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ORIGINAL RESEARCH

Changes of Gut Microbiome in Adolescent Patients with Chronic Spontaneous Urticaria After Omalizumab Treatment

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Purpose: Omalizumab is a humanized anti-immunoglobulin (Ig) E monoclonal antibody that is effective in treating some patients with chronic spontaneous urticaria (CSU) who do not respond to antihistamines. Gut microbiome plays a role in the pathogenesis of allergies and autoimmune diseases. Here, we investigated differences in the gut microbiome of adolescent CSU patients before and after omalizumab treatment, which has not been previously reported.

Patients and Methods: Ten adolescent CSU patients were given 300 mg omalizumab subcutaneously in three treatments at 4-week intervals. Urticaria Activity Score (UAS7) was applied to evaluate the efficacy of each omalizumab treatment during follow-up. Fecal samples were collected before and 12 weeks after the first treatment. Total DNA of the gut microbiota in all fecal samples were extracted. The 16S rRNA gene-targeted sequencing technology was used for the analysis of the diversity and distribution of gut microbiome, followed by bioinformatics analysis.

Results: UAS7 scores decreased significantly after each treatment compared with the baseline (all $P < 0.0001$). There were five well-controlled responders and five non-responders after three treatment sessions of omalizumab. The dominant bacteria phyla in all fecal samples were Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. Alpha diversity analysis showed no significant difference before and after treatment ($P > 0.05$), whereas beta diversity analysis revealed a significant difference in the bacterial abundance before and after treatment ($P < 0.01$). The relative abundance of Alphaproteobacteria and Betaproteobacteria at the class level and *Burkholderia, Rhodococcus*, and *Sphingomonas* at the genus level decreased significantly after treatment (linear discriminant analysis > 4, $P < 0.05$). The functional prediction results showed that the dioxin and xylene degradation pathways were more abundant before treatment.

Conclusion: Omalizumab is effective in treating CSU and the abundance of Alphaproteobacteria and Betaproteobacteria was reduced after treatment, which may help improve the treatment outcomes in adolescent CSU patients.

Keywords: chronic spontaneous urticaria, adolescent patients, omalizumab, gut microbiome, 16S rRNA gene-targeted sequencing

Introduction

Chronic spontaneous urticaria (CSU) is a particular urticaria characterized by itchy wheals that last for at least 6 weeks without obvious triggers, and second generation H1-antihistamines are used as its first-line treatment.[1] However, the majority of patients with CSU do not respond to antihistamines. Several studies have confirmed the effectiveness of omalizumab in treating CSU.[1,2] It can be used to treat recalcitrant CSU in adolescents older than 12 years who are resistant to conventional therapy.[3,4] Autoimmunity is considered as one of the most frequent causes of CSU. Type I and II autoimmunity (ie, cytokenergic immunoglobulin (Ig) Es and IgG autoantibodies to IgE or high-affinity FcεRI receptor,

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respectively) have been implicated in the pathogenesis of CSU.[5] Moreover, some pro-inflammatory cytokines such as IL-6 and IFN-γ could be relevant effectors in the autoimmune pathogenesis underlying CSU.[6] The characteristic spontaneous wheals are caused by the reaction of autoantibodies to high-affinity IgE receptors or to IgE on the surface of mast cells or basophils.[7,8] Omalizumab is a humanized anti-IgE monoclonal antibody that selectively binds to free IgE, decreasing its serum level and reducing the number of high-affinity IgE receptors on mast cells, basophils, and dendritic cells.[9,10] Additionally, omalizumab can downregulate the transcription of genes that are related to mast cell and leukocyte infiltration, skin repair, oxidative stress, and vascularization. As a result, the genetic signature of lesional skin in patients with CSU could be improved.[10] In addition, the peripheral blood basophil counts are increased because of reduced recruitment to the skin by omalizumab.[11] Furthermore, omalizumab may help to regulate the defective basophil IgE receptor pathways.[12] In brief, the mechanism by which omalizumab improves urticaria has not been fully elucidated.[13]

It has been reported that the imbalance in autoimmunity, inflammation, and coagulation is the main mechanism for the pathogenesis of chronic urticaria.[14,15] There is evidence suggesting the gut microbiome plays a part in the pathogenesis of allergies and the regulation of the immune system.[16] Therefore, gut microbiome composition may contribute to the pathogenesis of CSU.[17] For example, fecal samples from healthy controls had a significantly higher relative abundance of *Lactobacillus* than that from patients with chronic urticaria.[18] The microbial population decreased in the CSU group compared with the healthy control one. Patients with CSU exhibited increased beta diversity in the abundance of Enterobacteriaceae, whereas that of Bacteroides, *Faecalibacterium, Bifidobacterium*, and Ruminococcaceae was significantly reduced in patients with CSU.[19] Moreover, Liu et al found a significant reduction in the abundance of *Subdoligranulum* and *Ruminococcus bromii* in patients with CSU or symptomatic dermographism, which has potential diagnostic value.[20] We found that the gut microbiota in adult patients with CSU in China has been studied in much more detail than that of adolescent patients.[17,19,21] Additionally, there have been no reports comparing the gut microbiota of adolescent patients with CSU before and after omalizumab treatment. Therefore, this present study aimed to determine whether the diversity and composition of the gut microbiota have been modulated in adolescent patients after omalizumab treatment.

