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Research article

Intestinal microbiota and probiotic intervention in children with bronchial asthma

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ABSTRACT

Objective: This study aims to understand the differences in intestinal flora, expression of helper T cells, allergy-related indicators, and cytokine levels between children with bronchial asthma and healthy children. The study seeks to clarify the effectiveness and safety of probiotic preparations in the treatment of bronchial asthma in children, and to provide new methods for the treatment of bronchial asthma.

Methods: A total of 66 pediatric patients aged 3–6 years with bronchial asthma and 35 healthy children undergoing physical examination during the same period were enrolled, designated as the asthma group and the healthy group, respectively. The asthma group was further divided into the probiotic group and the non-probiotic group based on whether probiotics were used. The gut microbiota, serum IgE antibody levels, cytokines (IL-4, IL-5, IL-9, IL-13 levels), proportions of helper T cells (Th1, Th2), and hypersensitive C-reactive protein were measured and compared among the groups.

Results: Children with bronchial asthma had decreased abundance and reduced diversity of intestinal flora compared to the healthy group. At the genus level, the asthma group showed increased abundance of Bacteroides and decreased abundance of Faecalibacterium and Veillonella; The probiotic group demonstrated a significantly higher improvement in the abundance of these genera before and after treatment compared to the non-probiotic group ($P < 0.05$). Compared to the healthy group, children with asthma had elevated levels of serum IgE, IL-4, IL-5, IL-9, and IL-13, as well as a decreased Th1/Th2 ratio, all of which showed statistical differences ($P < 0.05$). After treatment, all immune indicators improved. Specifically, the probiotic group exhibited a more significant decrease in serum IgE, IL-4, and IL-13 levels compared to the non-probiotic group ($P < 0.05$).

Conclusion: Children with bronchial asthma exhibit dysbiosis of intestinal flora, characterized by an increased abundance of the Bacteroides and decreased abundance of the Faecalibacterium and Veillonella. This imbalance in intestinal flora increases the risk of allergic diseases. Probiotics can

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effectively improve dysbiosis of intestinal flora, contributing to the balance of immune function in children, and can be used as an adjunct therapy for the treatment of bronchial asthma.

1. Introduction

Bronchial asthma is the most common chronic airway disease in children, characterized by abnormal immune responses, leading to reversible airflow obstruction, airway hyperresponsiveness (AHR), and other clinical reactions [1]. The occurrence of bronchial asthma in children involves various mechanisms such as immune, neural regulation, and genetic factors, all of which contribute to the initiation of airway inflammation, the persistence of chronic inflammation, and airway remodeling [2]. The proposal of the "Hygiene Hypothesis" [3] suggests a correlation between early exposure to environmental factors and the increase or decrease in the incidence and development of asthma and allergies. As an extension of this hypothesis, the "Microbial Community Hypothesis" also indicates that changes in the bacteria present in the human gut during the early stages of life due to factors such as antibiotic use, infections, or diet disrupt the normal microbial mechanisms that promote immune tolerance, leading the immune system towards a state that promotes allergic diseases and asthma [4].

In recent years, probiotics have been widely used to alter and improve intestinal flora. By giving probiotics, adult asthmatic patients will benefit from better respiratory parameters [5]. However, clinical studies on the treatment of bronchial asthma in children are relatively rare, and its clinical prevention and treatment effects are not clear. Children, unlikely to adults, are naïve to intestinal flora, have fewer underlying diseases, and less antibiotics exposure. Therefore, research on children intestinal flora with asthma is valuable.

This study aims to understand the differences in intestinal flora, expression of helper T cells, allergy-related indicators, and cytokine levels between children with bronchial asthma and normal children. It aims to provide a basis for laboratory diagnosis of pediatric bronchial asthma. Additionally, the study aims to observe the effects of probiotic intervention on the improvement of gut microbiota and regulation of immune status, thereby clarifying the effectiveness and safety of probiotic intervention in the treatment of pediatric bronchial asthma.

2. Methods

2.1. Selection of research Subjects and Grouping

We collected 66 pediatric patients aged 3–6 years with bronchial asthma, all of whom met the diagnostic criteria for childhood bronchial asthma in China and did not have any exclusion criteria for this study. Additionally, 35 healthy children undergoing physical examinations during the same period were selected as the healthy group. The asthma group was further divided into the probiotic group and the non-probiotic group based on the treatment method. Both groups received basic treatment with inhaled budesonide, with the probiotic group also taking a combination of intestinal probiotics concurrently. The contents of this probiotic were Lactobacillus reuteri GL-104, Lactobacillus paracasei, Lactobacillus rhamnosus, Lactobacillus acidophilus GL-206, and Bifidobacterium longum. Participants of probiotic group was given a pack of powdered form probiotic twice daily for consecutive six months. There were no statistically significant differences in gender and age among the groups, as shown in Table 1.

