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Hydrogen inhalation: A novel approach to alleviating allergic rhinitis symptoms by modulating nasal flora

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ABSTRACT

Background: Allergic rhinitis (AR) is an allergic reaction dominated by the Th2 immune response in the nasal mucosa. The bacterial infection process affects the balance between Th1 and Th2 immune responses, and the level of exposure to environmental flora is closely related to the development of AR. Hydrogen (H_2) is a medical molecule with anti-inflammatory and antioxidant properties. This study aimed to explore the possible mechanism of action of H_2 on AR through its ability to regulate the balance of nasal flora.

Methods: Serum eosinophil count (EOS), immunoglobulin E (IgE) concentration, visual analog scale (VAS), total nasal symptom score (TNSS), and rhinoconjunctivitis quality of life questionnaire (RQLQ) were observed before and after hydrogen inhalation in AR patients. Skin prick test (SPT) was used to determine allergen sensitisation. Community composition and relative abundance of nasal flora were examined before and after hydrogen inhalation and in normal subjects using 16S rRNA gene sequencing.

Results: There were no adverse reactions during and after hydrogen inhalation in AR patients, with a favorable safety profile and significant improvements in VAS, TNSS, EOS, and IgE ($P < 0.05$). Cavity flora 16S rRNA gene sequencing showed higher abundance of Ruminococcus and Erysipelotrichaceae flora in the nasal cavity of AR patients than in normal subjects, and their abundance could be down-regulated after H_2 inhalation. H_2 significantly increased the abundance of Blautia_faecis and negatively correlated with VAS, TNSS, EOS, and IgE.

Conclusions: H_2 may improve symptoms in AR patients by modulating the distribution of nasal flora. Trials with larger sample sizes are required to further test this hypothesis.

Trial registration: This trial was registered in the China Clinical Trial Registry (Registration No. ChiCTR2200062253).

Keywords: Allergic rhinitis, H_2, Nasal flora, Blautia_faecis, Erysipelotrichaceae, Ruminococcus

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INTRODUCTION

Allergic rhinitis (AR) is an IgE-mediated type 1 allergic reaction to specific external allergens, which causes a corresponding inflammatory state of the nasal cavity characterized by 4 main symptoms: profuse watery nasal discharge, nasal congestion, nasal itchiness, and sneezing.[1] Seasonal and perennial AR is a global health problem with a global prevalence of between 10% and 40%, affecting between 2% and 25% of children and up to 40% of adults.[2] The composition of microorganisms in the nasal cavity of healthy individuals is involved in maintaining the normal immune response of the nasal mucosa,[3,4] and an imbalance in its flora would be at risk for the development of AR.[5,6] The microbiota influences the neonatus from gestation onwards through the maternal microbiota and immune response.[7] Lacking contact with maternal vaginal flora during cesarean section increases the risk of AR in children.[7] A dysbiotic microbiota is not only capable of disrupting the respiratory epithelial barrier,[8,9] but it can also migrate to interepithelial and subepithelial areas and promote the development of tissue microinflammation.[5,10] The airway microbiota can also influence host metabolism and homeostasis in vivo, including epithelial cell growth and repair, inflammation, and immune responses, thereby influencing the onset and progression of chronic diseases.[5,6,9]

Hydrogen gas (H_2) is a special anti-inflammatory and antioxidant factor, which has the advantages of easy diffusion, fast action, and no significant adverse effects.[11,12] H_2 inhibits the phosphorylation of tyrosine associated with the mast cell Fc epsilon RI signaling pathway, inhibits its downstream signaling, and alleviates type I hypersensitivity in mouse skin.[13-16] Moreover, H_2 has been shown to mitigate liver injury through inhibition of the LPS/TLR4/NF-κB pathway, leading to enhanced intestinal flora and reinforcement of the intestinal barrier.[18] Additionally, H_2 can decrease the infiltration of inflammatory cells like eosinophils and lymphocytes in mucous and submucous membranes by elevating levels of superoxide dismutase (SOD),[17] thereby alleviating oxidative stress and allergic symptoms associated with AR.[19] Although these findings indicate that the microbiota might have a pivotal role in the effect of H_2 on AR subjects, there is still a lack of direct evidence. Consequently, we conduct this study to identify specific bacterial groups that are signatures and predictors for a sustained response to H_2 therapy, attempting to elaborate the action mechanisms of H_2 from the perspective of human nasal microbiota.

In this study, patients with AR were administered high concentrations of H_2 via nasal inhalation. The study observed changes in visual analog scale (VAS),[20] total nasal symptom score (TNSS),[21] and rhinoconjunctivitis quality of life questionnaire (RQLQ)[22] scores, as well as clinical indexes including serum eosinophil count (EOS) and immunoglobulin E (IgE) levels, before and after treatment. Additionally, the study analyzed the impact of H_2 on the distribution of nasal flora in AR patients using 16S rRNA gene sequencing to further investigate the mechanism of H_2 in treating AR.

MATERIALS AND METHODS

Inclusion and exclusion criteria

Inclusion criteria for the AR group

1) The diagnosis of AR was confirmed by a hospital clinician in conjunction with clinical manifestations, serum IgE test results, and allergen skin prick test (SPT), and was in accordance with the Chinese Guidelines for the Diagnosis and Treatment of Allergic Rhinitis (2022, Revised Edition)
2) Age ≥18 years, able to give informed consent
3) The patients participating in the study need to stop antibiotics and steroid hormones for more than 4 weeks, including intranasal steroid hormones

Exclusion criteria

1) Combined sinusitis, nasal polyps, or nasal tumors
2) Combined malignant tumors

3) Combined severe cardiac, hepatic, and renal functional disease
4) Coagulation abnormalities
5) Illiteracy, poor self-awareness and cooperation
6) Comorbid mental illness or severe communication disorders

RESEARCH POPULATION

Thirty cases of AR patients who attended the rhinology outpatient clinic of the hospital from April 2022 to July 2023 were selected as the test group. All were patients with simple persistent AR without comorbidities with other allergic diseases. Thirty healthy individuals who had a physical examination during the same period were selected as the healthy control (HC) group. The experimental groups were divided into HI pre and HI post groups according to pre and post-hydrogen inhalation. The patients in the test group were 6 males and 24 females, aged 18–47 years, with a mean age of 30 years. The control patients were 8 males and 22 females, aged 18–47 years, with a mean age of 30 years. Comparison of general information such as gender, age and BMI between the 2 groups of patients, the differences were not statistically significant ($P > 0.05$) and were comparable. The study protocol complied with the ethical guidelines of the Declaration of Helsinki.[23]

Collection of specimens

Strictly following the aseptic operation, the subjects were placed in the supine position, the skin around the mouth and nose was sterilized with iodine vapor before sampling, and under the guidance of the nasal endoscope, disposable sterile cotton swabs were placed to the area of the common nasal tract, rotated at least 6 full turns until the swabs were saturated, bilateral nasal cavity were collected, and then removed and placed in liquid nitrogen vials, and then placed in the refrigerator at −80 °C within 15 min until the DNA was extracted.

Hydrogen inhalation treatment

H_2 with a concentration of 66.6% was prepared by a hydrogen-oxygen nebulizer (SHANGHAI ASCLEPIUS MEDITEC CO., LTD., model AMS-H-03), and the AR patients inhaled hydrogen for 3 h per day for 4 consecutive weeks.

