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Characteristics of functional constipation and analysis of intestinal microbiota in children aged 0–4 in Zunyi region

Shungang Gong¹, Zhengbo Yu¹, Yuan Ding¹, Yue Wang¹, Xi Li¹ and Shengli Gu^{1*}

Abstract

Background Functional constipation (FC) significantly impacts children's health. This study investigates the prevalence and microbiota characteristics of FC in children aged 0–4 years in Zunyi area.

Methods From October to December 2023, 2039 children aged 0–4 years in Zunyi were selected using stratified sampling and cross-sectional survey methods. A questionnaire based on Rome IV diagnostic criteria was used. Twenty-nine children with FC were randomly selected as the functional constipation group (FCG), and 26 healthy children, matched for age, sex, and area, were selected as the control group (CG).

Results A total of 2051 questionnaires were collected, with 2039 valid responses. Among them, 151 children had FC, resulting in a prevalence rate of 7.4%. The prevalence rates in boys and girls were 6.6% and 8.5%, respectively, with no significant gender difference ($P > 0.05$). Alpha diversity analysis revealed higher richness and diversity of intestinal flora in the FCG compared to the CG. At the phylum level, Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes were dominant in both groups. The FCG showed a higher relative abundance of Firmicutes, Actinobacteria, and Proteobacteria compared to the CG ($P < 0.05$).

Conclusions The prevalence of FC in children aged 0–4 years in Zunyi is 7.4%. Disease characteristics vary with age and living environment but are unrelated to gender. The gut microbiota of children with FC shows significant alterations, with higher diversity and specific phyla abundance.

Keywords Children, Functional constipation, Gut microbiota, Rome IV criteria, Zunyi

Introduction

Functional constipation (FC) is one of the most common gastrointestinal functional disorders in pediatrics, characterized by reduced frequency of defecation, difficulty in defecation, dry stools, abdominal pain, and a series of gastrointestinal symptoms. This condition seriously

affects the physical and mental health of children, especially infants and young children, impacting their growth and development, reducing their quality of life, and causing varying degrees of anxiety in parents [1]. Despite this, more than 95% of children with FC do not have corresponding organic lesions.

Currently, the diagnosis of FC is primarily based on the Rome IV criteria [2]. The global prevalence of FC in children is 14.4% [3], while in Chinese children aged 2 to 18 years, it is 6.0% [4]. Although children with FC generally do not develop serious complications, they may experience emotional and physical discomfort, which adversely

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affects their families. Long-term constipation increases the family's medical burden and impacts health-related quality of life. A large-scale domestic study suggests that symptoms of FC persist into adulthood in approximately one-third of affected children [5].

The etiology of childhood FC remains unclear and may be associated with various factors such as diet, psychology, physiology, family, social environment, and intestinal microbiota [6–10]. However, there has been no systematic investigation or reporting on the prevalence and associated characteristics of FC among children aged 0–4 years in the Zunyi region to date. The intestinal microbiota, as an important component of children's gastrointestinal health, plays a significant role in the pathogenesis of childhood FC.

This study investigates the prevalence of FC among children aged 0–4 years in the Zunyi region, providing a basis for the prevention and treatment of this disease in the area and facilitating further clinical work. By examining the differences in abundance, diversity, and other aspects of intestinal microbiota between fecal samples from children with FC (FCG) and a control group (CG), this study aims to provide evidence for further exploration of the pathogenesis of FC.

Materials and methods

Survey objects

The survey subjects are children aged 0 to 4 in the Zunyi area, with the survey period spanning from October 2023 to December 2023. Inclusion criteria: (1) Age ≤ 4 at the time of the survey; (2) Registered residents of Zunyi who have lived locally since birth; (3) Informed consent from guardians. Exclusion criteria: (1) Refusal to participate in the survey for any reason at any stage; (2) Suffering from organic gastrointestinal diseases; (3) Congenital spinal deformities; (4) Neurological disorders affecting normal gastrointestinal function; (5) Genetic metabolic diseases affecting normal gastrointestinal function; (6) Underwent gastrointestinal surgery from birth to the time of investigation. The ethical and informed consent application for this study was approved by the Ethics Committee of the Third Affiliated Hospital of Zunyi Medical University, Approval No.: Ethics Review (2022)-1-205. This study have been deposited in the publicly accessible Sequence Read Archive (SRA) database at the National Center for Biotechnology Information (NCBI). The specific accession number is PRJNA1136411. All included research subjects understood the content of this study and signed informed consent forms. (Please see Supplementary file “Questionnaire Survey”)

Sample size estimation

Based on the 2021 census data, the population of children aged 0–4 in Zunyi area was selected as the study

population. Due to the lack of disease prevalence data for children with FC in this region, the total disease prevalence of FC in children aged 2–18 in China, which is 6%, was adopted as the expected disease prevalence of FC in children aged 0–4 in this region. Thus, with $P=0.06$, $\alpha=0.05$, using the sample calculation formula of $N = \mu_{\alpha}^2 \times P \times q / (\delta^2)$, the total sample size was estimated to be 1526 individuals. Considering potential sample loss and incomplete information, the sample size was increased to 2039 cases. According to the population census data of Zunyi in 2021, the total number of children aged 0–4 in each county, district, and city (county-level city) was taken as the population. The sample size was calculated based on the above sample formula, and the ratio of sample size to the population is defined as the sampling ratio. According to the sampling ratio, the number of individuals to be sampled in each region was determined as follows: Honghuagang District 340, Huichuan District 248, Bozhou District 329, Tongzi County 247, Daozhen County 104, Meitan County 145, Xishui County 308, and Renhuai City 288.

