

Gut microbiome alterations in patients with wheat-dependent exercise-induced anaphylaxis

Zhirong Du, Xiang Gao, Jia Yin*

Department of Allergy, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China
Beijing Key Laboratory of Precision Medicine for Diagnosis and Treatment of Allergic Disease, Beijing 100730, China
National Clinical Research Center for Dermatologic and Immunologic Diseases, Beijing 100730, China

ARTICLE INFO

Keywords:

Wheat-dependent exercise-induced anaphylaxis
Microbiota
Food allergy

ABSTRACT

The intestinal microbiota plays a critical role in food allergy development. However, little is known regarding the structure and composition of the intestinal microbiota in patients with wheat-dependent exercise-induced anaphylaxis (WDEIA). We examined the gut microbiota alterations in patients with WDEIA and the microbiota's association with WDEIA. Fecal samples were collected from 25 patients with WDEIA and 25 healthy controls. Environmental exposure factors were obtained, serum total IgE, IgE specific to wheat, gluten, and ω -5 gliadin were measured. Fecal samples were profiled using 16S rRNA gene sequencing. The relative abundances of the bacterial genera *Blautia* ($P < 0.05$), *Erysipelatoclostridium* ($P < 0.01$), *Akkermansia* ($P < 0.05$) and *Lachnospiraceae_NK4A136_group* ($P < 0.05$) were significantly increased, while those of *Lactobacillus* ($P = 0.001$) and *Dialister* ($P < 0.05$) were significantly decreased in subjects with WDEIA. The microbial diversity did not differ between WDEIA patients and healthy controls. IgE specific to ω -5 gliadin was positively associated with the *Oscillospira* ($r = 0.48$, $P < 0.05$) and negatively associated with *Leuconostoc* ($r = -0.49$, $P < 0.05$). Total IgE levels were significantly negatively correlated with *Bifidobacterium* ($P < 0.05$). The gut microbiome compositions in WDEIA patients differed from those of healthy controls. We identified a potential association between the gut microbiome and WDEIA development. Our findings may suggest new methods for preventing and treating WDEIA.

1. Introduction

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a life-threatening distinct IgE-mediated wheat allergy manifesting as pruritus, urticaria, angioedema, dyspnea, upper respiratory tract obstruction, gastrointestinal symptoms, hypotension, vascular collapse, and anaphylactic shock [1]. WDEIA patients experience an allergic reaction when wheat ingestion is combined with augmenting cofactors, such as exercise, alcohol and nonsteroidal anti-inflammatory drugs; however, these patients can tolerate ingesting wheat products without these cofactors [2]. The WDEIA etiology remains elusive [2]. Studies have indicated that increased histamine release via blood flow redistribution and transient plasma hyperosmolality, increased bioavailability of allergens through increased gastrointestinal permeability, which may play a role in this disorder [2,3]. No effective treatment is currently available, and further episodes can only be prevented by consuming a wheat-free diet or avoiding exercise after wheat ingestion.

Increasing evidence shows that intestinal dysbiosis is associated

with the development of allergic diseases [4,5]. Previous studies suggested that the intestinal microbiota plays key roles in activating mucosal immunity and programming oral tolerance [6,7]. Sustained tolerance to intestinal symbiosis helps establish and maintain food tolerance in the host, and intestinal flora alterations can lead to allergic diseases [8]. Diversity of the gut microbiota has been inversely associated with the development of food sensitizations, while specific taxa are thought to protect against food allergies [9,10]. Germ-free mice become susceptible to dietary antigens by developing T_H2 cell and IgE responses [11]. Apart from direct immunologic effects, the microbiota may control intestinal barrier functions [4]. For example, *Clostridia* colonization increased the expression of barrier-promoting interleukin-22 and decreased intestinal permeability [10]. Conversely, Scherf et al. [2] investigated how cofactors affected gliadin absorption and found that gliadin absorption in the gut depended less on cofactors than previously hypothesized, and no differences occurred in the intestinal microbiota under different interventions. However, all volunteers in that study were healthy, whereas the present study enrolled WDEIA

* Corresponding author at: Department of Allergy, Peking Union Medical College Hospital, No. 1 Shuaifuyuan Street, Wangfujing, Beijing 100730, China.
E-mail address: doctoryinjia@163.com (J. Yin).

<https://doi.org/10.1016/j.intimp.2020.106557>

Received 26 March 2020; Received in revised form 25 April 2020; Accepted 30 April 2020

Available online 07 May 2020

1567-5769/© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

patients [2]. Our previous study showed that the average age of WDEIA onset was 34 years, and these patients had previously tolerated wheat combined with exercise, which differs from the onset of traditional food allergies [12]. The mechanisms that lead to the changes in wheat tolerance in WDEIA patients is unclear. Diet, the commensal microbiota and the interactions between them may cause deviations from the default state of mucosal immune tolerance, thus resulting in food allergies. We hypothesized that the gut microbiota alteration is associated with the onset of WDEIA. We aimed to identify differences in the gut microbial compositions between healthy controls and WDEIA patients. To date, this is the first study to focus on the association between the microbiome and WDEIA.