Observation indicators

The severity of clinical symptoms in AR patients before and after H_2 treatment was scored using the VAS,[20] TNSS,[21] and RQLQ.[22]

VAS score: Patients were scored according to their symptoms (nasal congestion, runny nose, itchy nose, sneezing) in the last week using a 0–10 cm analog scale: 0 cm: 0 points, no symptoms; 1–3 cm: 1–3 points, mild; 4–6 cm: 4–6 points, moderate; 7–10 cm: 7–10 points, severe.

TNSS score: 0–3 points (0 = no symptoms; 1 = mild; 2 = moderate; 3 = severe): mild: no symptoms causing obvious discomfort; moderate: symptoms causing discomfort but not affecting daily life or interfering with sleep; severe: symptoms interfering with daily life activities and sleep status, adding up the points of each symptom to get the total score is the TNSS score.

RQLQ score: It consists of 7 dimensions and 28 items, each dimension is scored separately and the total score is the RQLQ score.

To observe the clinical efficacy of H_2 in the treatment of AR patients. Symptom relief rate = (pre-treatment VAS score − post-treatment VAS score)/pre-treatment VAS score, Symptom relief rate >65% is regarded as Significant, 25%–65% is regarded as effective, <25% is regarded as ineffective, and the total effective = Significant + effective.

The levels of IgE (IU/ml) and EOS (×109/L) blood laboratory indices in AR patients before and after H_2 treatment were tested.

Skin prick test (SPT) was performed according to standard practice with saline as the negative control solution and 0.1% histamine hydrochloride as the positive control solution. Each drop was pricked vertically with a sterilized, disposable, allergenic, single-tip metal puncture needle and the skin was punctured (as long as it does not bleed). The needle was lifted and discarded after 1 s and the drop was wiped away after 2–3 min. The result was read out after 10–20 min but the reaction should be observed several times in the meantime: if there was a yellowish dermatomal

papule surrounded by erythema, then there had been a positive test reaction.

Microbiota analysis

The 16S rRNA gene was sequenced from the total nasal secretions of the subjects. Amplicon libraries were prepared using the library construction kit The NEBNext Ultra II DNA Library Prep Kit (Cat. No. E7645B). The constructed libraries were quantified by Qubit (Qubit@ 2.0 Fluorometer) and Q-PCR, and after the libraries were qualified, they were subjected to PE250 up-sequencing using NovaSeq6000. The 16S rRNA data were analyzed using the QIIME2 (Version QIIME2-202202) software, and the DADA2 module was used for noise reduction to quantify sequences with 100% similarity to an actionable threshold for classification units, thus obtaining the final ASVs (AmpliconSequence Variants). Species annotation was performed using QIIME2 software with the Silva 138.1 database. The default parameter for the QIIME2 analysis process minimum threshold is 0.7. Based on the analysis of the operational categorization units, the α-diversity indices Chao1 and Shannon were calculated using random sampling of sample sequences, and sparse curves were plotted. Unbiased UniFrac analysis (Lozupone and Knight, 2005) was used to determine if there were significant differences in microbial communities between samples.

Statistical methods

The data were statistically analyzed using SPSS 22.0 software. Measurements are expressed as x±s using a *t*-test or rank-sum test; Count data were expressed as cases or rates using the χ 2 test; Rank information was tested using the rank-sum test. $P < 0.05$ was considered a statistically significant difference. The symbols *, ** and *** indicate P values of <0.05, <0.01 and <0.001, respectively.

RESULT

Baseline characteristics

There were no statistically significant differences in age ($P = 0.96$), gender ($P = 0.54$), and BMI ($P = 0.83$) between patients in the experimental and healthy control groups (Table 1).

Data analysis of clinical efficacy indicators before and after H_2 treatment

The changes in VAS, TNSS, and RQLQ indexes before and after hydrogen inhalation in AR patients were compared. The results showed that VAS[20] and TNSS[21] scores were significantly reduced with statistical significance ($P = 0.005$, $P = 0.003$), and no statistically significant difference was seen in RQLQ scores ($P = 0.692$) (Fig. 1). After 4 weeks of H_2 intervention in 30 AR patients who participated in this experiment, the effect of H_2 treatment was significant in 12 of them, with an average symptomatic relief rate of 81.25%, efficacious in 9 patients, with an average symptomatic relief rate of 38.67%, and ineffective in 9 patients, with an average symptomatic relief rate of 15.33%. This suggests that nasal inhalation of hydrogen in AR patients has significant clinical efficacy in the treatment of AR.

The RQLQ scores were not significantly different before and after treatment, and we took into account that the RQLQ adds several non-nasal symptomatic factors to the VAS and TNSS scores, including emotional as well as activity, sleep, and other complex factors.

Index	Group	HC n = 30	AR n = 30	P
Sex	Male	8	6	0.54
	Female	22	24	
Age	18–47	30	30	0.96
BMI(kg/m^2)	≤23.9	13	12	0.83
	24–27.9	14	16	
	≥28	3	2	

Table 1. Baseline analysis between the HI pre and HC groups

Comparison of laboratory indicators

Comparison of EOS, and IgE indexes in AR patients after H_2 intervention showed a significant decrease in EOS and IgE scores, and the mean values of the differences were −0.145 and −142.8, which were statistically significant (P = 0.002, P = 0.013) (Fig. 1). This suggests that inhalation of H_2 reduces EOS and IgE levels in the blood of AR patients and improves allergic symptoms in AR patients.

Alpha diversity analysis

A total of 9204 alpha diversity analyses (ASVs) were obtained from the 3 groups by 16S rRNA assay, of which the number of ASVs possessing the same number was 1378 in the 3 groups, the number of ASVs specific to the AR group was 2403, the number of ASVs specific to the HI post group was 1738, and the number of ASVs specific to the HC group was 1632. The HC and HI post groups had significantly fewer specific ASVs than the HI pre group (Fig. 2A).

Comparison of alpha diversity of nasal flora in HI pre, HI post, and HC groups. The Chao1 index (P = 0.031, P = 0.001), observed_otus index (P = 0.029, P = 0.001), and Shannon index (P = 0.033, P = 0.011) in the HI pre group were higher than those in the HC and HI post groups, and the differences were all statistically significant. From this, we can see that the α-diversity of the flora in the HI post group was similar to that of the HC group (Fig. 2B, C, D). This suggests that the balance of nasal flora in AR patients after H_2 intervention is regulated to more closely resemble the level of flora abundance in normal subjects.

Beta diversity analysis

LEfSe analysis

At the family level, the main high-abundance groups in the HI pre group were Chitinophagaceae, Oscillospiraceae, Eubacterium_coprostanoligenes_group, Clostridia_vadinBB60_group, and Nocardioidaceae; the main high abundance groups in the HI post group were Enterobacteriaceae, Mitochondria; the main high abundance groups in the HC group were Corynebacteriaceae, Propionibacteriaceae, Rhodocyclaceae, Erwiniaceae.

