Research Square

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The structural differences of intestinal flora between people with functional diarrhea and the general population

Shuo Ma
Inner Mongolia Medical University
Lifang SHI
Affiliated Hospital of Inner Mongolia Medical University
Zhixin Wang
Inner Mongolia Medical University
Xiaoqian Zhang
Inner Mongolia Medical University
Sujuan Li
Affiliated Hospital of Inner Mongolia Medical University
Mingliang Hao
Inner Mongolia Medical University
Shiqi Wang
grace1817@163.com

Inner Mongolia Medical University

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Abstract

Objective To analyze the characteristics of intestinal flora in patients with Functional Diarrhe (FDr), explore the correlation between different bacterial genera and FDr, as well as its predictive and diagnostic value for FDr, and provide new ideas for the prevention and treatment of FDr.

Method This study adopted a case-control design. Questionnaire surveys and fecal sampling analyses were conducted on patients with functional diarrhea and normal family members without gastroenteric-related diseases. The microbial diversity of fecal samples in each group was sequenced by 16S full-length rRNA using the third-generation Pacbio sequencing platform. Obtain DNA sequences and other data to conduct species difference analysis and study the association strength of each factor with the onset of FDr.

Result A total of 64 samples were included, among which 32 were from the functional diarrhea group (case group) and 32 from the normal population group (control group).The results showed that the number of exposed pairs in the case group was significantly higher in terms of spicy diet and postmeal abdominal pain. Species composition analysis revealed that at the phylum level, the abundance ratio of actinomycetes in the case group increased, while that of Firmicutes decreased. At the genus level, the genus Bifidobacterium increased, while the genera Bacteroides and Faecalibacterium decreased. At the species level, Bifidobacterium pseudomonas was elevated, while Faecobacter procellarum was decreased. The diversity and uniformity of the case group decreased, while the richness and coverage of the intestinal flora in the two groups were basically the same. LefSe analysis screened out 41 differential bacterial genera. Among them, the relative abundance of Prevotella type 9, Prevotella Korella, etc. in the case group was higher than that in the control group, while the relative abundance of Bacteroides monocytoides, Clostridium difficile group 1, etc. was lower than that in the control group.

Conclusion There are significant differences in the intestinal flora structure between the population with functional diarrhea and the normal population.

Introduction

Functional Diarrhe (FDr) is a common subtype of abnormal functional gastrointestinal disorders/abnormal brain-gut interaction. Its clinical features include continuous or repeated excretion of loose or liquid stools, without accompanied by abdominal discomfort symptoms such as abdominal pain. According to the Rome IV diagnostic criteria, functional diarrhea can be diagnosed when more than 25% of a patient's stools present as non-formed and the possibility of diagnosing irritable bowel syndrome is ruled out. This standard also requires that the duration of related symptoms should meet the dual-phase standard: the total disease course should be no less than 6 months, and the diagnostic conditions should be continuously met within the last 3 months. There are no underlying organic diseases, no bacterial, viral or parasitic infections, but it is solely caused by gastrointestinal dysfunction[1, 2]. FDr has seriously affected the quality of life of patients and triggered a series of health

problems. In a cross-sectional survey conducted in China in 2012, the average incidence rate of FDr in China was 1.54%[3]. In a joint survey sample in 2020, the combined prevalence of functional diarrhea, a functional disorder of the lower digestive tract, was 4.7% worldwide[4]. The prevalence of functional diarrhea in China is lower than that in Western countries, but it remains at a relatively high level within Asia[5]. The pathogenesis of FDr has not been fully elucidated at present, but its occurrence may be related to changes in gastrointestinal motility, abnormalities in the brain-gut axis, alterations in the intestinal flora and visceral hypersensitivity[6].

This study aims to compare the composition and structure differences of intestinal microbiota between patients with functional diarrhea and healthy control populations through 16S rRNA gene sequencing technology, reveal the potential association between microbiota disorder and functional diarrhea, and provide microbiological basis for clinical diagnosis or intervention.

Methods

Participants' selection

The cases in this study were derived from patients with functional diarrhea who visited the Department of Gastroenterology of the Affiliated Hospital of Inner Mongolia Medical University in 2024. The controls were derived from family members of the patients who had no functional gastrointestinal disorders, no diarrhea or bacterial infections. Based on the matching of ethnic groups and ages within ± 5 years, the case group and the control group were respectively formed. A total of 32 people were sampled from each group. The 16s sequencing samples were fecal samples from patients with functional diarrhea and healthy controls. Due to the high degree of individual differences in the gut microbiota and the lack of a clear experimental endpoint in gut microbiota research, based on literature reviews and previous studies on the gut microbiome, a sample size of 10 per group is sufficient for 16s sequencing analysis[7]; Meanwhile, the estimation of the sample size can be evaluated through sparse curves. When the curve tends to flatten, it indicates that the sample size is sufficient and reasonable. In this study, the results of the sparse curve tend to flatten, indicating that the current sample size of sequencing can meet the analysis requirements.