2. Methods

2.1. Study design

To investigate the gut microbiomes of WDEIA patients, we performed 16S rRNA sequencing of 50 fecal samples (25 individuals with WDEIA and 25 healthy controls) enrolled at Peking Union Medical Hospital (PUMCH) from 2017 to 2018. WDEIA was diagnosed via the following criteria: (i) anaphylaxis occurred only when physical activity occurred within 6 h of wheat ingestion; (ii) ω -5 gliadin-specific or gluten-specific IgE were positive; (iii) consuming a wheat-free diet prevented anaphylaxis; and (iiii) anaphylaxis could occur in the presence of other cofactors, such as nonsteroidal anti-inflammatory drugs, aspirin, and alcohol. Anaphylaxis is determined according to the World Allergy Organization (WAO) systemic allergic reaction grading system (Grade 4 and 5) [13]. Potential risk factors for WDEIA, including residential area, early-life use of antibiotics and keeping pets, were determined. Serum-specific IgE antibodies to wheat, gluten, and ω -5 gliadin were measured using the ImmunoCAP system (Phadia AB, Uppsala, Sweden). Positive results were defined as ≥ 0.35 kU_A/L. Healthy controls were recruited from individuals on regular physical check-ups in the same hospital. All the control subjects were healthy individuals who had no history of allergic rhinitis, allergic asthma, food allergy, atopic dermatitis and any other allergic manifestations. Antibiotic use by participants in the past 7 days was an exclusion criterion for stool collection. The ethic committees of PUMCH approved this study. All participants were informed of this study and signed written informed consent.

2.2. Sample collection and DNA extraction

Participants collected approximately 2 g of fresh fecal sample into 5-ml stool containers at Peking Union Medical College Hospital. Samples were frozen, transported immediately and stored at -80°C until further analysis. Frozen stool samples were defrosted on ice. DNA was extracted from the feces using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) per the manufacturer's instructions. The amount of DNA was determined using a NanoDrop ND-2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The genomic DNA purity and quality were determined via 1.0% agarose gel electrophoresis.

2.3. 16S rRNA gene sequencing

The V3–V4 regions of the 16S rRNA gene were amplified with the forward primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR of the 16S rRNA was performed in 25- μl triplicates containing 12.5 μl 2 $\times$ Taq PCR MasterMix, 3 μl bovine serum albumin (2 ng/ μl), 2 primers (5 μM), 2 μl template DNA, and 7.5 μl ddH₂O. The cycling parameters were 95 $^{\circ}\text{C}$ for 5 min, followed by 28 cycles of denaturation at 95 $^{\circ}\text{C}$ for 45 s, annealing at 55 $^{\circ}\text{C}$ for 50 s, and elongation at 72 $^{\circ}\text{C}$ for 45 s with a final extension at 72 $^{\circ}\text{C}$ for 10 min. PCR was repeated three times per sample

to decrease reaction-level biases. Three PCR products were mixed per sample, separated by 2% agarose gel electrophoresis, purified using a QIAquick Gel Extraction Kit (QIAGEN, Germany), quantified using real-time PCR, and sequenced at Beijing Allwegene Tech, Ltd. (Beijing, China). High-throughput sequencing was performed on a MiSeq platform at Allwegene Company (Beijing). After the run, image analysis, base calling and error estimation were performed using Illumina Analysis Pipeline Version 2.6.

2.4. Data processing

Raw sequence data were analyzed using the pipeline tools Quantitative Insights Into Microbial Ecology (QIIME, v1.8) and Mothur. The overlapping paired-end reads were merged into a sequence using FLASH, with a minimum 10-bp overlap length. Data were decontaminated by removing sequences shorter than 50 bp, those with a low-quality level (<20), and those that did not exactly match the primer sequences or barcode tags. All high-quality reads were clustered into operational taxonomic units (OTUs) at 97% sequence similarity via USEARCH (v8.1). All sequences were classified into taxonomic groups using the Ribosomal Database Project (RDP) Classifier tool [14]. Alpha and beta diversity analyses were performed using QIIME v1.8. Differences in relative abundances of the phylotypes were determined via linear discriminant analysis (LDA) effect size (LEfSe). LDA scores calculated by LEfSe method were used to assess the most differentially abundant taxa between two groups, with a significance level of $\alpha = 0.05$ and an LDA threshold of 2.0. To compare the overall fecal microbiome structure in all subjects, principal component analysis was conducted using QIIME.

2.5. Statistical analysis

Statistical analyses were performed in SPSS 25.0 (SPSS Inc., Chicago, IL, USA). Continuous variables are expressed as the mean $\pm$ standard deviation and compared via Student's *t*-test. Categorical variables are expressed as percentages and compared using a chi-square test. Metastats analysis was performed to determine significant differences in bacterial compositions between the two groups. Statistical dependence between continuous variables was analyzed via Spearman's rank correlation. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Population characteristics

Table 1 shows the clinical characteristics of the WDEIA patients. The average age of the WDEIA group was 32.6 ± 11.7 years, and approximately half were men. The median duration of the disease was 5.25 (0.25–54) months. In total, the patients had a median of 2 (1–6) anaphylaxis episodes. Of these anaphylactic reactions, 35.7% (20/56) were Grade 4 severity, 64.3% (36/56) were Grade 5 severity according to the WAO grading system. 20% (5/25) of WDEIA patients had other allergic disorders, consisting of two with allergic rhinitis and three with immediate-type food allergy to other foods. The causative foods were crab ($n = 1$) and shrimp ($n = 2$). The median level of total IgE was 219 (39.1–1286) kUA/L. The positive rate of specific IgE to wheat, gluten, and ω -5 gliadin was 68% (17/25), 96% (24/25), and 96% (24/25) respectively. Table 2 shows the baseline characteristics of the 50 participants. The WDEIA group was matched with the control group. Patients with WDEIA were less likely to keep pets than were the controls (56% vs 24%, respectively, $P = 0.02$); otherwise, the two populations were similar (Table 2).

Table 1
Clinical characteristics of WDEIA patients.