At the genus level, the main high-abundance groups in the HI pre group were

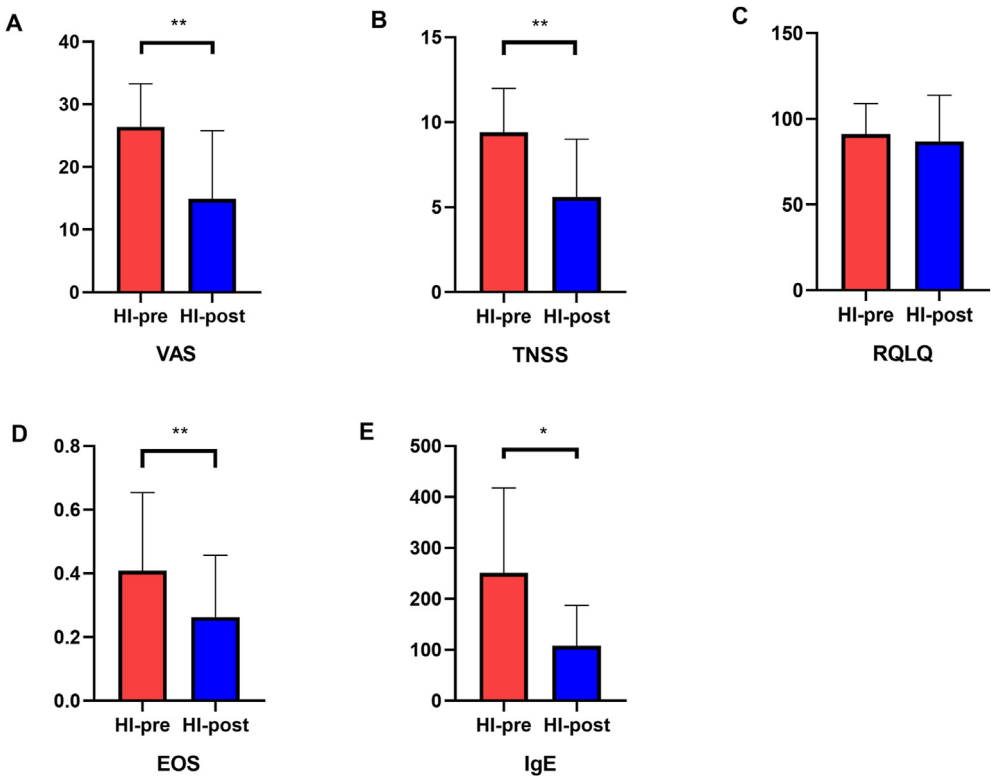

Fig. 1 Comparison of VAS, TNSS, RQLQ, EOS, and IgE between the HI pre and HI post groups

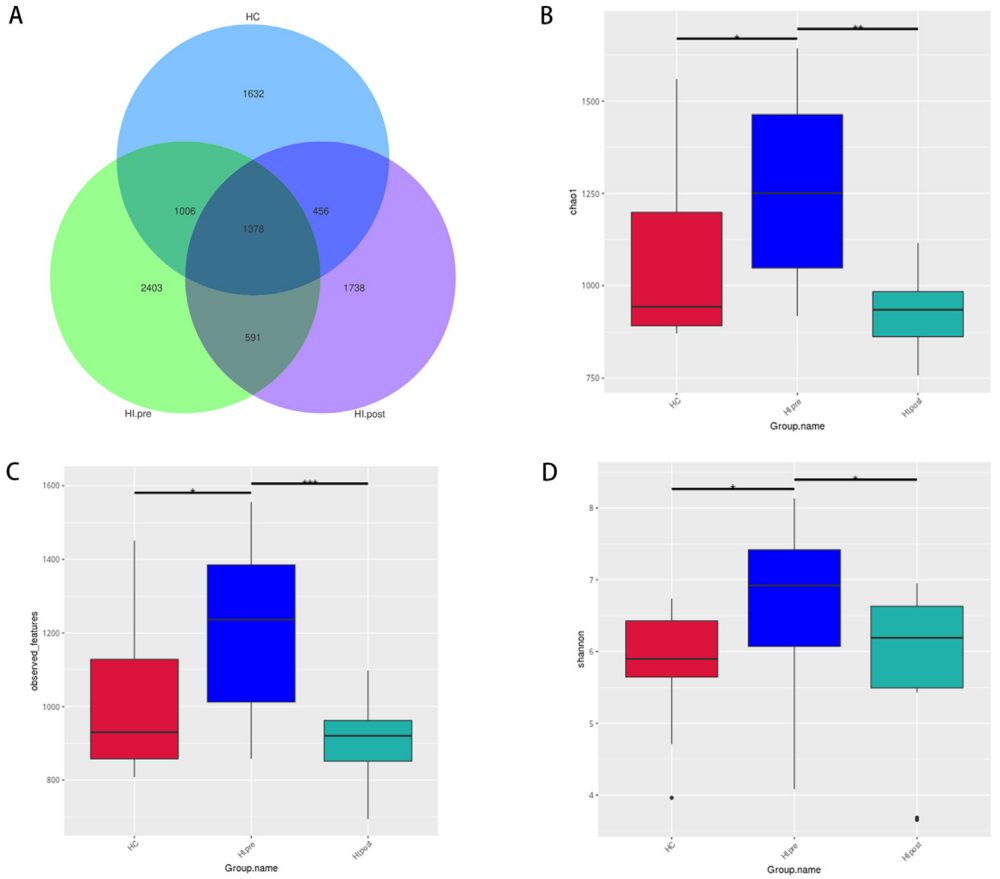

Fig. 2 Comparison of Alpha diversity of nasal flora between the HI pre, HI post, and HC groups

Eubacterium_coprostanoligenes_group, Muribaculaceae, Clostridia_vadinBB60_group, Nocardioides; the main high-abundance groups in the HI post group were Escherichia_Shigella, Moraxella, Mitochondria; the main high-abundance groups in the HC group were Cutibacterium, Peptoniphilus (Fig. 3).

The above results show that there is a significant difference in the distribution of nasal flora between

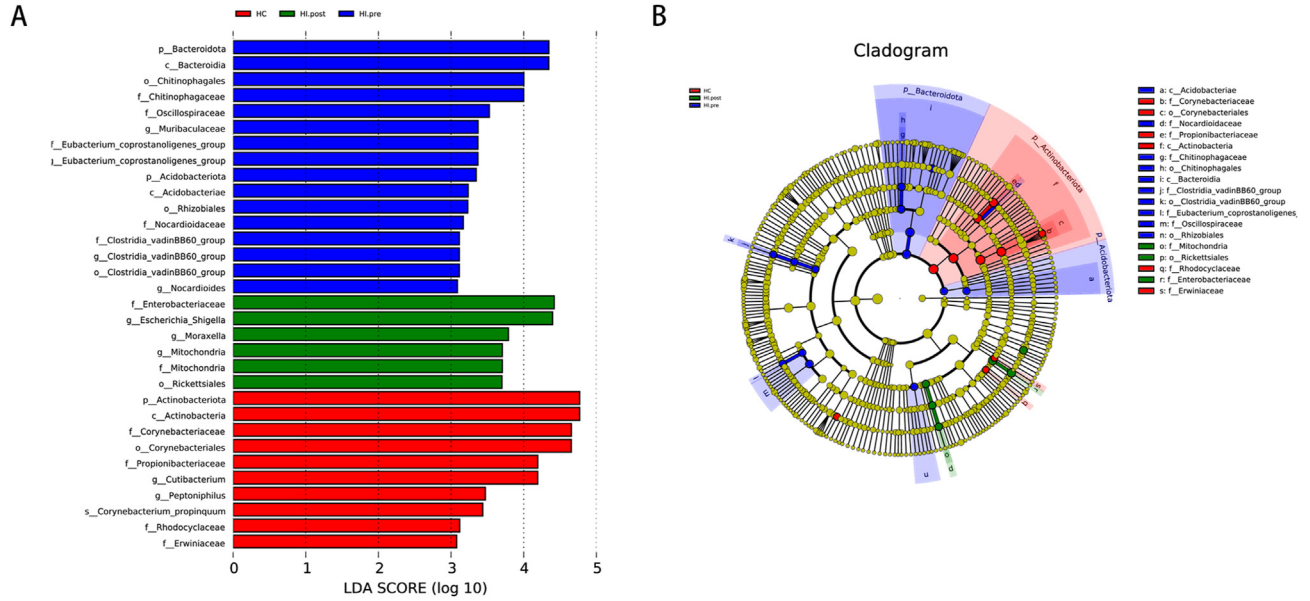

Fig. 3 High abundance of nasal flora between the HI pre, HI post, and HC groups

AR patients and normal subjects, and that there is a significant difference in the distribution of nasal flora in AR patients before and after H_2 treatment, and that H_2 can influence the distribution of nasal flora in AR patients.