2.2. Intestinal microbiota detection

We used second-generation high-throughput sequencing technology to detect the gut microbiota of the enrolled children. Specifically, the V3–V4 regions of the 16S rRNA gene were amplified using primers, and the sequencing was performed on the Illumina NovaSeq platform. This approach allowed us to identify the microbial species present in the samples. Subsequently, we analyzed the alpha diversity of the gut microbiota using the QIIME2 software platform. Alpha diversity analysis assesses species diversity within individual samples using a series of statistical indices to evaluate the richness and diversity of microbial species. Richness measures the number of species within a single sample, while diversity indices quantify the heterogeneity of the microbial community.

2.2.1. Calculation of community richness index

Chao1: The Chao1 algorithm estimates the number of operational taxonomic units (OTUs) in a sample by calculating the index of OTUs detected only once or twice in the community, providing an estimate of the actual number of species present in the community. Chao1 is commonly used in ecology to estimate total species richness and was first proposed by Chao (1984).

Table 1
Age and gender between the asthma group and the healthy group.

	(n = 66) Asthma group		Healthy group (n = 35)
	Probiotic group (n = 34)	Non-probiotic group (n = 32)	
Gender (M/F)	16/18	14/18	19/16
Age	4.44 ± 1.03	4.44 ± 0.93	4.71 ± 1.03

Data were analyzed by chi-square test.

Table 2
Microbial diversity between the asthma group and the healthy group.

	Asthma group (n = 66)	Healthy group (n = 35)	p value
Shannon[a]	4.91 ± 1.04	5.42 ± 0.91	0.016
simpson△	0.88 ± 0.12	0.92 ± 0.04	0.080
Chao1△	965.58 ± 345.46	1235.93 ± 545.95	0.015
ace△	920.62 ± 266.91	1224.40 ± 527.43	0.003

Note: Data are presented as means±SDs and M(P25,P75).T-test and Mann-Whitney *U* test was used for analysis.Indicates non-normally distributed data and was analyzed using the Mann-Whitney *U* test.

[a] a Indicates normally distributed data and was analyzed using independent sample *t*-test. △ indicates non-normally distributed data and was analyzed using the Mann-Whitney U test

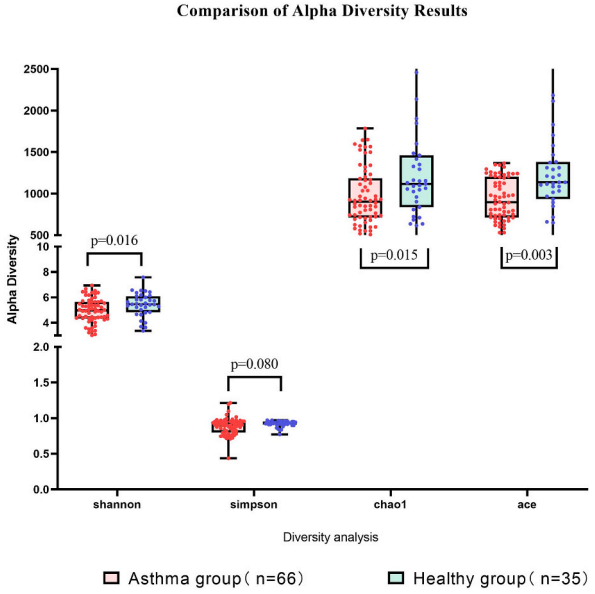

Fig. 1. Comparison of Alpha Diversity Results in asthma group and healthy group. This figure compares the α diversity of intestinal flora between the healthy group and the asthma group, including shannon index, simpson index, chao1 index and ace index, among which ace index showed the most significant difference between the two groups.

ACE: This index is used to estimate the number of OTUs in the community, and it includes OTUs with fewer than 10 sequences in its calculations, thus estimating the actual number of existing species in the community. It is one of the commonly used indices in ecology to estimate the total number of species, and it is distinct from the Chao1 algorithm.

2.2.2. Calculation of community diversity index

Shannon [6]: The Shannon-Wiener index takes into account both the richness and evenness of a community. A higher Shannon index value indicates higher diversity in the community.

Simpson [7]: The Simpson diversity index is used to assess the diversity of the microbial community. A higher Shannon index value indicates higher diversity in the community.Generally, the Shannon index emphasizes community richness and rare OTUs, while the Simpson index focuses on evenness and dominant OTUs within the community.

2.3. Serum immune index detection

2.3.1. Serum IgE antibody detection

The serum IgE antibody is detected using the specific protein analysis system manufactured by Beckman Coulter. Approximately 3 ml of non-anticoagulant blood was collected via routine venipuncture and centrifuged at 2500–3000 rpm for 6–10 min to separate the serum. The serum samples were then analyzed using the designated machine.

