Throat microbiota alterations in patients with hereditary angioedema

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ABSTRACT

Background: Hereditary angioedema (HAE) is a rare disease characterized by recurrent episodes of cutaneous and submucosal edema. The clinical course of HAE is heterogeneous and unpredictable. There are no reliable indicators associated with angioedema attacks and severity. Throat microbiota plays vital roles in the maintenance of human health, while its association with HAE is barely understood. Therefore, this study aimed to investigate the alteration of throat microbiota and its correlation with attacks severity in HAE patients.

Methods: Throat swab samples were collected from HAE patients and their healthy family members, and then subjected to 16S rRNA sequencing. Alpha and beta diversity were used to examine the diversity and structure of bacterial communities. The relative abundance of individual bacteria was compared between study groups to determine the discriminant taxa. Spearman's correlation and linear regression were applied to analyze the correlation between throat bacteria and attacks severity.

Results: Irrespective of the study groups, the throat microbiome was predominantly occupied by Firmicutes, Bacteroidetes, Proteobacteria, and Fusobacteria. The recent onset of laryngeal edema is associated with the altered composition of microbiome community in HAE patients. The relative abundance of Bacteroidetes and Prevotellaceae was significantly increased in patients with recent episodes of laryngeal edema, compared to patients without recent episodes of laryngeal edema. Additionally, HAE attack severity scores positively correlated with the relative abundance of Bacteroidetes.

Conclusion: We reported alterations of the throat microbial communities in HAE patients and explored the correlation between bacteria and edema severity, which may shed light on understanding the disease course and developing new therapeutic strategies for HAE.

Keywords: Disease severity, Dysbiosis, Hereditary angioedema, Laryngeal edema, Microbial community

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INTRODUCTION

Hereditary angioedema (HAE) is an autosomal dominant disorder with a prevalence of approximately 1:50,000 worldwide.[1] HAE commonly manifests with recurrent episodes of cutaneous and submucosal edema. The upper airway, gastrointestinal tract, and extremities are the most commonly affected sites.[2] Laryngeal edema can cause asphyxia, which is the leading cause of death in patients with HAE.[3] Gastrointestinal edema manifests as a sudden onset of severe abdominal pain and is often misdiagnosed as acute abdomen, leading to unnecessary surgery.[4] The clinical course of HAE attacks is heterogeneous and unpredictable.[5] Even among members of the same family with the same mutation, the frequency, location and severity of HAE attacks are highly variable.[6,7] Some patients experience frequent life-threatening HAE episodes, while others experience only a mild course or even remain asymptomatic for life.[8,9] The cause of clinical heterogeneity in HAE remains unclear. Therefore, factors related to the activity and severity of the disease need to be assessed.[10]

The underlying mechanism of type 1 HAE (HAE-1) and type 2 HAE (HAE-2), respectively, is C1 inhibitor (C1-INH) deficiency, and C1-INH dysfunction. Both mechanisms result from mutations in *SERPING1*. C1-INH regulates multiple proteases involved in the complement, kallikrein-kinin system, coagulation, and fibrinolytic pathways.[11] In HAE-1/2, the kallikrein-kinin system is over-active and produces bradykinin, leading to swelling.[11] Although *SERPING1* mutations are the major genetic defect of HAE-1/2, there is little correlation between the type of *SERPING1* mutations and the phenotype of HAE.[5,12] Subsequent studies attempted to link *SERPING1* mutations to disease severity but yielded conflicting results.[13,14]

A new concept in exploring disease-related biomarkers is that the microbial community may act as a whole and influence the progression of multiple diseases.[15] Microbial communities are crucial for the maintenance of human health.[16] Several studies have confirmed that alterations in the oral and gut microbiota are associated with a variety of diseases, including obesity,[17,18] rheumatoid arthritis,[19,20] COVID-19,[21] and disorders of the skin,[22] oral cavity,[23] and digestive system.[24] Recently, our previous study reported alterations in the gut microbiome of HAE patients and found a decrease in the abundance of Firmicutes and an increase in the abundance of Proteobacteria in patients with recent gastrointestinal edema.[25] However, there is insufficient evidence to confirm the relationship between gut microbiota and laryngeal edema.[25]

The National Institutes of Health Human Microbiome Project (HMP) has revealed that a wide diversity of microbial flora harbors in the human throat.[26] However, the characteristics of throat microbiome in HAE patients and the relationship between throat microbiome and edema episodes, especially laryngeal edema episodes, are not studied in previous research. Therefore, the purpose of this study is to characterize the microbial community in the throat of HAE patients and to explore specific taxa associated with HAE activity and attack severity.

