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Research Article
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Exploring the links between gut microbiome changes and irritable bowel syndrome in Han populations in the Tibetan Plateau

Xingguang ZHANG[1,2], Wei XU[2], Weilong ZHONG[1], Wencheng ZHANG[2], Cheng YANG[2], Lisa DUAN[2], Haiyan NIU[2], Yanmei DONG[2], Taotao LIU[2], Shihai XIA[2✉], Bangmao WANG[1✉]

[1]*Department of Gastroenterology and Hepatology, Tianjin Medical University General Hospital, Tianjin Institute of Digestive Diseases, Tianjin Key Laboratory of Digestive Diseases, Tianjin 300052, China*
[2]*Department of Gastroenterology, Characteristic Medical Center of Chinese People's Armed Police Force, Tianjin Key Laboratory of Hepatopancreatic Fiberosis and Molecular Diagnosis & Treatment, Tianjin 300162, China*

Abstract: The gut microbiome shows changes under a plateau environment, while the disbalance of intestinal microbiota plays an important role in the pathogenesis of irritable bowel syndrome (IBS); however, the relationship between the two remains unexplored. In this work, we followed up a healthy cohort for up to a year before and after living in a plateau environment and performed 16S ribosomal RNA (rRNA) sequencing analysis of their fecal samples. Through evaluating the participants' clinical symptoms, combined with an IBS questionnaire, we screened the IBS sub-population in our cohort. The sequencing results showed that a high-altitude environment could lead to changes in the diversity and composition of gut flora. In addition, we found that the longer the time volunteers spent in the plateau environment, the more similar their gut microbiota composition and abundance became compared to those before entering the plateau, and IBS symptoms were significantly alleviated. Therefore, we speculated that the plateau may be a special environment that induces IBS. The taxonomic units g_*Alistipes*, g_*Oscillospira*, and s_*Ruminococcus_torques*, which had been proved to play important roles in IBS pathogenesis, were also abundant in the IBS cohort at high altitudes. Overall, the disbalance of gut microbiota induced by the plateau environment contributed to the high frequency of IBS and the psychosocial abnormalities associated with IBS. Our results prompt further research to elucidate the relevant mechanism.

Key words: Gut microbiome; Plateau environment; Irritable bowel syndrome

1 Introduction

Tibet Autonomous Region in China, with an average altitude of over 4000 m, features the typical characteristics of a plateau environment with low temperature, low atmospheric and oxygen partial pressures. Tibetans have a unique genetic composition and developed special lifestyles and dietary habits that have allowed them to adapt to the special environment of the plateau. In recent years, easy access to transportation has led to an increasing number of people traveling from the plains to the plateau, challenging their mental and physical health, which often resulted in altitude illness. Gastrointestinal disorders such as anorexia, stomach pain, dyspepsia, gastroesophageal reflux, and diarrhoea are common and possibly overlooked as transient conditions. Hypoxia and cold stress can induce acute gastric mucosal lesions, which worsens with increasing altitude (He et al., 2004; Wu et al., 2007). Diarrhoea has an incidence of 10%–14% among highland travellers and workers as a type of common disease, which involves the dysbiosis of gut microbiome related to the harsh environment (Basnyat and Starling, 2015). High altitude also affects the human immune system, disrupting mucosal immunity by weakening the mucosal barrier of the intestine (Khanna et al., 2018).

The ratio of resident bacteria to human cells is close to 1:1. This microbiota is also called the "second genome" of the human body (Sender et al., 2016). A

✉ Bangmao WANG, mwang02@tmu.edu.cn
Shihai XIA, xshhcx@sina.com
Bangmao WANG, https://orcid.org/0000-0002-4702-9711
Shihai XIA, https://orcid.org/0000-0002-3748-4841

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healthy microbiome composition plays a vital role in building and activating the immune system to fight pathogens (Altveş et al., 2020). The interplay of different genetic and environmental factors may be important in shaping the composition and function of gut microbiome in high-altitude populations (Li et al., 2020; Liu K et al., 2020). With increasing altitude, body mass index (BMI), and age, there are significant differences in microbial diversity and richness among different regions of Tibet in terms of gut microbiome; for example, facultative anaerobes were more abundant in Tibetan people's guts (Lan et al., 2017). Also, the microbiome of high-altitude populations had higher energy efficiency and was rich in butyric acid-producing bacteria, suggesting that both altitude and genetic/cultural background had significant effects on microbial composition (Li et al., 2016). The high-altitude environment rapidly shaped the intestinal microbiome of newly settled Han population, which became increasingly like those of Tibetans and adapted to life on the plateau (Jia et al., 2020).

Irritable bowel syndrome (IBS) is a functional bowel disease characterized by abdominal discomfort or pain and altered bowel habits. The mechanism of IBS has not been fully elucidated but is currently thought to involve a combination of factors causing abnormal bowel–brain interactions, among which the gut microbiome is important in the aetiology of IBS. Compared with healthy individuals, patients with IBS displayed a dysbiosis of intestinal microorganisms (Labus et al., 2019; Pittayanon et al., 2019), which indirectly suggests that dysbiosis is a possible factor in the aetiology of IBS (Crouzet et al., 2013). Population studies are more relevant for developing treatments as there are significant differences between animal models of IBS and human IBS in terms of gut microbiome, mucosal immune system, diet, socialization, and psychological status (Liu et al., 2014; Vannucchi and Evangelista, 2018). The prevalence of IBS meeting the Rome IV criteria in the Chinese population was 2.3% via an internet survey and 1.4% from household surveys (Sperber et al., 2021), but it was 11.9% and 16.4% in Lhasa (altitude 3650 m) and the highland cold region, respectively, higher than that in the plain region (Yu et al., 2019; Wang et al., 2020).

In this study, we screened the IBS sub-population by evaluating the clinical symptoms. In addition, fecal samples were collected and the gut microbiome was analyzed using 16S ribosomal RNA (rRNA) sequencing. We found that a high-altitude environment can lead to significant changes in the gut microbiome, and there was an evident relationship between the changed gut microbiome and IBS. We explored the aetiological mechanism of gut microbiome changes in IBS and provided a theoretical basis for the application of probiotic drug therapy in IBS patients.

2 Study cohort and methods

This was an observational study, and hence the investigators did not interfere with any clinical diagnosis or treatment during the follow-up, sample collection or questionnaires. In our microbiome analyses, we conformed to well-accepted scientific ideas and rationality in the experimental design and inclusion criteria. The participants maintained basic consistency in their diet, living conditions, lifestyle, and nature of work, largely eliminating differences due to these important factors. One hundred and twenty-two male Han Chinese participants coming from an altitude of 100–1000 m were recruited, of which 92 participants (labelled as GH group) migrated to an average altitude of approximately 3650 m of the Lhasa area in the Tibetan Plateau (hereinafter plateau), and another 30 participants (labelled as J.0 M group) as the control group migrated to the Jinan District in Tianjin with an altitude of 10–100 m (hereinafter plain). Further 25 male Tibetan participants (labelled as Z group) coming from different areas of the Tibetan Plateau with altitudes of 3000–4500 m were recruited and migrated to Lhasa. The Tibetans were followed up at 3, 6, and 12 months, which stages were respectively labelled as Z.3 M, Z.6 M, and Z.12 M groups. The Han Chinese who entered Lhasa were followed up at 3, 6, and 12 months, which stages were respectively labelled as GH.3 M, GH.6 M, and GH.12 M groups. During the one-year follow-up, the numbers of participants were 92 in the GH group and 25 in the Z group at 3 months, 88 in the GH group and 9 in the Z group at 6 months, and 81 in the GH group and 9 in the Z group at 12 months (Fig. 1).