2.3.2. Cytokine level detection

Cytokine levels (IL-4, IL-5, IL-9, IL-13) were detected using flow cytometry technology. 25 μl of anticoagulated plasma, mixed capture microspheres, and 25 μl of fluorescent markers were added to the flow tube and for incubated for 2.5 h. After centrifugation and washing, the samples were analyzed using the designated machine. Post-detection, the cytokine concentrations were determined

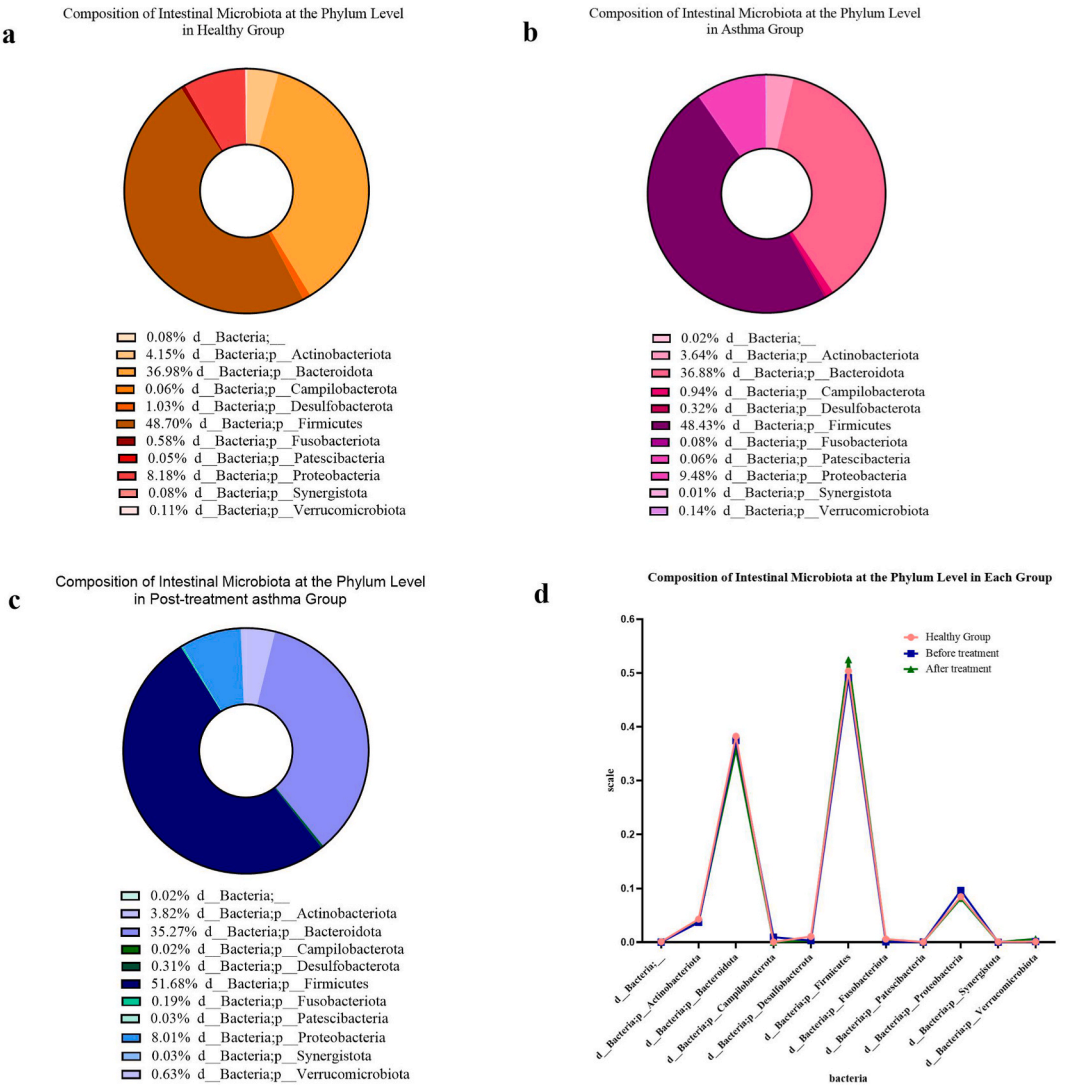

Fig. 2. Composition of Intestinal Microbiota at the Phylum Level in Each Group. Fig. 2-a, b and c represent the composition of bacteria at the phylum level in the healthy group, asthma group and asthma treated group, respectively. Fig. 2-d represents the comparison of the phyla level bacterial composition of each group.

by comparing them with standard curves using analysis software.

2.3.3. Detection of helper T cell (Th1, Th2) ratio

Based on the surface markers characteristic of Th1/Th2 cell clusters, we will set up the experimental plan. Th1 cells are surface-marked as $CD4^{+}CXCR3^{+}CCR6^{-}$, while Th2 cells are surface-marked as $CD4^{+}CXCR3^{-}CCR6^{-}$. We will use APC to label CXCR3, PE to label CCR6, and PE-cy7 to label CD4. Through flow cytometry technology, we will obtain the ratio of Th1 to Th2 cells.

