestinal flora in patients with advanced *S. japonicum* infection, and the impact of this change on the patient's physiology and pathology deserves further study.

Various diseases often lead to diversity changes in the gut microbiota, and the diversity of gut microbiota is also closely related to the prognosis of diseases [46]. The gut microbiota often exhibits high alpha diversity among healthy people, while in a disease state, the alpha diversity of the intestinal flora often decreases, which will lead to the disease-related flora becoming the predominant bacteria and will enhance pathogenicity [47]. A previous study indicated that the intestinal flora of mice infected with *S. japonicum* showed low alpha diversity and increased beta diversity, which was consistent with our findings based on human specimens [23]. However, the reduction in bacterial diversity was less severe in patients who underwent splenectomy.

We further explored the effect of splenectomy on the physiological function of intestinal microbiota in patients with advanced *S. japonicum* infection. Our results showed that the physiological function of intestinal microbiota in patients with advanced *S. japonicum* infection who underwent splenectomy was closer to that of healthy people than patients who did not receive splenectomy. This suggested that splenectomy might effectively reverse some physiological functions which had been changed by *S. japonicum* infection. Based on the KEGG database, we found that the functional changes in patients with advanced *S. japonicum* infection were mainly focused on the disorder of energy metabolism and

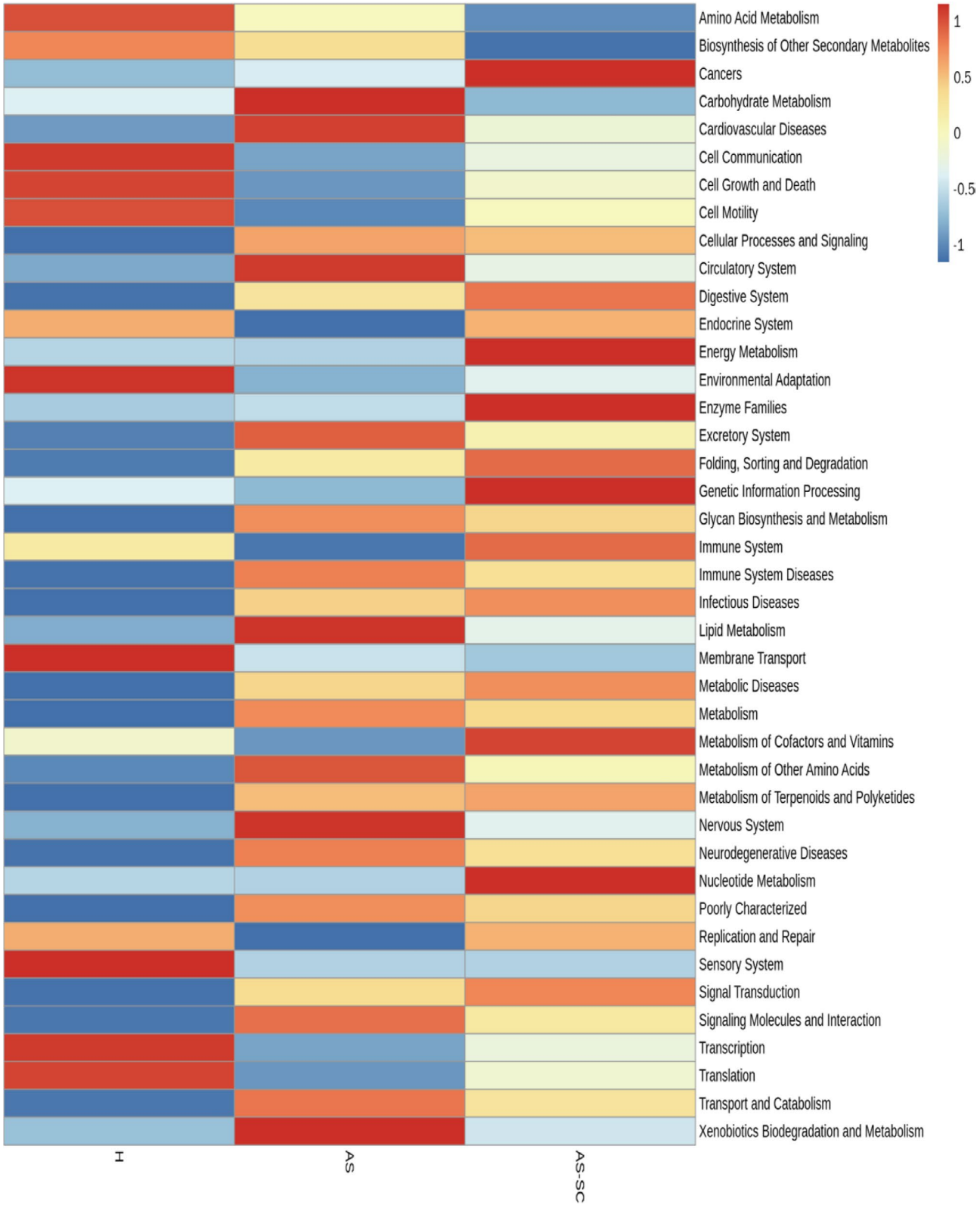

FIGURE 6 | Heat map of genes which remarkably change in three groups of patients. AS, advanced *S. japonicum* infection; AS-SC, advanced *S. japonicum* infection undergoing splenectomy; H, healthy people.

processes and signalling. In patients with advanced *S. japonicum* infection, a large number of hepatic parenchymal and non-parenchymal cells were in a pathological state and various cell signal transduction actions were often affected due to the severe degree of liver fibrosis [19]. Meanwhile, the liver function of patients with advanced *S. japonicum* infection entered a state of decompensation, and the energy metabolism of the liver was seriously affected [13]. The KEGG results were in good agreement with the clinical stage and clinical performance of those patients. In addition, we found that the function of different gut microbiota