Of the 81 Han participants, 13 with IBS, 12 with nonspecific gastrointestinal symptoms, and 56 that remained healthy were included. Subsequently, 11 asymptomatic participants were randomly selected from the 56 healthy participants as the control group, and thereby

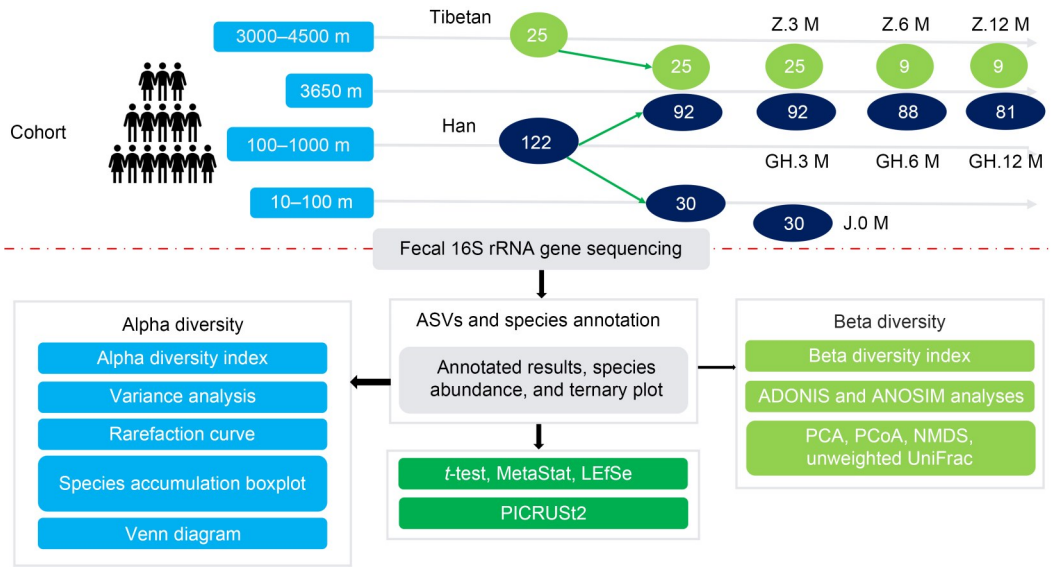

Fig. 1 Prospective population experimental design and fecal analysis flowchart. The fecal samples were collected and subjected to 16S ribosomal RNA (rRNA) gene sequencing followed by diversity analysis, bacterial composition analysis, functional prediction, and differential analysis. ASVs: amplicon sequence variants; LEfSe: linear discriminant analysis (LDA) effect size; ADONIS: permutational multivariate analysis of variance; ANOSIM: analysis of similarities; PCA: principal component analysis; PCoA: principal co-ordinates analysis; NMDS: non-metric multi-dimensional scaling.

36 Han Chinese participants were finally included in the analyses. However, four cases were excluded due to ineligible fecal samples at 12 months. Twenty-five Tibetans in the Z.3 M group, 9 Tibetans in the Z.6 M group, 9 Tibetans in the Z.12 M group, and 15 Han Chinese in the J.0 M group were also included in the data analysis. Compared with the Z.3 M and J.0 M groups, the heart rate per minute was significantly higher in GH.3 M (P=0.009). Compared with the Z.3 M and GH.3 M groups, finger oxygen saturation was significantly higher in the J.0 M group (P<0.001). However, there were no significant differences between the GH.3 M, Z.3 M, and J.0 M groups in terms of age, BMI, education, or dietary habits (P>0.05; Table 1).

All participants completed the Patient Health Questionnaire-15 (PHQ-15) and Stress Response Questionnaire (SRQ). They maintained exercise 4–5 times per week for 30–60 min each time, slept 8–10 h per day, and did not take antibiotics or probiotics during the study period. All fecal samples were collected in special preservation tubes to ensure adequate mixing with the preservation solution, and then stored and transported for 16S rRNA gene sequencing in a timely manner (Ningbo Aijie Conning Biotechnology Co., Ltd., Ningbo, China; product record No. 20200417). The 16S rRNA gene sequencing protocol was carried out at Novogene Co., Ltd., Beijing, China (H101SC20122539), and the detailed methods were provided in the supplementary file (16S rRNA gene sequencing).

3 Results

3.1 Subject baseline characteristics

The detailed schematic of the study is shown in Fig. 1. A total of 162 fecal samples were sequenced on an Illumina NovaSeq platform, amplicon sequence variants (ASVs) were generated, and a total of 17 446 044 high-quality sequences (raw data) were obtained. After stitching, filtering, and noise reduction of the raw data, 14 567 917 high-quality sequences (Nochime; average 104 321, ranging between 46 585 and 114 175 reads per sample) were used for species annotation and analysis (Table S1). The final numbers of ASVs obtained for analysis were 1845 in the J.0 M group, 9885 in GH.3 M, 2710 in GH.6 M, 1691 in GH.12 M, 6396 in Z.3 M, 1293 in Z.6 M, and 983 in Z.12 M. The Venn diagram showed that the J.0 M, GH.3 M, and Z.3 M groups had 618 shared ASVs, while the GH.3 M group had 5880 unique ASVs, the Z.3 M group had 2482, and the J.0 M group had 1030. The GH.3 M, GH.6 M, and GH.12 M groups had 749 shared ASVs, while the GH.3 M group had 8433 unique ASVs, the GH.6 M group had 1231, and the GH.12 M group had 687.

Table 1 Comparison of general clinical data between the GH and Z groups living in the plateau and the control J group living in the plain

Group	Age (years)	BMI			Academic education		Dietary habits			Heart rate (beats/min)	Finger oxygen saturation (%)
		Mean±SD (kg/m^2)	18.5–23.9 kg/m^2	24.0–27.9 kg/m^2	High school	University	More meat	More vegetables	A balance of both		
GH											
GH.3 M (*n*=36)	20.08±1.46	22.03±1.92	31	5	15	21	4	2	30	71.56±10.91	89.28±3.23
GH.6 M (*n*=36)	20.08±1.46	22.03±1.92	31	5	15	21	4	2	30	71.56±10.91	89.28±3.23
GH.12 M (*n*=32)	20.09±1.44	22.01±1.99	28	4	13	19	4	2	26	71.59±11.32	89.27±3.23
Z											
Z.3 M (*n*=25)	19.92±1.93	21.31±1.75	22	3	16	9	4	1	20	64.52±8.14	90.96±3.49
Z.6 M (*n*=9)	20.44±2.29	20.71±1.41	8	1	6	3	1	1	7	64.33±8.35	90.66±3.84
Z.12 M (*n*=9)	20.44±2.29	20.71±1.41	8	1	6	3	1	1	7	64.33±8.35	90.66±3.84
J											
J.0 M (*n*=15)	19.40±1.45	22.91±1.84	10	5	7	8	2	1	12	64.00±5.43	97.47±1.92
Comparison	GH.3 M vs. Z.3 M vs. J.0 M				GH.3 M vs. Z.6 M vs. J.0 M					GH.3 M vs. Z.3 M vs. J.0 M	
Statistical value	F=0.934	X^2=3.094, *df*=2			X^2=1.824, *df*=2		X^2=0.373, *df*=4			F=7.148	F=36.961
P value	0.398	0.213			0.402		0.985			0.009	<0.001

Data are expressed as mean±standard deviation (SD) or number. $P<0.05$ represents statistical significance (Chi-square test or *F*-test). BMI: body mass index; *df*: degree of freedom.

Meanwhile, the Z.3 M, Z.6 M, and Z.12 M groups had 545 shared ASVs, while the Z.3 M group had 5444 unique ASVs, the Z.6 M group had 393, and the Z.12 M group had 284 (Fig. 2). The Venn diagram showed that after the participants migrated to Lhasa, the number of total or unique ASVs was the highest at 3 months for both Han and Tibetans and it showed a consistent decline over time at 6 and 12 months.

3.2 Diversity of the human gut microbiome in the plateau environment

The rarefaction curve and species accumulation boxplot illustrated that the amount of sequenced data was reasonable and that the sampling was sufficient for data analysis (Fig. S1). The Shannon index analysis showed that the alpha diversity of the GH.3 M group was higher than those of the GH.6 M and GH.12 M groups ($P=1.846948\times10^{-4}$ and $P=7.840129\times10^{-7}$, respectively). Interestingly, however, the alpha diversity was very close or slightly decreased in the GH.12 M group compared with the J.0 M group ($P=5.996\,522\times10^{-1}$; Fig. 3a). Compared with the GH.3 M group, the Chao1 index significantly declined from GH.6 M to GH.12 M ($P=6.448\,412\times10^{-8}$ and $P=8.725\,816\times10^{-10}$, respectively). Compared with the Z.3 M group, the Chao1 index gradually decreased and the difference was significant in the Z.12 M group ($P=3.54\,539\times10^{-4}$; Fig. 3b). The observed operational taxonomic units (OTUs) tended to decrease gradually with the prolongation of the plateau stay both in the GH and Z groups and showed similar results as the Chao1 index (Fig. 3c).