Materials and Methods

Patients

Ten adolescent patients (aged 12–18 years, 5 males and 5 females) with CSU who lived in Tianjin City in the year prior to sample collection visited the Dermatology Department of Tianjin First Central Hospital and were treated with omalizumab (Novartis Pharma Stein AG) between January 2020, and February 2021 were retrospectively evaluated. All patients and their legal guardians provided written informed consent for their data to be published in the article. The study adhered to the principles of the Declaration of Helsinki of the World Medical Association (1964), and ethical approval for this study was granted by the Ethics Committee of Tianjin First Central Hospital (No. YLC2019014). "Spontaneous appearance of wheals, angioedema, or both for longer than 6 weeks due to unknown causes" were used as diagnostic criteria for CSU. The calculation of the urticaria activity score (UAS7) was based on the EAACI/GA²LEN/EDF/WAO guideline.[22] Personal factors were recorded, including sex; age; course of the disease. The laboratory investigations included white blood cell, platelet, and eosinophil counts; neutrophil, lymphocyte, and monocyte percentages; erythrocyte sedimentation rate (ESR); C-reactive protein (CRP); D-dimer; total IgE, antinuclear antibodies (ANA); and *Helicobacter pylori* test, anti-thyroglobulin antibody, and thyroid peroxidase antibody results. The exclusion criteria included: antibiotic, probiotic, or prebiotic application within 3 months before fecal sample collection; any known disease, such as autoimmune diseases, gastrointestinal diseases, allergic rhinitis, asthma, eczema; intake of cheese, yogurt, or pickles 3 days prior to fecal sample collection; and inability to collect fecal samples as required.

Treatment Regimens

The patients were treated with omalizumab subcutaneously at a dosage of 300 mg for three sessions at 4-week intervals.[1,2] Fecal samples were collected before and 12 weeks after the first treatment session.

Follow-Up and Efficacy Evaluation

A total of three follow-up sessions were conducted at 4, 8, and 12 weeks after the first treatment session. The patients were asked to record the occurrence of wheals and pruritus every day, and the UAS7 was calculated by a dermatologist at baseline evaluation and during each follow-up session (Figure 1). After the treatment of omalizumab, the well-controlled response was defined as UAS7 ≤ 6 and the complete response was defined as UAS7 = 0.[23] The patients with UAS7 > 6 were considered as non-responders.[24]

DNA Extraction and PCR Amplification

The E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, US) was employed for the extraction of the microbial DNA in the fecal samples according to the manufacturer's protocol. We confirmed the quality of the extracted DNA by 1% agarose gel electrophoresis. Primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used for the amplification of the V3-V4 hypervariable regions of the bacterial 16S rRNA gene in a thermocycler PCR system (GeneAmp 9700, ABI, USA). The reaction conditions were as follows: initial denaturation at 95°C for 3 minutes; followed by 27 cycles of 95°C for 30s, 55°C melting for 30s, and 72°C for 45s; and a final extension step of 72°C for 10 min. A 2% agarose gel was prepared for the extraction of the resulting PCR products. Then, the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) was employed for the purification of PCR products. Finally, the QuantiFluor™-ST (Promega, USA) was used for the quantification of PCR products according to the manufacturers' protocols. After the purified amplicons were pooled, they were sequenced on an Illumina MiSeq platform (Illumina, San Diego, USA) based on the standard operational procedures.

Bioinformatics and Statistical Analysis

The "Atacama soil microbiome tutorial" of Qiime2docs, together with customized program scripts (https://docs.qiime2.org/2019.1/), was used to perform the analysis. Briefly, the QuimeTools import program in the QIIME™ 2 system was used to operate the format imported with raw FASTQ files. After the filtration, trim, denoising, and merging of the demultiplexed sequences of each fecal sample, the chimeric sequences were recognized and removed using the QIIME™ 2 dada2 plugin to acquire the feature table of amplicon sequence variants (ASV). The QIIME™ 2 feature-classifier plugin was then applied to align the ASV sequences with a pre-trained Greengenes 13_8 99% database (trimmed to the V3-V4 region bound by the 338F/806R primer pair) to produce the taxonomy table. The mitochondrial sequences of contamination were filtered using the QIIME™ 2 feature table plugin. The linear discriminant analysis effect size (LEfSe) and

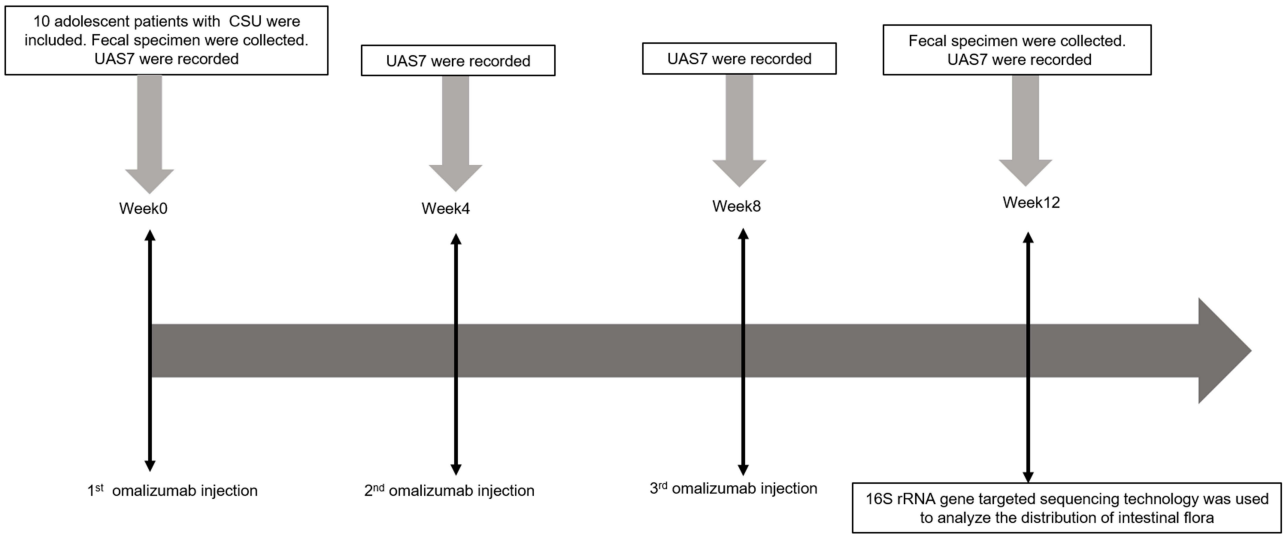

Figure 1 Study flowchart of materials and methods.
Abbreviations: CSU, chronic spontaneous urticaria; UAS, urticaria activity score.

differential gene expression analysis based on the negative binomial distribution (DEseq2) were employed to identify the microbiota with significantly different abundances before and after omalizumab therapy. The core diversity plugin within QIIME™ 2 was used to calculate the diversity metrics. The observed operational taxonomic units (OTUs), Chao1 richness estimator, Shannon diversity index, and Simpson index were used to describe the microbial richness and evenness of alpha diversity within a single fecal sample.

