

## Intestinal Microbes-based Analysis of Immune Mechanism of Childhood Asthma

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### ABSTRACT

This work was developed to explore the relationship between intestinal microflora composition and immune function changes in children with asthma and to provide theoretical references for clinical diagnosis and treatment. Forty-eight children with asthma who received standardized treatment in the outpatient department of pediatric respiratory asthma in Children's Hospital were selected as the research objects, which were rolled into 24 cases of S0 group (complete control) and 24 cases of S1 group (incomplete control group). In addition, ten healthy children with general data matching the research objects were selected as a blank control (D0 group). The intestinal microbial composition and immune function indexes of each group were detected. The results showed that there were differences in the intestinal microbes of the three groups of children with Bifidobacterium, Megasphaera, Oscillibacter, Bilophila, and f\_Ruminococcaceae. Among them, the proportions of Bifidobacterium, Megasphaera, and f\_Ruminococcaceae in the intestinal microbes of the children in the S1 group were notably less than those in the S0 and D0 groups. The proportion of these three bacterial genera in the S0 group was also considerably smaller than that in the D0 group ( $P < 0.05$ ). In addition, the CD3+ levels of children in the S1 group were notably lower than those in the S0 and D0 groups, while the CD4+ and CD4+/CD3+ were higher than the S0 and D0 groups ( $P < 0.05$ ). The differences between CD3+, CD4+, and CD4+/CD3+ in the S0 and D0 groups were not considerable ( $P < 0.05$ ). The proportions of Bifidobacterium, Megasphaera, f\_Ruminococcaceae, and Parasutterella in children with intestinal microbes were significantly positively correlated with CD3+ levels ( $P < 0.05$ ), and significantly negatively correlated with CD4+ and CD4+/CD3+ levels ( $P < 0.05$ ). In short, children with different levels of asthma control had a certain degree of flora disorder and decreased immune function in the intestinal flora. The decrease in the relative abundance of Bifidobacterium, f\_Ruminococcaceae of Firmicutes, and Parasutterella of Riken Bacteria, and the increase in the relative abundance of Oscillatoria meant the decline of the immune function of the children.

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### Introduction

Childhood asthma is a common disease in children (0-14 years old). It is a form of recurrent cough, wheezing, and dyspnea. It is accompanied by reversible and obstructive chronic respiratory diseases with airway hyperresponsiveness (1,2). Mild asthma attacks in children are often characterized by cough and chest tightness. Severe attacks in children may appear irritability, sitting breathing, shrugging wheezing, three concave signs, nasal alar agitation, and lips blue. The disease usually breaks out or worsens at night and early morning (3-5). In recent years, the incidence of asthma in children is increasing, especially in children aged 3-6 years. Current treatment approaches for asthma mainly control the development of the disease, reduce or

alleviate acute attacks, achieve symptom control, and reduce future risks (6). Although asthma can't be cured, most patients with asthma can get good control of asthma symptoms and good quality of life through standardized treatment (7,8). However, clinical data showed that there are still many children with asthma due to the failure of timely treatment or non-standard treatment, resulting in poor control of asthma symptoms and even the deterioration of the disease, the development of adult asthma and protracted, lasting lifetime. Some patients even lose the ability to do physical activity completely.

In recent years, with the development of the emerging field of microecology, more and more scholars found that intestinal flora is closely related to the occurrence and development of asthma (9). The

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intestinal tract is the largest mucosal immune organ and micro-ecosystem of the human body. These microbiotas not only participate in a variety of physiological functions of the body, inhibit pathogenic bacteria, play the role of mucosal barrier and defense, but also induce the immune system to produce an anti-inflammatory response and inhibit the occurrence of autoimmune and allergic diseases (10,11). Under physiological conditions, the intestinal flora is symbiotic with the host to maintain a dynamic balance. Once the bacterial community is disturbed, it may lead to disease. Related studies suggested that microflora can not only affect intestinal immune function but also affect distal organs through itself or metabolites (12,13). In addition, the study of T lymphocyte differentiation and transcription has become the main direction of exploring the immune pathogenesis of asthma. Among them, the Th1/Th2 imbalance theory has been regarded as the leading core theory of asthma. Recent studies also suggested that Th17 cells, regulatory T lymphocytes, and dendritic cells are involved in the pathogenesis of asthma immunology, that is, the immune imbalance mechanism of Th17/Treg (14). Other studies linked disorders of intestinal microbes to a variety of lung diseases, including allergic reactions, asthma, and cystic fibrosis.

To sum up, there are many studies on asthma and intestinal flora, asthma, and probiotics. However, there are few studies on the relationship between asthma control levels and intestinal flora, and it is necessary to further analyze the molecular mechanism of both. Therefore, forty-eight children with asthma who received standardized treatment in the outpatient department of pediatric respiratory asthma in Children's Hospital were selected as the research objects, which were rolled into 24 cases of S0 group (complete control) and 24 cases of S1 group (incomplete control group). In addition, ten healthy children with general data matching the research objects were selected as a blank control (D0 group). The intestinal microbial composition and immune function indexes of each group were detected.

