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Efficacy and Safety of Transcutaneous Auricular Vagus Nerve Stimulation in Patients With Constipation-Predominant Irritable Bowel Syndrome: A Single-Center, Single-Blind, Randomized Controlled Trial

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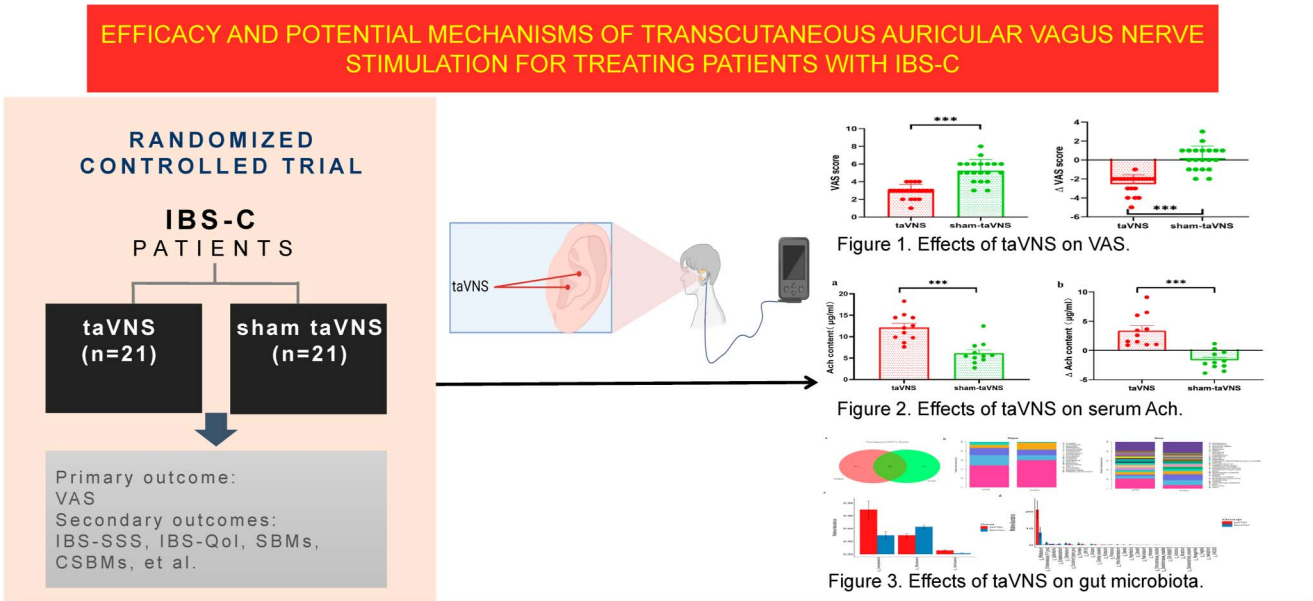
INTRODUCTION: **Transcutaneous auricular vagus nerve stimulation (taVNS) is a promising therapy for irritable bowel syndrome (IBS). The aims of this clinical trial were to evaluate the influence of taVNS on autonomic functions, rectal sensation, and acetylcholine (Ach) levels and to explore potential mechanisms involving gut microbiota and metabolic profiles.**



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METHODS: **This study was a single-center, single-blind, randomized controlled trial executed at the First Affiliated Hospital of USTC, Anhui, China. Individual patients' constipation-predominant IBS (IBS-C)-related symptoms and mental health were assessed and scored using questionnaires at baseline and at week 4. Levels of serum Ach and nitric oxide, anorectal manometry, and heart rate variability were assessed both before and after the therapy. Fecal samples from each group were assessed to compare the gut microbiota, short-chain fatty acids, and gut microbiota-derived tryptophan metabolites.**