MATERIALS AND METHODS

Study subjects and sample collection

This study enrolled 36 C1-INH related HAE patients. The diagnosis of HAE was made in accordance with the US Hereditary Angioedema Association (HAEA) medical advisory board 2020 guidelines for the management of hereditary angioedema[27] and the international World Allergy Organization/European Academy of Allergy and Clinical Immunology (WAO/EAACI) guideline for the management of hereditary angioedema – the 2021 revision and update.[28] Seventeen healthy controls were chosen from the family members of the included HAE patients. All healthy controls were free of edema symptoms and had negative laboratory test results for HAE. All patients and healthy controls met the following criteria: (1) no use of antibiotics within the 2 months prior to throat swab collection; (2) no use of antiseptic mouthwash within the 24 h prior to throat swab collection; and (3) no other oral, pharyngeal, and respiratory diseases, including dental problems, tonsillitis, upper respiratory tract infections, or cancer within the 3 months prior to sample collection.

Throat swabs were collected by a trained physician. The posterior pharynx and each tonsillar region were swabbed 3 times separately with nylon flannel swab, and the swabs were immediately placed in sterile tube (Creative

Microbiologicals, Ltd., Suzhou, China) and frozen at −80 °C. Four patients underwent follow-up sampling to assess microbiome alterations before and after the danazol treatment. The study was approved by the Research Ethical Committee of the Peking Union Medical College Hospital. Each participant completed a written informed consent.

Patient grouping

Demographic and clinical characteristics were obtained from medical records and questionnaires. A face-to-face questionnaire survey was administered to obtain data from each participant, including sociodemographic factors (age, sex, and ethnicity), edema episodes (location, time, and severity), treatment, lifestyle (drinking, smoking, exercise, and eating habit), antibiotic use, and other diseases (hypertension, diabetes, cirrhosis, depression, Crohn's disease, and ulcerative colitis). For the detailed electronic version of the questionnaire, see Supplementary Table 1.

Patients were grouped according to the pattern of recent episodes. First, to evaluate the association between throat flora and laryngeal edema, patients were divided into 3 groups according to whether they recently suffered from laryngeal edema, as follows: (1) suffered from laryngeal edema within the previous month (LE.1m); (2) had a history of laryngeal edema but had no episode within the previous month (NLE.1m); and (3) never had laryngeal edema (NLE). Second, to evaluate the association between throat flora and gastrointestinal edema, patients were also divided into 3 groups: (1) suffered from gastrointestinal edema within the previous month (GE.1m); (2) had a history of gastrointestinal edema but had no episode within the previous month (NGE.1m); and (3) never had gastrointestinal edema (NGE). Third, to evaluate the association between throat flora and skin edema, patients were divided into 2 groups: (1) suffered from skin edema within the previous month (SE.1 m), and (2) had no skin edema in the previous month but had a history of skin edema (NSE.1 m).

Hereditary angioedema attack severity score

Visual analog scale (VAS) was used to assess the severity in patients who had an episode at any site in the month prior to sampling. The VAS consists of a straight line on a continuous scale from 0 to 100 mm. When evaluating the severity of skin, gastrointestinal, and laryngeal edema episodes, 0 mm represents no symptoms and 100 mm represents the most severe edema, the most severe abdominal pain and asphyxia requiring tracheotomy, respectively. Patients were asked to rate the severity of their most recent episode by completing VAS score. If the patient had only skin edema in the last time, VAS score was used to represent the severity of symptoms; If the patient had gastrointestinal or laryngeal edema last time, symptom severity was represented by 2 × VAS score, as this can be very painful or even fatal.

Extraction of genome DNA and polymerase chain reaction amplification

Total genome DNA was isolated from throat swabs using the CTAB method. DNA concentration and purity were monitored on 1% agarose gels and then diluted to 1 ng/μL. The V3–V4 regions of the bacterial 16S ribosomal RNA (rRNA) were amplified by polymerase chain reaction (PCR) used the universal primer 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′). PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). The PCR products were mixed with the same volume of 1X loading buffer (containing SYB Green), and then the mixture was detected by electrophoresis using 2% agarose gel. The PCR products were purified using the Qiagen Gel Extraction Kit (Qiagen, Germany).