Metastat analysis

At the family level, the relative abundance of Acidaminococcaceae ($P = 0.026$, $P = 0.007$), SC-I-84 ($P = 0.009$, $P = 0.022$), TRA3-20 ($P = 0.022$, $P = 0.007$) was significantly higher in the HI pre group than in the HC and HI post groups, and the difference was statistically significant. The relative abundance of nasal flora in AR patients after H_2 intervention was close to normal levels (Fig. 4B–D).

At the genus level, Chitinophaga was significantly less abundant in the HI pre group and was a significantly different bacterium between the HC, HI pre and HI post groups ($p < 0.05$) (Fig. 4A). H_2 intervention resulted in a significant increase in its relative abundance in the nasal cavity of AR patients ($p < 0.05$). Erysipelotrichaceae ($P < 0.001$, $P = 0.003$), and Steroidobacter ($P < 0.001$, $P = 0.038$) showed a significant increase in abundance in the HI pre group and was the bacterium that differed significantly

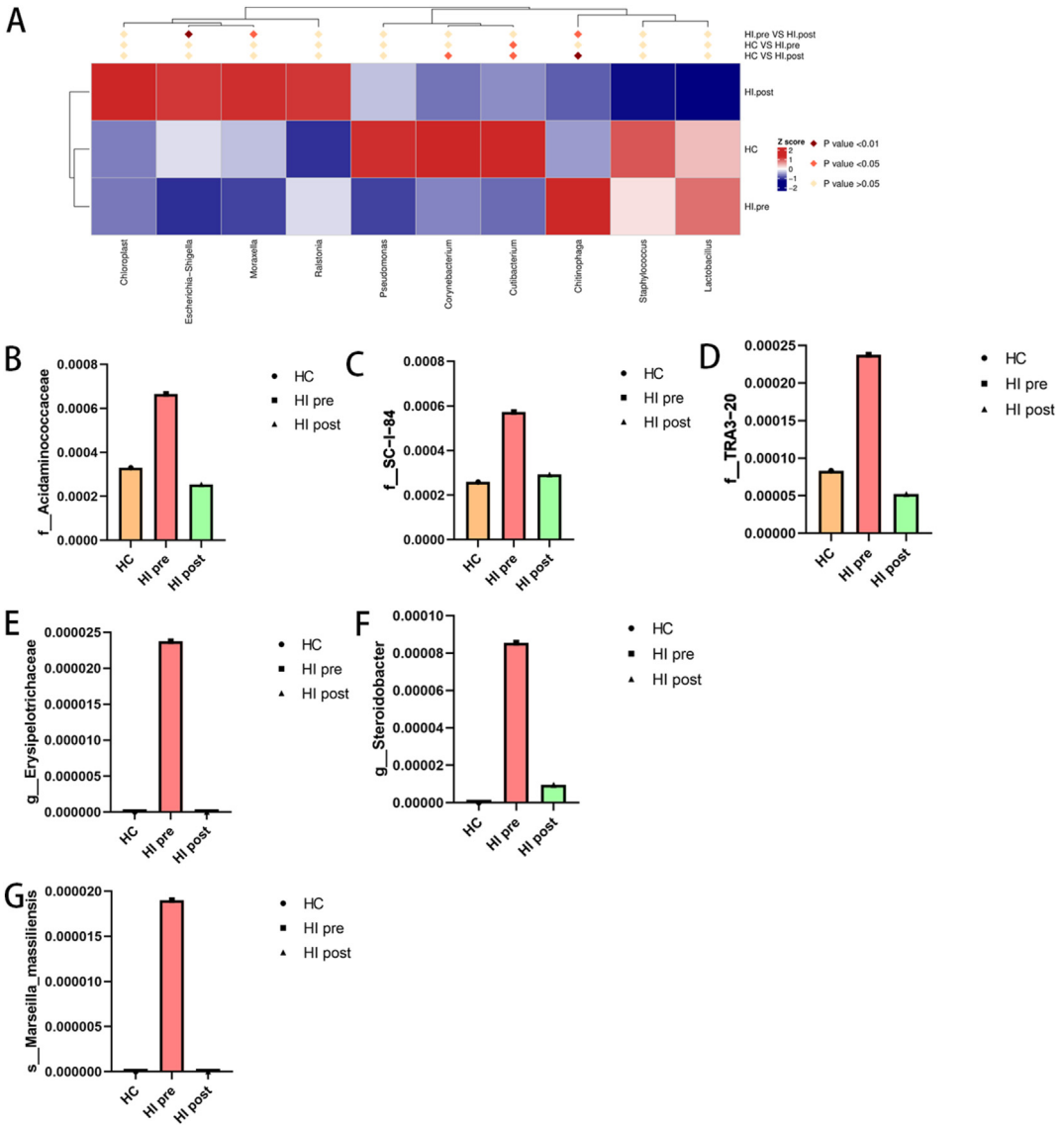

Fig. 4 Bacteria with statistically significant differences at the family, genus, and species level between the HI pre and HI post groups, as well as the HC group

between the HI pre and HC and HI post groups (Fig. 4E and F). H_2 intervention resulted in a significant reduction in the relative abundance of the above 2 genera in the nasal cavity of AR patients ($P = 0.003$, $P = 0.038$) (Fig. 4E and F).

At the species level, Marseilla_massiliensis was the bacterium with significant differences between the HI pre and HC groups and also between the HI pre and HI post groups. The relative abundance of Marseilla_massiliensis was significantly increased ($P = 0.008$) in the HI pre group compared to the HC group. Whereas, the relative abundance of Marseilla_massiliensis was significantly lower ($P = 0.008$) in the HI post group compared to the HI pre group (Fig. 4G). This suggests that by giving nasal inhalation of H_2 to patients with AR, the disruption of their nasal flora can be regulated.

At the family, genus, and species level, we have demonstrated that Acidaminococcaceae, SC-I-84, TRA3-20, Erysipelotrichaceae, Steroidobacter, and Marseilla_massiliensis play key roles in this process and that the down-regulation of the abundance of these flora facilitates the restoration of the regulation of the balance of the nasal flora in patients with AR toward the level of the nasal flora of normal subjects.

T-test analysis

At the phylum level, the main differentiating bacteria between the HI pre and HC groups were Actinobacteriota, Bacteroidota, Acidobacteriota, Verrucomicrobiota, Chloroflexi, and Gemmatimonadota, of which Bacteroidota, Acidobacteriota, Verrucomicrobiota, and Gemmatimonadota were also the main differential bacteria between the HI pre and HI post groups ($P = 0.019$, $P = 0.002$, $P = 0.005$, $P = 0.002$). Moreover, the relative abundance of these bacteria in the HI pre group was higher than that in the HC and HI post groups (Fig. 5A and B).