The structural differences of microbial communities across samples were investigated by Beta diversity distances which were calculated using the Bray-Curtis distance metric, then principal coordinate analysis (PCoA) was applied to make the beta diversity distances visualized. Furthermore, phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) was employed to predict the Kyoto Encyclopedia of Genes and Genomes (KEGG) Ortholog (KO) functional profiles.

Laboratory test results and UAS7 data were analyzed using GraphPad Prism 9.0 (Insightful Science Company, San Diego, CA, USA). A paired *t*-test or Wilcoxon test was employed to analyze the differences in the laboratory test results before and after treatment. One-way repeated measures analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test was used to calculate differences between multiple groups. Data are presented as the mean ± standard deviation; $P < 0.05$ was considered statistically significant.

Results

The clinical characteristics of adolescent patients with CSU before and after omalizumab treatment are presented in Table 1. Ten adolescent patients with a disease course ranging from 8–32 months were included in the study. There were no significant differences in the laboratory test results before and after treatment, except for the eosinophil, ESR, CRP, and total IgE levels. All of them were significantly lower after omalizumab treatment than before treatment, indicating the attenuation of activity in patients with CSU. ANA, *H. pylori*, anti-thyroglobulin, and thyroid peroxidase antibody test results were all negative before omalizumab treatment. UAS7 showed a significant improvement after each omalizumab treatment session ($P < 0.0001$) (Figure 2). There were five well-controlled responders and none complete responders after three treatment sessions of omalizumab (Table 2).

Venn diagram analysis was performed for clarification of the features of OTUs between the various groups. A total of 318 bacterial OTUs were common in patients with CSU before and after omalizumab treatment. The number of OTUs in the patients before and after treatment were 386 and 402, respectively (Figure 3). The alpha and beta microflora diversity was compared in patients with CSU before and after omalizumab treatment to estimate gut microbiota richness,

Table 1 Clinical Features of Adolescent Patients with Chronic Spontaneous Urticaria Before and After Omalizumab Treatment

Variables	Pre-Treatment M±SD/IQR	Post-Treatment M±SD/IQR
WBC ($\times 10^9$/L)	7.31 ± 1.98	6.60 ± 1.15
PLT ($\times 10^9$/L)	226.90 ± 64.89	235.3 ± 62.87
N (%)	59.15 ± 9.50	58.58 ± 10.16
Eo ($\times 10^9$/L)	(0.12, 0.32)	(0.03, 0.17)*
L (%)	27.83 ± 9.98	29.90 ± 10.39
M (%)	7.80 ± 2.041	8.56 ± 1.56
ESR (mm/h)	17.90 ± 11.24	8.70 ± 3.43*
CRP (mg/L)	7.31 ± 3.68	4.28 ± 3.14*
D-dimer (μg/L)	242.40 ± 143.20	305.90 ± 111.40
Total IgE (IU/mL)	(199.55, 682.85)	(20.08, 249.78)*

Note: *$P < 0.05$.

Abbreviations: WBC, white blood cell; PLT, platelet; N, neutrophil; Eo, eosinophil; L, lymphocyte; M, monocyte; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IgE, immunoglobulin E; M±SD, mean ± standard deviation; IQR, interquartile range.

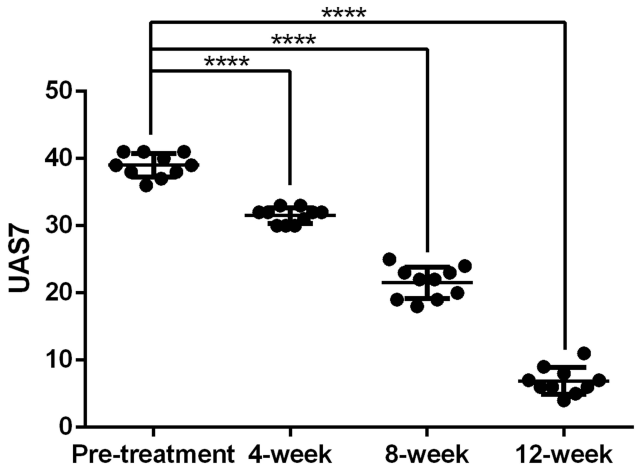

Figure 2 Changes in UAS7 at baseline and 4, 8, and 12 weeks after the first omalizumab treatment of CSU patients. (****$P < 0.0001$).
Abbreviation: UAS, urticaria activity score.

evenness, and abundance. Alpha diversity was evaluated according to the observed OTUs and Chao1, Shannon, and Simpson indices. In the present study, no significant differences were observed in OTUs (Figure 4A) and Chao1 (Figure 4B), Shannon (Figure 4C), and Simpson (Figure 4D) indices of adolescent patients with CSU before and after omalizumab treatment (all $P > 0.05$), indicating that bacterial community richness and evenness of gut microbiota did not differ between the groups.