Sequencing and data processing

Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA). Library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Illumina NovaSeq platform was used to sequence the library and yield 250 bp paired-end reads. After library preparation and sequencing, paired-end reads were assigned to samples according to their unique barcode. FLASH (V1.2.7) was used to merge paired-end reads and generate raw tags. Quality filtering was performed according to the QIIME (v1.9.1) quality control process and the UCHIME algorithm was used to remove chimeric sequences to obtain effective tags. The effective tags were clustered into operational taxonomic units (OUTs) at 97% sequence identity using Uparse software

| | HAE patients | | | Controls |
	Total	Without recent episode	With recent episode	
Samples	36	18	18	17
Age (mean ± SD)	41.9 ± 14.4	41.50 ± 17.99	42.39 ± 10.02	36.1 ± 19.5
Sex (%)				
Female	22 (61.1)	10 (55.6)	12 (66.7)	6 (35.3)
Male	14 (38.9)	8 (44.4)	6 (33.3)	11 (64.7)
Cigarette smoking				
Yes	4 (11.1)	1 (5.6)	3 (16.7)	3 (17.6)
No	32 (88.9)	17 (94.4)	15 (83.3)	14 (82.4)
Alcohol consumption				
Yes	5 (13.9)	2 (11.1)	3 (16.7)	3 (17.6)
No	31 (86.1)	16 (88.9)	15 (83.3)	14 (82.4)
Comorbidities				
Hypertension	2 (5.6)	2 (11.1)	0 (0.0)	2 (11.8)
Hyperlipidemia	3 (8.3)	2 (11.1)	1 (5.6)	1 (5.9)
Type of HAE (%)				
HAE-1	34 (94.4)	18 (100.0)	16 (88.9)	NA
HAE-2	2 (5.6)	0 (0.0)	2 (11.1)	NA
Laryngeal edema (%)				
Within one month	7 (19.4)	0 (0.0)	7 (38.9)	NA
Prior to one month	14 (38.9)	7 (38.9)	7 (38.9)	NA
Never	15 (41.7)	11 (61.1)	4 (22.2)	NA
Gastrointestinal edema (%)				
Within one month	4 (11.1)	0 (0.0)	4 (22.2)	NA
Prior to one month	20 (55.6)	10 (55.6)	10 (55.6)	NA
Never	12 (33.3)	8 (44.4)	4 (22.2)	NA
Skin edema (%)				
Within one month	15 (41.7)	0 (0.0)	15 (83.3)	NA
Prior to one month or never	21 (58.3)	18 (100.0)	3 (16.7)	NA
Long-term prophylaxis				
Yes	16 (44.4)	11 (61.1)	5 (27.8)	NA
No	20 (55.6)	7 (38.9)	13 (72.2)	NA

Table 1. The characteristics of study participants. *HAE, hereditary angioedema.NA, not applicable.SD, standard deviation*

(Uparse v7.0.1001). Representative sequences for each OTU were annotated using the Silva database version 132. In order to investigate phylogenetic relationship of different OTUs, and the differences in dominant species in different groups, multiple sequence alignment was performed using the MUSCLE software (Version 3.8.31). The OTU abundance information was normalized for subsequent analysis.

Bioinformatics and statistical analysis

Alpha diversity was applied to identify community diversity among different groups, and the Shannon index was calculated by QIIME software (version 1.9.1). Beta diversity on weighted unifrac was calculated by QIIME software (Version 1.9.1) and used to evaluate the differences in species complexity. Principal coordinate analysis (PCoA) based on a distance matrix of weighted unifrac was

used for data visualization. Analysis of similarities (ANOSIM) was used to determine statistical differences. For taxonomic assessment of microbiota, taxa were represented by a specific phylogenetic resolution (phylum, family, and genus). Only taxa with a relative abundance of at least 0.5% in any group were included in the analysis. For comparative statistic, a Benjamini-Hochberg false discovery rate (FDR) corrected p-value was calculated to correct for multiple testing, an adjusted p-value (q-value) < 0.05 was considered as statistically significant. Paired t-tests were used to assess microbiome alterations before and after treatment in the same patient. Linear discriminant analysis (LDA) effect size (LEfSe) was also applied to explore the most discriminatory taxa between groups, with an LDA score of 4.0 as the cut-off value.

In addition, the relationship between the severity of HAE episodes and the microbial flora was evaluated. Correlations between Shannon index and HAE attack severity score, as well as between the relative abundance of dominant phyla and HAE attack severity score, were established using the Spearman's correlation analyses. Besides, univariate and multivariate linear regression analyses were conducted to assess the association between species diversity, relative abundance of dominant phyla, and HAE attack severity score. Age (years), sex (male/female), smoking habit (yes/no), alcohol consumption (yes/no), and long-term prophylaxis (yes/no) were served as potential confounding factors and adjusted in the multivariate linear regression model. Statistical analyses were performed with R software, version 3.5.0 (R Foundation for Statistical Computing). A p-value less than 0.05 was considered as statistically significant.