## Materials and methods

### Research samples

Forty-eight children with asthma who received standardized treatment in the pediatric Respiratory

asthma outpatient department of Children's Hospital from December 1, 2019, to January 1, 2021, were selected as the research subjects. Clinical data such as age, sex ratio, body mass index (BMI), breastfeeding time, delivery mode, term, and chest CT were obtained. They were divided into 24 patients in the S0 group (complete control) and 24 patients in the S1 group (incomplete control) according to the level of control of asthma symptoms of the Global Asthma Prevention initiative. In addition, ten healthy children whose general data were matched with the subjects were selected as a blank control (D0 group). This study had been approved by the medical ethics committee of the hospital, and the children's families had been informed of this study and signed the informed consent.

Inclusion criteria: i) children aged 3-14 years; ii) children and their families who cooperated with the experiment; iii) children with complete general clinical data; iv) children who met the diagnostic criteria for children with bronchial asthma in China.

Exclusion criteria: i) children with primary heart disease; ii) children with liver, kidney, and other organ diseases; iii) children with primary immunodeficiency; iv) children with mental diseases; v) children with asthma caused by bronchopulmonary dysplasia and primary ciliary dyskinesia; vi) children who failed to follow the standardized treatment of asthma; vii) children who participated in clinical trials of other drugs within the last three months.

### Sample collection and extraction

I. Sample collection. Feces of all enrolled children were collected using a special stool collection device (provided by Hangzhou Jingbai (G-Bio) Biotechnology Co., LTD.). The middle part of the stool was collected according to the standard instructions and placed in the storage tube containing the preservation liquid. After sampling, the samples were kept in an anaerobic environment as far as possible and stored in a refrigerator at -80°C for fecal DNA extraction and detection.

II. DNA extraction (15). i) 200µL feces were placed in a centrifuge tube. 400µL Streptavidin buffer, 100µL normal saline, 20µL Proteinase K, and 0.3g grinding beads were added to the samples, shaken and mixed, and cultured at 65°C for 15 minutes. During the incubation period, the supernatant was extracted

into a new centrifuge tube after further shaking three times, vortication for 15 seconds, and centrifugation at 10,000 rpm for 5 minutes. ii) 15 $\mu$ L RNaseA was added, shaken and mixed, and left at room temperature for five minutes. Then, it was put on ice for five minutes and centrifuged at 10,000 rpm for five minutes. The supernatant was then transferred to a new 1.5mL centrifuge tube. The solution obtained in the previous step was added to an adsorption column CR2 and centrifuged at 10,000 rpm for 30 seconds. After the waste liquid was removed, CR2 was placed in a collection tube. iii) 400 $\mu$ L buffer GD was added to the adsorption column CR2 and centrifuged at 10,000 rpm for 30 seconds. Then, 500 $\mu$ L PW rinse solution was added to CR2 and centrifuged at 10,000 rpm for 30 seconds. After removing the waste liquid, CR2 was put into the collection tube. iv) The operation was repeated once, and CR2 was put back into the collection tube, and centrifuged at 10,000 rpm for 2 minutes. Then, the waste liquid was removed, and the CR2 was placed at 25 degrees Celsius for several minutes. v) CR2 was put in a new centrifuge tube. 40 $\mu$ L eluting buffer was added to the middle part of the adsorption membrane, left for 5 minutes at 25°C and centrifuged at 10,000 rpm for 3 minutes. Then, the solution was collected and put into a centrifuge tube.

III. Concentration purity and concentration determination (16). The recovered DNA fragment was detected by agarose gel electrophoresis and ultraviolet spectrophotometer with a concentration and purity of 28 degrees. Quality control: the total amount of DNA extracted should be greater than 10ng. An OD<sub>260</sub>/OD<sub>280</sub> ratio of 1.7-1.9 was considered acceptable.

### PCR amplification and product purification

I. The extracted genomic DNA was used as a template, and the regions to be tested were 16SrDNA V3-V4. PCR steps were as follows. The reaction procedure included pre-denaturation at 95°C for 3 minutes, denaturation at 95°C for 30 seconds; annealing at 55°C for 30 seconds, extension at 72°C for 45 seconds, terminal extension at 72°C for 5 minutes, and storage at 4°C.

II. Electrophoresis inspection. 1.5 $\mu$ L PCR product was taken for agarose gel electrophoresis, and then UV imaging was taken with a gel imaging system.