Questionnaire design

The questionnaire design referred to relevant domestic and international literature and was based on the Rome IV diagnostic criteria for functional constipation (FC). The content of the questionnaire has been repeatedly discussed and researched by the research group, statistically analyzed by experts, and its reliability and validity are reliable. Including basic information (age, gender, family history, feeding history, defecation habits, parental information, etc.), Rome IV diagnostic criteria, FC-related risk factors (including whether living in towns or villages, whether the primary caregivers are parents, whether parents' education level is below high school, whether parents have been working outside, whether the child has a history of constipation, whether the child has an allergy history, whether probiotics have been taken in the past month, whether parents had a history of constipation in childhood, whether siblings have a history of constipation, etc.).

FC diagnostic criteria

The diagnosis of childhood FC adopted the Rome IV G7 criteria, requiring at least 2 of the following within 1 month: (1) Bowel movements ≤ 2 times per week; (2) History of fecal retention; (3) Painful defecation or passage of hard stools; (4) Passage of large-diameter stools; (5) Presence of large fecal mass in the rectum. For toilet-trained children, two additional criteria were included: (6) Fecal incontinence at least once a week after toilet training; (7) Passage of large stools leading to toilet obstruction.

Questionnaire survey

Using a stratified sampling method, one county-level medical institution's pediatric health department and one kindergarten were selected as collaborating units from each administrative region. The research team provided uniform training to medical staff or teachers from the collaborating units to ensure consistency in questionnaire completion standards. Electronic questionnaires were distributed to parents of children aged 0 to 4 on-site, with professional guidance provided to parents one-on-one for completion. Completed questionnaires were promptly submitted and checked.

Intestinal flora analysis

Research objects

Twenty-nine children with FC from the survey were randomly selected as the functional constipation group (FCG). Twenty-six healthy children matched with the FCG in terms of age, gender, and region were selected as the control group (CG). Exclusion criteria included: (1) Hypothyroidism, congenital megacolon, constipation-predominant irritable bowel syndrome, and other diseases; (2) Neurogenic, metabolic, and drug-induced constipation; (3) Probiotic intake within the last month; (4) Use of antibacterial drugs within the last month.

Specimen collection and examination

Fresh fecal samples from the children were collected separately using aseptic techniques and stored in sterilized cryovials, then transferred and stored at -80°C within half an hour. DNA from the bacterial community in the precipitate was extracted using the QIAGEN stool mini kit, and DNA from the bacterial community in the supernatant was extracted using the Microbiome Kit. After amplification of bacterial 16 S rRNA using 16 S rDNA V3+V4 region primers, sequencing was performed on the Illumina Miseq platform, and sequencing results were compared to the RDP database to determine the bacterial composition of each sample.

Intestinal flora analysis

Quality control and filtering of paired-end reads were performed based on sequencing quality, followed by assembly according to the overlap relationship between paired-end reads to obtain optimized data. Sequence denoising methods (such as DADA2/Deblur) were used to process the optimized data, obtaining Amplicon Sequence Variant (ASV) representative sequences and abundance information. Based on the ASV representative sequences and abundance information, taxonomic analysis, community diversity analysis, and species difference analysis of the gut microbiota in the two groups were performed. Additionally, the relative abundance and diversity differences of the gut microbiota in the two

groups were analyzed to explore the correlation between FC and gut microbiota.

Statistical analysis

Statistical analysis of data was performed using SPSS 29.0 software. For continuous variables with normal distribution, data were expressed as mean $\pm$ standard deviation and compared using the t-test. For non-normally distributed data, the median (interquartile range) was used and compared using the Mann-Whitney test. Categorical variables were presented numerically, with proportions expressed as percentages, and comparisons between groups were made using the χ^2 test.

Results

General situation

In this survey, a total of 2051 questionnaires were distributed and 2051 were collected, resulting in a 100% response rate. Of these, 2039 questionnaires were valid, with an effective rate of 99.4%. In terms of gender, there were 1185 boys (58.1%) and 854 girls (41.9%). In terms of residence, 1312 (64.3%) lived in urban areas and 727 (35.7%) lived in towns/villages. In terms of age groups, there were 594 (29.1%) children aged 0–1 years (exclusive), 1012 (49.6%) children aged 1–3 years (exclusive), and 433 (21.2%) children aged 3–4 years.