2.3.4. Detection of high-sensitivity C-reactive protein

2 ml of blood with EDTA anticoagulant is collected, and the high-sensitivity C-reactive protein is detected using a fluorescence immunoassay analyzer.

2.4. Pulmonary function test

Using the MasterScreen PAED produced by Vyaire Medical GmbH, slow vital capacity (also known as maximal vital capacity, VC_{max}), forced expiratory volume in 1 s (FEV1), and $FEV1/VC_{max}$ ratio were measured before and after treatment in both the probiotic therapy group and the non-probiotic therapy group.

Table 3
Microbial abundance for the healthy group, asthma group, and post-treatment asthma group M(P_{25},P_{75}).

Microorganism	Healthy group (n = 35) (%)	Asthma group (n = 66) (%)	Post-treatment asthma group (n = 66) (%)
Bacteroides genus	31.123(13.26,41.01)△	34.887(26.45,43.37)	26.298(20.46,37.29)[a]
Faecalibacterium genus	16.522(12.10,24.07)△	6.140(4.27,11.31)	13.908(7.93,21.78)[a]
Veillonella genus	0.253(0.11,0.48)△	0.102(0.03,0.19)	0.198(0.03,0.41)
Prevotella species	1.023(0.25,2.53)	1.985(0.73,4.52)	1.326(0.44,3.90)
Bifidobacterium genus	1.413(0.45,3.27)	1.157(0.44,1.49)	1.870(0.47,4.88)[a]
Lactobacillus genus	0.023(0,0.63)△	0.303(0.09,0.81)	0.168(0.02,0.89)[a]
Clostridium genus	0.113(0.06,0.35)△	0.632(0.17,1.41)	0.781(0.24,1.13)[a]
Roseburia genus	0.387(0.15,1.35)	1.112(0.22,2.96)	0.725(0.18,3.89)
Fusobacterium genus	0.007(0,0.04)	0.003(0,0.03)	0.001(0,0.01)
Akkermansia muciniphila	0.013(0,0.22)	0.022(0,0.14)	0.041(0,0.24)
Prevotella genus	0.314(0.01,3.00)△	0.825(0.27,3.55)	1.112(0.32,19.75)
Ruminococcus genus	1.074(0.19,1.67)	1.249(0.33,3.06)	1.120(0.57,3.03)
Parabacteroides genus	1.855(0.57,2.62)△	1.285(0.44,2.36)	0.367(0.04,1.46)[a]
Alistipes genus	0.017(0,0.30)	0.138(0,0.72)	0.179(0.01,0.46)
Collinsella species	0.068(0.01,0.53)△	0.004(0,0.037)	0.025(0,0.07)
Coprococcus genus	0.143(0.60,0.34)△	0.072(0.01,0.26)	0.572(0.07,1.04)
Mucispirillum genus	0.288(0.15,0.66)	0.542(0.17,1.08)	0.496(0.23,1.20)
Spirochaetes genus	0.369(0.12,1.30)△	0.166(0.03,0.65)	0.055(0.003,0.13)[a]
Dialister genus	0.417(0.01,2.87)△	0.113(0.003,0.81)	0.980(0.10,2.11)
Collinsella genus	0.063(0.01,0.54)△	0.005(0,0.07)	0.042(0.01,0.20)[a]
Streptococcus genus	0.436(0.33,0.89)△	0.189(0.09,0.45)	0.110(0.06,0.19)[a]

Data are presented as M(P25,P75),and Mann-Whitney *U* test was used for analysis.Comparisons between groups were corrected by the Bonferroni method.
Abundance: Represents the proportion of various bacterial genera in the intestinal microbiota.
△ represents the healthy group compared with the asthma group, $P < 0.05$.
[a] △ represents the healthy group compared with the asthma group, $P<0.05$ [a]represents the post-treatment asthma group compared with the asthma group, $P<0.05$

2.5. Statistical analysis

SPSS 28.0 was used for analysis.The alpha diversity of gut microbiota will be represented as X ± SD, while bacterial abundance and immunological indicators will be presented as M (P25, P75). Shapiro-Wilk test (W test) was used to analyze the normality of data. If the data follows a normal distribution, the *t*-test will be used for comparison between two groups, and one-way analysis of variance (ANOVA) will be used for comparison among multiple groups. If the data does not follow a normal distribution, the Wilcoxon rank-sum test will be used for comparison between two groups, and the Mann-Whitney *U* test will be used for comparison among multiple groups. Bonferroni correction will be applied for inter-group comparison. Chi-square test will be used for comparison of categorical variables between groups.