At the family level, the main differentiating bacteria between the HI pre group and HC group were Propionibacteriaceae, Oscillospiraceae, Clostridia_vadinBB60_group, Acholeplasmataceae, and Gemmatimonadaceae. Among them, Clostridia_vadinBB60_group and Gemmatimonadaceae were also the main differentiated bacteria between the HI pre and HI post groups ($P = 0.022$, $P = 0.006$, $P = 0.006$, $P = 0.002$). Moreover, the relative abundance of these bacteria was higher in the HI pre group than in the HC and HI post groups (Fig. 5C and D).

At the genus level, the main differentiating bacteria between the HI pre and HC groups were Cutibacterium, Lachnospiraceae_NK4A136_group, Lachnospiraceae_UCG-001, Clostridia_vadinBB60_group. Anaeroplasma, Ruminococcus, Eubacterium_xylanophilum_group, where Clostridia_vadinBB60_group, Ruminococcus, Eubacterium_xylanophilum_group were also the main differential bacteria between the HI pre and HI post groups ($P = 0.022$, $P = 0.013$, $P = 0.047$), and the relative abundance of these bacteria was higher in the AR group than in both the HC and HI post groups (Fig. 5E and F).

At the species level, the main differential bacterium between the HI post and HI pre groups was Blautia_faecis ($P = 0.024$) (Fig. 5G). The abundance of this bacterium was significantly increased in the HI post group compared to the HI pre group, suggesting that H_2 intervention favors the colonization of the nasal cavity by this bacterium.

In addition, at the family and genus levels, we also found bacteria with significantly increased abundance due to H_2 intervention bacteria, respectively. At the family level, Enterobacteriaceae had significantly higher abundance in the HI post group than in the HI pre group ($P = 0.002$) (Fig. 5D). At the genus level, Escherichia_Shigella, and Curtobacterium had significantly higher abundance in the HI post group than in the HI pre group ($P = 0.003$, $P = 0.048$, $P = 0.005$) (Fig. 5F). This suggests that the improvement of nasal symptoms in AR patients after H_2 inhalation may be related to the redistribution of these flora.

Network

The values of key parameters of network diagrams such as network diagram diameter, degree of modularity, clustering coefficient, network diagram density, and degree of averaging in the HI pre, HI post, and HC groups were 0.26, 0.55, 0.06, 44.27, 3.47; 0.37, 0.44, 0.04, 26.62, 3.99; 0.39, 0.45, 0.04, 28.89 and 3.99. These key parameters of the network diagram were very similar between the HI post and HC groups, with the HI pre group being significantly higher than the HC group (Fig. 5H, I, 5J). H_2 regulation of nasal flora is an extremely complex process with complex

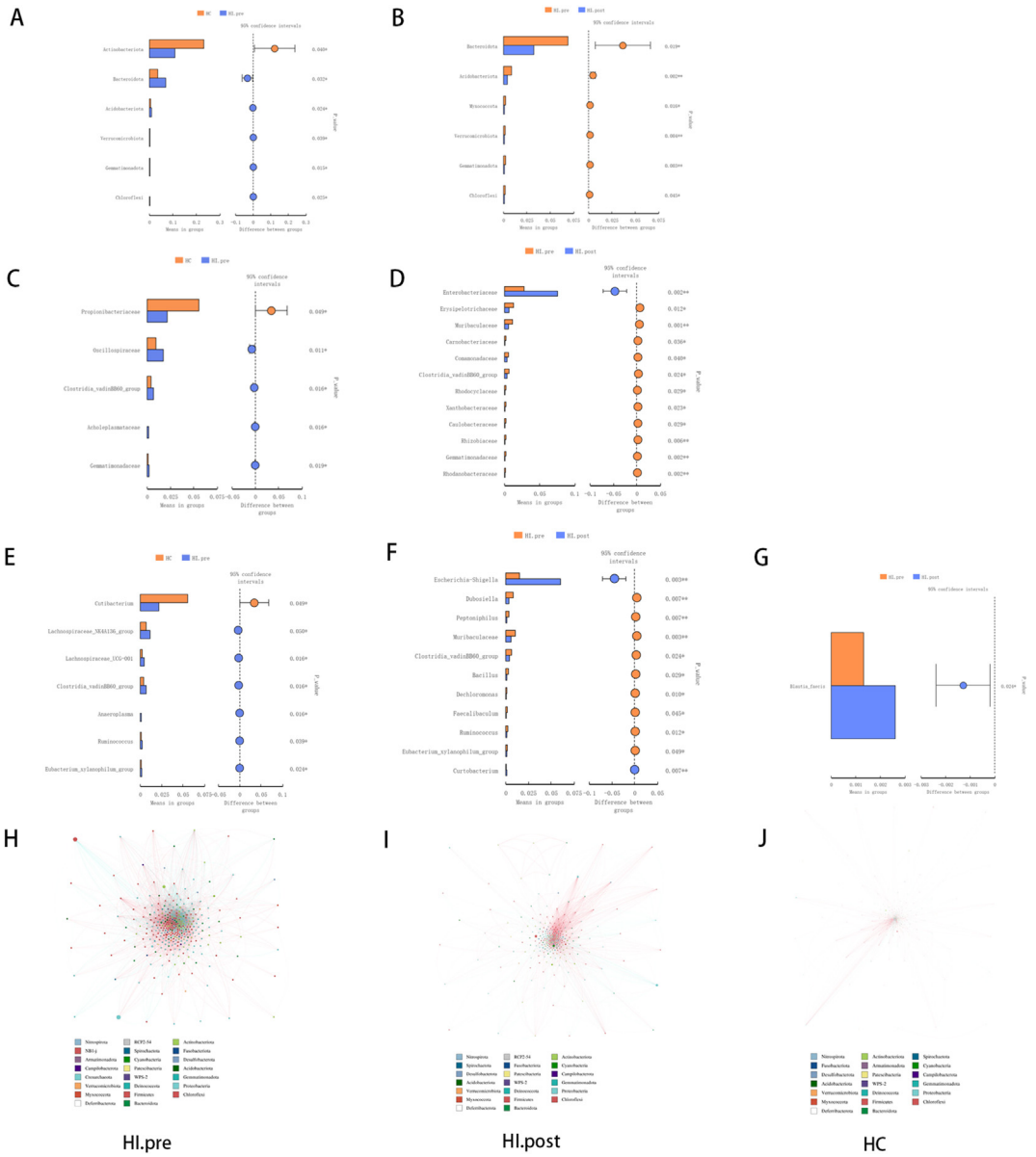

Fig. 5 Significantly different bacteria between the HI pre and HI post groups and between the HI pre and HC groups at the phylum, family, genus, and species level (A–G). Degree of modularity, clustering coefficients, and network graph density between the HI pre, HC, and HI post groups represented in the Network graph (H–J)

interrelationships between different bacteria, which may be either mutually reinforcing or inhibitory, and Network plots show that Blautia is positively correlated with WPS-2. Myxococcota was negatively correlated with Firmicutes and positively correlated with Proteobacteria. Actinobacteriota was positively correlated with Firmicutes. Bacteroidota is positively correlated with Actinobacteriota. Acidobacteriota is positively correlated with Firmicutes and Bcteroidota. Verrucomicrobiota is positively correlated with Bcteroidota, Proteobacteria. Chloroflexi is positively correlated with Acidobacteriota. gemmatimonadota is positively correlated with Verrucomicrobiota.