Characteristics of FC in children aged 0 to 4 years old in Zunyi area

Among the 2039 children surveyed, 151 met the diagnostic criteria for FC, resulting in a prevalence rate of 7.4% in the Zunyi area. The prevalence of FC was 6.6% (78/1185) among boys and 8.5% (73/854) among girls, with no statistically significant gender difference ($P > 0.05$). The prevalence of FC was significantly higher in children living in towns or villages (13.6%, 99/727) compared to those in urban areas (4.0%, 52/1312) ($P < 0.05$). Age-specific prevalence rates were 9.6% (57/594) for children aged 0–1 years, 6.3% (64/1012) for children aged 1–3 years, and 6.9% (30/433) for children aged 3–4 years, with statistically significant differences between age groups ($P < 0.05$).

FCG and CG intestinal flora analysis

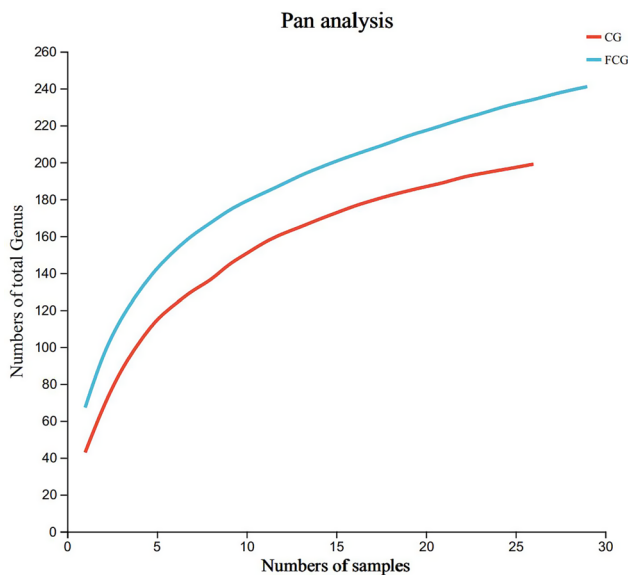
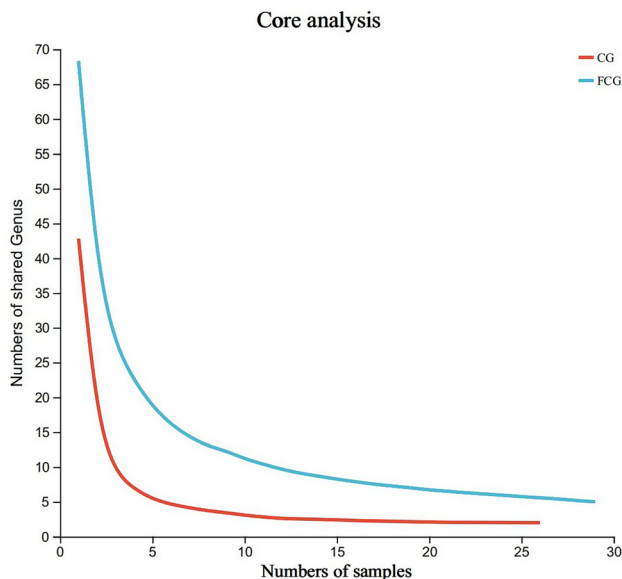
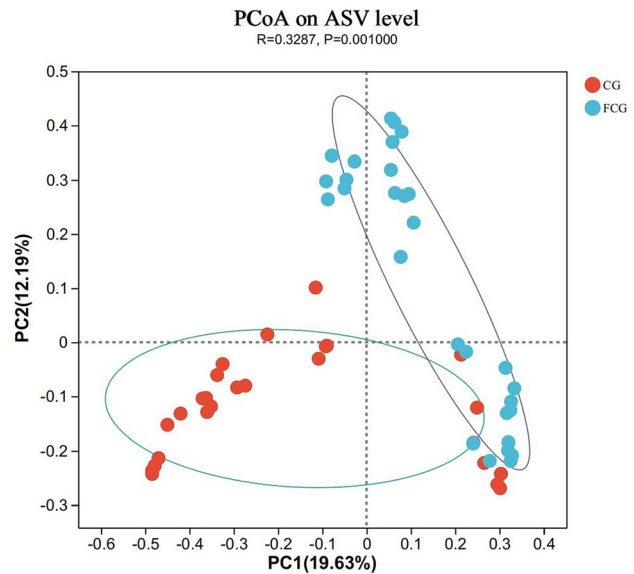
General information

The study included 29 children with FC (14 males and 15 females), aged 17 (11–34) months, and 26 healthy controls (12 males and 14 females), aged 23 (12) months. The mean age was 35 months. There were no statistically significant differences in age ($z = -0.379$, $P = 0.706$) or gender ($\chi^2 = 0.025$, $P = 0.875$) between the groups, as shown in Table 1.