3. Results

3.1. Comparative analysis of intestinal microbiota

3.1.1. Comparison of Alpha Diversity Results

Comparing the Shannon index, Simpson index, Chao1, and ACE between the asthma group and the healthy group, we found significant differences in the Shannon index, Chao1, and ACE ($P < 0.05$), with a particularly significant difference in the ACE index reflecting community richness ($P < 0.01$). For specific values, please refer to Table 2, and for data distribution, please see Fig. 1.

3.1.2. Composition of Intestinal Microbiota at the Phylum Level in Each Group

An analysis of the fecal samples sent for examination in this study at the phylum level reveals the ten most abundant bacteria, which was showed in Fig. 2(a–d). Among them, the Firmicutes, Bacteroidees, Proteobacteria, and Actinobacteria phyla accounted for higher proportions, with no significant difference between the groups.

3.1.3. Composition of Intestinal Microbiota at the genus level in each group

Through sequencing and software analysis of the intestinal microbiota, the abundance of common genera in each group was obtained. The statistics for the genera with higher abundance are shown in the following table (expressed as M(P25,P75)). Compared to the healthy group, the asthma group exhibited an increase in the abundance of the Bacteroides and Clostridium genera, and decrease in the abundance of Faecalibacterium, Veillonella and other bacteria. After treatment, the abundance of these genera approached the levels in the healthy group, which was showed in Table 3.

While analyzing the four most dominant bacteria among intestinal flora, the variation of the bacterial concentration was compared on the 66 asthmatic children. From Fig. 3(a–d), both probiotic and non-probiotic treated group showed decrease in Bacteroides and increase in Faecalibacterium and Veillonella concentration. However, the probiotic treated group had greater change than the non-

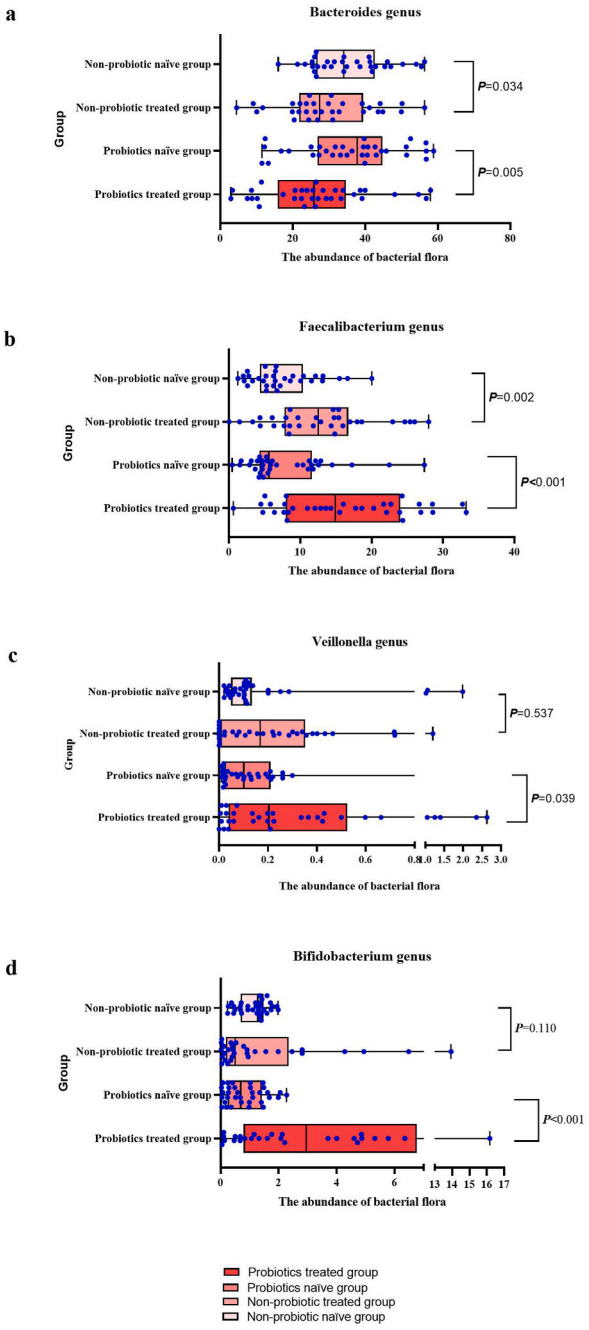

Fig. 3. Changes in the microbiota in the probiotic treatment group and the non-probiotic treatment group. Fig. 3-a, b, c, and d represent the comparison of Bacteroides, Faecalibacterium genus, Veillonella genus, and Bifidobacterium genus before and after treatment with probiotic rent and before and after treatment with non-probiotic group, respectively.

probiotic treated group. Additionally, Bifidobacterium genus significantly increased only after probiotic treatment ($P < 0.01$), whereas the change was not significant in the non-probiotic treatment group ($P > 0.05$).