RESULTS

A total of 36 HAE patients and 17 healthy controls were included in this study. Table 1 shows the characteristics of the study participants. The mean age of HAE patients was 41.9 ± 14.4 years. Among those patients, 4 (11.1%) were current smokers and 5 (13.9%) were regular drinkers; 2 (5.6%) had hypertension and 3 (8.3%) had hyperlipidemia. Half of the patients had an episode of HAE within the last month, 7 (38.9%) had recent laryngeal edema, 4 (22.2%) had recent gastrointestinal edema, and 15 (83.3%) had recent skin edema. In addition, 20

patients had never received long-term prophylaxis and 16 were receiving danazol for long-term prophylaxis. It should be clarified that currently, only danazol, tranexamic acid, and Lanadelumab are approved for long-term prophylaxis in China.[29] However, tranexamic acid is not recommended for long-term prophylaxis according to the international WAO/EAACI guideline,[28] and Lanadelumab was not yet available in the Chinese market during the time of this study. Therefore, danazol is the first choice for long-term prophylaxis in Chinese HAE patients over 16 years of age.[29] In addition, no patients in this study received C1-INH concentrate, ecallantide, or icatibant during an edema episode because these drugs were not approved for the treatment of acute HAE episodes in China at the time of the study. No patients received fresh frozen plasma.