The distance matrix heatmap showed significantly different coefficients of dissimilarity between the samples (Fig. 4a). Principal co-ordinates analysis (PCoA) revealed that the J.0 M, GH.3 M, and GH.6 M presented three representative groups. The Z.3 M group was observed to be close to or clustered in the area of the GH.3 M group, and the GH.12 M group was closest to the J.0 M and Z.12 M groups (Fig. 4b). Non-metric multi-dimensional scaling (NMDS) analysis presented

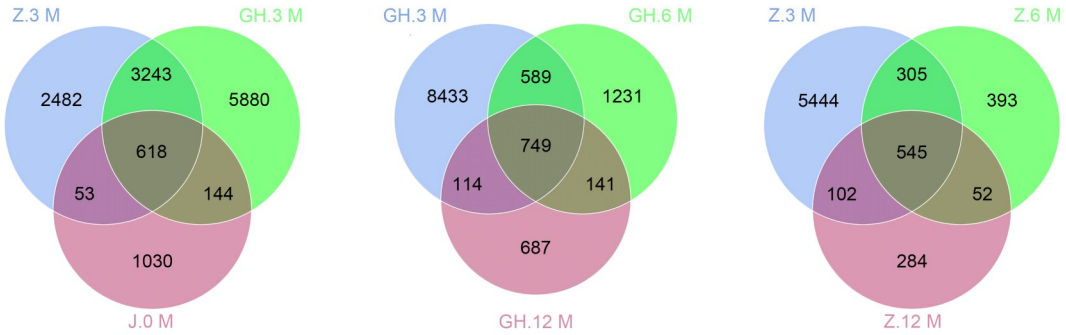

Fig. 2 ASVs for each group on the Venn diagram. Each circle in the figure represents a group of samples, the numbers in the overlapping part of circles and circles represent the numbers of ASVs shared between sample groups, and the numbers in the non-overlapping part represent the numbers of ASVs unique to the sample groups. ASVs: amplicon sequence variants.

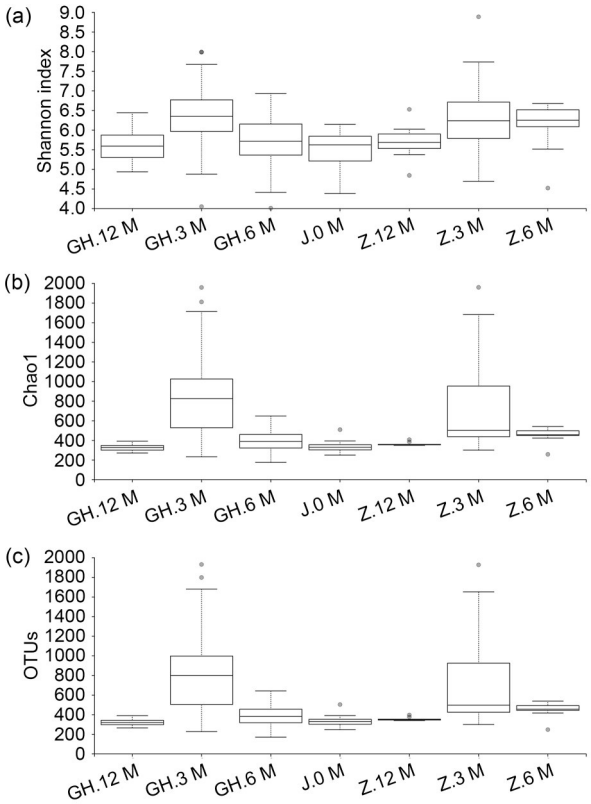

Fig. 3 Richness, diversity, and evenness of microbial communities among different groups. (a) The Shannon index was higher in the GH.3 M and Z.3 M groups than in the J.0 M group, and then gradually decreased toward the GH.12 M and Z.12 M groups and was finally close to that of the J.0 M group. (b, c) The Chao1 and observed index of OTUs showed similar results to the Shannon index. Discrete points in box plots reflect outliers for species diversity within the group. OTUs: operational taxonomic units.

similar degrees of species difference between groups as the PCoA analysis (Fig. 4c). Analysis of similarities (ANOSIM) analysis illustrated that the J.0 M group had significantly lower R values than the GH.3 M, GH.6 M, and GH.12 M groups (R=0.453 696, R=0.356 417, and R=0.717 596, respectively; all P=0.004 975), and there was a highly significant difference between GH.3 M and GH.6 M (R=0.498 653, P=0.004 975), so the GH.3 M and Z.3 M groups had higher R than the other groups and resembled the apex of the parabola, while the J.0 M, GH.12 M, and Z.12 M groups presented the two ends of the parabola (Fig. 4d).

Data analysis revealed that the diversity and richness of the gut microbiome of Han Chinese increased significantly at 3 months of migration to the plateau from the plains; it showed a significant decline at 6 months of observation, and at 12 months the decline approached a similar level as that before entering the plateau. Even for Tibetans who had lived on the plateau for a long time, the diversity and richness of the gut microbiome also changed with the environment and then, over time, they returned to levels close to those before the environmental change.

3.3 Effects of the plateau environment on the composition of human gut microbiome

3.3.1 Differences in gut microbiome composition between the GH.3 M, J.0 M, and Z.3 M groups

At the phylum level, the top 10 species were Firmicutes (47.57%), Bacteroidota (39.70%), Proteobacteria (6.40%), Actinobacteriota (4.85%), Fusobacteriota (0.33%), Acidobacteriota (0.15%), Cyanobacteria (0.25%), Verrucomicrobiota (0.24%), Desulfobacterota (0.18%), and Gemmatimonadota (0.06%) (Fig. 5a). There were 39 species in the GH.3 M group that were more abundant compared to the J.0 M group by the MetaStat method, and six of the top 10 phyla were

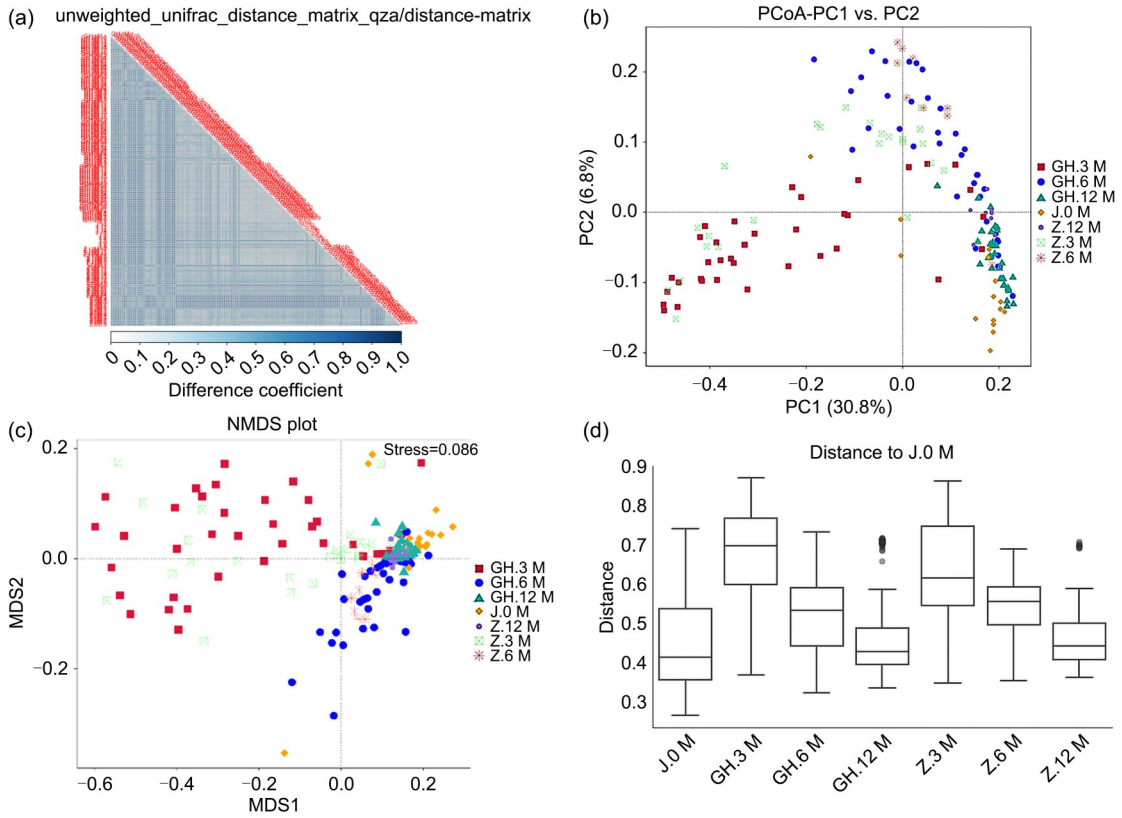

Fig. 4 Beta diversity analysis of microbial communities among different groups. (a) Heatmap with unweighted distance matrix showing the differences in the species diversity of samples. (b, c) PCoA and NMDS based on unweighted UniFrac distance illustrated that J.0 M, GH.3 M, and GH.6 M presented three representative groups. (d) ANOSIM function analysis showed that each group was significantly different from the other groups. PCoA: principal co-ordinates analysis; NMDS: non-metric multi-dimensional scaling; ANOSIM: analysis of similarities.