Table 1 General information of FCG and CG children

Variables	Functional constipation group (29 cases)	Normal group (26 cases)	χ^2/Z	<i>p</i> value
Gender			0.025	0.875
Male	14(48.3)	12(46.2)		
Female	15(51.7)	14(53.8)		
Age	17(11,34)	23(12,35)	-0.379	0.706

Note: Age is expressed as the median, and the unit is months

**Fig. 1** Pan species analysis at the genus level**Fig. 2** Core species analysis at the genus level**Fig. 3** Principal coordinate analysis based on sample ASV abundance between two groups

Sample size assessment

Pan/Core curve graphs were used to illustrate the impact of sample size on total and core species abundance. At the genus level, the Pan curve plateaued with increasing sample size, while the Core curve declined and also plateaued. These curves indicate that our sample size for FCG and CG was adequate, ensuring the reliability of the results (Figs. 1 and 2).

Diversity analysis of intestinal flora

Alpha diversity analysis

We analyzed the abundance and diversity of intestinal microbiota at the ASV level. The Ace, Chao, and Sobs indices in FCG were 223.913, 219.428, and 214.690, respectively, while in CG, they were 116.044, 113.617, and 110.885, respectively, indicating higher abundance in FCG ($P < 0.05$). The Shannon and Simpson indices were 3.092 and 0.106 in FCG, and 2.107 and 0.253 in CG, indicating richer diversity in FCG ($P < 0.05$).

PCoA principal coordinate analysis of two groups of samples

The Adonis test was used to determine whether there was statistical significance between the different groups. The results showed that samples from each group clustered separately, indicating a clear distinction. The explanatory rates of PC1 and PC2 in the PCoA coordinate axes were 19.63% and 12.19%, respectively. As shown in Fig. 3, the distribution trend of the FCG children samples was more scattered compared to the healthy children group. This indicates that when comparing the gut microbiota structure, there were larger differences among individuals in the FCG group, while the differences in the healthy control group were relatively smaller. The total distances

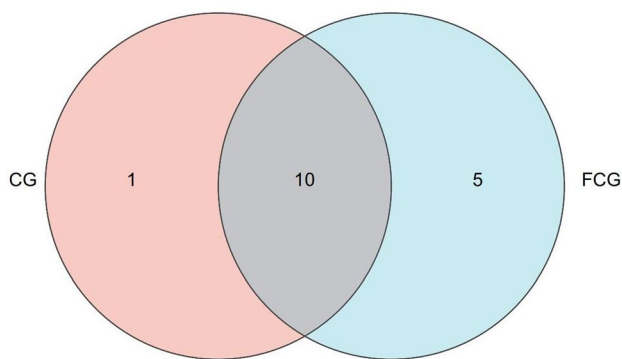


Fig. 4 Venn diagram analysis of phylum-level species between two samples



Fig. 5 Venn diagram analysis of species at genus level between two samples

between samples in FCG were relatively large, indicating larger differences in gut microbiota composition within the group. The overall distances between samples in CG were relatively small, suggesting smaller differences in

gut microbiota composition within the group. In addition, the distances between samples from the two groups were also substantial, indicating differences in microbial abundance between the two groups at $P < 0.05$. This indicates a high level of confidence in the test results.

Analysis of intestinal flora composition

Comparison of species differences

Venn diagrams at the phylum level indicated 5 unique taxa in FCG, 1 in CG, and an overlap of 10 taxa. At the genus level, FCG had 77 unique taxa, CG had 36, and there were 165 overlapping taxa. These results suggest significant differences in the intestinal microbial species between FCG and CG (Figs. 4 and 5).

FCG and CG intestinal microbial species and their relative abundance

At the phylum level, FCG was dominated by Firmicutes (44.70%), Bacteroidetes (25.96%), Actinobacteria (13.38%), and Proteobacteria (13.07%). CG was dominated by Actinobacteria (55.75%), Firmicutes (32.54%), Proteobacteria (7%), and Bacteroidetes (4.67%). At the genus level, dominant genera in FCG included Bifidobacterium, Escherichia-Shigella, and Lactobacillus; in CG, they included Bacteroides, Bifidobacterium, and Enterococcus (Fig. 6).

Analysis of species differences between the two groups

The Mann-Whitney test was used to analyze the differences in the relative abundance of species at the phylum and genus levels between two groups. Selecting the top 15 species with the highest combined abundance means between the two groups, statistical analysis revealed