The inclusion criteria for the case group were: (1) Meeting the Rome IV diagnostic criteria for functional diarrhea (persistent or recurrent loose or watery stools, no abdominal pain or discomfort, no underlying organic disease, no infection, caused by gastrointestinal dysfunction, symptoms lasting for at least 6 months and meeting the criteria in the last 3 months); (2) Be over 18 years old; (3) Participate voluntarily and sign the informed consent form. The exclusion criteria for the case group were: (1) Previous history of gastrointestinal surgery; (2) Use antibiotics and probiotics for treatment within 3 months; (3) The inclusion criteria for the control group that disagreed with fecal retention were: (1) No gastrointestinal functional diseases; (2) No diarrhea or bacterial infection; (3) Be over 18 years old; (4) Voluntarily participate and sign the informed consent form; The exclusion criteria for the control group were: (1)

Previous history of gastrointestinal surgery; (2) Use antibiotics and probiotics for treatment within 3 months; (3) Those who do not agree to retain their feces. This study has been approved by the Ethics Committee of Inner Mongolia Medical University (Ethics Number: YKD202403025).

Data Collection

Review the outpatient medical records of the Department of Gastroenterology at the Affiliated Hospital of Inner Mongolia Medical University from January 1, 2024 to December 31, 2024, mainly extracting cases of functional diarrhea with a clear diagnosis. And for the patients with functional diarrhea collected, controls were selected from their families based on the same ethnicity and age within ± 5 years. Questionnaire surveys were conducted with the selected research subjects and subsequent fecal sample collections were carried out.

Definition

Definition of Drinking: Referring to the "Chinese Dietary Guidelines (2022)", drinking alcohol at least once a week for a continuous or cumulative period of six months is defined as having a drinking behavior. Spicy diet: Eating fresh chili peppers directly; Add fresh/dried chili peppers, chili oil/sauce/sauce when cooking. The above-mentioned behaviors occurred at least three times a week on average over the past six months. Greasy diet: Consuming high-fat foods, including fried foods, animal fats, and high-fat dishes, etc. The above-mentioned behaviors occurred at least three times a week on average over the past six months.

Statistical analysis

All data analyses were conducted using SPSS 25.0 software. Quantitative data conforming to the normal distribution were expressed as "mean ± standard deviation" ($x \pm s$), and count data were described using composition ratios. Paired chi-square test was used for statistical inference between the two groups. All statistical analyses were conducted using two-sided tests, with the significance level set at $\alpha = 0.05$. The collected fecal samples were subjected to 16S rRNA sequencing and biological analysis. Then, species classification and identification were carried out and an evolutionary tree was constructed to obtain the microbial species composition spectrum. The richness, diversity characteristics and uniform distribution of species within a specific habitat are measured by means of alpha diversity.

Results

Basic Information: Among the cases, there were 14 males and 18 females. In the control group, there were 18 males and 14 females. The gender ratio of male to female was 1:1. The average age of the case group was 42.19 ± 18.95 years old, and that of the control group was 42.13 ± 18.05 years old. In both the

case group and the control group, the ethnic group was mainly Han, both accounting for 27 (49.1%), as shown in Table 1.

Table 1
Baseline Data of the Survey Subjects

		Case group	Control group
Gender	Male	14(43.8%)	15(46.9%)
	female	18(56.3%)	17(53.1%)
Age (years)		42.19 ± 18.95	42.13 ± 18.05
Ethnicity	Han Ethnicity	27(84.4%)	27(84.4%)
	Manchu	2(6.3%)	2(6.3%)
	Mongolian and others	3(9.4%)	3(9.4%)
Drinking alcohol	yes	19(59.4%)	20(62.5%)
	no	13(40.6%)	12(37.5%)
Spicy diet	yes	15(46.9%)	5(15.6%)
	no	17(53.1%)	27(84.4%)
Greasy diet	yes	16(50.0%)	7(21.9%)
	no	16(50.0%)	25(78.1%)
Anxiety	yes	8(25.0%)	4(12.5%)
	no	24(75.0%)	28(87.5%)
Depression	yes	3(9.4%)	0(0.0)
	no	29(90.6%)	32(100.0%)
Postprandial abdominal pain	yes	18(56.3%)	8(25.0%)
	no	14(43.8%)	24(75.0%)
Bloating	yes	13(40.6%)	9(29.1%)
	no	19(59.4%)	23(71.9%)

Univariate analysis of Factors Related to Diarrhea: The factors related to diarrhea include: alcohol consumption, spicy diet, greasy diet, anxiety, depression, postprandial abdominal pain, abdominal distension, etc. The results of the paired chi-square test are shown in Table 2. According to the P value, OR value and 95% confidence interval, it can be known that the number of pairs exposed to spicy diet, greasy diet and postprandial abdominal pain in the case group was significantly higher than that in the control group (OR = 3.5, P = 0.031; OR = 3.25, P = 0.049; OR = 6.0, P = 0.013).