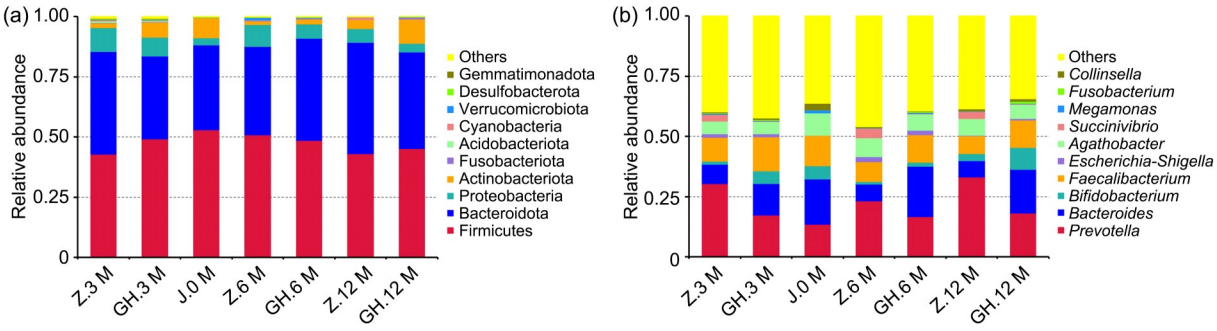

Fig. 5 Bacterial composition of different groups at the phylum (a) and genus (b) levels, where the horizontal coordinates show the different groups, the different colors of the vertical coordinates indicate the relative abundance corresponding to different bacteria, and "Others" refer to the sum of the relative abundance of all other bacteria except the top 10 taxa.

Acidobacteriota, Desulfobacterota, Fusobacteriota, Gemmatimonadota, Proteobacteria, and Verrucomicrobiota. There were only environmental differences linked to altitude between the J.0 M and GH.3 M groups, confirming the change in the composition of the gut microbiome after living in the plateau for 3 months. There were 13 phyla with statistically significant differences between the Z.3 M and GH.3 M groups, and three of the top 10 phyla in the GH.3 M group were more frequent than those in the Z.3 M group, such as Actinobacteriota, Desulfobacterota, and Firmicutes, whereas Bacteroidota in the Z.3 M group was more abundant compared to the GH.3 M group. The living environment, diet, and work of Tibetan and Han people

after entering Lhasa were basically the same, and the difference between GH.3 M and Z.3 M in gut microbial composition might be because of the genes responsible for adapting to the new environment. The relative abundances of the top 10 phyla were compared between the GH.3 M, Z.3 M, and J.0 M groups by linear discriminant analysis (LDA) effect size (LEfSe). The results showed that Firmicutes and Actinobacteriota in the J.0 M group were more abundant compared to the GH.3 M and Z.3 M groups, and Bacteroidota and Proteobacteria in the Z.3 M group were more abundant compared to the J.0 M and GH.3 M groups. Firmicutes/Bacteroidota (F/B) was significantly higher in the J.0 M and GH.3 M groups than in the Z.3 M group (P=0.006), but there was no significant difference between the J.0 M and GH.3 M groups.

At the genus level, the top 10 taxa were as follows: *Prevotella* (21.80%), *Bacteroides* (13.24%), *Bifidobacterium* (3.80%), *Faecalibacterium* (10.76%), *Escherichia-Shigella* (1.07%), *Agathobacter* (6.72%), *Succinivibrio* (1.50%), *Megamonas* (0.42%), *Fusobacterium* (0.29%), and *Collinsella* (0.89%) (Fig. 5b). LEfSe analysis showed that *Bacteroides*, *Bifidobacterium*, *Agathobacter*, and *Collinsella* were more abundant in the J.0 M group than in the GH.3 M and Z.3 M groups, while *Prevotella* and *Succinivibrio* were significantly enriched in the Z.3 M group. Compared with the GH.3 M and J.0 M groups, *Prevotella*/*Bacteroides* (P/B) was significantly higher in the Z.3 M group. The LEfSe analysis showed that Bacilli at the class level were significantly higher in the GH.3 M group than in the J.0 M group. Differences in the top 10 taxa of the three groups were presented on the ternary plot and determined by LEfSe (Fig. 6).

The comparison of gut microbes of Han Chinese who had lived in the plateau for 3 months with those of Tibetan and Han Chinese co-inhabitants living on the plain confirmed that genes and plateau environmental factors play crucial roles in the compositional alteration of gut microbes.

3.3.2 Differences in gut microbiome composition between the J.0 M, GH.3 M, GH.6 M, and GH.12 M groups

The environment in which the J.0 M group had been living in the plain was similar to that of the GH group before entering the plateau; that is, the results of the J.0 M, GH.3 M, GH.6 M, and GH.12 M groups represented the changes in gut microbiome of Han Chinese at 0, 3, 6, and 12 months after entering the plateau, respectively. During the one-year observation period, among the top 10 bacterial phyla, six (Proteobacteria, Acidobacteriota, Cyanobacteria, Verrucomicrobiota, Desulfobacterota, and Gemmatimonadota) showed an increasing trend in relative abundance from the J.0 M group to the GH.3 M group, followed by a decreasing trend from the GH.3 M group to GH.12 M group. Firmicutes showed a decreasing trend from the J.0 M group to the GH.12 M group, with the J.0 M group exhibiting higher abundance than the GH.12 M group. Bacteroidota showed an increasing trend from the GH.3 M group to the GH.6 M group and a decreasing trend to the GH.12 M group. However, Fusobacteriota consistently exhibited a increasing trend with significant differences from the J.0 M group to the GH.12 M group (Fig. 7). At the genus level, the relative abundances of six (*Prevotella*, *Bacteroides*, *Bifidobacterium*, *Faecalibacterium*, *Agathobacter*, and *Collinsella*) of the top 10 bacteria from the J.0 M group to the GH.12 M group either increased or decreased, and returned to a level in the GH.12 M group close to that in the J.0 M group, with no statistically significant difference. *Bifidobacterium* and *Collinsella* showed a statistically significant decrease from the J.0 M group to the GH.6 M group and then a gradual increase from the GH.6 M group to the GH.12 M group. Notably, *Succinivibrio* was absent in people before entering the plateau, while it increased significantly and remained stable thereafter (Fig. 8).

Regardless of the taxonomic level, the composition of a higher proportion of gut microbes changed adaptively during 3–6 months of entering the plateau environment in Han Chinese and then reverted over months 6–12 back to the condition seen before entering the plateau. Therefore, it is possible that genes, immunity, and other factors play important roles in maintaining the balance of gut microbiome.

3.3.3 Differences in gut microbiome composition between the Z.3 M, Z.6 M, and Z.12 M groups

The ratio of F/B increased significantly from Group Z.3 M to Group Z.6 M (F=13.399, P=0.023), and then it decreased gradually from Group Z.6 M to Group Z.12 M and dropped back to nearly that of Group Z.3 M, with no statistically significant difference. Comparing the Z.3 M, Z.6 M, and Z.12 M groups