The top four taxa in relative abundance at the phylum level included Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria both before and after treatment (Figure 5A). The top four taxa in relative abundance at the class level included Clostridia, Bacteroidia, Betaproteobacteria, and Actinobacteria before treatment; and Clostridia, Bacteroidia, Gammaproteobacteria, and Bacilli after treatment (Figure 5B). The top four taxa in relative abundance at the order level included Clostridiales, Bacteroidales, Burkholderiales, and Sphingomonadales before treatment; and Clostridiales, Bacteroidales, Enterobacteriales, and Bifidobacteriales after treatment (Figure 5C). The top four taxa in relative abundance at the family level included Ruminococcaceae, Lachnospiraceae, Bacteroidaceae, and Prevotellaceae before treatment; and Ruminococcaceae, Bacteroidaceae, Lachnospiraceae, and Prevotellaceae after treatment (Figure 5D). The top two taxa in relative abundance at the genus level included *Faecalibacterium* and *Bacteroides* before treatment; and *Bacteroides* and *Prevotella* after treatment (Figure 5E). The top two taxa in relative abundance at the species level before and after treatment were *Prausnitzii* and *Copri*, respectively (Figure 5F).

Beta diversity analysis revealed significant differences in the bacterial community composition of adolescent patients with CSU before and after omalizumab treatment ($P < 0.01$). A PCoA scatter diagram based on the Bray-Curtis data between samples is presented in Figure 6A. The results revealed independent clustering of patients with CSU before and after treatment. The relative abundance of bacteria at different taxonomic levels in the patients before and after treatment was compared using LEfSe and DEseq2 analyses (Figure 6B, Table 3). The taxa with significant differences (linear discriminant analysis LDA > 4 with P <0.05) included Alphaproteobacteria and Betaproteobacteria at the class level; Sphingomonadales, Actinomycetales, and Burkholderiales at the order level; Burkholderiaceae, Nocardiaceae,

Table 2 Efficacy of Omalizumab Treatment During the Study

Patients	Week 4 N(%)	Week 8 N(%)	Week 12 N(%)
Non-responders	10(100)	10(100)	5(50)
Well-controlled responders	0(0)	0(0)	5(50)
Complete responders	0(0)	0(0)	0(0)

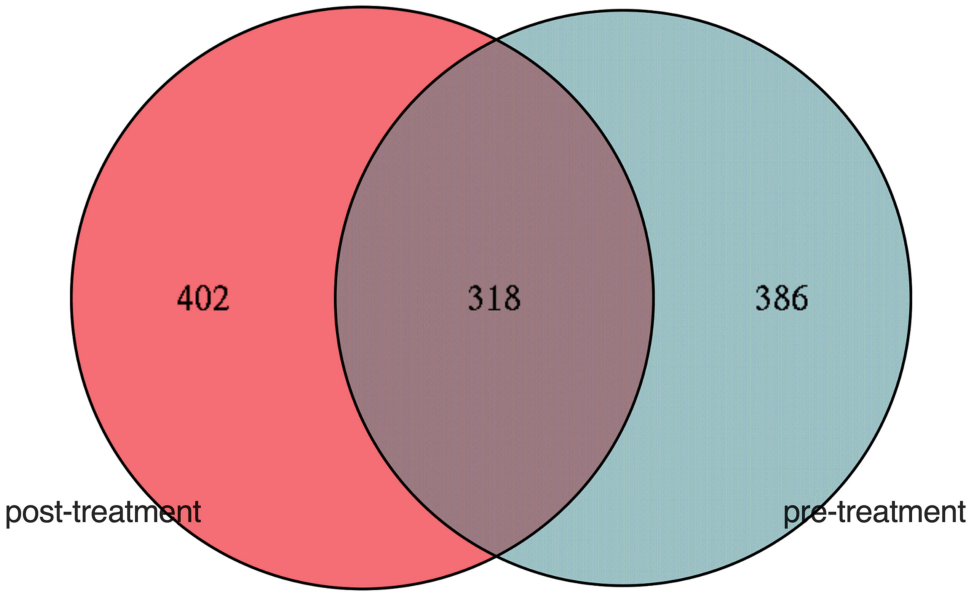

Figure 3 Venn diagram demonstrating operational taxonomic units common or unique to omalizumab pre-treatment and post-treatment groups.

Sphingomonadaceae, and Oxalobacteraceae at the family level; and *Burkholderia, Rhodococcus*, and *Sphingomonas* at the genus level. Before omalizumab treatment, patients with CSU were characterized by a higher abundance of all the taxa mentioned above.

Functional prediction revealed that alterations of the gut microbiota before and after treatment may lead to changes in pathways with high abundance involved in dioxin and xylene degradation (Figure 7), which were more abundant in adolescent patients with CSU before omalizumab treatment.

Discussion

In our research, 10 adolescent patients with CSU were treated with three sessions of omalizumab at 4-week intervals. We found that omalizumab helped to relieve urticarial activity and was effective for treating adolescent patients with CSU who are resistant to antihistamines. Notably, the eosinophil, ESR, CRP, and total IgE levels decreased, fewer allergic symptoms of CSU were seen and the UAS7 score decreased dramatically after each treatment as shown in Figure 2. Five patients were well controlled after three treatment sessions of omalizumab (Table 2).The richness, evenness, and composition of the gut microbiota of the 10 patients with CSU before and after omalizumab treatment were compared based on 16S rRNA gene profiling. We found no significant difference in alpha diversity in the patients before and after treatment (Figure 4), suggesting that the richness and evenness of the gut flora were not changed after omalizumab treatment. Zhang et al detected that there was no significant difference between the alpha diversity of patients with CSU and healthy individuals.[21] Therefore, it could be inferred that the alpha diversity of the gut microbiota was not correlated with improvement in outcomes or complete remission of CSU after treatment. We discovered that the top four taxa in relative abundance at the phylum level before treatment were Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria, highly corroborating the results presented by Eckburg et al and Wang et al[17,25] Our results showed that the ranking of abundances of the top four gut flora detected in patients with CSU before omalizumab treatment remained same after treatment (Figure 5A).