Table 2
Paired chi-square test for diarrhea-related factors in two groups

Influencing factors	Case group(+) Control group(+)n	Case group (+) Control group (-)n	Case group (-) Control group (+)n	Case group (-) Control group (-)n	χ^2	*OR*(95%*CI*)	*P*
Drinking alcohol	11	8	9	4	0.423		1.000
Spicy diet	1	14	4	13	1.719	3.5(1.15–10.63)	0.031
Greasy diet	3	13	4	12	0.183	3.25 (1.06–9.97)	0.049
Anxiety	1	7	3	21	0.000		0.344
Depression	0	3	0	29	-		-
Postprandial abdominal pain	6	12	2	12	1.524	6.0(1.34–26.79)	0.013
Abdominal distension	5	8	4	15	1.157		0.388

Multivariate regression analysis of diarrhea-related factors: Factors with statistically significant results ($P < 0.05$) in the univariate analysis were included as independent variables for comprehensive analysis in the multivariate regression. The results showed that spicy diet (OR = 8.952, 95%CI:) Postprandial abdominal pain (1.150-69.678) and postprandial abdominal pain (OR = 12.682, 95%CI: 1.327-121.159) are risk factors for functional diarrhea, as shown in Table 3.

Table 3
Multivariate regression analysis of Influencing Factors of Functional Diarrhea

Variable	*P*	*OR*	*OR*95%*CI*
Spicy diet	0.036	8.952	1.150-69.678
Postprandial abdominal pain	0.027	12.682	1.327-121.159

Sequencing Depth Analysis:Through Rarefaction Curve analysis (Rarefaction Curve Analysis) Fig. 1, it can be seen that in this study, when the sequencing depth reached 40,000 reads, the rarefaction curve tended to flatten, indicating that the sequencing depth of the two groups of samples had reached saturation. Further increasing the sequencing depth is no longer meaningful. Therefore, the sequencing results are sufficient to reflect the diversity of the current samples, and the species richness is adequate.

Analysis of Alpha Diversity Differences in Gut Microbiota: Alpha diversity is based on the calculation and analysis of samples by OTU to reflect the diversity of gut microbiota species communities in the research subjects. This study adopted seven indicators, namely Ace index, Chao1 index, Observed species index, Good's coverage index, Pielou's evenness index, Shannon index and Simpson index, to analyze the characteristics of microbial communities. As can be seen from Table 4, the diversity and uniformity of the case group decreased, while the richness and coverage of the intestinal flora in the two groups were basically the same.

Table 4
Alpha Diversity Analysis

Index type	P value
Ace	0.158
Chao1	0.158
Observed species	0.084
Good's coverage	0.291
pielou evenness	0.006
shannon	0.004
simpson	0.008

Analysis of Gut Microbiota Structure: Based on the phylum, genus, and species classification of the gut microbiota of the two groups of research subjects, cluster analysis was conducted on the composition of the microbial community, and the differences in microbiota abundance were observed. The results showed that: At the phylum level, the top 10 bacterial phyla with the highest abundance in both groups of research subjects were exactly the same. Compared with the control group, the abundance proportion of Actinobacteriota in the case group was significantly increased, while that of Firmicutes was significantly decreased, as shown in Fig. 2.

At the bacterial genus level, the top 10 bacterial phyla with the highest abundance in both groups of samples remained the same. Compared with the control group, the abundance proportion of Bifidobacterium in the case group was significantly increased. The abundance ratios of Bacteroides and Faecalibacterium were significantly reduced, as shown in Fig. 3.

However, at the strain level, compared with the control group, the abundance ratio of Bifidobacterium pseudocatenulatum in the case group was significantly increased, while the abundance ratio of Faecalibacterium prausnitzi was significantly decreased, as shown in Fig. 4.

Differential Microbiota analysis: When the score of Linear discriminant analysis (LDA) is greater than 3. At this level In the case group, there were Prevotella 9, Prevotella copri, Bifidobacterium faecale, and Streptococcus lutetiensis, Bacteroides coprocola, Clostridium indolis, Lachnospira multipara,

Bacteroides faecalis The relative abundance of coprophilus and Parasutterella excrementihominis was relatively high and the difference was statistically significant (P < 0.05). While the control group included Bacteroides_uniformis, Clostridium sensu stricto 1, Phocaeicola dorei, and Clostridium saudiensis saudiense, Clostridium tertium, uncultured rumen bacterium (uncultured rumen bacterium), Ruminococcus The relative abundance of bicirculans was relatively high and the difference was statistically significant (P < 0.05). To sum up, Prevotella type 9, Prevotella copri, Bifidobacterium faecale, Streptococcus lutetiensis, Bacteroides coprocola, Clostridium indolis, Lachnospira multipara, Bacteroides faecalis coprophilus and Parasutterella excrementihominis may serve as potential biomarkers of the intestinal flora in patients with functional diarrhea, as shown in Fig. 5.