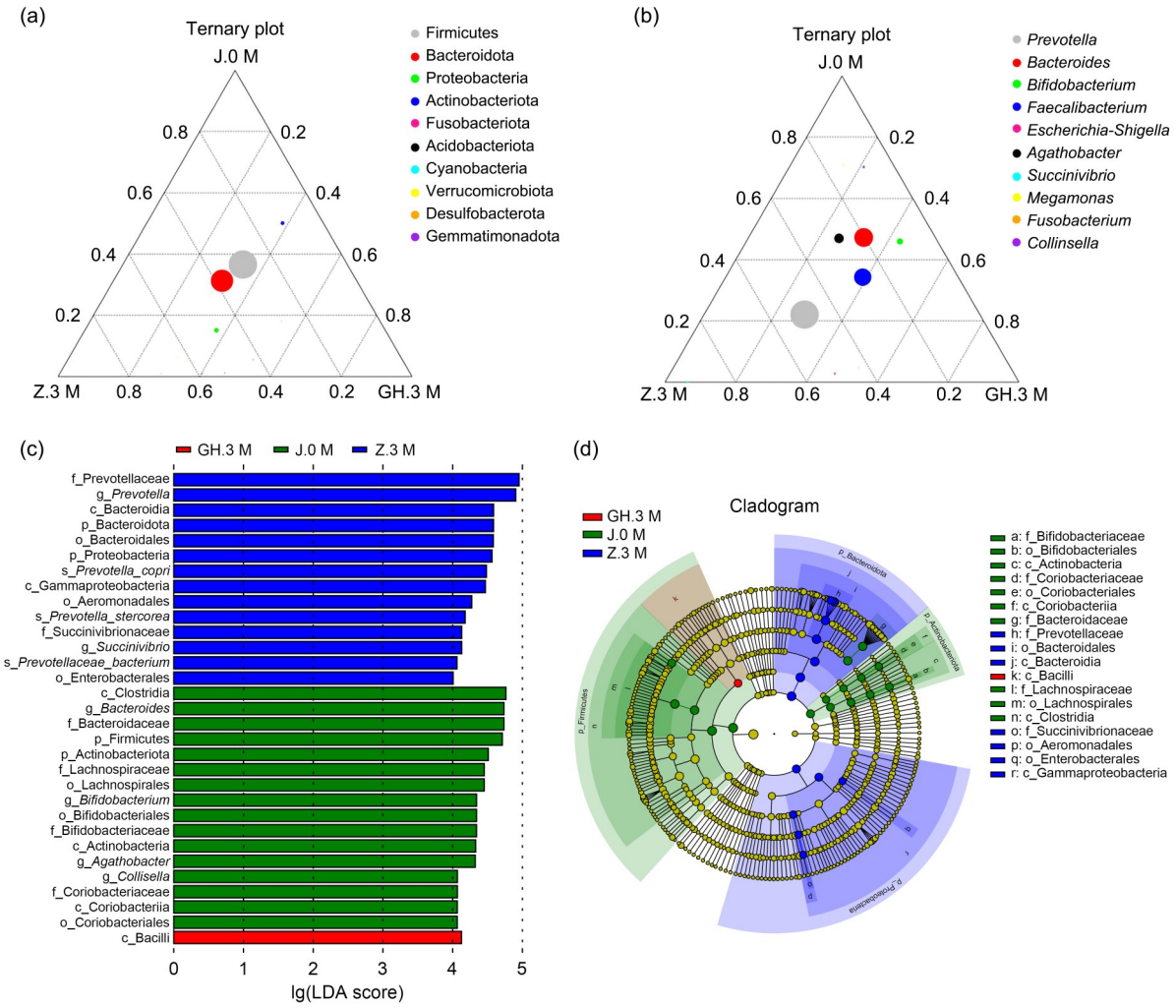

Fig. 6 Differences in the top 10 taxa of the three groups presented on the ternary plot and determined by linear discriminant analysis (LDA) effect size (LEfSe). (a) At the phylum level, Firmicutes and Actinobacteriota were significantly more abundant in the J.0 M group, and Bacteroidota and Proteobacteria were significantly enhanced in the Z.3 M group. (b) At the genus level, *Bacteroides*, *Bifidobacterium*, *Agathobacter*, and *Collinsella* were significantly higher in the J.0 M group, whereas *Prevotella* and *Succinivibrio* were significantly more abundant in the Z.3 M group. (c) Taxa with LDA scores greater than the set value (set to 4 by default) were illustrated in the histogram of the LDA value distribution. (d) Evolutionary branching diagram radiating from inner to outer circles represent taxonomic levels from phylum to species, with differential species as biomarkers indicated in different colors.

by LEfSe analysis, p_Actinobacteriota and g_*Alloprevotella* in the Z.12 M group were higher than those in the Z.3 M and Z.6 M groups. Compared with the Z.3 M and Z.12 M groups, *Escherichia-Shigella* was significantly enriched in the Z.6 M group. We performed continuous dynamic observation at 3, 6, and 12 months in the Tibetan population under altered dietary patterns, and identified altered gut microbial composition, but mostly for taxa with low relative abundance. Moreover, it was noteworthy that the ratio of F/B indicated an altered adaptation.

3.4 Relationship between the human gut microbiome and IBS in the plateau environment

3.4.1 Differences in gut microbiome composition between different groups

At the 12-month follow-up, the complete data of 81 participants were collected, and 11 participants were excluded due to incomplete data. We found 13 cases diagnosed with IBS, with an annual incidence of 16.05%; among them, 8 had diarrhoea predominant irritable bowel syndrome (IBS-D) and 5 presented mixed

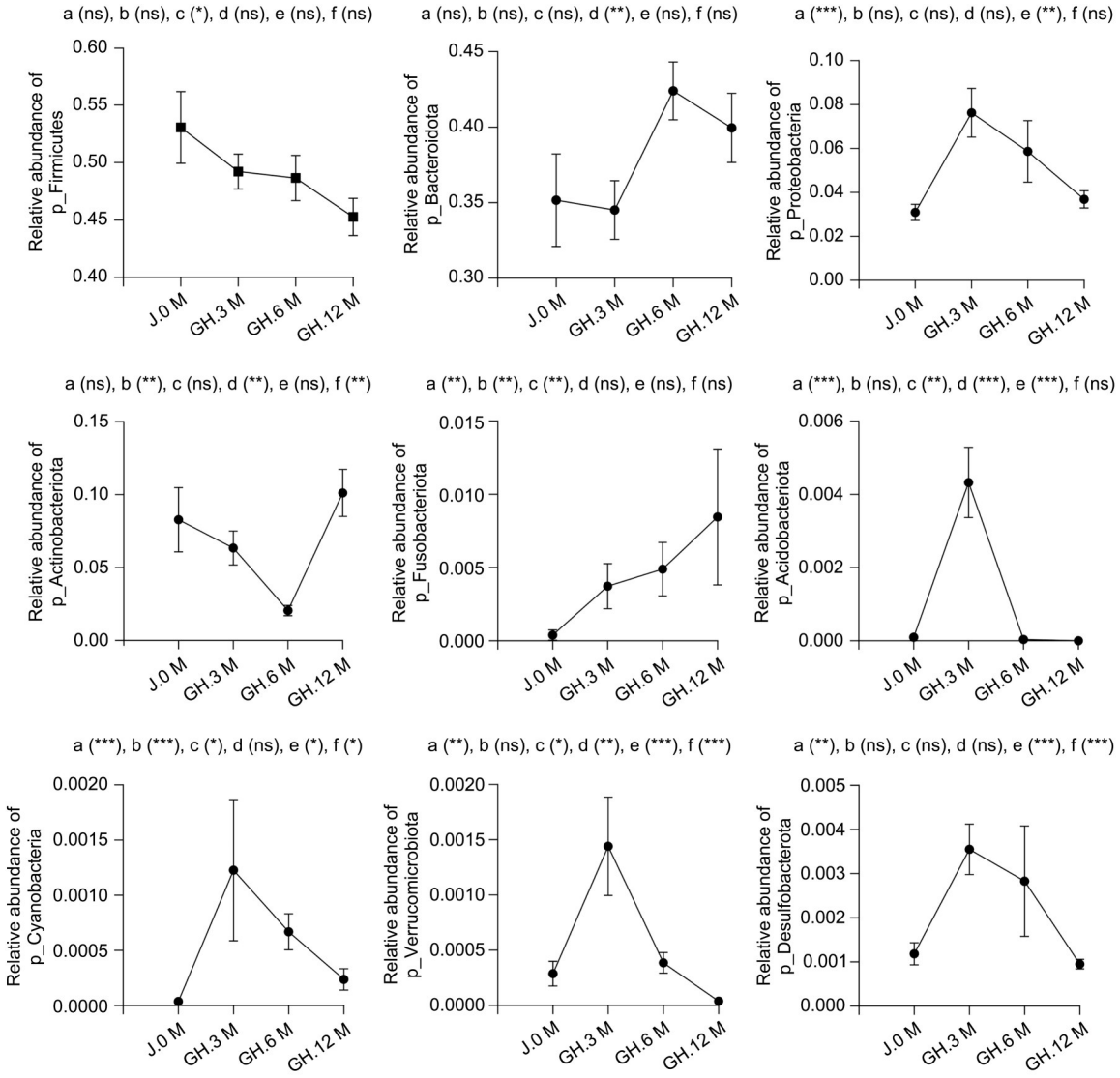

Fig. 7 Trends in the relative abundances of the top 9 bacteria between groups at the phylum level during the one-year observation period. A line graph was used to show the change in the relative abundance of bacteria at different time. The statistical differences obtained by the MetaStat method were depicted in the annotations at the top of the figure: a (J.0 M vs. GH.3 M), b (J.0 M vs. GH.6 M), c (J.0 M vs. GH.12 M), d (GH.3 M vs. GH.6 M), e (GH.3 M vs. GH.12 M), and f (GH.6 M vs. GH.12 M); * P<0.05, ** P<0.01, *** P<0.001 and ns, not significant (P>0.05). Values are expressed as mean± standard deviation (SD), n=15 for J.0 M, n=36 for GH.3 M and GH.6 M, and n=32 for GH.12 M.

irritable bowel syndrome (IBS-M). A total of 38 fecal samples collected at 3, 6, and 12 months were analyzed in the IBS group. Among these 81 participants, 11 asymptomatic participants were randomly selected and a total of 30 fecal samples at 3, 6, and 12 months were collected to form the normal control (NC) group for control analysis. Additionally, 12 patients with non-specific gastrointestinal symptoms between IBS and NC were selected for inclusion in the study and named as WC, which included 3 cases of constipation, 3 cases of gastrointestinal discomfort, 2 cases of dyspepsia, 2 cases of abdominal pain, and 2 cases of diarrhoea. There were no statistically significant differences in alpha diversity or beta diversity between the IBS and NC groups. The relative abundances of p_Latescibacterota, c_Vampirovibrionia, c_Latescibacterota, o_Gastranaerophilales, o_Latescibacterota, f_Butyricicoccaceae, f_Gastranaerophilales, f_Campylobacteraceae, f_Latescibacterota, g_*Lachnospiraceae_UCG-010*, g_*Butyricicoccus*, g_*Gastranaerophilales*, g_*Oscillospira*, g_*Latescibacterota*, s_*Ruminococcus_torques*, s_*Candidatus_Gastranaerophilales*, and s_*Thauera_terpenica* in the