III. Purification. i) The mixed DNA purification magnetic beads of equal volume were added to the PCR product, blown and mixed, and left at room temperature for three-five minutes. ii) The samples were placed on a magnetic rack for three minutes, then the supernatant was removed, and 150 $\mu$ L 80% ethanol was added. iii) The previous step was repeated, the ethanol was completely removed, and the sample was dried in air at room temperature. 25 $\mu$ L NF Water was added to blow and mix the eluting product, which was then left for three minutes. 25 $\mu$ L supernatant was soaked into a new tube and stored at -15°C for later use.

### Bioinformatics analysis

Illumina Miseq platform was used for sequencing, and sample data were separated from offline data according to the Barcode sequence (17) and PCR amplification primer sequence. After the Barcode and primer sequences were truncated, the reads of each sample were spliced using FLASH, and the spliced sequences obtained were Raw Tags data. The data was processed by strict quality control and filtering to get high-quality Clean Tags data. Then, the obtained data were compared with the database, and the chimera sequence was removed. Finally, the Effective Tags were harvested, that were, the optimized sequence.

Non-repeating sequences were extracted from the acquired data, OTU containing only one sequence was removed, and OTU clustering was performed for all high-quality sequences according to 97% similarity. In the clustering process, chimerism was removed to obtain the representative sequence of OTU.

RDP classifier was used to carry out taxonomic analysis on OTU representative sequences whose similarity was less than 97%. After the obtained data was compared with that in the database, species were annotated at the level of the kingdom, phylum, class, order, family, genus, etc. Then, the community composition of each sample was analyzed statistically.

The relative content of fecal microbes in OTU sequence number was detected, and Alpha diversity analysis was performed for each child sample.

### Statistical methods

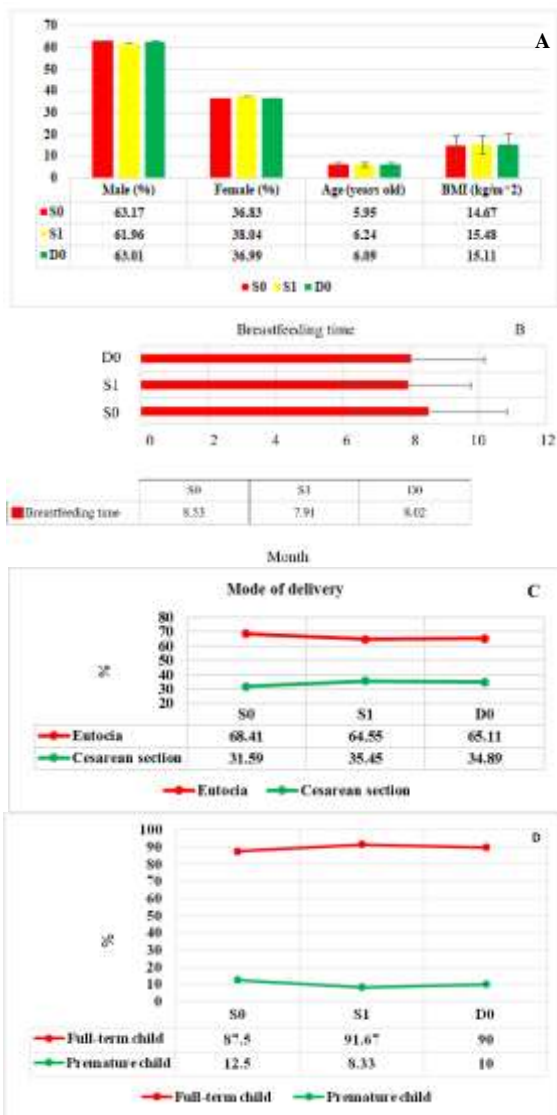
Data processing in this study was analyzed by SPSS 19.0. The measurement data were expressed as

mean ± standard deviation ( $\bar{x}\pm s$ ) and the counting data as a percentage (%). Pearson was used to analyzing the correlation between the two. One-way analysis of variance was used for pairwise comparison.  $P<0.05$  was considered statistically significant.

**Results and discussion**

**Comparison of clinical data of three groups of children**

Figure 1 showed the comparisons of the clinical data of the three groups of tested children. There were no substantial differences in the age, sex ratio, BMI, breastfeeding time, delivery method, and full-term data of children among the S0, S1, and D0 groups ( $P<0.05$ ).