Alpha diversity demonstrates the distribution of gut flora within a sample, whereas beta diversity is often used to compare the diversity and similarity of gut flora between two different samples. We found that the beta diversity of gut microbiota was significantly different in adolescent patients with CSU before and after omalizumab treatment. The two groups clustered independently in the PCoA diagram (Figure 6A). We then analyzed the specific differences in the microbial communities at different taxonomic levels and found that the relative abundances of Alphaproteobacteria and Betaproteobacteria at the class level that belong to the phylum Proteobacteria, decreased after omalizumab treatment.

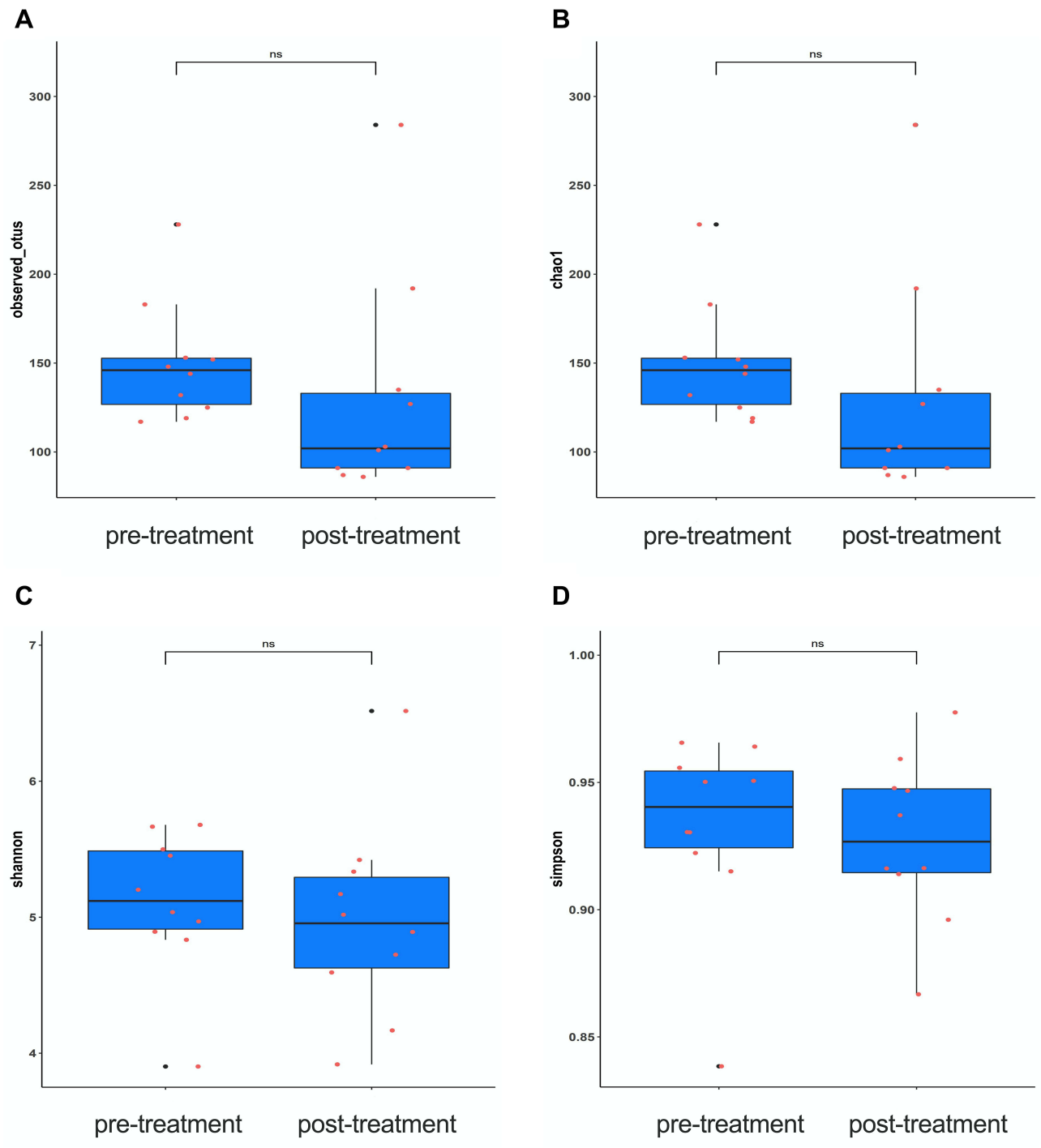

Figure 4 Differences in alpha diversity between adolescent CSU patients before and after omalizumab treatment. (**A**) Alpha diversity evaluated according to the observed OTUs indices. (**B**) Alpha diversity evaluated according to the Chao1 indices. (**C**) Alpha diversity evaluated according to the Shannon indices. (**D**) Alpha diversity evaluated according to the Simpson indices.
Note: $P > 0.05$.
Abbreviation: ns, non-significant.

Although Proteobacteria are commonly found in the human intestine, changes in their relative abundance can cause microbiota dysbiosis, even are related to diseases. It was reported that the abundance of Proteobacteria was higher in patients with asthma and allergic diseases.[26,27] Moreover, Lu et al found that Proteobacteria increased in patients with chronic urticaria.[28] Zhang et al found that the relative abundance of Proteobacteria, including Alphaproteobacteria and