in these patients with splenectomy was susceptible to Shigellosis, and their carbohydrate, vitamin, cofactor and riboflavin metabolic pathways were significantly changed. The spleen plays an important role in both innate immunity and adaptive immunity [48]. Although splenectomy may increase the risk of infection, there are no relevant retrospective studies to confirm whether there are more infectious events in patients with advanced *S. japonicum* infection. To summarise, compared with the healthy group, the biological function of gut microbiota of patients with advanced *S. japonicum* infection changed significantly. Even for patients with advanced *S. japonicum* infection, the changes in the biological functions of the flora were different depending on whether they had undergone splenectomy.

5 | Conclusions

This study systematically compared the intestinal flora characteristics of healthy people (H group), patients with advanced *S. japonicum* infection (AS group) and patients after splenectomy (AS-SC group). The results showed that the three groups of flora structures were separated. Functional prediction showed that the intestinal flora functions of patients with advanced *S. japonicum* infection and patients after splenectomy for advanced *S. japonicum* infection had different changes. By exploring the effect of splenectomy on intestinal flora in patients with advanced *S. japonicum* infection, it provides inspiration for further research and transformation.

Author Contributions

This experiment was conceived and designed by C.Z., P.Z. and Y.M. The data were processed by C.Z. The biological specimen processing was completed by C.Z. The patient recruitment and screening were completed by P.Z. The article was written by C.Z. Y.M. provided financial support for the project.

Acknowledgements

We would like to thank Professor Sun Yihong and Professor Wang Xuefei for their encouragement and recognition of Dr. Zhou in his growth.

Ethics Statement

The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments. All experiments in this study were approved by the Clinical Research Ethics Committee of the Third XiangYa Hospital of Central South University. All participants signed an informed consent form, and patient privacy was fully protected.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The sequencing data of the 16S rRNA gene and metagenome have been deposited in the NCBI Sequence Read Archive under the project number PRJNA1014933.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/pim.70008.

References

1. L. G. Song, X. Y. Wu, M. Sacko, and Z. D. Wu, "History of Schistosomiasis Epidemiology, Current Status, and Challenges in China: On the Road to Schistosomiasis Elimination," *Parasitology Research* 115, no. 11 (2016): 4071–4081.