**Figure 1.** Comparison of clinical data of three groups of tested children. (A: the age, gender, and BMI of the child; B: the breastfeeding time of the child; C: the child’s delivery ways; D: whether the child was full-term)

Figure 2 showed the chest CT images of a 5-year-old female child with asthma. Tubular and cystic bronchiectasis was seen, mainly in the upper lobe. Mucus obstruction and mediastinal and hilar lymph node lesions were observed. The diagnosis was bronchial asthma with mediastinal emphysema. Figure 3 showed the chest CT images of a 10-year-old male child, with proximal bronchiectasis in both upper lobes, especially in the left upper lobe, showing the glove sign. The lesions were mainly branched and lacked pleomorphism. Tuberculosis was not considered. The lesion was confined to the hilum and did not support congenital branching. A typical allergic bronchopulmonary aspergillosis was considered. Figure 4 showed the chest CT image of a healthy 10-year-old male child. There was no increase, thickening, and disorder in a broncho-vascular bundle of two lungs. No abnormal density shadows were observed in both lungs. No abnormally enlarged lymph nodes were observed in the mediastinum. The trachea and left and right main bronchus were unobstructed. No thickening or adhesion was observed in the bilateral pleura. The position of the heart and great vessels were normal, no abnormal dilatation and stenosis were observed, and the bone density of thoracic osteoblasts was normal in each group.



**Figure 2.** Chest CT images of a 5-year-old female child with asthma.



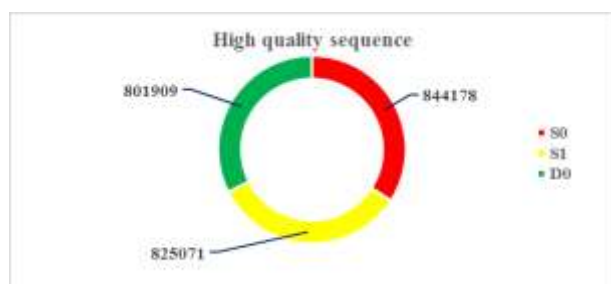
**Figure 3.** Chest CT images of a 10-year-old male child with asthma.



**Figure 4.** Chest CT images of a 10-year-old male healthy child.

### Sequencing analysis results of intestinal microbes in children

The stool samples of all subjects were sequenced, and a total of 2,471,158 high-quality sequences were obtained. In Figure 5, there were 844,178 high-quality sequences in children in the S0 group, 825,071 high-quality sequences in children in the S1 group, and 801,909 high-quality sequences in children in the D0 group. The difference between the three groups was not substantial ( $P>0.05$ ).



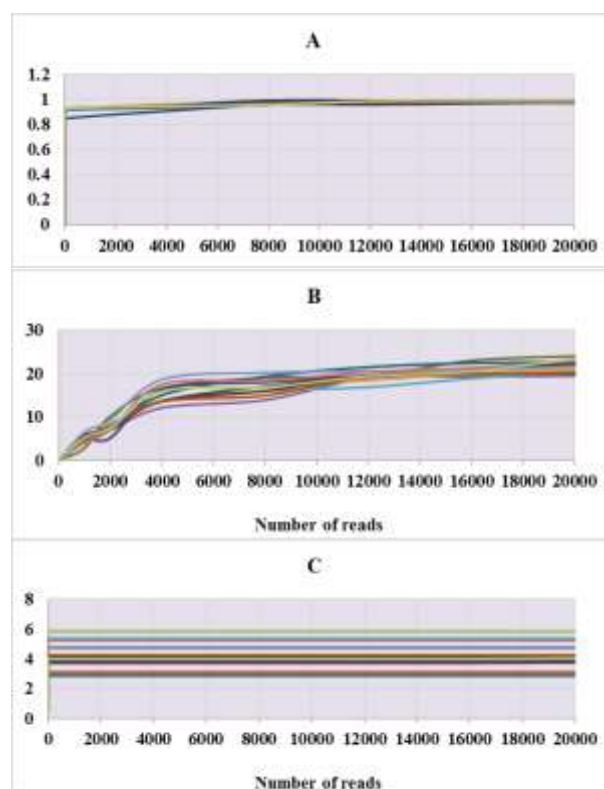
**Figure 5.** High-quality sequencing results of stool samples from the three children.

Figure 6 showed the intestinal microbial serial dilution curve of all children's samples. The good's coverage index of all children's samples was above 97%, and the curve gradually stabilized with the increase in sequencing volume and entered a plateau. The PD\_whole\_tree index of all children's samples increased as the amount of sequencing increased, until it stabilized and entered a plateau. In addition, the Shannon index of all children's samples quickly stabilized as the amount of sequencing increased, which then entered a plateau.

### Diversity analysis of intestinal microbe Alpha in children

Figure 7 showed the Alpha diversity analysis of intestinal microbes in children. The number of OUT, Chao1 index, and Shannon index of children in the S1

group were considerably smaller than those of children in the S0 and D0 groups, and the difference was considerable ( $P<0.05$ ). The Chao1 index of children in the S1 group was remarkably lower than that of children in the D0 group ( $P<0.05$ ). However, the number of OUT and Shannon index of children in the S0 group were not significantly different from those of children in the D0 group ( $P>0.05$ ).