Patient no.	Age (years)	Sex	Duration of disease (months)	No. episodes	Atopy status	Total IgE (kUA/L)	Specific IgE (kUA/L)		
							wheat	gluten	ω-5 gliadin
1	25	M	6	2	None	408	0.96	1.33	6.14
2	30	F	3	1	None	291	0.68	7.34	16.8
3	17	M	5.25	4	None	758	1.59	11.7	7.7
4	25	F	2	1	None	166	0.39	1.55	6.96
5	45	M	2	3	None	201	0	0.41	2.61
6	47	F	1	1	None	143	0.1	0.64	7.01
7	32	F	24	4	AR	739	6.83	31.2	41.7
8	53	F	54	6	None	119	0.25	2.66	5.92
9	33	M	10.5	2	None	306	1.03	3.1	9.31
10	16	F	8	3	None	211	0.27	2.34	6.9
11	34	F	13	3	None	407	1.39	4.42	29.9
12	19	M	1.5	1	None	39.1	0	2.27	5.25
13	17	F	4	1	AR	99.1	3.32	0.55	0
14	22	M	24	3	None	134	0.04	0.08	1.35
15	31	M	0.25	1	None	1286	0.84	10.8	11.1
16	27	F	24	2	None	1017	1.76	8.04	17.7
17	55	M	1	1	None	193	0.45	1.67	7.37
18	37	F	6	3	None	53.8	0	0.5	0.39
19	41	M	36	3	FA	608	0.54	5.33	16.9
20	50	M	0.5	1	None	1170	1.2	7.57	14.7
21	22	F	32	3	None	180	0.78	2.81	7.72
22	38	M	3	2	FA	914	3.07	3.51	16.7
23	46	F	2	1	None	48.8	0.47	0.64	1.84
24	23	F	29	3	None	219	0.26	2.08	8.88
25	32	M	3	1	FA	742	0.53	2.42	11.2

AR, allergic rhinitis; FA, food allergy to non-WDEIA-causative foods; WDEIA, wheat-dependent exercised-induced anaphylaxis.

Table 2
Characteristics of the study population.

Variables	Control subjects	Patients with WDEIA	P-value*
Age (years)	33.3 (10.64)	32.6 (11.7)	0.85
Male sex	12 (48)	12 (48)	1.0
Pets exposure			0.02
Yes	14 (56)	6 (24)	
No	11 (44)	19 (76)	
Residential area			0.56
Country	9 (36)	14 (56)	
City	16 (64)	11 (44)	
Use of antibiotics			0.74
Frequent	5 (20)	7 (28)	
Seldom	20 (80)	18 (72)	

Number (%) or mean (SD) reported.

* Chi-square test for categorical variables, T test for continuous variables.

3.2. Intestinal microbiota compositions in WDEIA patients and controls

In total, 2,566,791 sequences were successfully obtained from the 50 fecal samples, and 87.2% were high-quality sequences clustered into 531 OTUs. Each sample contained 44,789 high-quality sequences on

average, and the number of high-quality sequences did not significantly differ between the groups ($P = 0.90$). To identify the intestinal microbiota, we conducted a taxonomic analysis by the RDP classifier. Thirteen phyla were found in all samples. Fig. 1 displays the total microbial taxonomic distribution per sample at the phylum and genus levels. Although the taxonomic compositions had high interindividual variation, there are several dominant taxa at both the phyla and genus levels.

OTUs were compared with a Venn diagram, and 434 common OTUs were identified, representing 87.15% (434/498) and 92.93% (434/467) of the total OTUs in the WDEIA and control groups, respectively. The dominant phyla were Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria, accounting for over 99% of the total phyla in both the controls and WDEIA subjects (Fig. 2A). The dominant genera of the intestinal microbiota were *Bacteroides* and *Faecalibacterium*, followed by *Eubacterium_rectale_group* and *Blautia* in both groups (Fig. 2B). Among the shared dominant genera in both groups, *Bacteroides* belongs to the phylum Bacteroidetes, and *Faecalibacterium*, *Eubacterium_rectale_group* and *Blautia* belong to Firmicutes. The dominant unique OTUs identified in the control group were *Dialister*, *Parabacteroides*, *Sutterella*, *Megasphaera*, and *Lactobacillus*, while those identified in the WDEIA group were *Lachnospiraceae_NK4A136_group* and *Akkermansia*.

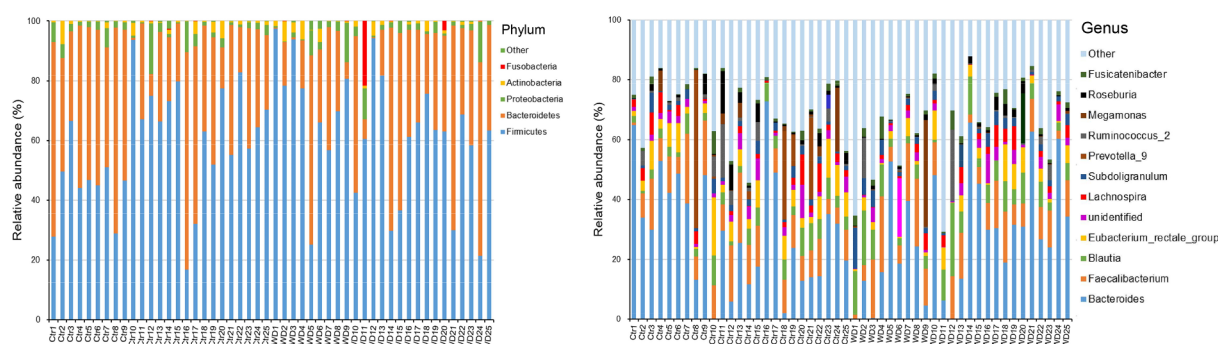


Fig. 1. Community bar-plot analysis shows relative abundance of fecal microbiota in each sample at the phylum and genus levels. Ctr: control.