2. Q. Li, N. Zhao, M. Liu, et al., "Comparative Analysis of Proteome-Wide Lysine Acetylation in Juvenile and Adult *Schistosoma japonicum*," *Frontiers in Microbiology* 8 (2017): 2248.

3. J. Wu, W. Xu, Z. Ming, H. Dong, H. Tang, and Y. Wang, "Metabolic Changes Reveal the Development of Schistosomiasis in Mice," *PLoS Neglected Tropical Diseases* 4, no. 8 (2010): e807.

4. A. G. Ross, P. B. Bartley, A. C. Sleigh, et al., "Schistosomiasis," *New England Journal of Medicine* 346, no. 16 (2002): 1212–1220, https://doi.org/10.1056/NEJMra012396.

5. F. Llanwarne and H. Helmby, "Granuloma Formation and Tissue Pathology in Schistosoma Japonicum Versus *Schistosoma mansoni* Infections," *Parasite Immunology* 43, no. 2 (2021): e12778.

6. C. Zhou, J. Li, C. Guo, et al., "Comparison of Intestinal Flora Between Patients With Chronic and Advanced Schistosoma Japonicum Infection," *Parasites & Vectors* 15, no. 1 (2022): 413.

7. C. Li, G. Ren, W. Deng, et al., "Prevalence and Incidence of Advanced Schistosomiasis and Risk Factors for Case Fatality in Hunan Province, China," *Acta Tropica* 217 (2021): 105862, https://doi.org/10.1016/j.actatropica.2021.105862.

8. A. Teixeira-Carvalho, O. A. Martins-Filho, Z. A. Andrade, J. R. Cunha-Mello, R. A. Wilson, and R. Correa-Oliveira, "The Study of T-Cell Activation in Peripheral Blood and Spleen of Hepatosplenic Patients Suggests an Exchange of Cells Between These Two Compartments in Advanced Human Schistosomiasis Mansoni Infection," *Scandinavian Journal of Immunology* 56, no. 3 (2002): 315–322.

9. A. Manzella, K. Ohtomo, S. Monzawa, and J. H. Lim, "Schistosomiasis of the Liver," *Abdominal Imaging* 33, no. 2 (2008): 144–150.

10. R. A. Isidro and L. Zhao, "Evolving Understanding of Noncirrhotic Portal Hypertension," *Surgical Pathology Clinics* 16, no. 3 (2023): 549–563.

11. F. Klotz, "Portal Hypertension and Schistosomiasis: 'An Originally Killing Entity'," *Bulletin de la Societe de Pathologie Exotique* 96, no. 3 (2003): 191–195.

12. Y. Li, D. Yu, Y. Li, et al., "A Study and Analysis of the Deaths due to Advanced Schistosoma Japonicum Infection in the Dongting Lake Area of China," *Journal of Tropical Medicine and Hygiene* 96, no. 2 (1993): 128–130.

13. W. Wu, A. Feng, and Y. Huang, "Research and Control of Advanced Schistosomiasis Japonica in China," *Parasitology Research* 114, no. 1 (2015): 17–27.

14. N. N. Nathan, D. J. Philpott, and S. E. Girardin, "The Intestinal Microbiota: From Health to Disease, and Back," *Microbes and Infection* 23, no. 6–7 (2021): 104849.

15. S. M. Jandhyala, R. Talukdar, C. Subramanyam, H. Vuyyuru, M. Sasikala, and R. D. Nageshwar, "Role of the Normal Gut Microbiota," *World Journal of Gastroenterology* 21, no. 29 (2015): 8787–8803.

16. Y. Jiang, Z. Yuan, Y. Shen, et al., "Alteration of the Fecal Microbiota in Chinese Patients With Schistosoma Japonicum Infection," *Parasite* 28 (2021): 1.

17. H. Huang, Z. Ren, X. Gao, et al., "Integrated Analysis of Microbiome and Host Transcriptome Reveals Correlations Between Gut Microbiota

and Clinical Outcomes in HBV-Related Hepatocellular Carcinoma," *Genome Medicine* 12, no. 1 (2020): 102.