Moreover, the relative abundance of Actinobacteriota, Bacteroidota, Acidobacteriota, Verrucomicrobiota, Chloroflexi, and Gemmatimonadota were significantly higher in the HI pre group, suggesting that these bacteria play an important role in the pathogenesis of AR. This suggests that these bacteria play an important role in the pathogenesis of AR and may have a biomarker role in the diagnosis of AR. The distribution of nasal flora in AR patients after H_2 treatment tended to be consistent

with that of the HC group, suggesting that it plays an important role in regulating the balance of nasal flora (Fig. 5H, I, 5J).

Correlation analysis between nasal bacterial flora and environmental factors

Correlation analysis using Spearman's correlation coefficient revealed significant positive or negative correlations between the relative abundance of different nasal flora and different environmental factors, and that patients with AR have unique microbial profiles associated with various aspects of the disease.

At the phylum level, WPS-2, Synergistota, and Sumerlaeota became significantly negatively correlated with RQLQ ($P = 0.021$, $P = 0.044$, $P = 0.029$). Verrucomicrobiota was significantly and positively correlated with block, snot, itch, sneeze, VAS, TNSS, EOS, IgE ($P = 0.033$, $P = 0.003$, $P = 0.024$, $P = 0.011$, $P = 0.006$, $P = 0.015$, $P = 0.044$, $P = 0.025$). Acidobacteriota, Gemmatimonadota, and Chloroflexi were significantly positively correlated with IgE ($P = 0.004$, $P = 0.011$, $P = 0.003$) (Fig. 6A).

At the family level, Lachnospiraceae, Ruminococcaceae, Bacillaceae, Oscillospiraceae, and Beijerinckiaceae were negatively correlated with RQLQ ($P = 0.02$, $P = 0.019$, $P = 0.007$, $P = 0.011$, $P = 0.047$). Enterobacteriaceae is negatively correlated with block, snot, itch, VAS, and TNSS ($P = 0.011$, $P = 0.002$, $P = 0.004$, $P < 0.001$, $P = 0.002$). Micrococcaceae are positively correlated with snot, sneeze, VAS, TNSS, EOS, and IgE ($P = 0.018$, $P = 0.044$, $P = 0.045$, $P = 0.048$, $P = 0.019$, $P = 0.039$). Muribaculaceae was positively correlated with snot, itch, sneeze, VAS, and IgE ($P = 0.006$, $P = 0.021$, $P = 0.012$, $P = 0.008$, $P = 0.007$) (Fig. 6B).

At the genus level, Escherichia. Shigella was negatively correlated with block, snot, itch, sneeze, VAS, and TNSS ($P = 0.002$, $P = 0.005$, $P = 0.008$, $P = 0.007$, $P < 0.001$, $P = 0.003$). Moraxella is negatively correlated with snot, itch, sneezing, and VAS ($P = 0.045$, $P = 0.021$, $P = 0.027$, $P = 0.02$). Blautia is negatively correlated with block, RQLQ ($P = 0.035$, $P = 0.014$). Lachnospiraceae_NK4A136_group is positively correlated with snot, itch, sneeze, VAS, and IgE ($P = 0.005$, $P = 0.008$, $P = 0.026$, $P = 0.029$, $P = 0.011$). Dubosiella is positively correlated with block, snot, itch, sneeze, VAS, TNSS ($P = 0.021$, $P = 0.013$, $P = 0.03$, $P = 0.015$, $P = 0.005$, $P = 0.029$, $P = 0.53$). Muribaculaceae were positively correlated with snot, itch, sneeze, VAS, and IgE ($P = 0.008$, $P = 0.023$, $P = 0.017$, $P = 0.01$, $P = 0.009$) (Fig. 6C, E-L).

At the species level, Blautia_faecis is negatively correlated with block, snot, sneeze, VAS, TNSS, RQLQ ($P = 0.002$, $P = 0.036$, $P = 0.027$, $P = 0.01$, $P = 0.014$, $P = 0.011$). Weissella_viridescens is negatively correlated with block, snot, VAS, TNSS, EOS, IgE ($P < 0.001$, $P = 0.04$, $P = 0.025$, $P = 0.014$, $P = 0.031$, $P = 0.019$). Enterococcus_cecorum, Tyzzerella_sp, and Blautia_caecimuris were negatively correlated with RQLQ ($P = 0.003$, $P = 0.015$, $P = 0.003$). (Fig. 6D).

The above results indicate that nasal flora of AR patients is significantly associated with nasal symptoms as well as IgE, and EOS. Up-regulation of relative abundance of Acidobacteriota, Verrucomicrobiota, Chloroflexi, Gemmatimonadota, Chitinophagaceae, Eubacterium_coprostanoligenes_group, Nocardioidaceae, and Muribaculacea bacterial flora may contribute to the development of nasal symptoms in AR patients. In contrast, up-regulation of the relative abundance of Enterobacteriaceae, Escherichia. Shigella, Moraxella, Blautia_faecis, and Blautia_caecimuris flora may play a role in suppressing nasal symptoms in AR patients. H_2 can affect the patients' nasal symptoms and blood parameters by modulating the abundance of specific flora in the nasal cavity.

Functional analysis

We analyzed the differences in colony function between the 3 groups by T-test. Volcano plots showed significant differences in the abundance of different bacterial functional classes in the HI pre, HC, and HI post groups (Fig. 7A and B). This suggests that the function of nasal flora in AR patients is significantly different from that of normal subjects and that H_2 intervention can influence the function of nasal flora in patients with AR.

KEGG showed that the functional abundance of the LacI family transcriptional regulator (K02529) and ParB family (K03497) was higher than normal