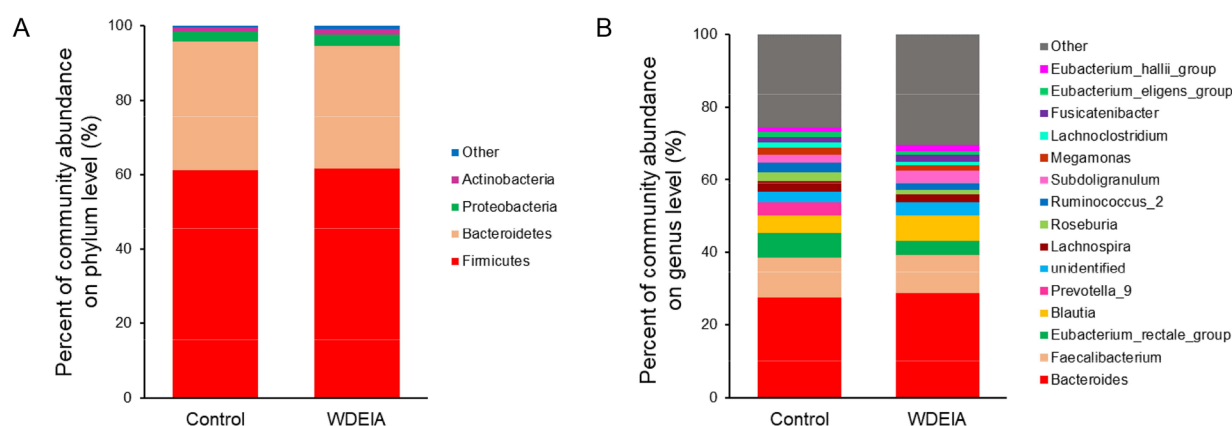


Fig. 2. Relative taxa abundance of fecal microbial community in WDEIA group and control group. (A) Comparison of relative taxa abundance between WDEIA and healthy control group at phylum level. (B) Comparison of relative taxa abundance between WDEIA and healthy control group at genus level.

3.3. Differences in specific microbes of WDEIA patients and controls

Principal coordinates analysis (PCoA) based on the weighted UniFrac distance and unweighted UniFrac distance both showed that the structure of intestinal microbiota between the WDEIA and the controls was similar.

To identify different bacterial taxon biomarkers between patients with WDEIA and healthy controls, LEfSe was used to find species that differed significantly in abundance between the groups. A cladogram was used to display all microbiota structures from the phylum and genus levels (Fig. 3A). The greatest differences in fecal microbiota taxa between the WDEIA patients and controls were identified by an LDA score > 2 (Fig. 3B). At the phylum level, the relative abundance of Verrucomicrobia ($P = 0.017$) was lower in the control fecal microbiome than in the WDEIA patients. At the family level, the relative abundance of Lactobacillaceae ($P = 0.001$) was significantly decreased, while Verrucomicrobiaceae ($P = 0.017$) and Erysipelotrichaceae ($P = 0.01$) were significantly increased in the WDEIA patients compared with the controls. At the genus level, the relative abundances of *Blautia* ($P = 0.018$), *Erysipelatoclostridium* ($P = 0.008$), *Akkermansia* ($P = 0.017$) and *Lachnospiraceae_NK4A136_group* ($P = 0.017$) were significantly increased, while those of *Lactobacillus* ($P = 0.001$) and *Dialister* ($P = 0.015$) were significantly decreased in WDEIA patients.

3.4. Microbial diversity did not significantly differ between WDEIA patients and controls

The Chao1 index was used to evaluate species richness, and the Shannon index was used to estimate the average phylogenetic diversity of the fecal microbiota in patients and controls. No significant difference was found between the groups (mean Chao1 diversity indices, 164 vs 165, $P = 0.90$; mean Shannon diversity indices, 0.70 vs 0.75; $P = 0.47$).

3.5. Correlation between the fecal microbiota and clinical parameters

Correlations between the intestinal microbiota and clinical indices, including serum total IgE and serum IgE specific to wheat, gluten and ω -5 gliadin, were determined via Spearman's rank correlation analysis (Fig. 4). Serum total IgE was positively correlated with *Paraprevotella* ($r = 0.46$, $P < 0.05$) and negatively correlated with *Bifidobacterium* ($r = -0.44$, $P < 0.05$). The specific IgE to wheat was positively associated with *Peptococcus* ($r = 0.51$, $P < 0.05$) and *Ruminococcaceae_UCG-009* ($r = 0.59$, $P < 0.01$) and negatively correlated with *Bilophila* ($r = -0.45$, $P < 0.05$), *Parabacteroides* ($r = -0.48$, $P < 0.05$) and *Sutterella* ($r = -0.44$, $P < 0.05$). Both the genera *Candidatus_Soleaferrea* and *Oscillospira* were positively associated with the specific IgE to gluten ($r = 0.71$, $P < 0.001$; $r = 0.53$, $P < 0.05$) and the specific IgE to ω -5 gliadin ($r = 0.72$, $P < 0.001$; $r = 0.48$,