Microbial alterations related to laryngeal edema

Alpha diversity as measured by the Shannon index did not differ significantly between the study groups (Fig. 1A, Kruskal–Wallis test, $p = 0.17$). However, PCoA indicates a distinct spatial separation between patients with and without recent laryngeal edema (Fig. 1B, ANOSIM, R = 0.28, $p = 0.009$). The relative abundance histogram shows that the dominant phylum in all groups were Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria, which accounted for more than 95% of bacterial sequences (Fig. 1C). Then, we analyzed the differential taxa between different groups. At the phylum level, the relative abundance of Bacteroidetes was significantly increased in patients with recent laryngeal edema compared to patients without recent laryngeal edema (Fig. 1D, $q = 0.018$ for LE.1m vs. NLE.1m). We further calculated the Firmicutes/Bacteroidetes (F/B) ratio to reflect dysbiosis. Patients with recent laryngeal edema had a slight decrease in F/B ratio, but this decrease in F/B ratio did not significantly differ from other study groups (Fig. 1E). At the family level, the relative abundance of Prevo-tellaceae was significantly increased in patients with recent laryngeal edema compared to patients without recent laryngeal edema (Fig. 1F, $q = 0.009$ for LE.1m vs. NLE.1m). These findings were further validated by the LEfSe analysis. Taxa including p_Bacteroidetes, c_Bacteroidia, o_Bacteroidales, and f_Prevotellaceae were increased in patients with recent laryngeal edema (LE.1m) with LDA scores >4 (Fig. 2A and B).
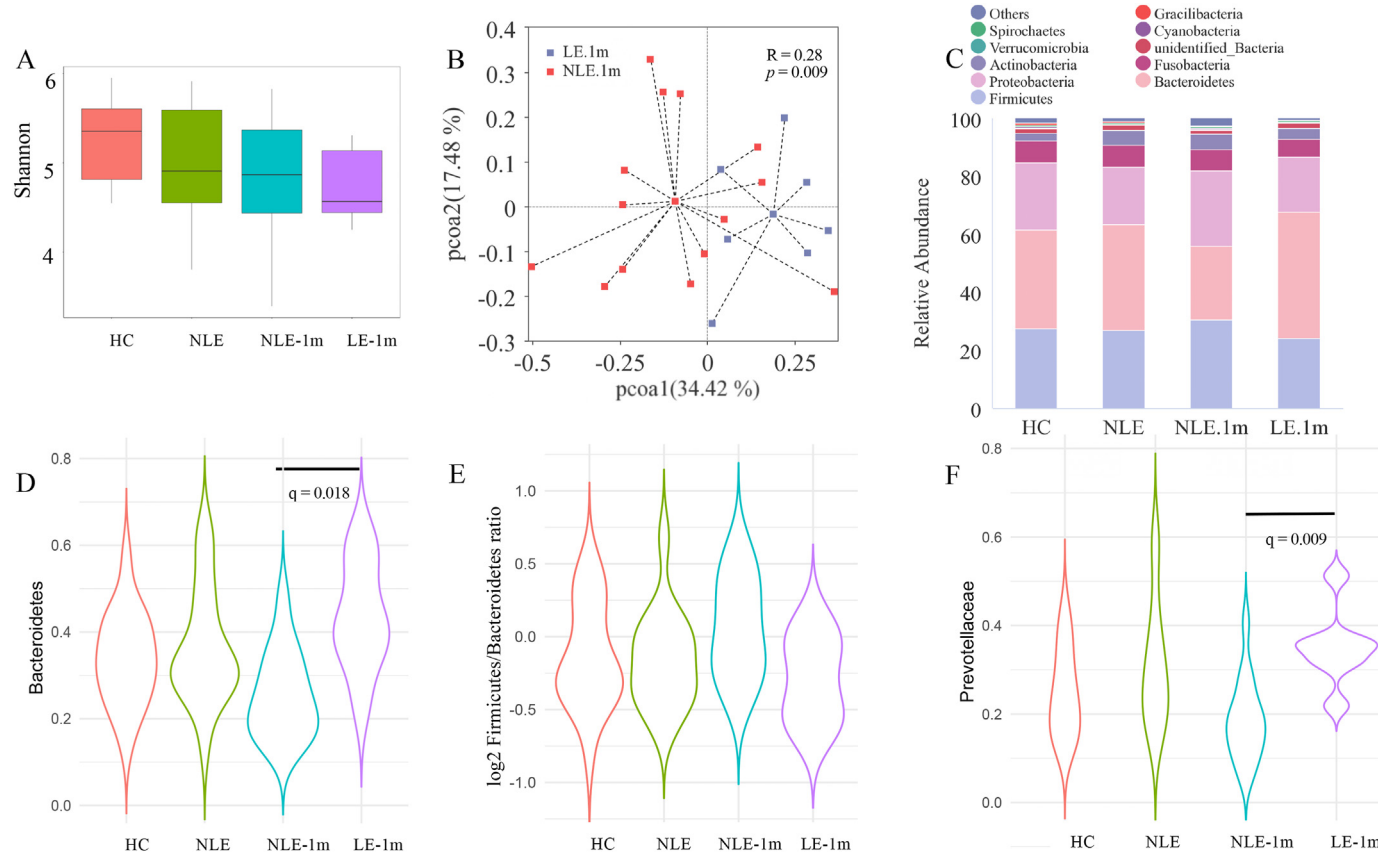
Fig. 1 Microbial alterations related to laryngeal edema. **(A)** Alpha diversity measured by the Shannon index (Kruskal–Wallis test, $p = 0.17$); **(B)** PCoA of bacterial beta diversity based on the weighted UniFrac distances (ANOSIM); **(C)** species relative abundance histogram at the phylum level; **(D)** the relative abundances of Bacteroidetes (Wilcoxon, q-value = FDR corrected p-value); **(E)** log2 Firmicutes/Bacteroidetes ratio; **(F)** the relative abundances of Prevotellaceae (Wilcoxon, q-value = FDR corrected p-value). **Abbreviations:** HC, healthy controls; LE.1m, patients suffered from laryngeal edema within the previous month; NLE.1m, patients had a history of laryngeal edema but had no episode within the previous month; NLE, never had laryngeal edema