18. M. Camilleri, K. Madsen, R. Spiller, B. Van Greenwood- Meerveld, and G. N. Verne, "Intestinal Barrier Function in Health and Gastrointestinal Disease," *Neurogastroenterology and Motility* 24, no. 6 (2012): 503–512, https://doi.org/10.1111/j.1365-2982.2012.01921.x.

19. Z. Liu, L. Zhang, Y. Liang, and L. Lu, "Pathology and Molecular Mechanisms of Schistosoma Japonicum-Associated Liver Fibrosis," *Frontiers in Cellular and Infection Microbiology* 12 (2022): 1035765.

20. D. Lin, Q. Song, J. Liu, et al., "Potential Gut Microbiota Features for Non-Invasive Detection of Schistosomiasis," *Frontiers in Immunology* 13 (2022): 941530.

21. H. Zhu, Y. Liu, S. Li, et al., "Altered Gut Microbiota After Traumatic Splenectomy Is Associated With Endotoxemia," *Emerging Microbes & Infections* 7, no. 1 (2018): 197.

22. Y. Liu, J. Li, Y. Jin, et al., "Splenectomy Leads to Amelioration of Altered Gut Microbiota and Metabolome in Liver Cirrhosis Patients," *Frontiers in Microbiology* 9 (2018): 963.

23. Y. Hu, J. Chen, Y. Xu, et al., "Alterations of Gut Microbiome and Metabolite Profiling in Mice Infected by Schistosoma Japonicum," *Frontiers in Immunology* 11 (2020): 569727.

24. C. Guo, P. Zhang, J. Li, et al., "The Characteristics of Intestinal Microbiota in Patients With Chronic Schistosomiasis Japonica-Induced Liver Fibrosis by 16S rRNA Gene Sequence," *Frontiers in Microbiology* 14 (2023): 1276404.

25. J. Hu, C. Wang, X. Huang, et al., "Gut Microbiota-Mediated Secondary Bile Acids Regulate Dendritic Cells to Attenuate Autoimmune Uveitis Through TGR5 Signaling," *Cell Reports* 36, no. 12 (2021): 109726.

26. R. C. Edgar, "UPARSE: Highly Accurate OTU Sequences From Microbial Amplicon Reads," *Nature Methods* 10, no. 10 (2013): 996–998.

27. J. G. Caporaso, J. Kuczynski, J. Stombaugh, et al., "QIIME Allows Analysis of High-Throughput Community Sequencing Data," *Nature Methods* 7, no. 5 (2010): 335–336.

28. L. J. Wang, C. Y. Yang, W. J. Chou, et al., "Gut Microbiota and Dietary Patterns in Children With Attention-Deficit/Hyperactivity Disorder," *European Child & Adolescent Psychiatry* 29, no. 3 (2020): 287–297.

29. S. C. Chang, S. F. Lin, S. T. Chen, et al., "Alterations of Gut Microbiota in Patients With Graves' Disease," *Frontiers in Cellular and Infection Microbiology* 11 (2021): 663131.

30. Z. P. Xu, H. Chang, Y. Y. Ni, et al., "Schistosoma Japonicum Infection Causes a Reprogramming of Glycolipid Metabolism in the Liver," *Parasites & Vectors* 12, no. 1 (2019): 388.

31. L. Zheng, L. Wang, Y. Hu, et al., "Higher Frequency of Circulating Vδ1 γδT Cells in Patients With Advanced Schistosomiasis," *Parasite Immunology* 43, no. 10–11 (2021): e12871.

32. H. Jiang, J. Zhou, M. Xia, et al., "Life Expectancy and Healthy Life Expectancy of Patients With Advanced Schistosomiasis in Hunan Province, China," *Infectious Diseases of Poverty* 12, no. 1 (2023): 4.

33. F. Q. Luo, J. X. Liu, G. Pan, P. Wang, G. J. Ding, and W. C. Deng, "Splenectomy and Esophagogastric Devascularization for Advanced Schistosomiasis With Cavernous Transformation of Portal Vein," *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi* 25, no. 6 (2013): 649–651.