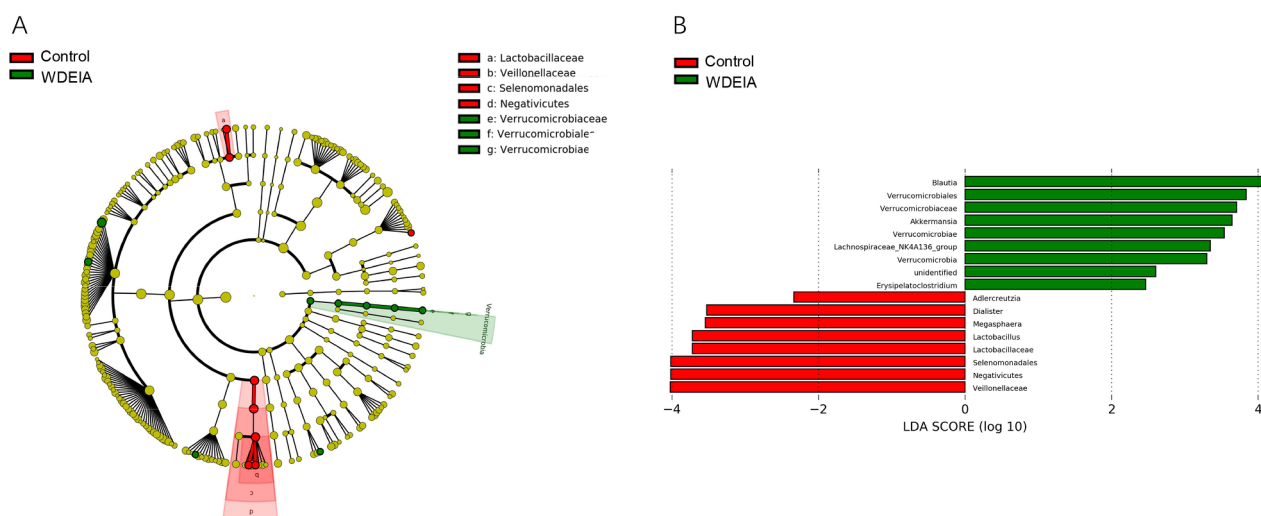


Fig. 3. Bacterial difference identification in fecal microbiota in WDEIA group and control group. (A) The taxonomic cladogram displayed the enriched taxa in WDEIA and control fecal microbiota. Each node represents a specific taxonomic type (red: control group; yellow: nonsignificant; and green: WDEIA group). The diameter of each circle is proportional to the relative abundance of the taxon. (B) Histogram of the LDA scores between WDEIA and control groups (LDA score > 2).

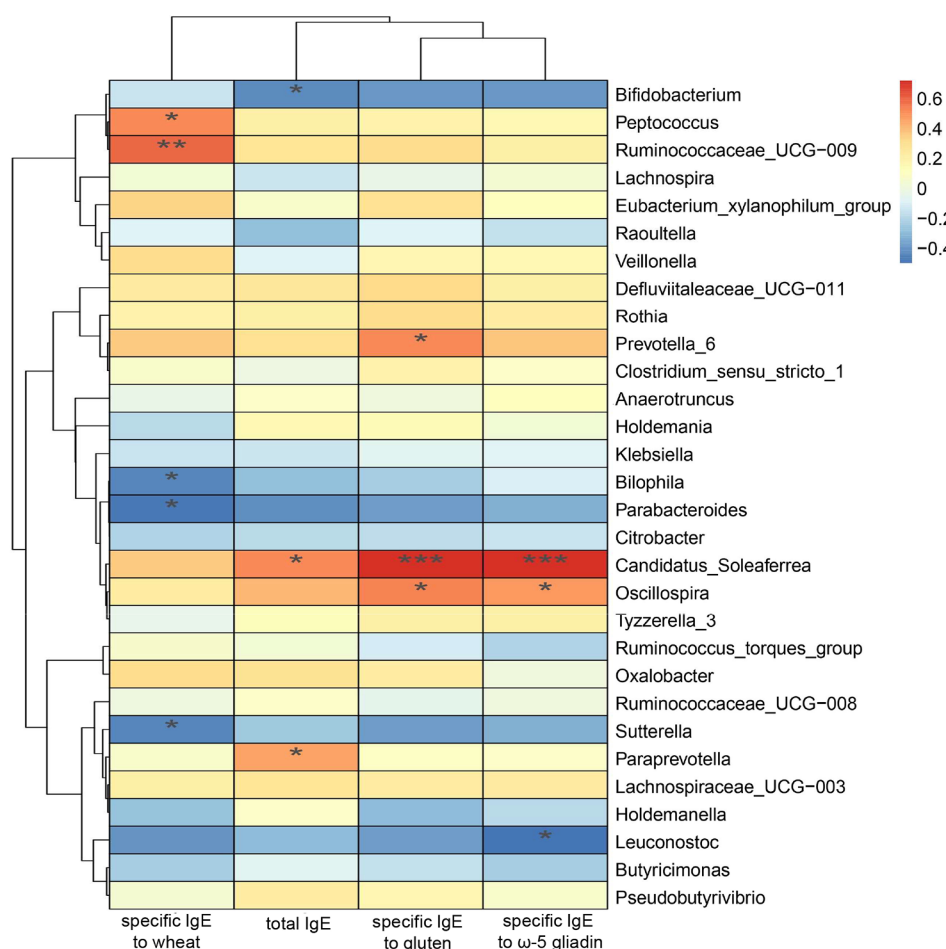


Fig. 4. Heatmap of spearman correlation analysis between the gut microbiota of WDEIA and the clinical parameters. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

$P < 0.05$). The specific IgE to ω-5 gliadin was negatively associated with *Leuconostoc* ($r = -0.49$, $P < 0.05$). No correlation was found between the identified gut microbes and environmental factors, including residential area, pet exposure and early-life antibiotics.

4. Discussion

In this study, the family-level richnesses of Verrucomicrobiaceae and Erysipelotrichaceae were higher, and that of Lactobacillaceae was lower in the fecal microbiomes of patients with WDEIA compared with those of the control group. At the genus level, *Blautia*, *Erysipelatoclostridium*, *Akkermansia* and *Lachnospiraceae_NK4A136_group* were enriched in WDEIA patients, while *Lactobacillus* and *Dialister* were enriched in the controls. Intestinal microbiome diversity did not differ between the two groups. We also found that clinical parameters might be associated with the gut microbiota.

To our knowledge, this is the first study to investigate the relationship between the microbiome and WDEIA development. Most previous studies focused on examining associations between the gut microbiome and food allergies, including milk, eggs, and peanuts [15]. Few studies have targeted the microbiome of WDEIA subjects [2]. As a life-threatening disease, investigating the associated intestinal microbiome in WDEIA patients may illustrate new methods for its treatment and prevention.