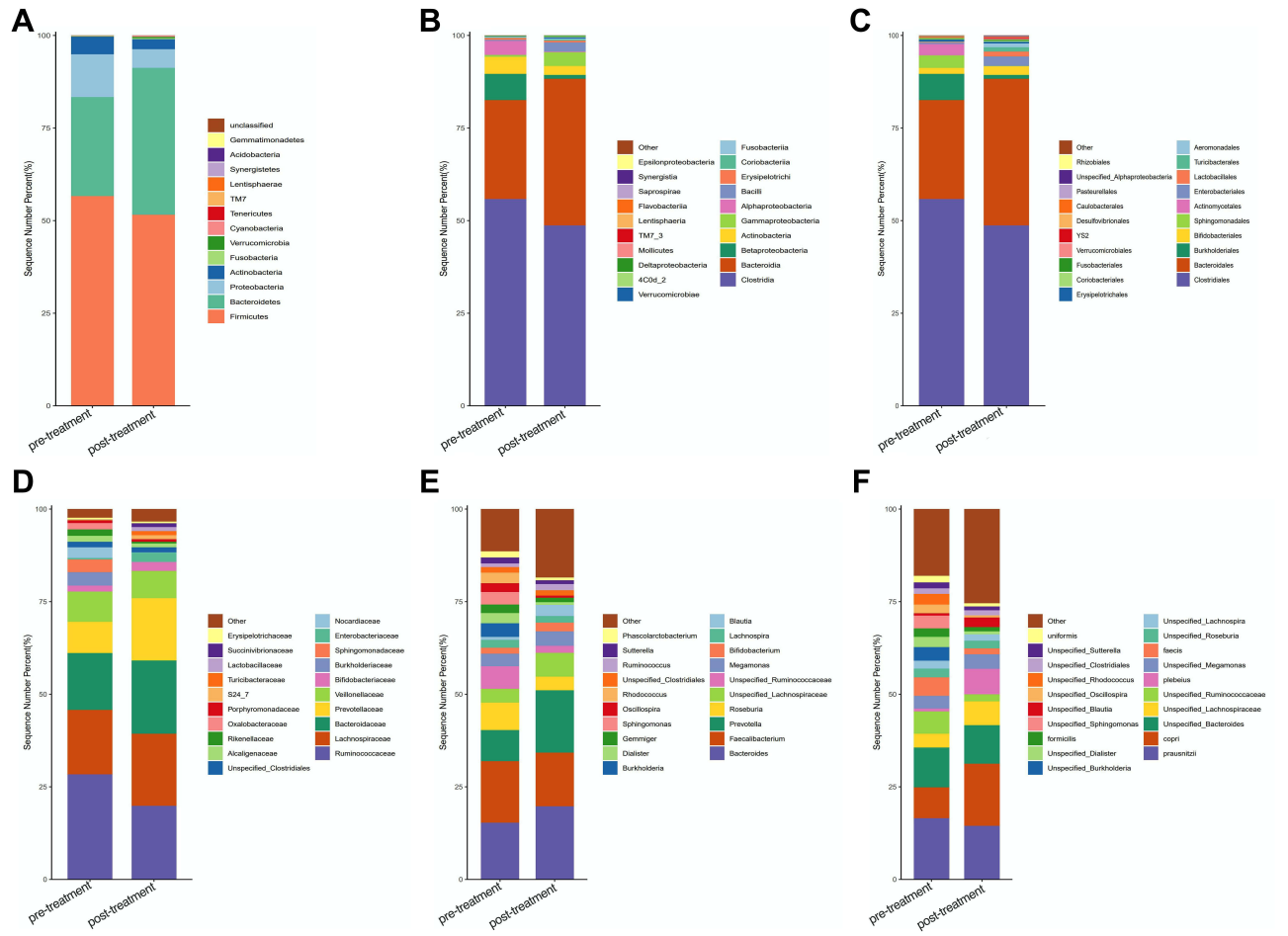

Figure 5 Gut microbiota composition in patients with CSU before and after treatment. (**A**) The top 13 taxa in relative abundance at the phylum level; (**B**) The top 20 taxa in relative abundance at the class level; (**C**) The top 20 taxa in relative abundance at the order level; (**D**) The top 20 taxa in relative abundance at family level; (**E**) The top 20 taxa in relative abundance at the genus level; (**F**) The top 20 taxa in relative abundance at the species level.

Betaproteobacteria, was significantly higher in patients with CSU compared to healthy controls.[21] Research suggests that the abundance of Proteobacteria is associated with the development of inflammatory skin diseases. When the abundance of Proteobacteria increased, the permeability of the gut mucosa was enhanced, thereby allowing the bacteria to enter the gut mucosa and cause damage to the gut mucosal barrier, leading to inflammatory skin diseases.[29] In our study, we speculated that a higher abundance of Alphaproteobacteria and Betaproteobacteria, belonging to Proteobacteria, may have an important role in CSU etiology and that the reduction of both taxa post-treatment indicated an improvement in urticarial severity. Notably, other studies have reported that the abundance of Proteobacteria could be altered by drug action as well. For example, highly active antiretroviral therapy reduced these bacteria in HIV-positive patients. Furthermore, atorvastatin increased the abundance of Proteobacteria in high-fat-diet rats and patients with atherosclerosis.[30] As a bacteria of the gut microbiome that is regulated by drugs, Proteobacteria is an excellent therapeutic target. Therefore, we speculate that Alpha- and Betaproteobacteria are potential therapeutic targets for omalizumab, as the reduction in these bacteria may be a marker of CSU improvement. More mechanistic studies are necessary to confirm that omalizumab can decrease the abundance of Proteobacteria, reducing the occurrence and improving chronic urticaria. Moreover, Burkholderiales with significantly higher abundance were found in the low IgE asthma group than in the high IgE asthma group at the order level. As a result, the experts inferred that a potential correlation between the relative abundance of Burkholderiales order and total serum IgE from allergic diseases may exist and need further investigation.[31] In contrast, Tsai et al showed that, compared with healthy controls, there was more abundant of the genus *Rhodococcus*, belonging to the order Actinomycetales, and the abundance of *Rhodococcus* was