Discussion

Previous studies have found significant differences in the gut microbiota between patients with functional gastrointestinal disorders and healthy individuals, and these differences may serve as potential biomarkers or new therapeutic targets for functional gastrointestinal disorders in the future[8]. However, there are currently no studies on the correlation between patients with functional diarrhea and the intestinal flora. Therefore, in this study, the full-length 16S rRNA genes of the gut microbiota in 32 pairs of patients with functional diarrhea and healthy controls were analyzed by high-throughput sequencing. Univariate paired chi-square tests were conducted on the factors related to functional diarrhea. The results showed that exposure factors such as spicy diet, greasy diet, and postprandial abdominal pain had statistically significant differences between the two groups. Existing studies have shown that capsaicin in spicy foods can abnormally activate the vanilloid receptor subtype 1 (VR1), accelerating colonic peristalsis, leading to loose stools and causing diarrhea symptoms[9]. Li Huayan et al[10]. observed the phenomenon of loose stools by stimulating capsaicin receptors in the colon of rats. In addition, unhealthy eating habits, such as excessive intake of greasy and high-fat foods, may have a negative impact on the intestinal mucosal barrier, thereby causing diarrhea. Due to the small sample size of this study, no association between alcohol consumption and diarrhea was found. However, existing research indicates that alcohol can damage the microbial environment in the intestinal tract and activate intestinal mucosal mast cells, inducing a low-grade inflammatory response in the intestinal mucosa and thereby triggering FDr[11]. Lin Renbin[12]found that alcohol consumption can lead to a decline in the biodiversity of the intestinal flora and affect the overall composition of the microbial community.

At the phylum level, compared with the control group, the abundance of Firmicutes in the case group was significantly reduced, while the abundance of actinomycetes showed a significant upward trend. The Firmicutes phylum is mainly composed of Gram-positive bacteria, aerobic and anaerobic bacteria, and includes a variety of beneficial bacteria that can produce short-chain fatty acids (such as butyric acid), which play an important role in maintaining intestinal health. In people with diarrhea, due to changes in the intestinal environment, the growth of these beneficial bacteria may be inhibited, leading to a reduction in the overall abundance of Firmicutes. For instance, Faecobacter prostrata is a beneficial bacterium of the Firmicutes phylum with anti-inflammatory effects, and its abundance is usually reduced

in patients with inflammatory bowel disease[13]. Actinomycetes are generally regarded as beneficial Gram-positive bacteria, such as Bifidobacterium, which are more abundant in healthy people but may vary in patients with diarrhea. If diarrhea is caused by certain factors leading to a reduction in beneficial bacteria such as Bifidobacterium, other genera of actinomycetes may increase relatively, thereby making the overall abundance of actinomycetes higher in the case group[14].

At the genus level, compared with the control group, the abundance of Bifidobacterium in the case group increased, while the abundance of Bacteroides and Faecobacterium decreased. Diarrhea may lead to an imbalance in the intestinal flora, and the abundance of certain genera of bacteria will change accordingly. Therefore, the genus Bifidobacterium may have growth advantages in certain specific intestinal environments, thereby increasing its abundance[15]. Beneficial bacteria such as Faecobacter praxis in the genus Faecobacter can produce short-chain fatty acids, which play an important role in maintaining intestinal health. In people with diarrhea, due to factors such as intestinal inflammation, their abundance may decrease[16].

At the species level, compared with the control group, the abundance ratio of Bifidobacterium pseudosmall chain in the case group was significantly increased, while the abundance ratio of Faecobacter proscenium was significantly decreased. The changes of these two bacterial species are consistent with those of the genera at the genus level. Faecobacter prostrata is not only a potential anti-inflammatory bacterium but also a representative symbiotic bacterium of the Firmicutes phylum, with the ability to produce SCFAs. Studies have shown[17] that Faecobacter propioniae and its metabolites have a protective effect on colitis in mice, improve intestinal ecological imbalance, increase bacterial diversity and the abundance of SCFas-producing bacteria.

The Alpha diversity Index, as a key indicator for measuring the characteristics of species in locally uniform habitats, covers multiple dimensions such as species diversity, richness, and uniformity. This study indicates that species diversity and evenness have declined in the case group.

In conclusion, spicy diet and postprandial abdominal pain are risk factors for functional diarrhea. The diversity and uniformity of the case group have decreased, while the richness and coverage of the intestinal flora in the two groups are basically the same. From the case group, Prevotella type 9, Prevotella Korella, Bifidobacterium fecal, Streptococcus Paris, Bacteroides faecalis, Clostridium indole, Trichospira multocida, Bacteroides faecalis and Palasatella faecalis were screened out as potential intestinal flora marker species for patients with functional diarrhea.

Declarations

Authors' contributions

Shuo Ma collected the data and drafted the manuscript. Shiqi Wang supervised the study and reviewed the final version of the manuscript. Zhixin Wang participated in data collectio.Xiaoqian Zhang

contributed to literature review, figure preparation, and manuscript formatting.Lifang Shi participated in experimental procedures.Sujuan Li participated in experimental proceduresMingliang Hao participated in data collection.All authors reviewed the manuscript.