3.2. Comparison of immunological indicators between asthma group and healthy group

By comparing the serum IgE antibody levels, IL-4, IL-5, IL-9, IL-13 levels, hypersensitive C-reactive protein (hCRP), and the Th1/Th2 ratio between the asthma group and the healthy group, it was found that, compared to the healthy group, the asthma group had elevated serum IgE antibody levels, IL-4, IL-5, IL-9, IL-13 levels, and a decreased Th1/Th2 ratio (Table 4). All of these differences were

Table 4
Immunological Indifcators between the asthma group and the healthy group.

Immunological Indicators	Asthma group (n = 66)	Healthy Group (n = 35)	Probiotic treated group (n = 34)	Non-probiotics treated group (n = 32)
IgE antibody (IU/ml)	302.95(129.10,524.93)	54.70(25.40,142.60)	180.50(96.75,300.50)	154.00(58.85,342.50)
IL-4 (pg/ml)	4.055(2.53,5.99)	2.160(1.30,3.32)	2.610(1.19,4.85)	2.880(1.84,4.83)
IL-5 (pg/ml)	1.060(0.64,1.56)	0.370(0.22,0.64)	0.395(0.23,0.57)	0.365(0.24,0.72)
IL-9 (pg/ml)	0.425(0.20,0.70)	0.200(0.04,0.41)	0.270(0.08,0.40)	0.270(0.14,0.45)
IL-13 (pg/ml)	0.505(0.30,0.78)	0.210(0.07,0.38)	0.265(0.16,0.40)	0.295(0.15,0.43)
hCRP (mg/L)	0.800(0.60,1.30)	0.700(0.060,1.00)	0.820(0.062,1.09)	0.800(0.60,1.02)
Th1/Th2	10.05 ± 2.95 %	13.32 ± 9.94 %	13.78 ± 2.35 %	12.98 ± 2.02

Data are presented as M(P25,P75),and Mann-Whitney *U* test was used for analysis.
Abbreviation.
Hypersensitive C-reactive protein (hCRP).
IL Interleukin.
IgE Immunoglobulin E.

statistically significant ($P < 0.05$). The difference in hCRP between the two groups was not statistically significant ($P > 0.05$). Fig. 4 (a–g) demonstrated decreasing serum level of above immunological markers on asthmatic treated group. Particularly, the levels of serum IgE, IL-4 and IL-13 in the probiotic treated group were significantly lower than those in the non-probiotic treated group ($P < 0.05$).

3.3. Comparison of pulmonary function before and after treatment between the probiotic therapy group and the non-probiotic therapy group

Analyzing reliable pulmonary function data, it was found that the probiotic therapy group showed greater improvement in FEV1/VC_{max} compared to the non-probiotic therapy group. There was a significant difference in FEV1/VC_{max} before and after treatment in the probiotic therapy group. Please refer to Table 5 for details.

4. Discussion

Asthma is one of the most common chronic respiratory diseases worldwide, characterized primarily by recurrent wheezing, shortness of breath, and sometimes accompanied by chest tightness or coughing. It is also characterized by airway hyperresponsiveness and variable airflow limitation and airway remodeling [8]. The occurrence of pediatric bronchial asthma has been escalating annually. Yet, challenges including hormonal side effects, the steep expenses associated with monoclonal antibodies, and age-related limitations [9,10] have constrained therapeutic and recovery options for children afflicted with bronchial asthma. Such constraints not only narrow down treatment alternatives for young asthma patients but also levy substantial financial strains on families and the broader community.In light of comprehensive research into the connection between gut microbiota, immune system modulation, and allergic conditions [11–13], adjusting the gut microbial environment to enhance bronchial asthma presents a novel approach for managing pediatric bronchial asthma.

Intestinal bacteria are composed of microorganisms residing in the human digestive tract.The ratios and makeup of these bacteria are dynamic, with different species either competing or cooperating, playing a pivotal role in the gut's microenvironment [11]. This study revealed that the diversity of intestinal flora in children with bronchial asthma was lower than that in the healthy children. In the ace index reflecting community richness, the asthma group was significantly lower than the healthy group, indicating that the intestinal flora in children with asthma has decreased in both species and quantity. This suggests a reduction in the number of gut microbes in children with asthma, a finding that is consistent with Hevia's research on the gut microbiota of 21 long-term adult asthma patients [14].

In this study, the sample demonstrated consistent bacterial proportions at the phylum level. It's been documented that the human gut hosts an immense bacterial population, with estimates reaching up to 10^14 individual bacteria. This population, which includes phyla like Firmicutes, Bacteroidetes, and Actinobacteria, displays substantial inter-individual variability [15]. At the genus level, the group with asthma exhibited a higher prevalence of the Clostridium and Bacteroides genera, while witnessing a decline in the Faecalibacterium and Veillonella genera. Following treatment, the levels of these genera neared those of the control group. Meanwhile, the probiotic treated group also showed an increase in the genus Bifidobacterium. Veal's research suggested that the early colonization of susceptible Bacteroides at three weeks old, combined with an uptick in the overall count of anaerobic bacteria, could predispose individuals to develop asthma later in life [16].