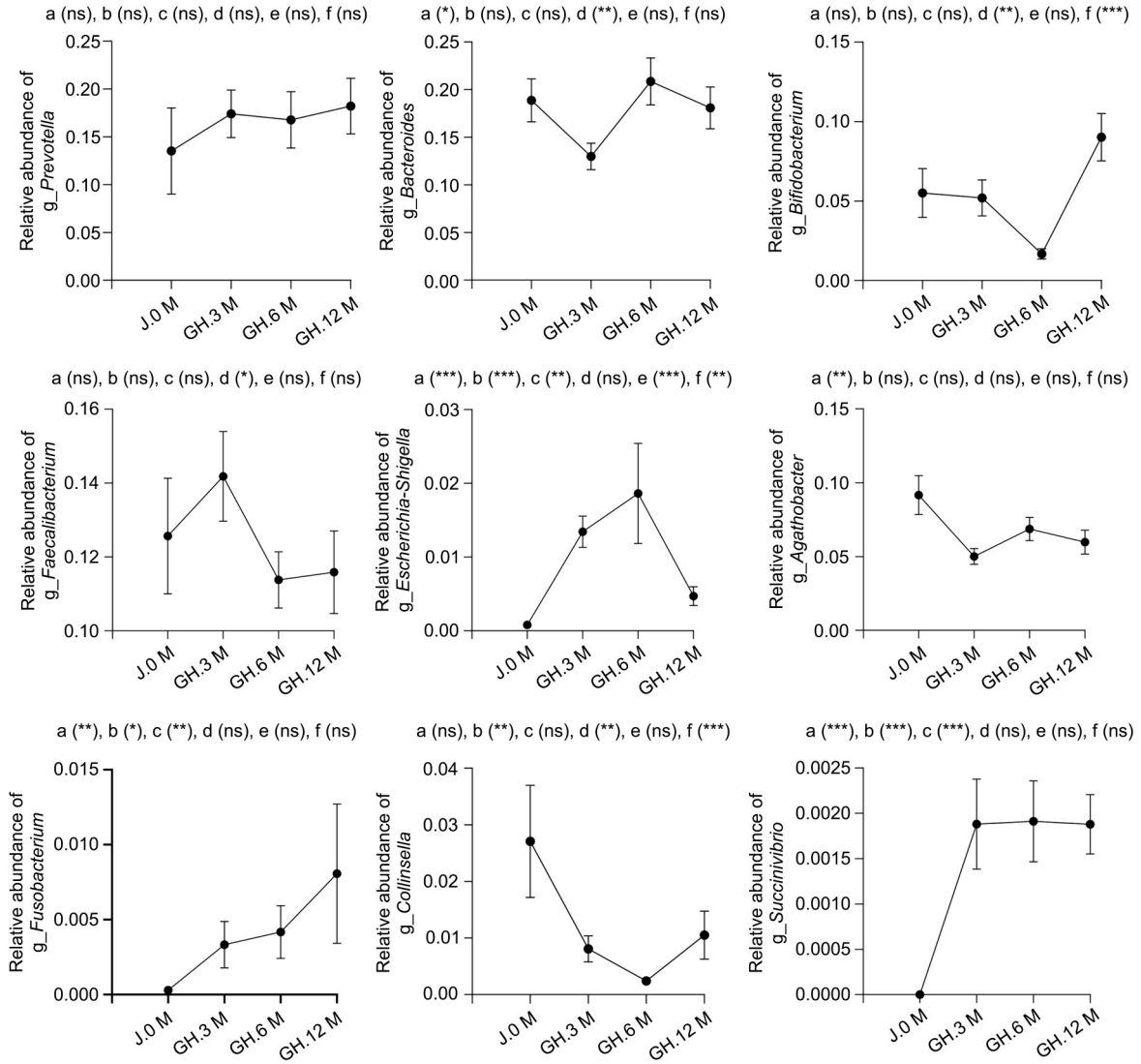

Fig. 8 Trends in the relative abundances of the top 9 bacterial taxa between groups at genus levels during the one-year observation period. A line graph was used to show the change in the relative abundance of bacteria at different time. The statistical differences obtained by the MetaStat method were depicted in the annotations at the top of the figure: a (J.0 M vs. GH.3 M), b (J.0 M vs. GH.6 M), c (J.0 M vs. GH.12 M), d (GH.3 M vs. GH.6 M), e (GH.3 M vs. GH.12 M), and f (GH.6 M vs. GH.12 M); * P<0.05, ** P<0.01, *** P<0.001 and ns, not significant (P>0.05). Values are expressed as mean± standard deviation (SD), n=15 for J.0 M, n=36 for GH.3 M and GH.6 M, n=32 for GH.12 M.

IBS group were higher than those in the NC group. At the same time, c_Clostridia, o_Oscillospirales, f_Victivallaceae, f_Ruminococcaceae, g_*Faecalibacterium*, g_*Coprococcus*, g_*Eubacterium_ventriosum*, g_*Merdibacter*, g_*UCG-009*, s_*Coprococcus_eutactus*, s_*Ruminococcus_torques*, s_*Coprococcus_catus*, and s_*Lachnospiraceae_bacterium* were more abundant in the IBS group than in the WC group. The relative abundances of g_*Ruminococcus_torques*, g_*Peptococcus*, and s_*Clostridium_paraputrificum* were higher in the NC group than in the WC group (Fig. 9). By comparing the differences in microflora between the IBS and NC groups, we confirmed that gut microbiome dysbiosis might be one of the aetiological factors for the development of IBS.

3.4.2 Functional alteration of the gut microbiome between different groups in response to the plateau environment

PICRUSt2 is a bioinformatics package for metagenomic function prediction based on marker genes (16S rRNA) for the IBS, NC, and WC groups. The

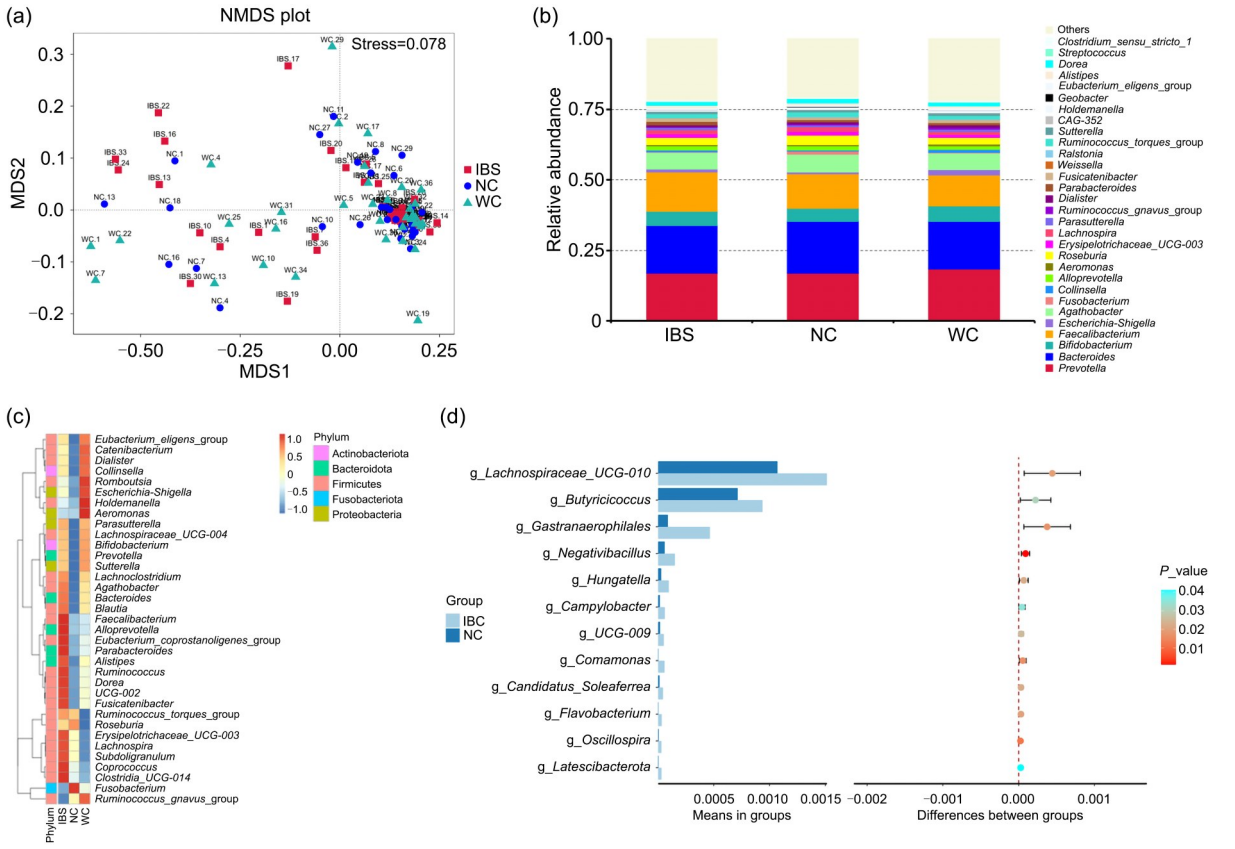

Fig. 9 Composition and differences in bacterial taxa between the three study groups. (a) NMDS based on the unweighted UniFrac distances of microbial communities between samples. (b) Histogram of the top 30 relative abundances of taxa at the genus level for different groups. (c) A heatmap illustrating the top 35 species abundance clusters for each group at the genus level. (d) Differences between the IBS and NC groups at the genus level. NMDS: non-metric multi-dimensional scaling; IBS: irritable bowel syndrome; NC: normal control; WC: nonspecific gastrointestinal symptoms.