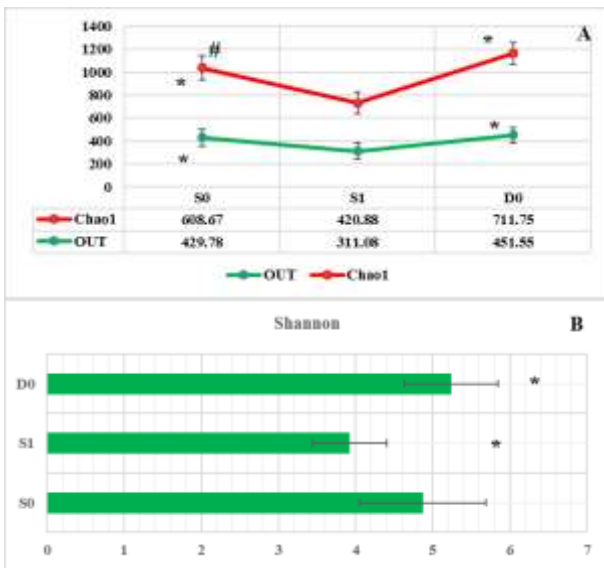


**Figure 6.** Dilution curve of an intestinal microbial sequence of all children's samples. (A: good's coverage index; B: PD\_whole\_tree index; C: Shannon index)

### The composition of intestinal microbes in the three groups of children at the phylum level

Figure 8 showed the composition of intestinal microbes at the phylum level in the samples of the three groups of children. The intestinal microbes of children were mainly Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia. The comparison among the groups showed that the intestinal microbes of the three groups of children's samples were not statistically different in Firmicutes, Bacteroidetes, Proteobacteria, and Verrucomicrobia ( $P<0.05$ ). The proportion of samples from children in the D0 group in the Actinomycota was remarkably greater than that in the S0 and S1 groups. In addition,

the proportion of actinomycete samples in the S0 group was remarkably greater than that in the S1 group, and the differences were substantial ( $P < 0.05$ ).



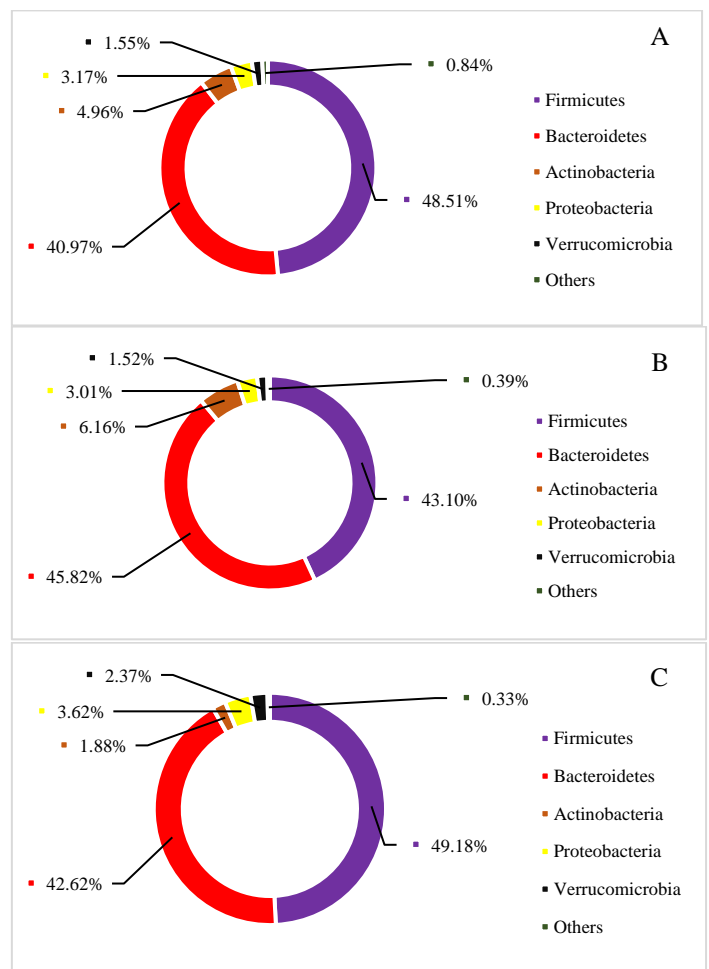
**Figure 7.** Alpha diversity analysis of the intestinal microbes in children. (A: the number of OUT and Chao1 index; B: the Shannon index). Note: \* indicated a considerable difference compared with the S1 group ( $P < 0.05$ ); # indicated a considerable difference compared with the D0 group ( $P < 0.05$ ).