Microbial alterations related to gastrointestinal edema and skin edema

As shown in supplementary Figure 1, when patients were grouped according to whether they had recently suffered from gastrointestinal edema, no significant differences in alpha diversity were found between the study groups. Patients with recent gastrointestinal edema had a slight but not statistically significant increase in the relative
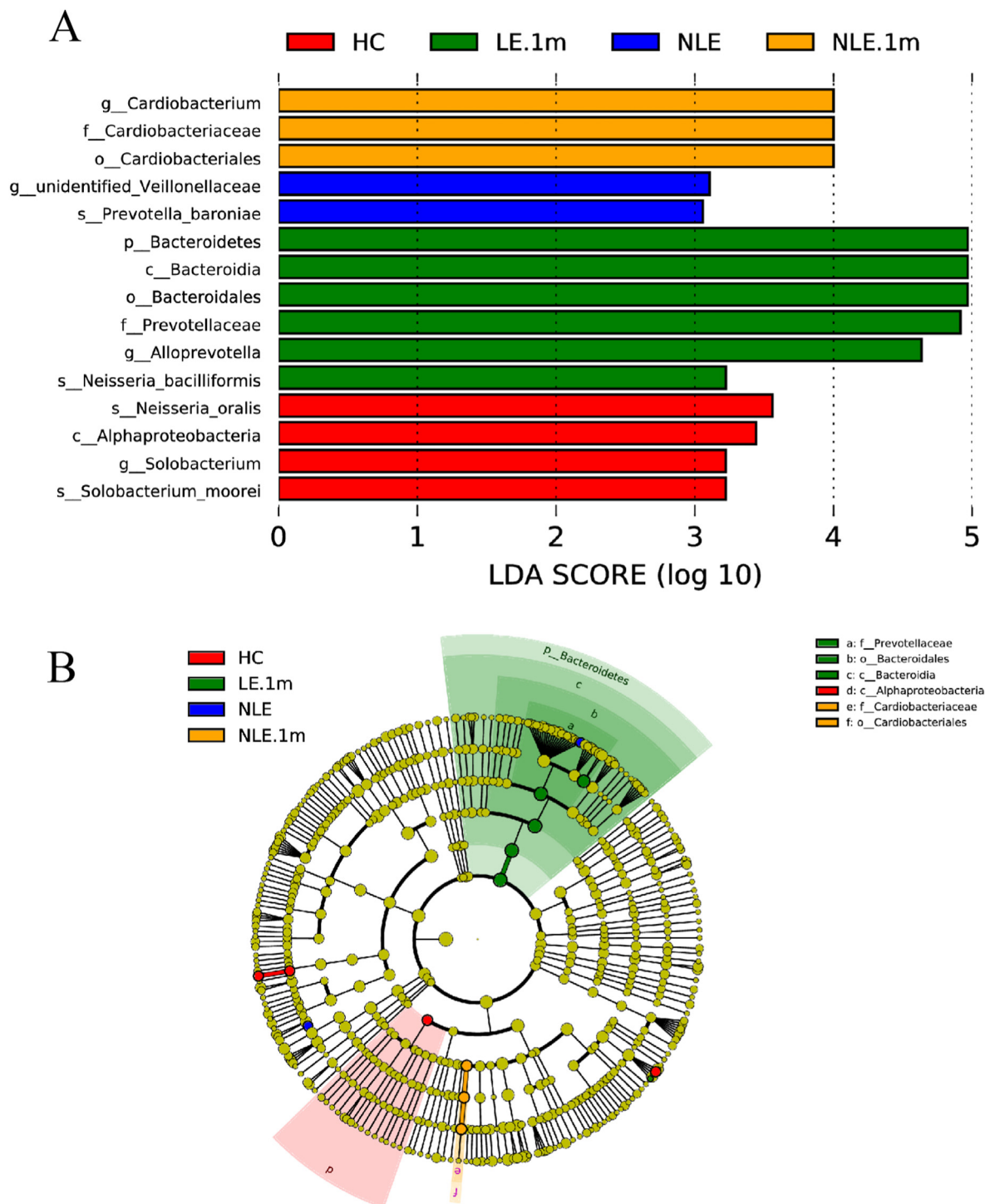

Fig. 2 LefSe analysis was applied to identify differentially abundant taxa. **(A)** LDA distribution histogram, the taxa with LDA scores more than 3 are shown; **(B)** LDA cladogram. **Abbreviations:** HC, healthy controls; LE.1m, patients suffered from laryngeal edema within the previous month; NLE.1m, patients had a history of laryngeal edema but had no episode within the previous month; NLE, never had laryngeal edema

abundance of Bacteroidetes, compared to patients without recent gastrointestinal edema. After FDR correction, no significant taxa changes were found in patients with recent gastrointestinal edema and in other study groups (Supplementary Figure 1). In addition, no significant changes in the diversity and structure of the microbial community were observed in patients with recent episodes of skin edema (Supplementary Figure 2). No significant taxa differences were found between groups after FDR correction (Supplementary Figure 2).

Relationship between recent episode and microbial flora

We then applied Spearman correlation and linear regression to further evaluate the relationship between Shannon index and HAE attack severity score, as well as between the relative abundance of dominant phyla and HAE attack severity score. The relative abundance of Bacteroidetes were positively correlated with attack severity score (r = 0.58, p = 0.01; Fig. 3B). Multivariate linear regression also indicates that the relative abundance of Bacteroidetes significantly associated with the severity of HAE. After adjusting for age, sex, smoking habit, alcohol consumption, and long-term prophylaxis, each 10% increase in the relative abundance of Bacteroidetes was associated with a 4.16-point increase in the HAE severity score, with a p = 0.001 (Table 2). However, no significant correlation was found between Shannon index and HAE attack severity score, or between the relative abundance of Firmicutes and HAE attack severity score (Fig. 3A and C, and Table 2).