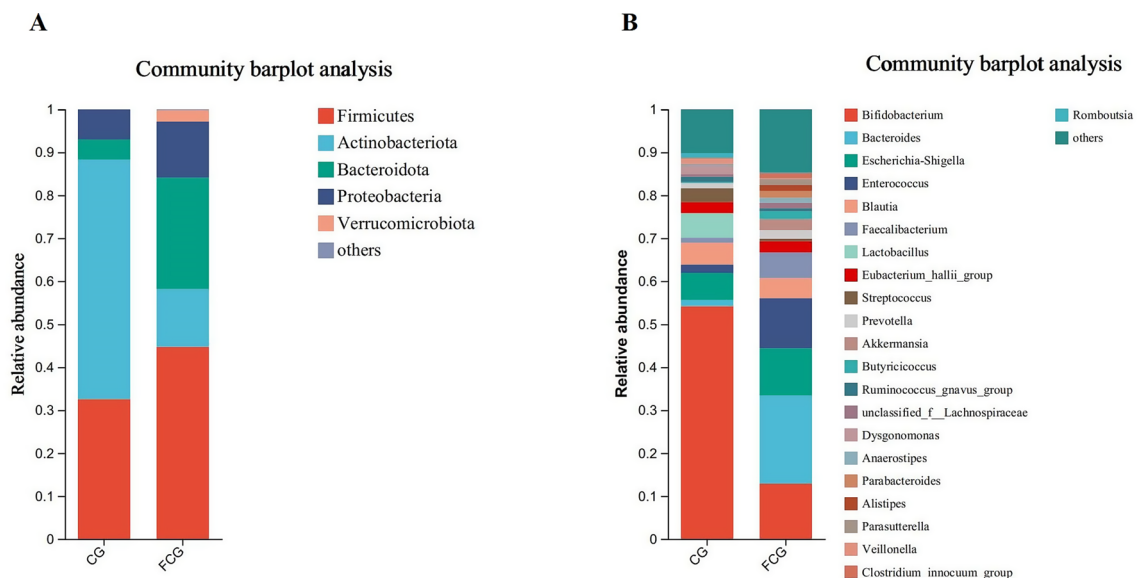


Fig. 6 A: Bar diagram on the Phylum level between two groups; B: comparing between two groups at the Genus level

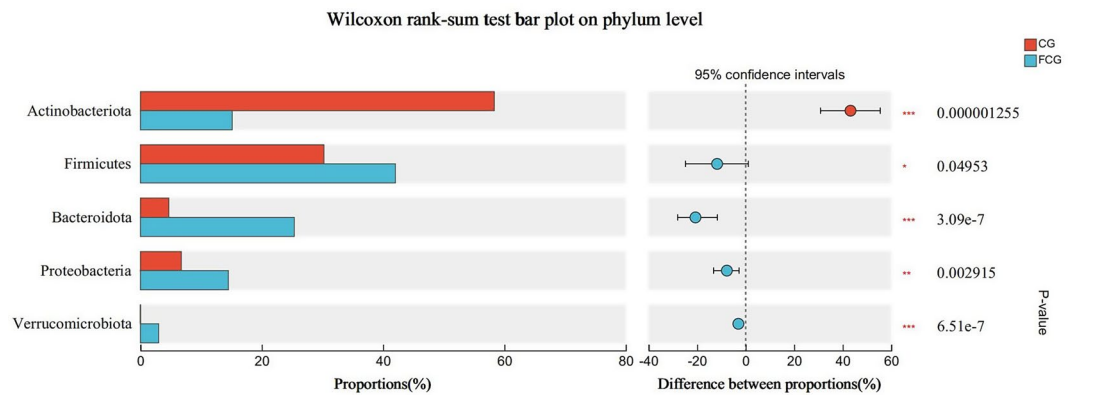


Fig. 7 Differences in relative abundance of species at the phylum level between two groups. *Note* The horizontal axis represents species names at different taxonomic levels, the vertical axis represents the percentage abundance of a species in a sample, and different colors represent different groups. The rightmost column shows the P-values, * $0.01 < P < 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$