The relative increase in the abundance of Clostridium and Bacteroides and the decrease in the relative abundance of Bifidobacterium and Lactobacillus are related to the development of allergic eczema or asthma. Various studies have revealed the effects of different genera on the occurrence of asthma, but there are differences in species, mainly due to the various influencing factors affecting the composition of the gut microbiota, such as feeding methods and levels of antibiotic exposure [13,17].

The main components of probiotics used in this study were Lactobacillus Roy GL-104, Lactobacillus paracasei, Lactobacillus rhamnosus, Lactobacillus acidophilus GL-206, and Bifidobacterium longum. Studies showed that Lactobacillus reuteri in intestinal flora is good to asthmatic patients. Lactobacillus reuteri can reduce airway inflammatory response and reduce the amount of IgE and

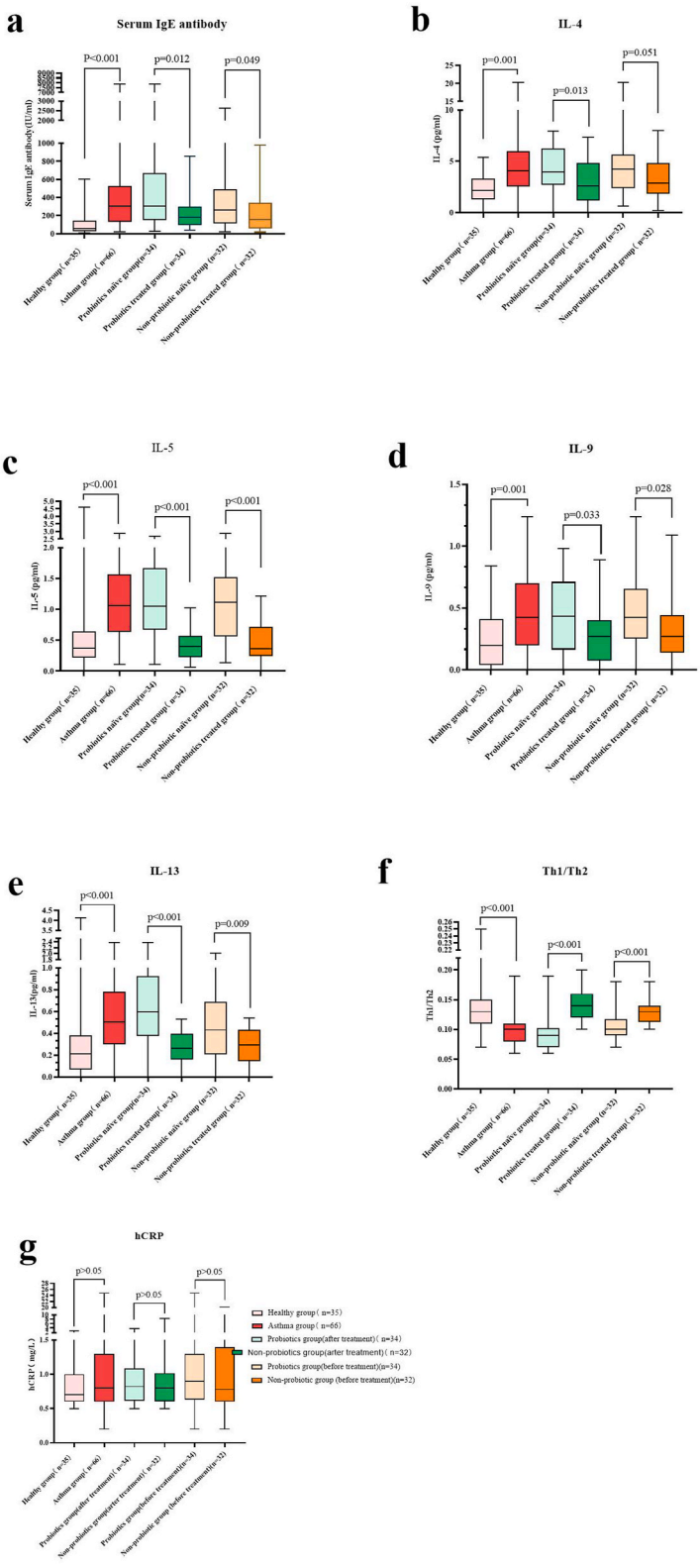

Fig. 4. Immunological indicators of Healthy group, asthma group, probiotics group and non-probiotics group.The levels of IgE antibody, IL-4, IL-5, IL-9, IL-13, hCRP and Th1/Th2 were changed among all groups, which has been shown in Fig. 4-a-g.