Our findings showed both similarities and differences compared with prior microbiome studies for other food allergies. The implicated disease taxa in other studies have been reported at varying taxonomic levels [16]. We found that Lactobacillaceae and *Lactobacillus* were enriched in healthy controls. Consistent with our results, Rezazadeh et al.

[17] found that the relative abundance of *Lactobacillus* and *Bifidobacterium* in fecal samples of healthy controls were significantly higher than that of patients with chronic urticaria. Burni et al. [18] demonstrated that *Lactobacillus rhamnosus* GG (LGG) supplementation enhanced the favorable immunoregulatory activities of an extensively hydrolyzed casein formula (EHCF) for allergies to cow's milk. *Lactobacillus* regulates immune functions by skewing toward a Th1 response, modulating the Th1/Th2 balance, inducing Treg cells and suppressing allergic responses [11,19]. LGG also enhanced tight-junction functions in human primary keratinocytes [20]. Conversely, Noval Rivas et al. [21] found enriched taxa from Lactobacillaceae and Lachnospiraceae in ovalbumin-sensitized I14raF709 mice versus wild-type mice. By comparing genetically similar populations with distinct atopic prevalences, Sepp et al. [22] reported higher *Lactobacillus* counts in the group with a higher atopy incidence. Biological effects of the microbiota may occur at the strain level; therefore, the impacts vary among strains within the same family. Moreover, many factors can influence microbiome study results, such as disparate food allergies, sample collection and processing [16].

In previous studies, Lachnospiraceae were enriched in the gut microbiomes of participants with food allergies versus controls [9,23]. Similarly, both *Blautia* and *Lachnospiraceae_NK4A136_group* of the Lachnospiraceae family, clustered in the WDEIA patients. In line with our study, Verrucomicrobia has been reported to be enriched in subjects with egg allergies [23]. A recent study [24] reported that *Veillonella* was involved in the asthma pathogenesis. No association was found between Veillonellaceae and WDEIA in our study, but the relative abundance of *Dialister* from Veillonellaceae was lower in the WDEIA group. *Dialister* had lower read counts in food allergy patients compared with that in

controls [25]. Consistent with our study, another study [26] found that the relative abundance of *Akkermansia* was higher in 12-month-old allergic infants than in healthy infants. However, a decrease in *Akkermansia muciniphila* in human intestines was found to be associated with an increased risk of atopic dermatitis [27]. The association between *Erysipelatoclostridium* and infant allergies changes dynamically [26]. Further studies are needed to investigate the functions of gut microbiota taxa in allergic diseases.

The gut microbiota plays an important role in the pathogenesis and course of allergic diseases [5,11]. WDEIA is an IgE-mediated disorder, and in this study, the serum total IgE level was negatively correlated with *Bifidobacterium*. *Bifidobacterium* can enhance barrier functions, modulate short-chain fatty acid (SCFA) metabolism and induce anti-inflammatory cytokine production [20,28]. SCFAs can regulate the size and function of the colonic Treg cell pool, while Treg cells suppress immediate hypersensitivity responses from mast cells [29]. Therefore, *Bifidobacterium* might protect against allergic diseases [28]. Specific IgE levels to wheat extract, gluten and ω -5 gliadin were detected for diagnosis. In our study, *Candidatus_Soleaferrea* and *Oscillospira* were positively associated with gluten-specific and ω -5 gliadin-specific IgE. *Oscillospira* was enriched in infants that become tolerant to cow's milk than those that remained allergic after treatment with EHCF + LGG [18]. *Oscillospira* produces butyrate via sugars released from host mucins or from fermentation products secreted by other bacterial species. However, the effects of *Oscillospira* in WDEIA remain unknown. It is reported that *Candidatus_Soleaferrea* is negatively correlated with the utilization efficiency of nitrogen in colon [30]. Currently, there have been limited reports on *Candidatus_Soleaferrea* and little is known about its function in allergic diseases. *Leuconostoc*, as an organic acid producer, was negatively correlated with ω -5 gliadin-specific IgE. *Leuconostoc* or its products may decrease the levels of ω -5 gliadin-specific IgE in WDEIA patients.

Previous studies showed that higher exposure to high levels of pets and pet allergens conferred protection against developing allergies [31]. Indoor household pets have been consistently linked to an altered home microbial environment, which may affect children's microbiological or immune development, including the development of atopy and overall IgE production [32]. Emerging data indicate that the presence of specific gut microbial taxa during infancy, such as *Bifidobacterium pseudolongum*, *Peptostreptococcaceae*, *Oscillospira*, *Ruminococcus* and *Cronobacter*, are associated with pet keeping [32]. However, these studies were performed on children, and the relevant intestinal microbial taxa varied greatly. Reported microbial alterations have been related to pet keeping. Interestingly, in our study, WDEIA patients were less likely to keep pets than were healthy controls, but we found no association between pet keeping and the gut microbiota.

Consistent with previous studies [25], the microbiome measures of diversity were similar between the controls and patients. However, controversial associations exist between microbial diversity and allergic diseases. Several studies reported inverse associations between intestinal microbial diversity and susceptibility to allergic diseases [5,33]. Azad et al. [5] reported an inverse association between gut microbial diversity at 3 months old and food sensitization at 1 year of age but not between gut microbial diversity at 12 months old and food sensitization. Similarly, Abrahamsson et al. [34] demonstrated that gut microbiota diversity was associated with atopic dermatitis at 1 month rather than 12 months of age. In early life, the gut microbiome is dynamic and dramatically shifts to an "adult-like" profile after solid foods are introduced [35,36]. Associations between gut microbial richness and allergies may be age-dependent. Therefore, introducing solid food likely obscures the relationship between the gut microbiota and allergies [15].