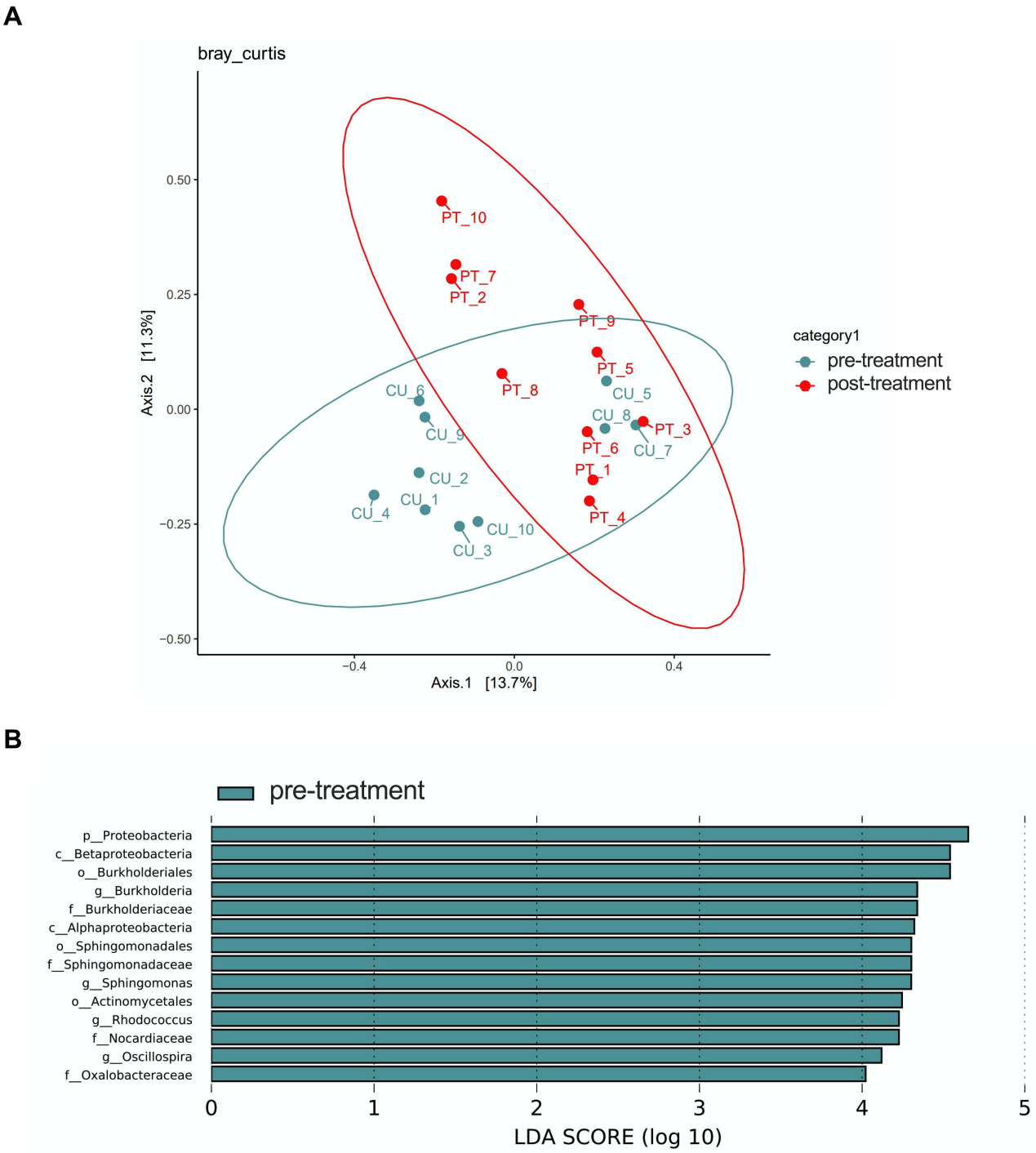

Figure 6 Beta diversity analysis based on PCoA and LEfSe. (**A**) PCoA of the bacterial community composition before and after treatment. (**B**) LEfSe analysis of the difference in the bacterial taxa relative abundance. Bacterial with Linear Discriminant Analysis (LDA) scores > 4.0 were considered significantly different.

positively correlated with total serum IgE levels in children with allergic conditions.[32] Finally, another study reported a higher abundance of the genus *Sphingomonas*, belonging to the Sphingomonadales order, in 45 food-sensitized infants compared to controls.[33] According to these results, there is an association between Burkholderiales, *Rhodococcus*, and *Sphingomonas* and allergic diseases. Furthermore, Hooper et al have reported that the commensal microbiota is responsible for systemic autoimmune and allergic diseases in animal models.[34] In our study, we found total IgE levels decreased significantly after omalizumab treatment. Therefore, we speculated that the gut microbiome might be

Table 3 The Taxa with Different Abundances Analyzed by Lefse and DEseq2

Levels	Control/Treatment log_2FoldChange
Class	
Alphaproteobacteria	4.66***
Betaproteobacteria	2.57**
Order	
Sphingomonadales	7.23****
Actinomycetales	6.60****
Burkholderiales	2.25***
Family	
Burkholderiaceae	7.80****
Nocardiaceae	7.96****
Sphingomonadaceae	7.49****
Oxalobacteraceae	6.99***
Genus	
Burkholderia	8.13****
Rhodococcus	8.38****
Sphingomonas	8.06****

Notes: Bacteria with LDA score > 4.0 and $P < 0.05$ were considered significantly discriminant. **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

associated with CSU which is caused by autoimmunity. In our study, we found that the abundance of the genera *Burkholderia, Rhodococcus*, and *Sphingomonas* decreased in patients with CSU after omalizumab treatment, which might help to alleviate the signs and symptoms of CSU.