Table 5
Comparison of FEV1/VC_{max} before and after treatment between the probiotic treated group and the non-probiotic treated group.

FEV1/VC_{max}	Probiotic treatment group (n = 8)	Non-probiotic treatment group (n = 20)
Before treatment (%)	88.75 ± 3.17	90.19 ± 9.41
After treatment (%)	100.05 ± 5.54	95.80 ± 9.56
P value	<0.001	0.048

Note: Data are presented as means±SDs. T-test was used for analysis.
This is a group of children aged 3 to 6 year-old. The unreliable pulmonary function test results were excluded regarding uncooperative participants.

Th2 in the peripheral blood [18]. Lactobacillus acidophilus can enhance the phosphorylation expression of cell tight junction related proteins and enhance the barrier function of intestinal epithelial cells [19]. Lactobacillus rhamnosus can protect the intestinal barrier by up-regulating the expression of tight junction proteins ZO-1 and occludin [20]. When it comes to innate immune cells, Bifidobacterium, Lactobacillus and Lactobacillus rhamnosus activate the phagocytic activity of macrophages, stimulate the secretion of phagocytic cells, and enhance the phagocytic effect [21].A comparison of two treatment plans found that probiotic treatment was superior in reducing Bacteroides and increasing Faecalibacterium, Bifidobacterium, and Veillonella. Especially, the increase in Bifidobacterium was significant, possibly related to the supplemented probiotic formula rich in bifidobacteria. Although exogenous probiotic intake positively impacts gut microbial restoration and balance, factors like antibiotic use and individual immune status can also affect its efficacy [22], making it crucial for probiotic supplementation and adjunctive treatment's success or failure. After the probiotics use, the symptoms of asthmatic patients had improved, by reduction of the hospital visits within an half year, reduction of the asthma attacks, and the decrease use of intravenous steroid during attacks.

With the deepening research into the association between gut microbiota and asthma, there is an increasing number of reports on the therapeutic effects of probiotics on allergic diseases. Probiotics not only regulate gut microbiota but also influence immune cells such as macrophages, dendritic cells, and T cells to suppress immune responses. Timely supplementation of oral probiotics has been found to have a certain preventive effect on acute asthma attacks [23]. In this study, it was observed that compared to the control group, the asthma group showed elevated levels of serum IgE, IL-4, IL-5, IL-9, and IL-13, and a decreased Th1/Th2 ratio. After treatment, all immunological parameters showed improvement, with probiotic therapy demonstrating a more significant effect in reducing serum IgE, IL-4 and IL-13 levels. These results further confirm the association between asthma occurrence and immune imbalance and dysfunction, while also providing new evidence for asthma diagnosis and the evaluation of probiotic therapy efficacy.

In this study, we aimed to collect pre- and post-treatment pulmonary function data from both the Probiotic treated group and the non-probiotic treated group of children. However, due to only partial completion of forced inhalation and exhalation maneuvers by some patients, along with frequent occurrences of insufficient expiratory force and premature exhalation, the reliable pulmonary function data we collected was limited. Despite this, we proceeded with statistical analysis of the reliable pulmonary function data and found significant differences in FEV1/VC_{max} between the probiotic treated group before and after treatment, with the magnitude of this difference being greater than that of the non-probiotic treated group. This provides some evidence for the beneficial effects of probiotics in improving asthma symptoms and assisting in asthma treatment. In future, we plan to increase the number of pulmonary function study cases to enhance the support for our experimental conclusions.

Limitation: This research was a preliminary observational study on early interventions. Its short timeframe, constrained sample size, and inability to completely account for daily environmental variations affect the gut microbiota and immune markers during subsequent assessments mean its findings have some restrictions. To strengthen these conclusions, it would be necessary to conduct more extensive research with a broader participant base and prolonged clinical monitoring.

In conclusion, probiotics may offer a new direction and hope for treating asthmatic children; more clinical trials and long-term research are needed.

Data availability statement

Data will be made available on request.

Ethics and consent

This study was approved with number KY2020PJ129 by Ningbo Medical Center Lihuili Hospital ethics committee. Complete written informed consent was obtained from the relative/guardian for the publication of this study and accompanying images.

CRediT authorship contribution statement

Xiaodan Chen: Conceptualization, Data acquisition, Writing – original draft. **Su-Boon Yong:** Writing – original draft. **Chin-Yuan Yii:** Writing – original draft, Writing – review & editing. **Bihong Feng:** Conceptualization, Design, Supervision. **Kai-Sheng Hsieh:** Supervision. **Qingcao Li:** Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bihong Feng reports financial support was provided by Zhejiang Provincial Health Commission of China (2021KY1040). Other authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34916.

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