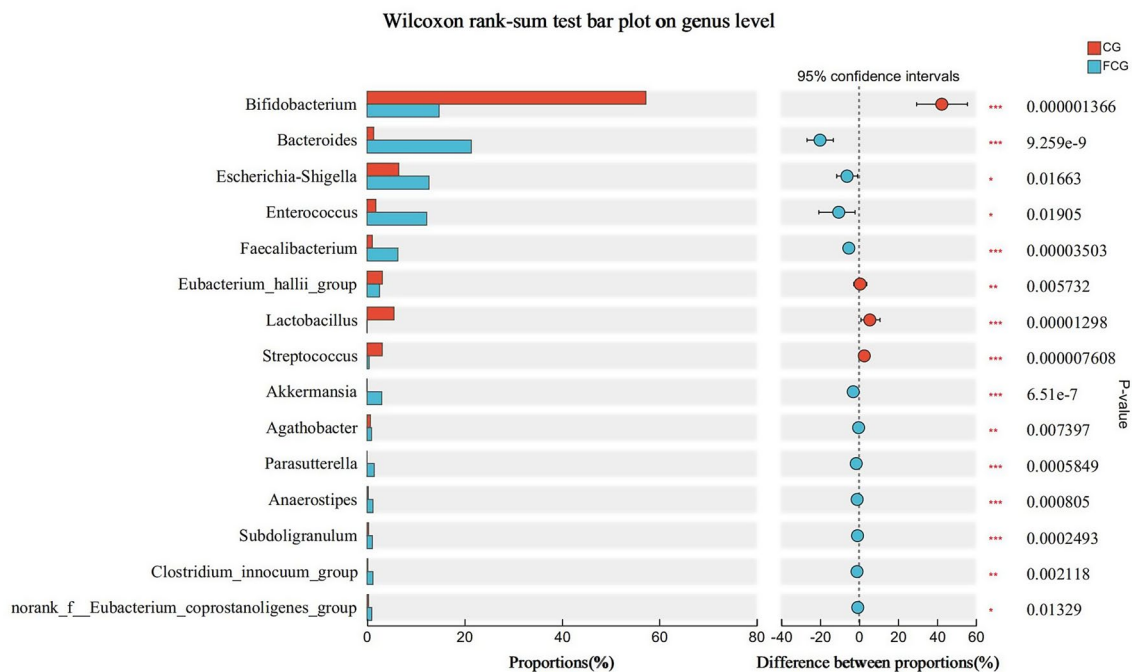


Fig. 8 Differences in relative abundance of species at the genus level between two groups. *Note* The horizontal axis represents species names at different taxonomic levels, while the vertical axis represents the percentage abundance of a certain species in the sample, with different colors indicating different groups. The P-value is shown on the far right, where * $0.01 < P < 0.05$, ** $0.001 < P \leq 0.01$, *** $P \leq 0.001$

significant differences in the four phylum levels between the two sample groups. As shown in Fig. 7, Firmicutes (Firmicutes), Bacteroidota (Bacteroidetes), and Proteobacteria (Proteobacteria) were dominant phyla in the FCG, while Actinobacteriota (Actinobacteria) was dominant in the CG. The differences between the two groups were statistically significant ($P < 0.05$). There were also differences at the genus level between the two sample groups. As shown in Fig. 8, dominant genera in the FCG included Bacteroides, Escherichia-Shigella, Enterococcus, Faecalibacterium, Akkermansia, etc. In the CG group, dominant genera included Bifidobacterium,

Eubacterium_hallii_group, Lactobacillus, and Streptococcus. The differences between the two groups were statistically significant ($P < 0.05$). Further LEfSe multi-level species difference discriminant analysis was conducted from phylum to genus level, with an LDA threshold of 4. The core differential classification unit analysis results of the two groups are shown in Fig. 9, where dominant microbial populations in the FCG included Bacteroidota, Enterococcus, Proteobacteria, Proteobacteria, Verrucomicrobiota, etc. Actinobacteriota, Bifidobacterium, Lactobacillus, Streptococcus, etc., were the main bacterial