detailed prediction process can be viewed on the web page description (https://github.com/picrust/picrust2/wiki). The 16S rRNA sequencing data from this experiment were used for functional prediction based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology (KO) database (https://www.genome.jp/kegg). The relative abundance cluster analysis of the top 35 functional annotations showed that the IBS group was very close to the NC group (Fig. 10a), while the WC group was a separate entity (Fig. S2). Functional differences between the various groups were analyzed by *t*-test, and 41 annotated functions were higher in the IBS group than in the NC group, including phosphoglycerate dehydrogenase (PHGDH), UDP-galactose-4-epimerase (GALE), DNA adenine methylase, oligogalacturonide transport system permease protein (togM), signal transduction, and uncharacterized proteins (Fig. 10b). The same functional differences were further illustrated on the volcanic distribution map (Fig. 10c).

3.5 Correlation of common mental disorders with gut microbiome in IBS in the plateau environment

Among patients who developed IBS after migration from the plains to Lhasa, the most significant symptoms were found at 6 months using the PHQ-15 score. The top 5 items in the PHQ-15 were items 10, 11, 1, 13, and 8 involving gastrointestinal discomfort and sleep disorders. Patients with IBS had a significantly higher PHQ-15 score rating at 6 months compared to 3 months (Z=−1.992, P=0.046) and 12 months (Z=−2.421, P=0.015) according to the Mann-Whitney U test (Table 2). The top 5 items in the SRQ of IBS patients were items 2, 1, 20, 4, and 19, which involved eating less, being easily stressed and anxious, being easily fatigued, having headaches, and decreased concentration. IBS patients had a significantly higher total SRQ score at 6 months than at 3 months by *t*-test (F=5.633, P=0.042) (Table 3). Spearman's rank correlation

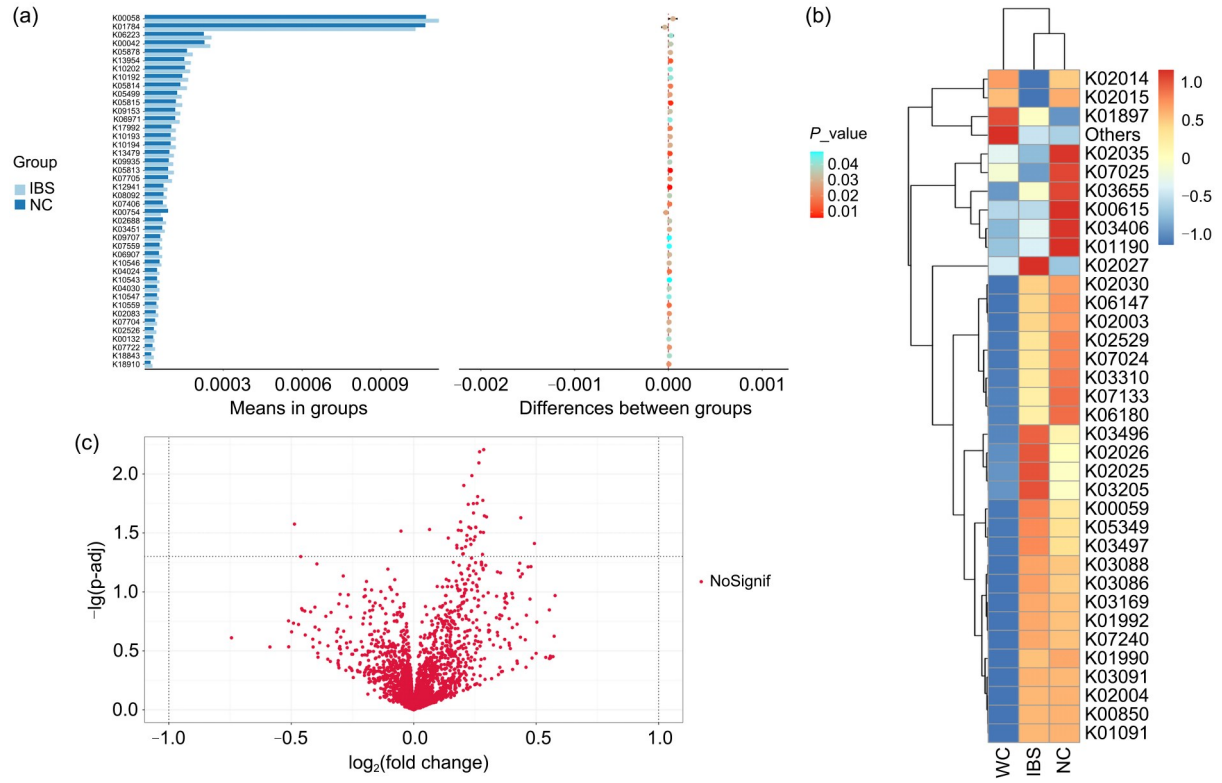

Fig. 10 Functional prediction of differential genes between the three groups based on KO database. (a) Differential gene function between the IBS and NC groups. (b) Heatmap of the top 35 functional annotation-based relative abundance between the three groups. (c) Each point in the volcano plot represents a gene function, and the horizontal axis represents the fold difference in the comparison group differential function, while the vertical axis corresponds to the *P*-value of the significant difference test between groups for the differential function. KO: Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology; IBS: irritable bowel syndrome; NC: normal control; WC: nonspecific gastrointestinal symptoms; p-adj: *P*-adjusted; NoSignif: no significance.

Table 2 PHQ-15 scores of different groups at 3, 6, and 12 months of living in the plateau environment

Group	*N* in GH.3 M				*N* in GH.6 M				*N* in GH.12 M			
	Total	Scoring			Total	Scoring			Total	Scoring		
		0–4	5–9	10–14		0–4	5–9	10–14		0–4	5–9	10–14
IBS	13	12	1	0	13	6	5	2	12	10	2	0
WC	12	11	1	0	12	10	2	0	12	11	1	0
NC	11	11	0	0	11	11	0	0	8	8	0	0

PHQ-15 contains 15 questions each scored 0–2; a total score of 0–4 indicates no somatic problems, 5–9 indicates mild somatic problems, 10–14 indicates moderate somatic problems, and 15 or more indicates severe somatic problems. PHQ-15: Patient Health Questionnaire-15; IBS: irritable bowel syndrome; NC: normal control; WC: nonspecific gastrointestinal symptoms; *N*: number of participants.

Table 3 SRQ of different groups at 3, 6, and 12 months of living in the plateau environment

Group	GH.3 M			GH.6 M			GH.12 M		
	N	SR		*N*	SR		*N*	SR	
		n	Mean±SE		*n*	Mean±SE		*n*	Mean±SE
IBS	13	4	4.50±2.08	13	7	14.14±12.73	12	3	4.33±2.51
WC	12	1	2	12	4	6.00±1.82	12	4	2.50±1.00
NC	11	0	0	11	0	0	8	0	0

The SR value represents the total stress response score. SRQ: Stress Response Questionnaire; IBS: irritable bowel syndrome; NC: normal control; WC: nonspecific gastrointestinal symptoms; *N*: number of participants; *n*: positive entries in the questionnaire; SE: standard error.

coefficient was applied to analyze the relationship between SRQ and the absolute abundance of gut microbiome at the genus level in IBS patients, and *Parabacteroides* was correlated with SRQ (r=0.651, P=0.006). Similarly, a total of more than 10 bacterial genera were also correlated with SRQ, such as *Sutterella* (r=0.535, P=0.024), *Alistipes* (r=0.394, P=0.029), *Phascolarctobacterium* (r=0.471, P=0.045), *Eubacterium_hallii_* group (r=0.468, P=0.046), *Anaerostipes* (r=0.598, P=0.012), *Butyricimonas* (r=0.544, P=0.022), *Odoribacter* (r=0.566, P=0.017), *Butyricicoccus* (r=0.562, P=0.018), and *Flavonifractor* (r=0.462, P=0.048). In addition, *Escherichia-Shigella*, *Succinivibrio*, and *Megamonas* were correlated with SRQ by Pearson's correlation coefficient analysis (r=0.661, P=0.005; r=0.667, P=0.004; and r=0.678, P=0.004). This study suggested that abnormal brain–gut axis interactions constituted by gut microbiome and common mental disorders might play an important role in the aetiological mechanisms underlying the development of IBS.