Current clinical research has reported that the intake of probiotics and/or prebiotics can modify gut microbiota.[35] Some experts have proved that *Bifidobacterium* and *Lactobacillus* are favorable in the improvement of CSU.[36] Namely, the relative abundance of *Lactobacillus* was significantly higher in fecal samples from healthy controls than in those from patients with chronic urticaria.[18] It has been revealed that probiotic bacteria could modulate the Th1/Th2 balance, suggesting that the development of inflammatory diseases, including allergies, could be prevented.[37] Researchers have reported the administration of *Bifidobacterium* and *Lactobacillus* as probiotics to alleviate inflammatory responses by ameliorating dysbiosis.[38,39] However, some studies have reported that the therapeutic efficacy of probiotic treatment differs among patients with chronic urticaria.[28] Specifically, Nettis et al evaluated the efficacy of probiotic therapy and found that *Lactobacillus* and *Bifidobacterium* were not advantageous for most patients with CSU.[40] According to our study, no differences in *Bifidobacterium* and *Lactobacillus* genera were seen in the patients before and after treatment, demonstrating that these bacteria in the gut could not be related with the improvement of CSU. Based on the evidence presented by the researchers above, a further study with large samples to investigate the efficacy of combined therapy with omalizumab and probiotics containing *Bifidobacterium* and *Lactobacillus* is warranted.In the present study, our functional prediction results mainly showed differences in the dioxin and xylene degradation pathways in patients before and after omalizumab treatment. Dioxins are found worldwide in the natural environment and accumulate in the food chain, mainly in the fatty tissues of animals. Xylene is an aromatic hydrocarbon which is a potential occupational hazard[41] and has been reported to potentially cause urticaria.[42,43] In contrast, no reports of urticaria associated with dioxins have been reported. We assumed that dioxin and xylene, which are not routinely tested as allergens, could be potential triggers for chronic urticaria. In our study, as CSU patients treated with omalizumab improved, the abundance of dioxin and xylene degradation pathways decreased. Therefore, a review of the contact history and allergen test results for dioxin and xylene is necessary to exclude the inducible urticaria before a diagnosis of CSU is made.

One limitation of this study was the small sample size and limited age group (ie, teenagers) of participants. Larger sample sizes, including participants of all ages, will produce more reliable results. Additionally, more advanced techniques, such as metagenomics, can further extend and verify the 16S rRNA sequencing analysis results, and

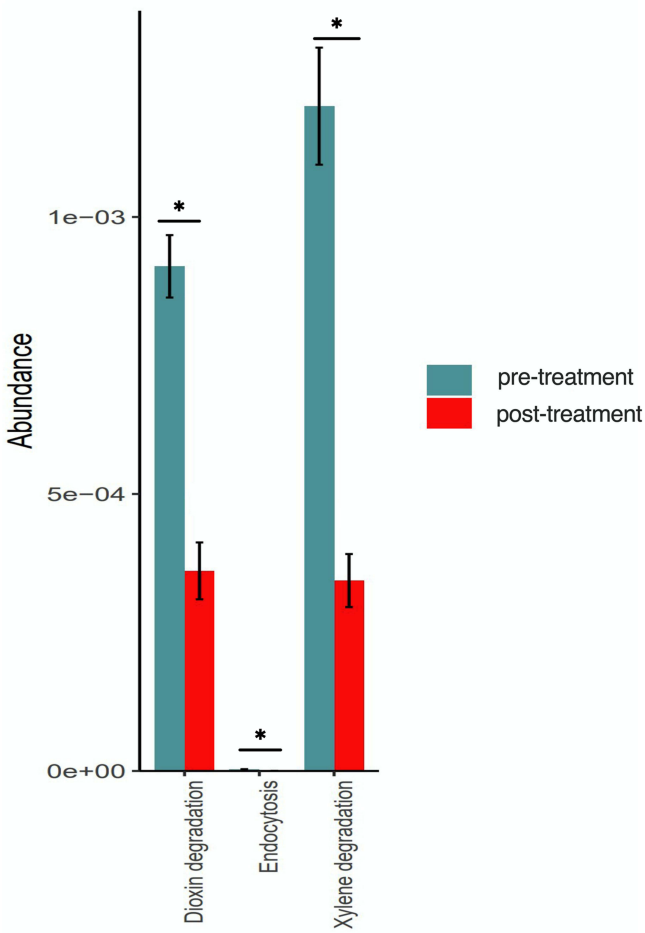

Figure 7 Differential pathway functional analysis between adolescent patients with CSU before and after omalizumab treatment. *$P < 0.05$.

metabolomics analysis can be used to find more detailed metabolic and functional profiles associated with gut microbiota after omalizumab treatment.

Conclusion

In conclusion, we preliminarily compared the elemental gut microbiota composition before and after omalizumab treatment using 16S rRNA gene sequencing of fecal samples from 10 adolescent patients with CSU. Significant differences were found in the relative abundance of gut microbiota before and after omalizumab treatment, especially Alpha- and Betaproteobacteria at the class level. Further functional prediction results revealed that the abundance of dioxin and xylene degradation pathways decreased after omalizumab treatment. Taken together, we inferred that the alleviation of CSU treated by omalizumab was accompanied with alterations in the gut microbiome, which may play a role in the improvement of CSU in adolescent patients.

Abbreviations

CSU, Chronic Spontaneous Urticaria; UAS, Urticaria Activity Score; Ig, Immunoglobulin; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; ANA, Antinuclear antibodies; ASV, amplicon sequence variants; LEfSe, linear discriminant analysis effect size; DEseq2, Differential gene expression analysis based on the negative binomial distribution; OTU, operational taxonomic units; PCoA, Principle coordinate analysis; PICRUSt, Phylogenetic investigation of communities by reconstruction of unobserved states; KEGG, Kyoto Encyclopedia of Genes and Genomes (KEGG); ANOVA, One-way repeated measures analysis of variance.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

Before undergoing the treatment, all patients and their legal guardians provided written informed consent for their data to be published in the article. The study adhered to the principles of the World Medical Association's Declaration of Helsinki, and ethics approval for this study was granted by the Ethics Committee of Tianjin First Central Hospital (No. YLC2019014).

Consent for Publication

Consent for publication was included in the informed consent obtained from each participant.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

Authors declare no competing interests for this study.

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