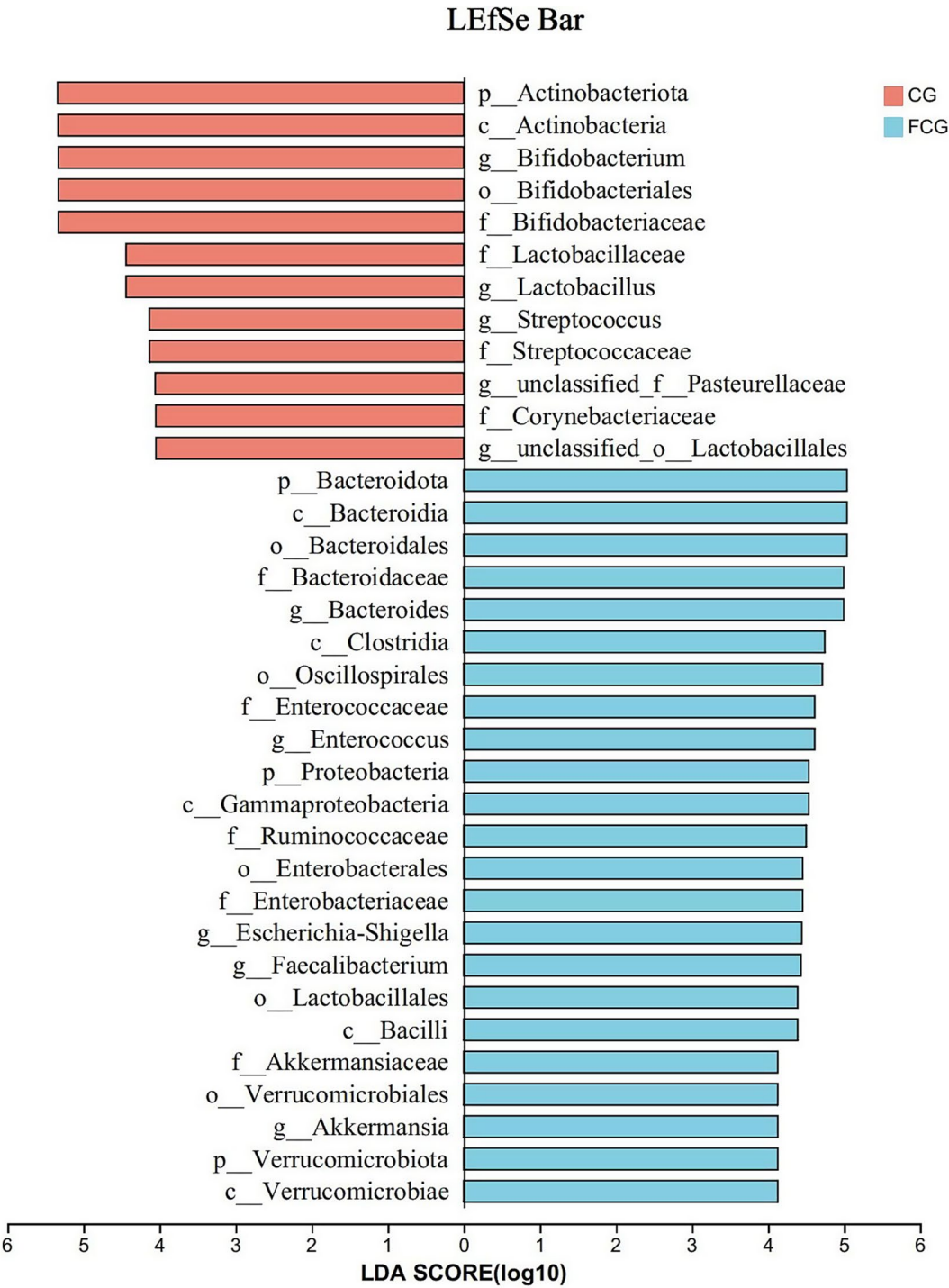


Fig. 9 LEfSe multi-level species difference discriminant analysis between two groups

species in the CG, playing important roles in intestinal motility, digestion, and absorption.

Discussion

FC is a common gastrointestinal disease in childhood, with no unified standard for its characteristics. Our survey shows that the incidence of FC in children aged 0–4 in Zunyi is 7.4%, slightly higher than the national rate of 6%. There is no significant difference in incidence between genders ($P > 0.05$). However, children in towns and rural areas have a higher incidence than those in urban areas ($P < 0.05$). This disparity is likely due to better medical care and early problem detection in urban areas, while rural children may lack proper child health care knowledge and supervision, increasing their risk of constipation [11]. The incidence of FC varies significantly among different age groups ($P < 0.05$), with the highest incidence in children aged 0–1 years, the lowest in those aged 1–3 years, and an intermediate level in those aged 3–4 years. Analyzing the reasons, infants usually start adding complementary foods around 6 months old. Complementary foods change the environment of the gastrointestinal tract, and the digestive system needs time to adapt to the newly added food, making constipation more likely to occur during this period [12, 13]. Whereas children aged 3–4 are in the preschool stage, facing kindergarten, changes in living environment, dietary habits, and defecation rhythms can easily lead to constipation.

In recent years, numerous studies have highlighted the significant role of gut microbiota in the onset of functional constipation (FC) in children, demonstrating notable alterations in the structure and abundance of gut microbiota in children with FC [1, 14–16]. This study conducted an Alpha diversity analysis on the gut microbiota of the FC group and a healthy control group. The results revealed significant differences in Ace, Chao, and Sobs indices between the FC group and the healthy control group ($P < 0.05$), indicating a higher richness of gut microbiota in children with FC compared to the healthy group. Additionally, the Shannon index in the FC group was significantly increased compared to the control group, further confirming higher community diversity in the FC group, consistent with previous research findings. Demoraes et al. [17] studied 79 children aged 3–6 years (39 with chronic constipation, 40 without constipation) and found that the levels of lactobacilli in stool samples per milligram were lower in children with chronic constipation compared to the control group children, which is consistent with the findings of this study. Furthermore, compared to the control group, there was a significant reduction in the genus *Bifidobacterium* in the FC group. *Bifidobacterium* is a well-recognized beneficial bacterium capable of inhibiting the growth and proliferation of other resident and pathogenic bacteria in the intestine

[18]. The higher abundance of gut microbiota in children with FC compared to children in the control group indicates significant alterations in the gut microbiota of children with functional constipation. This alteration is possibly related to a significant decrease in *Bifidobacterium* in the FC group, which may not effectively inhibit the growth of other bacteria, leading to a correlated increase in pathogenic bacteria. This finding further reveals the possible association between changes in gut microbiota and FC.

The gut is the most extensively colonized organ in the human body, predominantly inhabited by bacteria from the phyla Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria [19, 20]. This study reaffirms the composition of gut microbiota at the phylum level, consistent with previous research findings. Although there were no differences in phylum-level taxa, the FC group exhibited distinct proportions compared to the healthy control group, characterized by significantly elevated abundance of Firmicutes, Bacteroidetes, and Proteobacteria in the FC group, while Actinobacteria were significantly reduced compared to the healthy control group, with statistically significant differences between the two groups ($P < 0.05$). Meij et al. [21] prospectively recruited children with chronic constipation and analyzed their gut microbiota differences using PCR-based analysis, revealing increased abundance of Proteobacteria and elevated levels of the genus *Bacteroides* in constipated children. Another study indicated significant differences in fecal microbial structure between obese constipated children and healthy children, with a significantly higher ratio of Firmicutes to Bacteroidetes and increased abundance of Firmicutes [14], consistent with the findings of this study.