Based on the findings above, we propose a hypothesis to explain the association between the intestinal microbiota alteration and WDEIA development. *Lactobacillus* may protect against WDEIA development by maintaining proper host immune tolerance and enhancing barrier

function [19,20]. A previous study has demonstrated that the supplementation of propionate can decrease susceptibility to allergic airway inflammation and weaken the effectiveness of lung dendritic cells at reactivating Th2 responses [37]. Thus, *Dialister*, as a propionate-producing microbe, may also play a protective role in WDEIA development. We found that *Blautia* and *Lachnospiraceae_NK4A136_group* were enriched in WDEIA patients. However, there is no research reported on *Blautia* and *Lachnospiraceae_NK4A136_group* in allergic diseases, and their mechanisms in the development of WDEIA need further research.

A limited number of clinical trials have examined probiotics for the prevention or treatment of allergic diseases. It has been proven that supplementation of EHCF with LGG accelerated the cow's milk protein tolerance in infants [38]. Combined antenatal and postnatal supplementation with LGG can protect infants at risk for atopic diseases from subsequent allergic sensitization [39]. In our study, we found that *Lactobacillus* was enriched in healthy controls compared with WDEIA patients. Given the well-documented link between LGG and immune system [18,19], we hypothesize that the supplement of *Lactobacillus* might be beneficial for the prevention and treatment of WDEIA. *Dialister* may also play a protective role in WDEIA development, but there are no reports on the supplementation of *Dialister*. The biological effects of the microbiota may vary among strains and the microbiota associated with different food allergy subphenotypes are also distinct [16]. Further studies are required to evaluate the effects of probiotic supplements for WDEIA.

As the first study to investigate the relationship between fecal microbiota profiles and WDEIA, this study provides novel directions for exploring the WDEIA pathogenesis, which may yield better treatments for this disorder. This study indicated an association between the gut microbiome and WDEIA. However, our study had several limitations. Firstly, as WDEIA is a rare disease, only 25 fecal samples were collected from the WDEIA group. The inconspicuous difference between the groups may have been due to the small sample size and the sequencing method. Larger sample sizes and metagenomics studies are needed in future studies. Secondly, our findings indicated that several gut microbes were associated with WDEIA, but as a cross-sectional study, our results cannot show causation; thus, future studies should include prospective cohorts. Thirdly, there might be selection bias in the controls. Finally, only findings at higher taxonomic levels were reported, whereas immunomodulatory functions differed between bacterial strains within the same genus.

5. Conclusions

This study revealed different gut microbiome compositions between patients with WDEIA and healthy controls. Furthermore, the gut microbiome may be associated with WDEIA development. The distinct gut microbiotas in WDEIA patients identified in our study may provide novel directions for preventing and treating WDEIA, especially when deeper sequencing can identify specific strains within these taxa.

CRedit authorship contribution statement

Zhirong Du: Writing - original draft. **Xiang Gao:** Conceptualization, Data curation. **Jia Yin:** Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was funded by the National Natural Science Foundation

of China (grant numbers 81273277 and 81771726), the CAMS Innovation Fund for Medical Sciences (grant numbers 2016-12M-1-003), the National Key Research and Development Program of China (grant numbers 2016YFC0901500).