4 Discussion

In this study, we recruited Tibetan and Han volunteers to systematically evaluate the correlations between changes in the gut microbiome and IBS in people moving to the Tibetan Plateau environment. The analysis results revealed that changes in gut microbiome due to the high-altitude environment might induce the occurrence of IBS. In addition, we observed changes in the gut microbiome in the Tibetan people of the plateau within one year after changes in their living condition; hence, these changes might play a crucial role in the alteration of gut flora. The factors affecting the alpha diversity and beta diversity of the gut microbiome in Tibetans and Han Chinese may include diet, genetic background, low oxygen, and low temperature (Beam et al., 2021; Liang et al., 2021; Wang et al., 2022). Moreover, significant differences in microbial diversity and richness were observed among Tibetan regions at different altitudes (Lan et al., 2017). The increased alpha diversity of the gut microbiome could be related to ethnic factors and might enhance ecosystem stability (Liu K et al., 2020; Ma et al., 2021). However, another study showed that the plateau environment decreased the diversity of gut microbiome in people who lived on the plateau for either a short or a long period of time, and the diversity remained at low levels for a sustained period even after leaving the plateau life (Jia et al., 2020). For the first time, our team performed one-year continuous follow-up of healthy people that entered the plateau at four time points (before entering the plateau, and 3, 6, and 12 months after entering the plateau), which were selected for sample collection and questionnaire. Our results revealed that the plateau environment could influence changes in their gut microbiome; at the same time, their bodies adapted to the plateau hypoxic environment within 3 months, and the diversity and richness of gut microbiome increased. This was followed by a decline within 6 and 12 months, which may be associated with the body's autoimmunity and host genes.

In this study, at the phylum level, the relative abundances of six of the top 10 bacterial phyla (Proteobacteria, Acidobacteriota, Cyanobacteria, Verrucomicrobiota, Desulfobacterota, and Gemmatimonadota) in Han Chinese showed an increasing trend within 3 months from moving from plain to plateau, followed by a decreasing trend from 3 to 12 months. At the genus level, LEfSe analysis showed that *Bacteroides*, *Bifidobacterium*, *Agathobacter*, and *Collinsella* were more abundant in the J.0 M group than in the GH.3 M group. Bacilli at the class level and *Bacillus* at the genus level were significantly higher in abundance in the GH.3 M group than in the J.0 M group. Numerous studies have also found that alterations in the plateau environment significantly affected the abundances of these bacteria at the phylum or genus level, but these were limited to the differences of bacteria between pre- and post-environmental changes and lacked dynamic detection at multiple time points (Adak et al., 2013; Liu et al., 2017; Wu et al., 2017; Gomez-Arango et al., 2018; Jia et al., 2020; Liu FY et al., 2020; Han et al., 2021; Ma et al., 2021). We performed the analysis of gut microbiota by collecting fecal samples from multiple time points during the period of moving to the plateau, and thus we could assess the dynamic changes in gut microbiome. Acute plateau environmental changes greatly altered the abundance of some bacteria, but with the progress of time, the abundance of many bacteria became closer to levels before entering the plateau.

In this study, we found that compared with the NC group, the relative abundances of g_*Oscillospira* and s_*Ruminococcus_torques* were higher in the IBS group. Many studies have found a disordered intestinal

flora in IBS patients. In addition, Zhu et al. (2021) found that *Oscillospira*, *Bacteroides*, and *Prevotella* were valuable in the diagnosis of IBS-D. The genus *Oscillospira* was able to digest diets rich in complex plant polysaccharides to produce beneficial butyrate, and previous research has found their significantly higher prevalence in people on a Mediterranean diet than in those on a Western diet (Nagpal et al., 2018). It was experimentally confirmed that *Blastocystis* infection could mimic IBS-like symptoms in rats, where altered gut microbiome composition, increased relative abundance of the genus *Oscillospira*, and decreased productions of acetate, propionate, and butyrate were established (Defaye et al., 2020). *Ruminococcus torques* is abundant in the gut flora of IBS patients and has been associated with the generation of severe pain (Iacob et al., 2017; Hasan et al., 2019). A low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) diet reduced symptoms in IBS and increased the relative abundance of the mucus-degrading bacterium *Ruminococcus torques* (Halmos et al., 2015). These results are consistent with our findings; hence both g_*Oscillospira* and s_*Ruminococcus_torques* might play an important role in the pathogenesis of IBS.

In this study, during the one-year follow-up period, the PHQ-15 and SRQ total scores of IBS patients were significantly higher than those of normal healthy subjects and were the highest at 6 months, suggesting that IBS in the plateau environment was associated with somatization symptoms, anxiety, and depression. Studies have shown that IBS patients often comorbid with psychosocial abnormalities, which significantly affect their quality of life. Patients with IBS often present with depression or anxiety that is positively correlated with the severity and frequency of gastrointestinal symptoms; scores on the PHQ-15 (used to assess somatization) were significantly higher in patients with IBS than in normal healthy individuals (Pinto-Sanchez et al., 2015; Liu et al., 2021). The role of the brain-gut pathways in IBS is bidirectional. One survey found anxiety and depression to be significant predictors of developing IBS and those with confirmed IBS developed anxiety and depression at follow-up (Koloski et al., 2016). IBS is closely related to stress stimuli, and chronic stress can increase intestinal mucosal barrier permeability, causing endotoxaemia and intestinal or systemic low-grade inflammation (de Punder and Pruimboom, 2015). Stressed rats exhibited visceral hypersensitivity, decreased colonic occludin expression, decreased abundance of butyrate-producing bacteria such as *Lachnospiraceae*, and decreased butyrate metabolic pathways (Zhang et al., 2019). The SRQ comprehensively assesses a person's emotional state, somatic reactions, and behavior, with higher scores indicating a greater degree of stress reactivity (Yuan et al., 2020). In our results, *Alistipes* in IBS was correlated with SRQ. As a recently described genus of the Bacteroidetes phylum, *Alistipes* was closely related to dysbiosis, inflammation, and disease, and it was linked with mental signs of depression (Cobo et al., 2020; Parker et al., 2020). Increased abundance of unclassified *Alistipes* in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)+IBS cases was used as one of the specific biomarkers (Nagy-Szakal et al., 2017). The concentration of *Alistipes* spp. was negatively correlated with the IBS-Severity Scoring System (IBS-SSS) and the Fatigue Assessment Scale (FAS) score (El-Salhy et al., 2019). Our results were similar to those of Parker et al. (2020) and Nagy-Szakal et al. (2017) in this regard. In short, *Alistipes* might play an important role in the development of IBS.

5 Conclusions

Our results provide additional evidence that the plateau environment affects the gut microbiome through adaptive changes, which might induce the occurrence of IBS, providing a new avenue of research in the aetiology of this illness. Importantly, for the first time, we found that a plateau environment may be special in inducing IBS. In addition, the PHQ-15 and SRQ scores revealed psychological abnormalities in most patients with IBS. Meanwhile, the pathophysiological mechanism between IBS and the gut microbiome needs to be further elucidated.

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Author contributions

Xingguang ZHANG, Wencheng ZHANG, Cheng YANG, Yanmei DONG, Taotao LIU, Shihai XIA, and Bangmao WANG

conceived the project and revised the manuscript. Xingguang ZHANG and Lisa DUAN designed questionnaire. Xingguang ZHANG and Haiyan NIU collected the samples and metadata. Xingguang ZHANG, Wei XU, and Weilong ZHONG analyzed and interpreted the data and results. Xingguang ZHANG wrote the manuscript and created the figure and tables. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Xingguang ZHANG, Wei XU, Weilong ZHONG, Wencheng ZHANG, Cheng YANG, Lisa DUAN, Haiyan NIU, Yanmei DONG, Taotao LIU, Shihai XIA, and Bangmao WANG declare that they have no competing interests in the research.

All study protocols were approved by the Ethics Committee of the Characteristic Medical Center of the Chinese People's Armed Police Force with the approval identifier on December 21, 2021 (approval No. 2021-0015.1) and in accordance with established national and institutional ethical guidelines. All participants were informed of the purpose and significance of the study and signed the informed consent forms prior to the collection of fecal samples and questionnaires.

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Supplementary information

16S rRNA gene sequencing; Table S1; Figs. S1 and S2