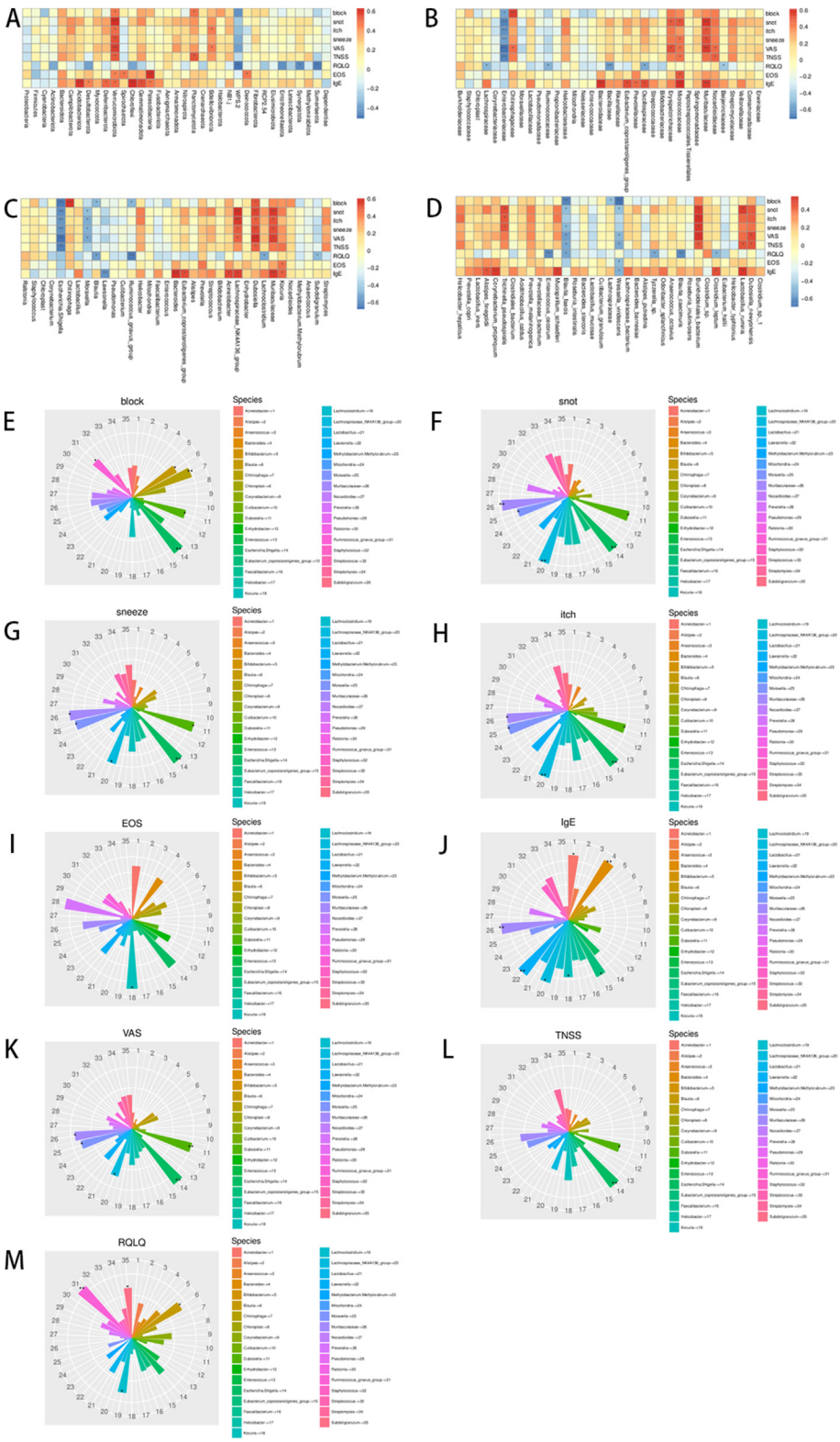

Fig. 6 Correlation analysis between nasal bacterial flora and environmental factors in the HI pre, HI post, and HC groups

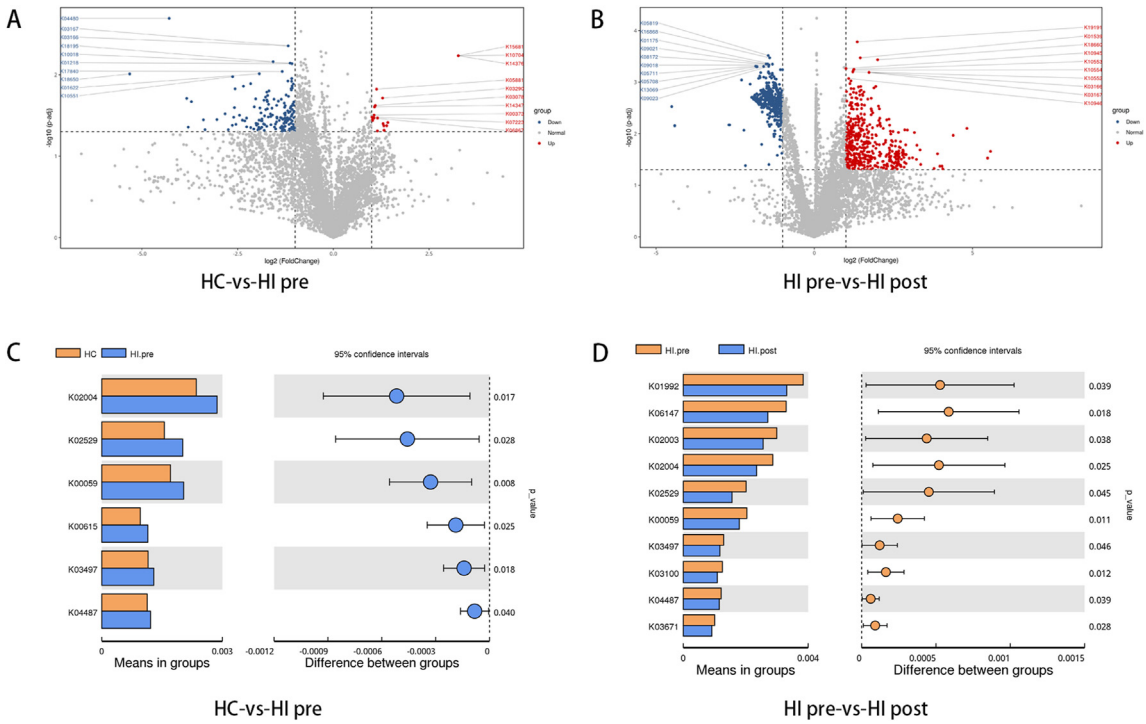

Fig. 7 Statistically significant differences in flora function between the HI pre, HI post, and HC groups

levels in AR patients ($P = 0.011$, $P = 0.012$) and was significantly down-regulated after hydrogen inhalation (Fig. 7C and D). This suggests that the H_2 intervention is able to promote the restoration of specific nasal flora function towards normal human levels by modulating their function.

DISCUSSION

The clinical incidence of AR is high, and the current clinical treatment is based on medication such as glucocorticoids and antihistamines, but the use of medication is always accompanied by more side effects and the emergence of drug resistance. We therefore explored a novel non-pharmacological treatment for AR with transnasal inhalation of high concentrations of H_2 (66.6%). A large number of cellular, animal model, and human clinical patient studies have successively demonstrated the potential therapeutic effects of H_2 on a wide range of diseases, including neurodegenerative diseases, metabolic diseases, cardiovascular and cerebrovascular diseases, inflammation, tumors, etc.[4] The airway microbiota can influence host metabolism and homeostasis in vivo, including epithelial cell growth and repair, inflammation, and immune responses, thereby influencing the onset and progression of chronic diseases.[5,6,9] The nasal microbiota plays a crucial role in the pathogenesis of AR.[5] Therefore, we utilized hydrogen in the treatment of AR patients and focused on the effect of hydrogen on the distribution of nasal flora in AR patients. In this experiment, AR patients were treated by nasal inhalation of high-concentration H_2 for 4 consecutive weeks, and after hydrogen inhalation for 3 h a day, nasal symptoms, as well as blood parameters, improved significantly, and the distribution of nasal flora was restored toward the level of normal after H_2 intervention, and the patients tolerated H_2 well during the treatment process, with no significant adverse reactions.

H_2 can effectively improve AR symptoms. Yu et al reduced oxidative stress and alleviated allergic symptoms in a mouse model of AR by increasing superoxide dismutase (SOD) levels with hydrogen-enriched water.[17] Fang S et al demonstrated that inhalation of high concentrations of H_2 (40%) reduced the infiltration of inflammatory cells, including eosinophils and lymphocytes, into the mucous membranes and submucosa.[19] After 7 consecutive days of hydrogen inhalation in AR

patients, the scores of VAS and TNSS decreased from 26.4 to 9.4 to 16.1 and 5.6, respectively, with statistically significant differences ($P < 0.01$), and the levels of EOS as well as serum total IgE also decreased significantly, which indicated that inhalation of H_2 had a significant efficacy in both clinical indicators as well as laboratory tests.