Microbial alterations after long-term prophylaxis

To test whether the treatment changed the throat microbiota, we first compared the microbiota of the 16 patients who received the treatment versus the microbiota of the 20 patients who did not. Among the 20 untreated patients, four provided samples before treatment and 2 months after danazol treatment. Therefore, we further explored changes of the throat microbial community in these 4 patients before and after danazol treatment. Supplementary Table 2 shows the clinical characteristics of these 4 patients. Supplementary Figures 3 and 4 show that in both approaches, the diversity and structure of the microbiota did not change significantly after treatment compared with pre-treatment. No significant differences in bacterial taxa were observed between the groups.

DISCUSSION

HAE is considered a heterogeneous disorder with complex manifestations. The clinical features of HAE, including age of onset, location of edema, frequency of attacks, and disease severity, vary widely.[5] Although *SERPING1* mutations are considered to be the genetic defect in HAE-1/2, so far individual mutations are neither sufficient to explain the patient's phenotype nor to predict the severity of edema episodes. Therefore, identification of factors associated with this heterogeneous clinical manifestation is urgently needed, as this may reflect underlying pathogenic pathways and provide opportunities for individualized treatment.
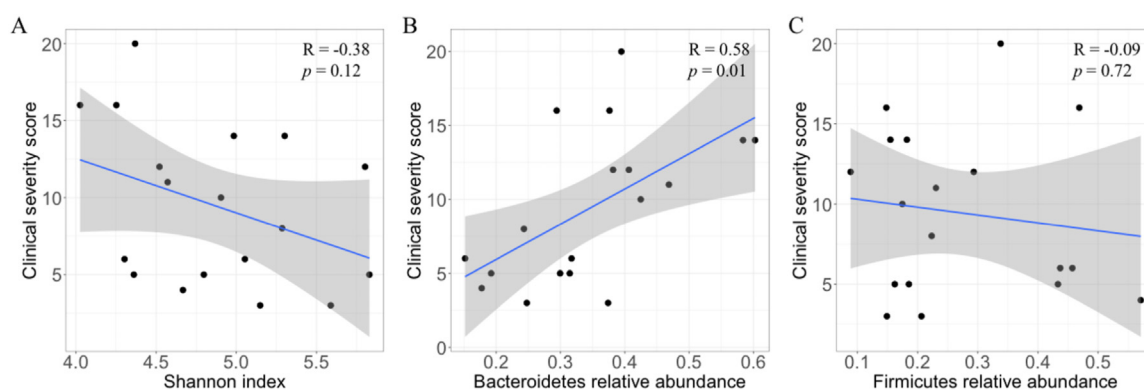

Fig. 3 **Spearman correlation between (A)** alpha diversity and HAE attack severity score; **(B)** the relative abundance of Bacteroidetes and HAE attack severity score; **(C)** the relative abundance of Firmicutes and HAE attack severity score

	Univariate linear regression				Multivariate linear regression[a]			
	β	95% CI		*p*	β	95% CI		*p*
Shannon index	−3.54	−8.23	1.15	0.13	−4.41	−10.39	1.57	0.13
Bacteroidetes	2.39	0.62	4.15	0.01	4.16	1.99	6.33	0.001
Firmicutes	−0.49	−2.40	1.41	0.59	−0.36	−3.54	2.82	0.81

Table 2. Linear regression between throat flora and clinical severity score. *CI, confidence interval. [a]Age, sex, smoking habit, alcohol consumption, and treatment were adjusted in multivariate linear regression*

We grouped patients according to the recent edema to explore possible mechanisms of HAE episodes associated with throat microbial flora. A one-month interval was chosen to define the recent edema because our previous study found that richness and diversity of microbial communities were significantly reduced among patients suffering from HAE attacks within the previous month.[25] In the present study, results showed that patients with recent episodes of laryngeal edema had a significantly altered throat microbial flora, as reflected by changes in community structure and potential bacterial marker species. The microbiomes of patients with and without recent episodes of laryngeal edema were divided into two clusters. The relative abundance of Bacteroidetes and Prevotellaceae was significantly increased in patients with recent laryngeal edema compared to patients without recent laryngeal edema. In addition, Spearman's correlation analysis and linear regression analysis indicated that the relative abundance of Bacteroidetes was positively correlated with the severity of HAE attacks. In this study, we did not find that danazol significantly influenced the composition of throat microbiota. However, the small sample size may have limited the possibility to detect statistical differences. Although all the results require extensive experimental validation, our results highlight the possibility that individual microbiota may be associated with HAE attacks and attacks severity.