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Data availability

The data that support the findings of this study are not publicly available due to ethical restrictions regarding participant privacy. Data are available from the corresponding author upon reasonable request and with permission from the ethics committee.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Inner Mongolia Medical University, the approval No. was YKD202403025.Consent was exempted from patients granted by the Institutional Review Board of Inner Mongolia Medical University in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

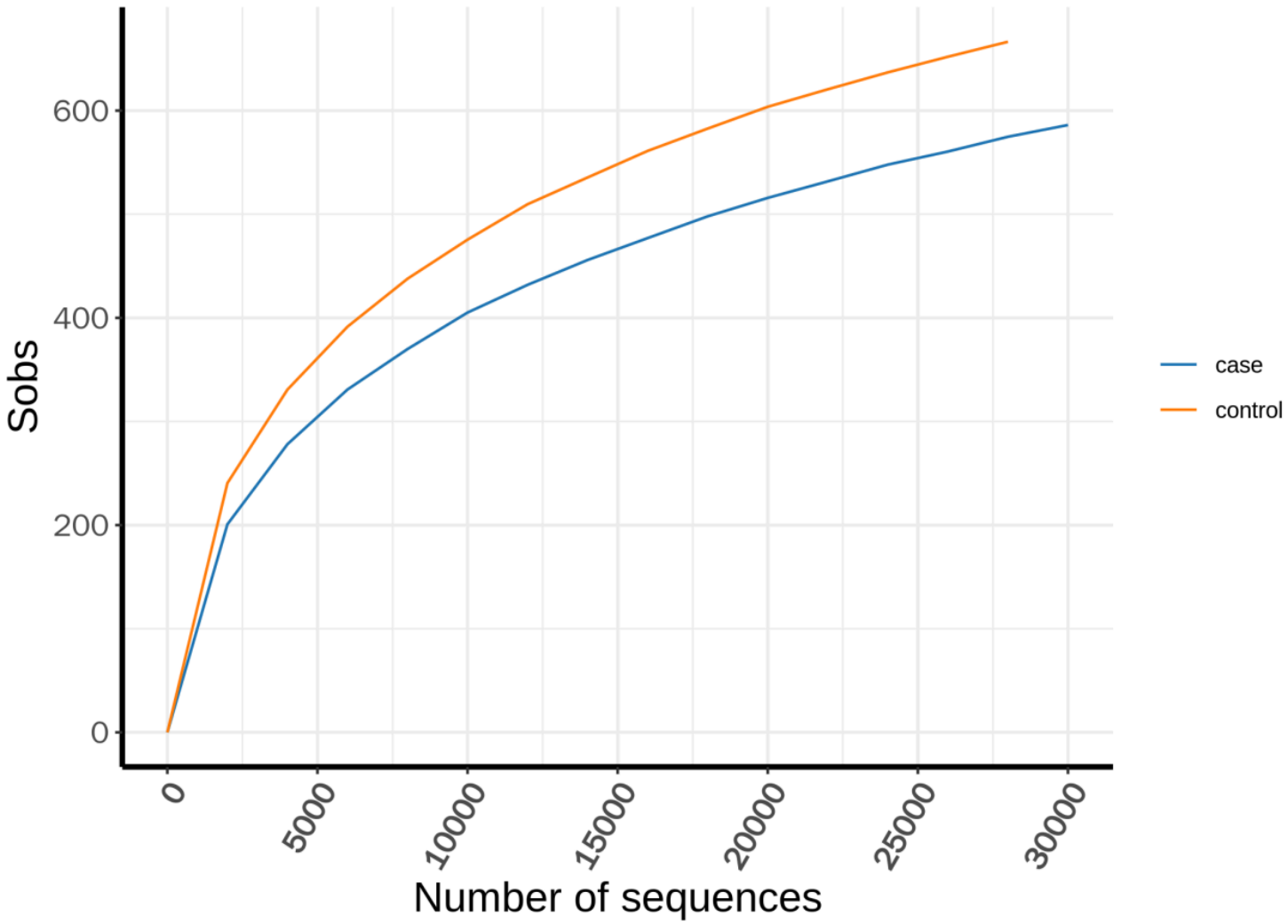


Figure 1

Shows the sparse curves of two groups of population samples

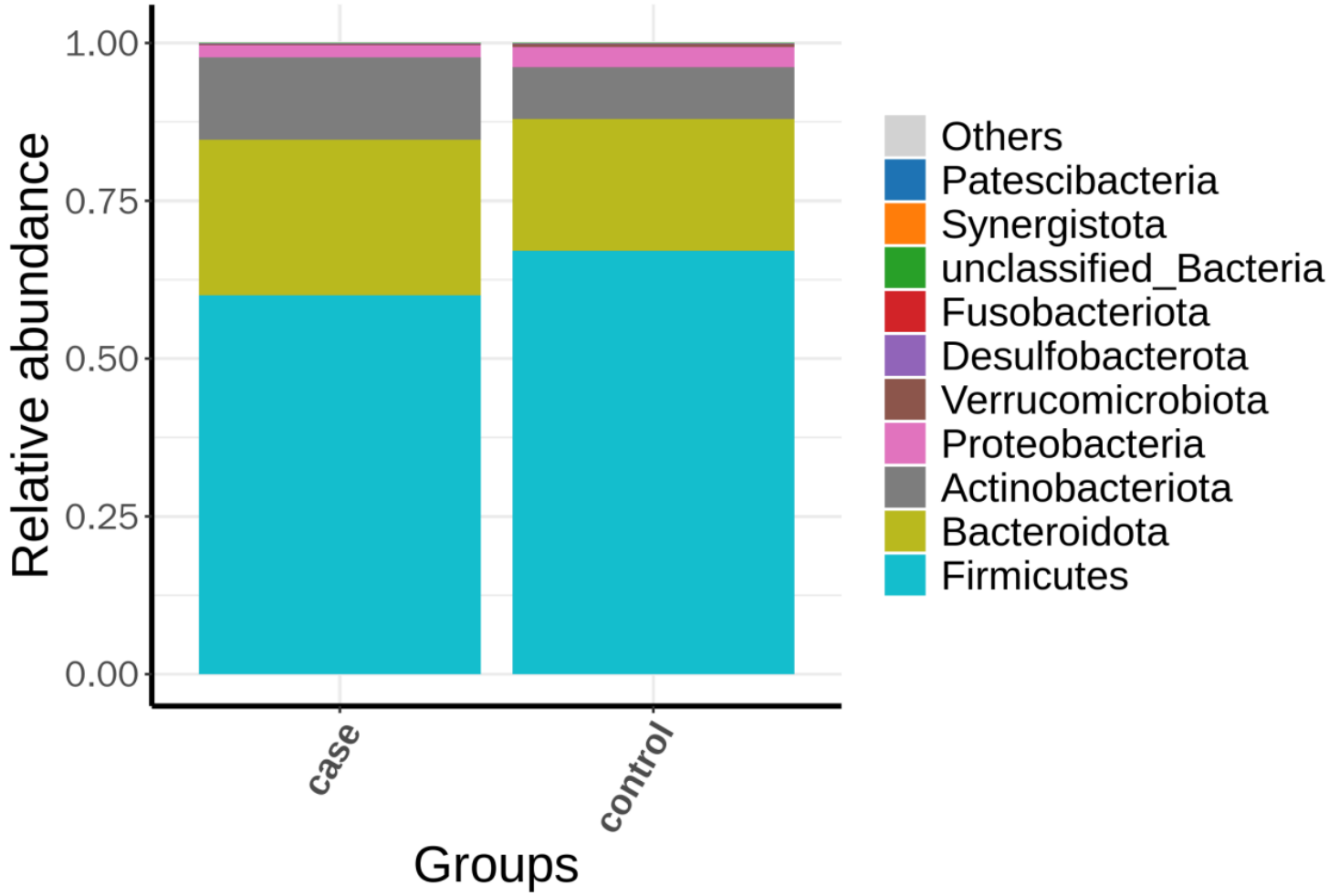


Figure 2

Bar charts of relative abundance of species at the phylum level in two groups

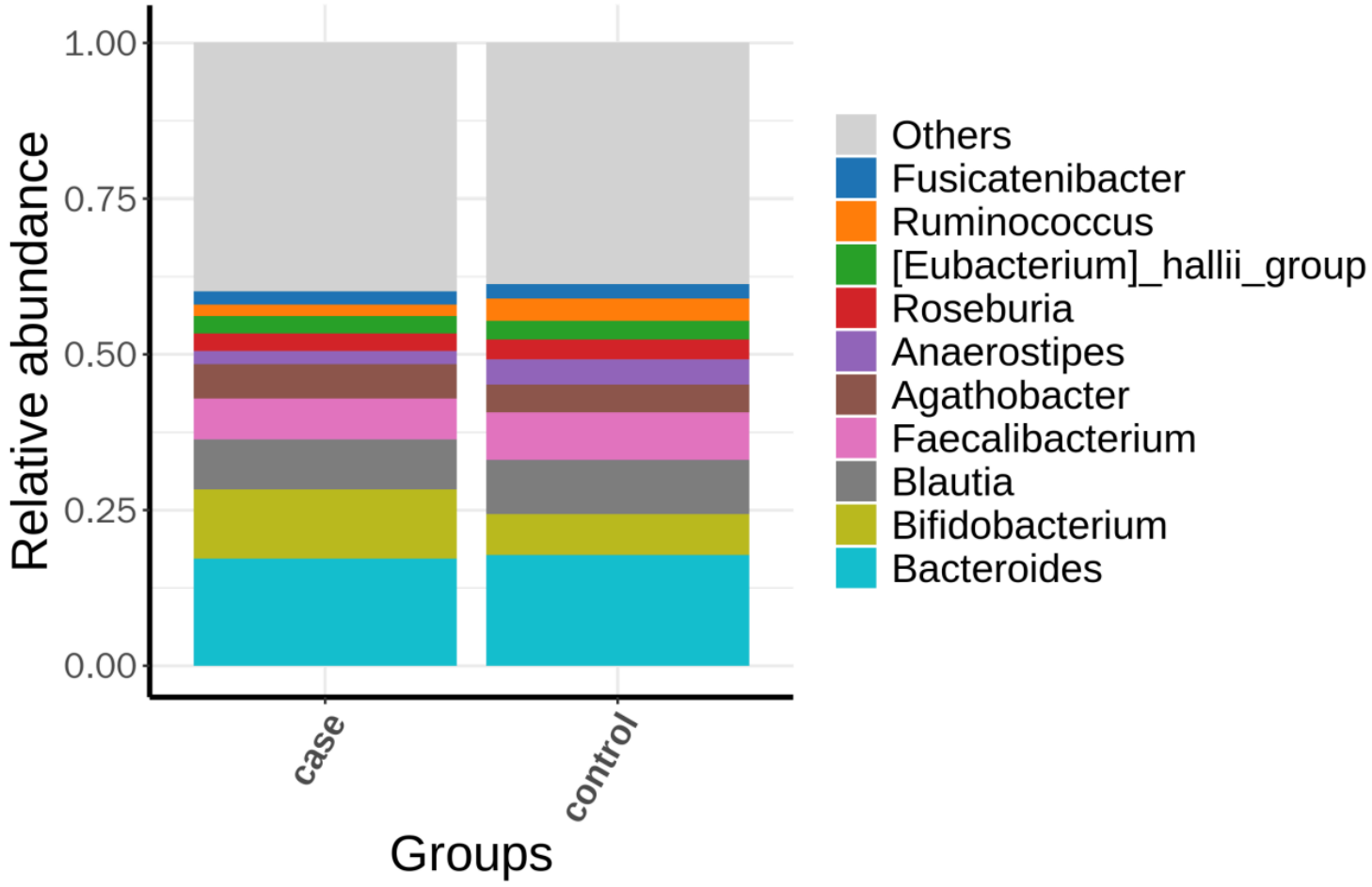