**The composition of the intestinal microbes of the three groups of children at the genus level**

Figure 9 showed the composition of the intestinal microbes at the genus level in the samples of the three groups of children. The intestinal microbes of the three groups of children were different in bacteria genera including Bifidobacterium, Megasphaera, Oscillibacter, Bilophila, and f\_Ruminococcaceae. The proportions of Bifidobacterium, Megasphaera, and f\_Ruminococcaceae in the intestinal microbes of children in the S1 group were notably less than those in the S0 and D0 groups. The proportion of these three bacterial genera in the S0 group was also considerably smaller than that in the D0 group ( $P < 0.05$ ). In addition, the proportions of Oscillibacter and Bilophila in the intestinal microbes of children in the S1 group were obviously greater than those in the S0 and D0 groups. The proportion of these two genera in the S0 group was also obviously greater than that in the D0 group ( $P < 0.05$ ).

In addition, the differences in the proportions of Alipies, Parasutterella, Barnesiella, Dorea, f\_Coriobacteriaceae, and f\_Bifidobacteriaceae in the

S0 group and D0 group were not considerable ( $P > 0.05$ ). However, the intestinal microbes of the S1 group were different from those of the S0 and D0 groups in terms of Alipites, Parasutterella, Barnesiella, Dorea, f\_Coriobacteriaceae, and f\_Bifidobacteriaceae. Among them, the proportions of Alipipes, Parasutterella, Barnesiella, and f\_Bifidobacteriaceae in the S1 group were notably less than those in the S0 and D0 groups, and the difference was considerable ( $P < 0.05$ ). The proportions of Dorea and f\_Coriobacteriaceae in the S1 group were obviously greater than those in the S0 and D0 groups ( $P < 0.05$ ).

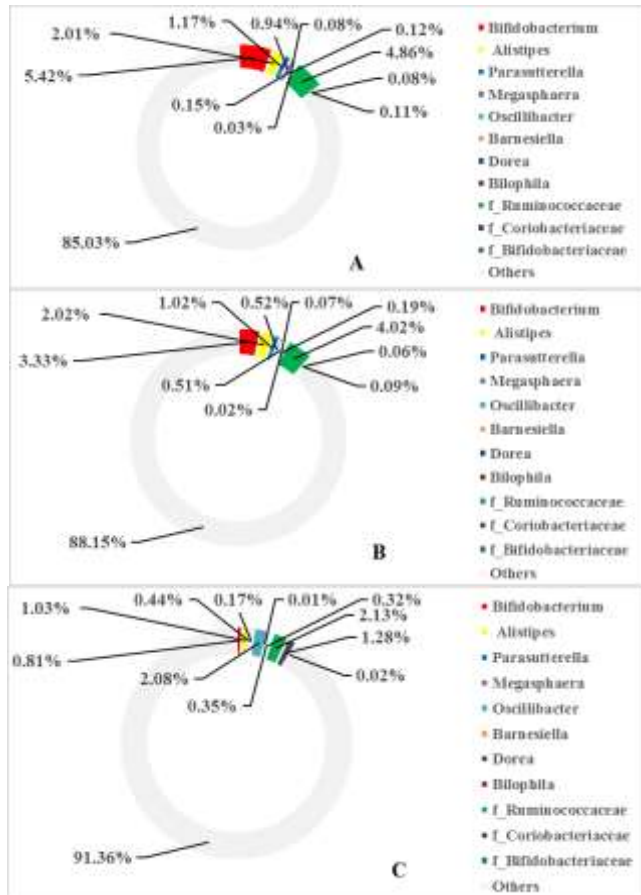


**Figure 8.** The composition ratio of the intestinal microbes in the three groups of children at the phylum level. (A: S0 group; B: D0 group; C: S1 group)

**Comparison of three groups of children’s immune function**

Figure 10 showed the comparison of the immune function of the three groups of children. The CD3+ levels of children in the S1 group were notably lower than those in the S0 and D0 groups, while the CD4+

and CD4+/CD3+ were higher than those in the S0 and D0 groups, with considerable differences ( $P<0.05$ ). The differences between CD3+, CD4+, and CD4+/CD3+ in S0 and D0 groups were not considerable ( $P<0.05$ ).



**Figure 9.** The composition of the intestinal microbes of the three groups of children at the genus level. (A: S0 group; B: D0 group; C: S1 group)

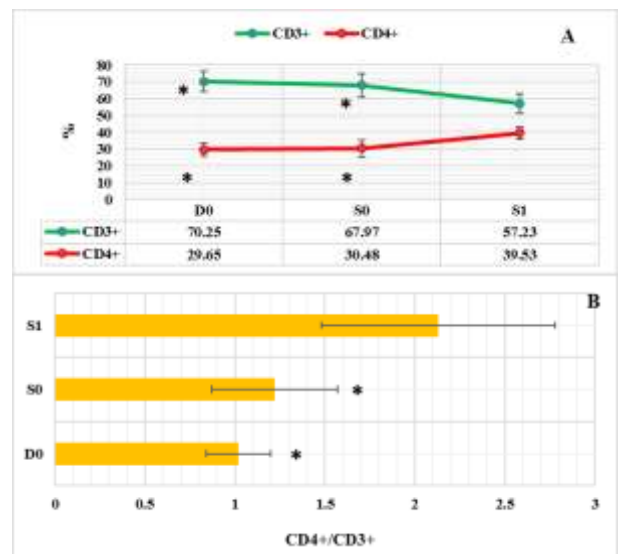
**Correlation analysis between the relative proportion of bacterial genera in children and their immune function**