Species in Bacteroidetes have been known to act as opportunistic pathogens outside the gastrointestinal tract. Many species in the phylum Bacteroidetes, including *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*, can interfere with the kallikrein-kinin system in human plasma.[30] These two species have been found to bind to high molecular weight kininogen, leading to a release of bradykinin.[30] The *Porphyromonas gingivalis*,[31] also belonging to the phylum Bacteroidetes, can directly liberate kinins from high molecular weight kininogen via produce cysteine proteases. In addition, many other bacterial pathogens may also over-activate the kallikrein-kinin system, increasing bradykinin production and thus inducing vascular leakage.[32] Bacteria such as Gram-positive group A *Streptococcus*[33] and *Staphylococcus aureus*[34] can directly release kinins from high molecular weight kininogen through produce cysteine proteases. Similarly, *Aeromonas sobria* can also directly cleave high molecular weight kininogen, leading to vascular leakage.[35] Some extracellular bacteria indirectly produce bradykinin by releasing proteinases that generate proteolytic activity of factor XII or plasma kallikrein.[36] The potential mechanism between microbial species and HAE remains to be verified by future experimental studies.

Although no additional studies have explored the changes of throat microbiome in HAE patients, our previous study reported the changes of gut microbiome in HAE patients. Patients with recent episodes of gastrointestinal angioedema showed a decrease in microbial diversity and richness. In addition, patients with recent episodes of gastrointestinal angioedema also showed a decrease in Firmicutes and an increase in Proteobacteria, suggesting a dysbiosis.[25] However, the evidence confirming the association between gut microbiota and laryngeal edema is not sufficient.[25] Therefore, in the present study, we explored changes of the throat microbiome in HAE patients and found a dysbiosis in patients with recent episodes of laryngeal edema.

This study has some limitations. First, because of the rarity of this disease, this study had a small sample size and was a single-center study. Therefore, multicenter studies with larger sample sizes are needed to further validate our results. Second,

although alterations in the microbiome may be associated with episodes of HAE, the causal relationship between the two remains unclear, and throat microbiota alterations might be the consequences of edema episodes. This should be explored in future prospective studies. Third, although we attempted to select healthy members from patients' families as controls to reduce the effect of dietary and geographical factors on the flora, not every patient was matched to a healthy control. These should be improved in future studies.

CONCLUSION

In conclusion, we reported alterations in the throat microbiome of HAE patients and performed a comprehensive analysis to assess the relationship between throat microbiomes and HAE episodes. The associations between throat microbiomes with laryngeal edema and disease severity in HAE patients may indicate the potential of microbiome-based interventions in the prevention and treatment of HAE episodes. Additional studies with larger sample sizes are needed to confirm these findings.

Abbreviations
HAE, hereditary angioedema; HAE-1, type 1 HAE; HAE-2, type 2 HAE; C1-INH, C1 inhibitor; LE.1m, patients who suffered from laryngeal edema within the previous month; NLE.1m, patients who had a history of laryngeal edema but had no episode within the previous month; NLE, patients who never had laryngeal edema; GE.1m, patients who suffered from gastrointestinal edema within the previous month; NGE.1m, patients who had a history of gastrointestinal edema but had no episode within the previous month; NGE, patients who never had gastrointestinal edema; SE.1m, patients who suffered from skin edema within the previous month; NSE.1m, patients who had no skin edema in the previous month but had a history of skin edema; VAS, visual analog scale; rRNA, ribosomal RNA; OUTs, operational taxonomic units; PCoA, principal coordinate analysis; ANOSIM, analysis of similarities; FDR, false discovery rate; LEfSe, Linear discriminant analysis effect size.

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

Authors' contributions
Xue Wang made substantial contributions to the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, and revising it critically for important intellectual content. Yang Cao made substantial contributions to the conception and design of the study and acquisition of data. Yuxiang Zhi made substantial contributions to the conception and design of the study, revising it critically for important intellectual content, and gave final approval of the version to be published.

Ethics, consent and permissions
All the subjects signed an informed consent under a research protocol approved by the Research Ethical Committee of the Peking Union Medical College Hospital (Approval number: HS-2402). All the subjects signed informed consent regarding publishing their data. No previously published graphic and tabular material was used in the manuscript.

Consent to publish
All authors reviewed the final version of the paper and agreed in writing to its publication in the World Allergy Organization Journal, as an open access article. All the subjects signed informed consent regarding publishing their data.

Confirmation of unpublished work
Our manuscript is original, has not been published before, is not currently being considered for publication elsewhere, and has not been posted to a preprint server.

Declaration of competing interest
The authors declare that they have no competing interests.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2022.100694.

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