H_2 has a role in regulating the distribution of flora. Numerous studies have shown that H_2 ameliorates disease by regulating flora balance in a variety of diseases. In the treatment of acute alcoholic liver injury, H_2 reduces oxidative stress and inflammation by blocking the activation of the hepatic LPS/TLR4/NF-κB pathway, which in turn attenuates hepatic injury, as well as improves intestinal flora and strengthens the intestinal barrier.[18] In patients with impaired fasting glucose (IFG), hydrogen-enriched water may ameliorate metabolic abnormalities and intestinal dysbiosis in patients with IFG by modifying the gut microbiota and altering the levels of biomarkers it produces.[24] Hydrogen-enriched water also shows potential for treating Alzheimer's disease by reducing hyperphosphorylated tau proteins and neuro progenitor fiber tangles, decreasing inflammatory responses, and improving disorders of energy metabolism in the brain and imbalances in the intestinal flora.[25] In our current study, it was found that transnasal inhalation of H_2 at concentrations up to 66.6% in AR patients significantly down-regulated the Alpha diversity and Beta diversity of the patients' nasal flora. Therefore, we can conclude that H_2 can modulate the nasal microbiota and contribute to the normalization of nasal flora.

H_2 affects the immune function of the nasal mucosa by regulating nasal flora. It has been shown that changes in the microbiota affect the immune function of the airway mucosa. Eosinophils, TH17 gene expression, and markers of allergic inflammation have all been associated with differences in the composition of the airway microbiota.[26] Gram-positive bacteria activate Th2-type immune responses by enhancing dendritic cell (DC) maturation and inflammatory Th1, Th2 and Th17 responses.[27] In our present study, the relative abundance of nasal flora of Ruminococcus was significantly higher in the AR group than in the Control and HI groups ($P < 0.05$). Ruminococcus is a Gram-positive anaerobic bacterium that effectively induces IL-17 production by $CD4^+$ T cells.[28] IL-17E in IL-17 is a chemokine of the Th2 type of immune response, regulates mucosal immunity, promotes Th2 cell differentiation and immune response reaction, exacerbates metamorphosis, and also affects the differentiation of Th17 cells.[29] Based on these findings, H_2 could suppress Th2-type immune responses and attenuate nasal allergic reactions by down-regulating the abundance of Ruminococcus.

H_2 has a role in regulating the balance of the bacterial flora. The abundance of Erysipelotrichaceae in the nasal cavity of AR patients was significantly higher than that of normal subjects ($p < 0.05$) and the relative abundance in the nasal cavity of AR patients was significantly decreased by H_2 intervention ($p < 0.05$). The relative abundance of Erysipelotrichaceae has positively correlated with tumor necrosis factor-alpha (TNF-α) levels, and an abnormal increase in TNF-α increases the permeability of the nasal mucosal barrier, destroy the nasal epithelial barrier, and induces the organism to make an excessive immune stress response to pathogens, allergens, and noxious stimuli, which can result in the occurrence of allergic symptoms such as nasal itchiness, sneezing, and runny nose.[30] Dubosiella, a species of Erysipelotrichaceae, was significantly and positively correlated with block, snot, itch, sneeze, VAS,[20] and TNSS[21] scores. Therefore, inhalation of H_2 can reduce the abundance of Erysipelotrichaceae, which decreases the concentration of TNF-α in the nasal cavity, improves the nasal epithelial barrier function, and effectively improves the symptoms of AR.

H_2 can influence the host nasal microenvironment by affecting the expression of genes related to carbohydrate metabolism in the flora. LacI family transcriptional regulator is an important component of the bacterial transcriptional regulatory network and is widely distributed in certain bacterial lineages that sense sugar effects and regulate carbohydrate utilization genes and hence carbohydrate metabolism.[31] Prediction of bacterial function by PICRUSt2 revealed that the LacI family transcriptional regulator was found to be highly expressed by bacteriophage functional genes in patients with AR, whereas the expression of this factor was markedly lacking in normal subjects and in patients after hydrogen inhalation. LacI family transcriptional regulator

can inhibit the expression of the galactose metabolism gene pathway in Lactococcus raffinolactis, which in turn affects the level of lactose metabolism.[32] The LacI family transcriptional regulator, which is highly expressed in the nasal flora of AR patients, can alter the carbohydrate metabolism level of the flora in the nasal cavity, affect the microenvironment of the nasal flora, and promote AR symptoms. H_2 can alleviate AR symptoms by inhibiting its expression level.

Blautia is widely distributed in the feces and intestines of mammals and can utilize H_2, CO_2, and carbohydrates as energy sources.[33] TJs are an important component of the connectivity complex between mucus, microbiota, surface fluid, and neighboring epithelial cells in the airway epithelial barrier,[34] and high glucose levels can lead to down-regulation of TJ proteins by decreasing the expression of connexin 43.[35] From this, we reasonably infer that Blautia promotes lipid and glucose metabolism and is involved in the improvement of glucose and lipid homeostasis, which in turn improves AR symptoms by regulating local metabolic levels, up-regulating TJ proteins, and enhancing the nasal epithelial barrier of AR patients against pathogenic bacteria. Blautia plays a role in maintaining the balance of the intestinal environment and preventing inflammation by up-regulating the concentration of regulatory T cells and circulating short-chain fatty acids (SCFAs), as well as by participating in biological processes such as iron scavenging and nutrient acquisition, chemical communication, and defense responses.[36-38] Our findings revealed that the relative abundance of Blautia in the nasal cavity of AR patients after H_2 inhalation was significantly increased, and Blautia_faecis was significantly negatively correlated with block, snot, sneeze, VAS, TNSS, and RQLQ. WPS-2 became significantly positively correlated with Blautia and WPS-2 became significantly negatively correlated with RQLQ. From this, we predicted that both WPS and Blautia are probiotics in the nasal cavity. H_2 can provide a greater energy source for Blautia in the nasal cavity, resulting in a significant increase in its abundance in the nasal cavity and up-regulation of TJ proteins, thus enhancing the nasal epithelial barrier function and reducing the invasion of harmful bacteria in the nasal cavity.

In summary, H_2 can be efficacious in the treatment of AR by regulating the abundance of specific flora and the expression of functional genes of the flora, affecting the Th2-type immune response and the epithelial barrier permeability of the nasal mucosa.

CONCLUSIONS

H_2 may improve symptoms in AR patients by modulating the distribution of nasal flora. Trials with larger sample sizes are required to further test this hypothesis.

Abbreviations

H2: Hydrogen; AR: Allergic rhinitis; EOS: Eosinophil count; IgE: Immunoglobulin E; VAS: Visual analog scale; TNSS: Total nasal symptom score; RQLQ: Rhinoconjunctivitis quality of life questionnaire; SPT: Skin prick test; ASVs: Amplicon sequence variants; IFG: Impaired fasting glucose; DC: Dendritic cell; SOD: Superoxide dismutase; TNF-α: Tumor necrosis factor alpha; SCFAs: Short-chain fatty acids.

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Availability of data and materials

Data will be made available on request.

Author contributions

Nan Wang mainly takes charge of writing the papers and finishing the experiments. Qianzi Ma mainly takes charge of sample collection and literature search. Tianwei Tang and Jiayuan Zhai mainly takes charge of sample collection sample collection. Yanlu Che, Junjie Liu and Yanan Sun mainly takes charge of literature search. Jingting Wang takes charge of directing the writing of this paper. Wanchao Yang plays a guiding role in directing the writing of this paper and providing research funding. All authors contributed to the article and approved the submitted version.

Ethics approval and consent to participate
The studies involving human participants were reviewed and approved by China Clinical Trial Registration Center, CHINA. The patients/participants provided their written informed consent to participate in this study. This trial was registered in the China Clinical Trial Registry (Registration No. ChiCTR2200062253).

Authors' consent for publication
We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. All authors approved the final manuscript and the submission to this journal.

Confirmation of unpublished work
We confirm our manuscript is original, has not been published before, is not currently being considered for publication elsewhere.

Declaration of competing interest
The authors declare no conflict of interest.

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