Analysis of species differences between the two groups shows that compared with healthy children, the FC group had significantly reduced numbers of *Bifidobacterium* and *Lactobacillus*, with statistically significant differences ($P < 0.05$). The above-mentioned genera are recognized probiotics, among which (*Bifidobacterium*) has various effects on human health such as biological barrier, nutrition, enhanced immunity, and improved gastrointestinal function. They also have antagonistic effects on various bacteria, inhibiting the growth and reproduction of foreign bacteria. While the genus *Lactobacillus* can improve the digestion and absorption of nutrients, produce various vitamins, and inhibit the reproduction of intestinal putrefactive and pathogenic bacteria. At the same time, it can decompose lactate generated from sugar in the intestine, increase intestinal acidity, thereby inhibiting the reproduction of intestinal pathogens, preventing protein hydrolysis, and can be used to treat digestive disorders. Another study has shown that the decrease in the quantity of *Lactobacillus* and *Bifidobacterium* leads to an increase in intestinal pH, preventing the development of

certain components of the microbiota, thus, changes in microbiota composition can easily lead to the occurrence of constipation [19].

The appropriate composition of gut microbiota maintains optimal butyric acid levels in the colon, ensuring proper intestinal motility. Optimal concentrations facilitate colonic smooth muscle contraction, supporting motility and preventing constipation. However, excessively high butyric acid levels inhibit muscle contraction and slow peristalsis. High butyrate concentrations may also inhibit mucin secretion by goblet cells and reduce stool volume by affecting water and electrolyte absorption [19, 22, 23]. This study found that *Faecalibacterium* abundance was significantly higher in children with FC compared to healthy children. *Faecalibacterium* is known to produce butyric acid, which plays a crucial role in maintaining intestinal health by protecting the mucosal barrier, reducing inflammation, and enhancing motility. Butyrate salts have a bidirectional effect on colonic motility, stimulating it at low concentrations and inhibiting it at high levels [24]. Therefore, dysbiosis leading to constipation may be linked to the metabolic products of the microbiota, particularly butyric acid, which may directly or indirectly contribute to childhood FC.

This study found a significant increase in the abundance of *Akkermansia* in the intestinal tract of FCG children, a bacterium that has been repeatedly identified as a statistically significant biomarker in FC children. The current study suggests a connection between this bacterium and diseases such as obesity, diabetes, and Alzheimer's disease [25–28]. Some domestic scholars have found in animal experiments that the abundance of *Akkermansia* genus is higher in the fecal microbiota of constipated mice. This may be related to its ability to degrade intestinal mucin, leading to fecal dryness and ultimately damaging the intestinal mucosal barrier [29, 30]. Other researchers have found that this bacterium belongs to the *Verrucomicrobia* phylum and specializes in mucin degradation and participates in the metabolism of human milk oligosaccharides [31]. It may reduce serotonin (5-HT) levels by upregulating the level of 5-HT transporter protein, thereby slowing intestinal transit and impeding stool expulsion, leading to constipation [32]. Meanwhile, the slowing of intestinal transit speed prolongs fecal retention time, causing water absorption and fecal dryness, leading to constipation. This vicious cycle may ultimately lead to the occurrence of functional constipation.

This study still has some limitations. Firstly, the cross-sectional design used in this study cannot observe the long-term changes and developmental trends of children's FC. Additionally, the questionnaire survey method used in this study has subjectivity in information acquisition and memory biases, which may lead to data uncertainty. Finally, this study did not consider other potential

influencing factors, such as genetic factors, environmental factors, etc., which impose certain limitations on the interpretation and generalization of the research results.

Conclusion

In conclusion, the incidence of FC in children aged 0 to 4 in Zunyi is related to long-term residential environment, and there are also differences between age groups. Analysis of intestinal flora indicates that there are statistically significant differences in the composition of intestinal flora between FC children and healthy children in this region, revealing the possible involvement of intestinal flora in the occurrence and development of FC in children.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12887-024-05383-w>.

Supplementary Material 1

Acknowledgements

Not applicable.

Author contributions

SG.G. made significant contributions to research design, specific experimental process management, data analysis, and manuscript writing; ZB.Y. made significant contributions to research design and data analysis; Y.D. and X.L. made significant contributions to experimental technical support and results section; Y.W. contributed to research design; SL.G. contributed to experimental operation and data collection; SG.G. and SL.G. made significant contributions to research design and manuscript review process. All authors reviewed the manuscript.

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Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

The ethical and informed consent application for this study was approved by the Ethics Committee of the Third Affiliated Hospital of Zunyi Medical University, Approval No.: Ethics Review (2022)-1-205. All included research subjects understood the content of this study and signed informed consent forms. All methods were carried out in accordance with Declaration of Helsinki.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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