34. C. Zhou, J. Li, C. Guo, et al., "Alterations in Gut Microbiome and Metabolite Profile of Patients With Schistosoma Japonicum Infection," *Parasites & Vectors* 16, no. 1 (2023): 346.

35. Q. F. Gui, H. L. Jin, F. Zhu, et al., "Gut Microbiota Signatures in Schistosoma Japonicum Infection-Induced Liver Cirrhosis Patients: A Case-Control Study," *Infectious Diseases of Poverty* 10, no. 1 (2021): 43.

36. G. D. Wu, J. Chen, C. Hoffmann, et al., "Linking Long-Term Dietary Patterns With Gut Microbial Enterotypes," *Science* 334, no. 6052 (2011): 105–108.

37. B. D. Kirby, W. L. George, V. L. Sutter, D. M. Citron, and S. M. Finegold, "Gram-Negative Anaerobic Bacilli: Their Role in Infection and Patterns of Susceptibility to Antimicrobial Agents. I. Little-Known Bacteroides Species," *Reviews of Infectious Diseases* 2, no. 6 (1980): 914–951.

38. I. Chattopadhyay, R. Dhar, K. Pethusamy, et al., "Exploring the Role of Gut Microbiome in Colon Cancer," *Applied Biochemistry and Biotechnology* 193, no. 6 (2021): 1780–1799.

39. L. Zhou, M. Zhang, Y. Wang, et al., "*Faecalibacterium prausnitzii* Produces Butyrate to Maintain Th17/Treg Balance and to Ameliorate Colorectal Colitis by Inhibiting Histone Deacetylase 1," *Inflammatory Bowel Diseases* 24, no. 9 (2018): 1926–1940.

40. R. M. Stilling, M. van de Wouw, G. Clarke, C. Stanton, T. G. Dinan, and J. F. Cryan, "The Neuropharmacology of Butyrate: The Bread and Butter of the Microbiota-Gut-Brain Axis?," *Neurochemistry International* 99 (2016): 110–132.

41. G. Li, J. Lin, C. Zhang, et al., "Microbiota Metabolite Butyrate Constrains Neutrophil Functions and Ameliorates Mucosal Inflammation in Inflammatory Bowel Disease," *Gut Microbes* 13, no. 1 (2021): 1968257.

42. C. G. Gonzalez, R. H. Mills, Q. Zhu, et al., "Location-Specific Signatures of Crohn's Disease at a Multi-Omics Scale," *Microbiome* 10, no. 1 (2022): 133.

43. X. Yuan, B. Chen, Z. Duan, et al., "Depression and Anxiety in Patients With Active Ulcerative Colitis: Crosstalk of Gut Microbiota, Metabolomics and Proteomics," *Gut Microbes* 13, no. 1 (2021): 1987779.

44. S. Han, L. Shang, Y. Lu, and Y. Wang, "Gut Microbiome Characteristics in IgA Nephropathy: Qualitative and Quantitative Analysis From Observational Studies," *Frontiers in Cellular and Infection Microbiology* 12 (2022): 904401.

45. X. Liang, S. Zhang, D. Zhang, et al., "Metagenomics-Based Systematic Analysis Reveals That Gut Microbiota Gd-IgA1-Associated Enzymes May Play a Key Role in IgA Nephropathy," *Frontiers in Molecular Biosciences* 9 (2022): 970723.

46. E. Z. Gomaa, "Human Gut Microbiota/Microbiome in Health and Diseases: A Review," *Antonie Van Leeuwenhoek* 113, no. 12 (2020): 2019–2040.

47. G. A. Kuziel and S. Rakoff-Nahoum, "The Gut Microbiome," *Current Biology* 32, no. 6 (2022): R257–R264.

48. R. E. Mebius and G. Kraal, "Structure and Function of the Spleen," *Nature Reviews. Immunology* 5, no. 8 (2005): 606–616.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.