From Table 1, the proportion of intestinal microbes in children with Bifidobacterium, Megasphaera, f\_Ruminococcaceae, f\_Bifidobacteriaceae, and Parasutterella was significantly positively correlated with CD3+ levels ( $P<0.05$ ). There was a considerable negative correlation between the proportion of Oscillibacter bacteria in the children’s intestinal microbes and the level of CD3+ ( $P<0.05$ ).

From Table 2, the proportion of intestinal microbes in children with Bifidobacterium, Megasphaera, f\_Ruminococcaceae, f\_Bifidobacteriaceae, and Parasutterella was significantly negatively correlated

with CD3+ levels ( $P<0.05$ ). There was a considerable positive correlation between the proportion of Oscillibacter bacteria in the children’s intestinal microbes and the level of CD3+ ( $P<0.05$ ).

From Table 3, the proportions of intestinal microbes Bifidobacterium, Megasphaera, f\_Ruminococcaceae, and Parasutterella were significantly negatively correlated with CD4+/CD3+ levels ( $P<0.05$ ). There was a considerable positive correlation between the proportion of the Oscillibacter genus and the level of CD4+/CD3+ in children with intestinal microbes ( $P<0.05$ ).



**Figure 10.** Comparison of the immune function of the three groups of children. Note: \* indicated that the difference was considerable compared with the S1 group ( $P<0.05$ ); # indicated that the difference was considerable compared with the D0 group ( $P<0.05$ ).

**Table 1.** Correlation analysis between relative proportion of bacteria and CD3+ level in children

Bacteria	CD3+	
	r	P
Bifidobacterium	5.203	0.033
Megasphaera	5.118	0.021
f_Ruminococcaceae	4.031	0.026
Oscillibacter	-3.846	0.047
Bilophila	-2.117	0.052
Alistipes	1.855	0.064
Parasutterella	2.031	0.039
Barnesiella	1.484	0.056
Dorea	-1.668	0.061
f_Coriobacteriaceae	-2.153	0.070
f_Bifidobacteriaceae	3.902	0.408

**Table 2.** Correlation analysis between relative proportion of bacteria and CD4+ level in children

Bacteria	CD4+	
	r	P
Bifidobacterium	-3.828	0.022
Megasphaera	-3.722	0.019
f_Ruminococcaceae	-4.011	0.045
Oscillibacter	3.776	0.031
Bilophila	1.205	0.074
Alistipes	-1.872	0.050
Parasutterella	-2.475	0.047
Barnesiella	-1.008	0.063
Dorea	2.158	0.061
f_Coriobacteriaceae	1.966	0.057
f_Bifidobacteriaceae	1.363	0.070

**Table 3.** Correlation analysis between relative proportion of bacteria and CD4+/CD3+ levels in children

Bacteria	CD4+/CD3+	
	r	P
Bifidobacterium	-4.468	0.013
Megasphaera	-3.915	0.025
f_Ruminococcaceae	-4.226	0.038
Oscillibacter	2.551	0.029
Bilophila	1.336	0.051
Alistipes	-1.908	0.056
Parasutterella	-3.371	0.042
Barnesiella	-1.449	0.061
Dorea	2.043	0.053
f_Coriobacteriaceae	2.115	0.066
f_Bifidobacteriaceae	1.490	0.055

In recent years, with the development of microecology and high-throughput technology, people have a deeper understanding of intestinal flora, and more and more scholars found that intestinal flora is closely related to asthma (18). As a possible risk factor affecting the occurrence of asthma, whether intestinal flora affects the control of asthma has not been clearly reported (19). Therefore, 48 children with asthma who received standardized treatment in the pediatric Respiratory asthma outpatient department of Children's Hospital from December 1, 2019 to January 1, 2021 were selected as the research subjects. They were divided into 24 cases S0 group (complete control group) and 24 cases S1 group (incomplete control group). In addition, ten healthy children with general data matching the patients were selected as a blank control (D0 group). Then, the intestinal microbiome composition and immune function indexes of each group were detected. It was found that there were no substantial differences in age, sex ratio, BMI, breastfeeding time, delivery modes, and term in S0,