References

- [1] K.A. Scherf, K. Brockow, T. Biedermann, et al., Wheat-dependent exercise-induced anaphylaxis, *Clin. Exp. Allergy* 46 (2016) 10–20.
- [2] K.A. Scherf, A.C. Lindenau, L. Valentini, et al., Cofactors of wheat-dependent exercise-induced anaphylaxis do not increase highly individual gliadin absorption in healthy volunteers, *Clin Transl Allergy* 9 (2019) 19.
- [3] N. Inomata, Wheat allergy, *Curr. Opin. Allergy Clin. Immunol.* 9 (2009) 238–243.
- [4] A.B. Blazquez, M.C. Berin, Microbiome and food allergy, *Transl Res* 179 (2017) 199–203.
- [5] M.B. Azad, T. Konya, D.S. Guttman, et al., Infant gut microbiota and food sensitization: associations in the first year of life, *Clin. Exp. Allergy* 45 (2015) 632–643.
- [6] B. Chen, L. Sun, X. Zhang, Integration of microbiome and epigenome to decipher the pathogenesis of autoimmune diseases, *J. Autoimmun.* 83 (2017) 31–42.
- [7] J.L. Round, S.K. Mazmanian, The gut microbiota shapes intestinal immune responses during health and disease, *Nat. Rev. Immunol.* 9 (2009) 313–323.
- [8] J. Tan, C. McKenzie, P.J. Vuillermin, et al., Dietary Fiber and Bacterial SCFA Enhance Oral Tolerance and Protect against Food Allergy through Diverse Cellular Pathways, *Cell Rep* 15 (2016) 2809–2824.
- [9] T. Feehley, C.H. Plunkett, R. Bao, et al., Healthy infants harbor intestinal bacteria that protect against food allergy, *Nat. Med.* 25 (2019) 448–453.
- [10] A.T. Stefka, T. Feehley, P. Tripathi, et al., Commensal bacteria protect against food allergen sensitization, *Proc Natl Acad Sci U S A* 111 (2014) 13145–13150.
- [11] B. Rodriguez, G. Priault, R. Bibiloni, et al., Germ-free status and altered caecal subdominant microbiota are associated with a high susceptibility to cow's milk allergy in mice, *FEMS Microbiol. Ecol.* 76 (2011) 133–144.
- [12] N. Jiang, J. Yin, L. Wen, et al., Characteristics of Anaphylaxis in 907 Chinese Patients Referred to a Tertiary Allergy Center: A Retrospective Study of 1,952 Episodes, *Allergy Asthma Immunol. Res* 8 (2016) 353–361.
- [13] L.S. Cox, M. Sanchez-Borges, R.F. Lockey, World allergy organization systemic allergic reaction grading system: is a modification needed? *J. Allergy Clin. Immunol. Pract.* 5 (58–62) (2017) e55.
- [14] J.R. Cole, Q. Wang, E. Cardenas, et al., The Ribosomal Database Project: improved alignments and new tools for rRNA analysis, *Nucleic Acids Res.* 37 (2009) D141–D145.
- [15] S. Bunyavanich, N. Shen, A. Grishin, et al., Early-life gut microbiome composition and milk allergy resolution, *J. Allergy Clin. Immunol.* 138 (2016) 1122–1130.
- [16] Y.J. Huang, B.J. Marsland, S. Bunyavanich, et al., The microbiome in allergic disease: Current understanding and future opportunities-2017 PRACTALL document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology, *J. Allergy Clin. Immunol.* 139 (2017) 1099–1110.
- [17] A. Rezazadeh, S. Shahabi, M. Bagheri, et al., The protective effect of Lactobacillus and Bifidobacterium as the gut microbiota members against chronic urticaria, *Int. Immunopharmacol.* 59 (2018) 168–173.
- [18] R. Berni Canani, N. Sangwan, A.T. Stefka, et al., Lactobacillus rhamnosus GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants, *ISME J.* 10 (2016) 742–750.
- [19] R. Aitoro, R. Simeoli, A. Amoroso, et al., Extensively hydrolyzed casein formula alone or with L. rhamnosus GG reduces beta-lactoglobulin sensitization in mice, *Pediatr. Allergy Immunol.* 28 (2017) 230–237.
- [20] R. Sultana, A.J. McBain, C.A. O'Neill, Strain-dependent augmentation of tight-junction barrier function in human primary epidermal keratinocytes by Lactobacillus and Bifidobacterium lysates, *Appl. Environ. Microbiol.* 79 (2013) 4887–4894.
- [21] M. Noval Rivas, O.T. Burton, P. Wise, et al., A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis, *J. Allergy Clin. Immunol.* 131 (2013) 201–212.
- [22] E. Sepp, K. Julge, M. Vasar, et al., Intestinal microflora of Estonian and Swedish infants, *Acta Paediatr.* 86 (1997) 956–961.
- [23] M. Fazlollahi, Y. Chun, A. Grishin, et al., Early-life gut microbiome and egg allergy, *Allergy* 73 (2018) 1515–1524.
- [24] J. Stokholm, M.J. Blaser, J. Thorsen, et al., Maturation of the gut microbiome and risk of asthma in childhood, *Nat. Commun.* 9 (2018) 141.
- [25] J.H. Savage, K.A. Lee-Sarwar, J. Sordillo, et al., A prospective microbiome-wide association study of food sensitization and food allergy in early childhood, *Allergy* 73 (2018) 145–152.
- [26] X. Shen, M. Wang, X. Zhang, et al., Dynamic construction of gut microbiota may influence allergic diseases of infants in Southwest China, *BMC Microbiol.* 19 (2019) 123.
- [27] Y.M. Park, S.Y. Lee, M.J. Kang, et al., Imbalance of gut streptococcus, clostridium, and akkermansia determines the natural course of atopic dermatitis in infant, *Allergy Asthma Immunol. Res.* 12 (2020) 322–337.
- [28] R.Y. Cheng, J.R. Yao, Q. Wan, et al., Oral administration of Bifidobacterium bifidum TMC3115 to neonatal mice may alleviate IgE-mediated allergic risk in adulthood, *Benef. Microbes* 9 (2018) 815–828.
- [29] G. Gri, S. Piconese, B. Frossi, et al., CD4+CD25+ regulatory T cells suppress mast cell degranulation and allergic responses through OX40-OX40L interaction, *Immunity* 29 (2008) 771–781.
- [30] L. Wang, K. Liu, Z. Wang, et al., Bacterial community diversity associated with different utilization efficiencies of nitrogen in the gastrointestinal tract of goats, *Front. Microbiol.* 10 (2019) 239.
- [31] H. Sbihi, R.C. Boutin, C. Cutler, et al., Thinking bigger: How early-life environmental exposures shape the gut microbiome and influence the development of asthma and allergic disease, *Allergy* 74 (2019) 2103–2115.
- [32] H. Kim, A.R. Sitarik, K. Woodcroft, et al., Birth mode, breastfeeding, pet exposure, and antibiotic use: associations with the gut microbiome and sensitization in children, *Curr. Allergy Asthma Rep.* 19 (2019) 22.
- [33] X. Hua, J.J. Goedert, A. Pu, et al., Allergy associations with the adult fecal microbiota: analysis of the American Gut Project, *EBioMedicine* 3 (2016) 172–179.
- [34] T.R. Abrahamsson, H.E. Jakobsson, A.F. Andersson, et al., Low diversity of the gut microbiota in infants with atopic eczema, *J. Allergy Clin. Immunol.* 129 (434–440) (2012) 440.e431–432.
- [35] T. Yatsunenko, F.E. Rey, M.J. Manary, et al., Human gut microbiome viewed across age and geography, *Nature* 486 (2012) 222–227.
- [36] C. Palmer, E.M. Bik, D.B. DiGiulio, et al., Development of the human infant intestinal microbiota, *PLoS Biol.* 5 (2007) e177.
- [37] A.R. Panzer, S.V. Lynch, Influence and effect of the human microbiome in allergy and asthma, *Curr. Opin. Rheumatol.* 27 (2015) 373–380.
- [38] R. Berni Canani, R. Nocerino, G. Terrin, et al., Effect of Lactobacillus GG on tolerance acquisition in infants with cow's milk allergy: a randomized trial, *J. Allergy Clin. Immunol.* 129 (580–582) (2012) 582.e581–585.
- [39] A. Huurre, K. Laitinen, S. Rautava, et al., Impact of maternal atopy and probiotic supplementation during pregnancy on infant sensitization: a double-blind placebo-controlled study, *Clin. Exp. Allergy* 38 (2008) 1342–1348.