Figure 3

Bar chart of relative abundance of two groups of species at the genus level

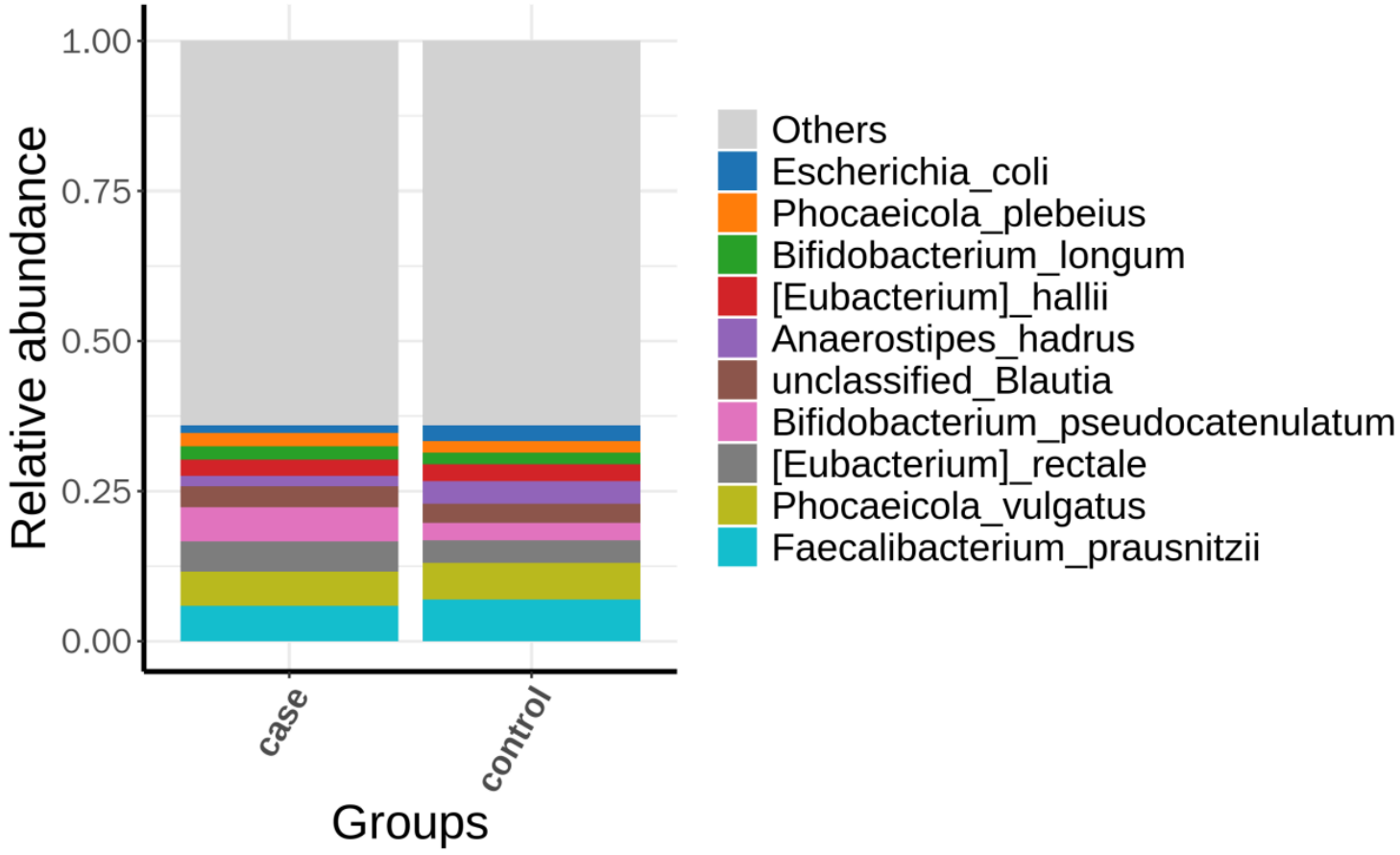


Figure 4

Bar chart of relative abundance of species at the two groups of species levels

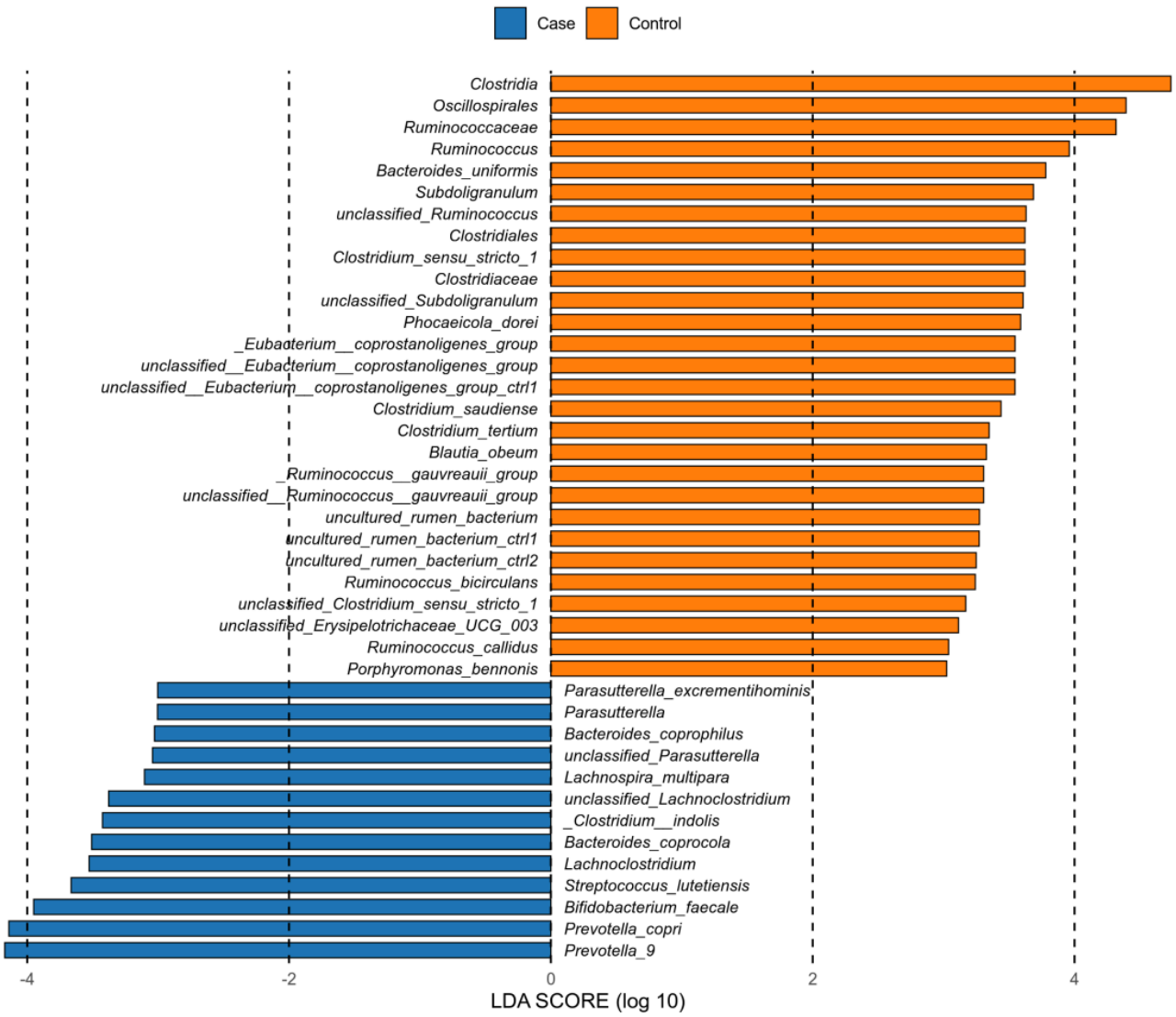


Figure 5

Bar chart of LDA value distribution