S1, and D0 groups ( $P<0.05$ ), which provided the feasibility for the follow-up research. The stool samples of all subjects were sequenced, and a total of 2,471,158 high-quality sequences were obtained. Among them, there were 844,178 high-quality sequences for children in the S0 group. There were 825,071 high-quality sequences in children in S1 group and 801,909 high-quality sequences in children in D0 group. Alpha diversity analysis found that the number of OUT, Chao1 index and Shannon index of the children in the S1 group were notably lower than those of the children in the S0 and D0 groups, and the difference was considerable ( $P<0.05$ ). This was similar to the findings of Johnson and Ownby (2017) (20), indicating that the abundance and diversity of intestinal flora were different between children with asthma and different control levels and healthy children. The decrease in species abundance and diversity of intestinal microbes may be related to the level of asthma control. In addition, there was no substantial difference in the number of OUTs and Shannon index of children in the S0 group compared with the children in the D0 group ( $P>0.05$ ). This meant that as the disease was well controlled, the level of the flora (abundance and diversity) may gradually improve, which was consistent with the homeostasis mechanism of the interaction between the host and the flora (21).

The intestinal microbes of children were mainly Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia. The comparisons among groups showed that the proportion of samples with Actinomycete from children in the D0 group was remarkably greater than that in the S0 and S1 groups, while the proportion of samples from the children in the S0 group was remarkably greater than that in the S1 group ( $P<0.05$ ). Since the considerable differences in the relative abundance of the three groups of samples of Actinomycetes were mainly due to the Bifidobacterium genus, the decrease in the relative abundance of Bifidobacteria may affect the level of asthma control (22). The intestinal microbes of the three groups of children were different in bacteria genera including Bifidobacterium, Megasphaera, Oscillibacter, Bilophila, and f\_Ruminococcaceae. Among them, the proportions of Bifidobacterium, Megasphaera, and f\_Ruminococcaceae in the



intestinal microbes of the children in the S1 group were notably less than those in the S0 and D0 groups. The proportion of these three bacterial genera in the S0 group was also considerably smaller than that in the D0 group, and the difference was great ( $P<0.05$ ), which indicated that there was a certain degree of flora disorder in the intestinal flora of children with different control levels of asthma (23).

The immune function indexes of the three groups of children were compared, and it was found that the CD3+ levels of children in the S1 group were notably lower than those in the S0 and D0 groups, while CD4+ and CD4+/CD3+ were higher than those in the S0 and D0 groups ( $P<0.05$ ). The differences between CD3+, CD4+, and CD4+/CD3+ in the S0 and D0 groups were not considerable ( $P<0.05$ ). CD3+ and CD4+ are the main cells that mediate cellular and humoral immunity of the body. The immune system of children with asthma was obviously disturbed, and with the control of asthma, the immune function of the children can return to normal (24). Pearson correlation analysis was carried out on the relative proportion of children's bacterial genera and their immune function indexes. The proportions of Bifidobacterium, Megasphaera, f\_Ruminococcaceae, and Parasutterella in children with intestinal microbes were significantly positively correlated with CD3+ levels ( $P<0.05$ ), and significantly negatively correlated with CD4+ and CD4+/CD3+ levels ( $P<0.05$ ). It meant that the decrease in the proportion of Bifidobacterium, f\_Ruminococcaceae of Firmicutes, and Parasutterella of Riken Fungi indicated the decline of the immune function of the children (25). The proportion of Oscillibacter bacteria in children's intestinal microbes was remarkably negatively correlated with CD3+ levels ( $P<0.05$ ), and was remarkably positively correlated with CD4+ and CD4+/CD3+ levels ( $P<0.05$ ). Therefore, the increase in the relative abundance of Oscillatoria may be related to the decline of the immune function of the children.

## Conclusions

In this study, 48 children with asthma who received standardized treatment in the pediatric Respiratory asthma outpatient department of Children's Hospital from December 1, 2019, to January 1, 2021, were selected as the study subjects. They were divided into 24 cases S0 group (complete control group) and 24

cases S1 group (incomplete control group). In addition, ten healthy children with general data matching the patients were selected as a blank control (D0 group). Then, the intestinal microbiome composition and immune function indexes of each group were detected. The results showed that the intestinal microflora of children with different levels of asthma control had a certain degree of microflora disorder and immune function decline. The decrease in the relative abundance of bifidobacterium, f\_Ruminococcaceae and Parasutterella of Firmicutes and the increase in the relative abundance of Acinetobacter meant a decrease in the immune function of children. Although it is learned that Bifidobacterium, f\_Ruminococcaceae, Parasutterella, and Oscillatoria are related to the immune function of the body, it is necessary to further prove the exact causal relationship between changes in the abundance of bacteria and immune function. In conclusion, this work provides a preliminary data reference for the relationship between intestinal microbes and changes in asthma immune function.

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## Conflict interest

The authors declare no conflict of interest.

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