RESULTS: **Between September 2023 and May 2024, 40 patients (n = 20 in both taVNS and sham-taVNS groups) completed the 4-week study by performing an intention-to-treat analysis. No differences in all parameters between taVNS and sham-taVNS groups at the baseline were found. The taVNS significantly improved the visual analog scale score ($P < 0.001$), IBS Severity Scoring System score ($P < 0.001$), weekly frequency of spontaneous bowel movements ($P < 0.001$), weekly frequency of complete spontaneous bowel movements ($P = 0.004$), Bristol Stool Form Scale score ($P < 0.001$), Hamilton Anxiety Scale score ($P < 0.001$), Hamilton Depression Scale score ($P < 0.001$), and IBS Quality of Life score ($P < 0.001$). Furthermore, taVNS improved rectal sensation in patients with IBS-C, including improvements in the threshold volume for initial sensation ($P = 0.033$), urge to defecate ($P = 0.022$), and rectoanal inhibitory reflex ($P = 0.002$). Moreover, taVNS elevated serum levels of Ach ($P = 0.005$) and reduced nitric oxide ($P = 0.016$) while also enhancing vagal activity ($P < 0.001$) as determined by spectral analysis of heart rate variability. Three patients in the taVNS group and 2 in the control group had adverse consequences, which were manageable. In addition, taVNS led to a significant rise in the level of the genus *Bifidobacterium* ($P = 0.038$) and increased levels of acetic ($P = 0.003$), butyric ($P = 0.011$), and propionic ($P = 0.005$) acids. It also decreased tryptophan metabolism content, including 3-hydroxyanthranilic acid ($P = 0.007$), anthranilic acid ($P = 0.026$), and L-tryptophan ($P = 0.002$).**

DISCUSSION: **The study manifested that noninvasive taVNS effectively improved constipation and abdominal pain symptoms in patients with IBS-C. The alleviation of IBS-C symptoms may be attributed to the integrative effects of taVNS on rectal functions, mediated through vagal, cholinergic, and multiomics mechanisms.**

KEYWORDS: transcutaneous auricular vagus nerve stimulation; constipation; irritable bowel syndrome; clinical trial

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/AJG/D501

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INTRODUCTION

Irritable bowel syndrome (IBS) is a prevailing condition characterized by recurring abdominal pain or discomfort, alterations in defecation patterns, and fecal characteristics, making it a common disease within the spectrum of disorders of gut-brain interaction (DGBI) (1). From a clinical perspective, IBS is characterized by its diversity and may be classified into 4 distinct subtypes: diarrhea predominant (IBS-D), constipation predominant (IBS-C), mixed presentation, and unspecified subtype (2). Recent epidemiological data indicate an approximate prevalence of 12.6% in Japan, China, and South Korea (3). In the United States, using the Rome IV criteria, the overall prevalence of IBS was found to be 6.1%, with a higher proportion of IBS-C cases compared with IBS-D (33.6% vs 28.1%) (4). Symptoms of IBS have been reported to affect patients' productivity for an average of 8.0 days each month. Among these patients, those with IBS-C are more prone to sexual avoidance, concentration difficulties, and self-consciousness compared with those with IBS-D (5).

Multiple factors and mechanisms, such as visceral hypersensitivity (VHS), altered brain-gut axis, impaired gut motility, gut microbiota imbalance, immune response, and psychiatric comorbidities, play essential roles in the pathophysiology of IBS (6,7). The current treatment options for IBS-C include making alterations to one's lifestyle, following specific diets, taking medicine, and undergoing psychiatric therapy (8). The US Food and Drug Administration (FDA) has granted acceptance for the use of linaclotide in IBS-C treatment, although it has been associated with side effects such as diarrhea, abortion, ectopic pregnancy, and pericoronitis (9). A nonpharmacological approach known as neuromodulation has emerged as a treatment avenue for DGBI (10). Our previous animal study found that transcutaneous auricular vagus nerve stimulation (taVNS) boosted IBS-C in mice by modulating the gut microbiota and interstitial cells of Cajal, whereas our clinical trial indicated that improvements in smooth muscle motility after taVNS could be related to vagus nerve-dependent mechanisms (11,12).

The gastrointestinal (GI) tract is innervated and dramatically modulated by the vagal nerve, manifesting that vagus nerve stimulation (VNS) shows promise as a possible therapy for illnesses of the DGBI (13). The vagal nerves, also known as the tenth cranial nerves, have a key involvement in controlling parasympathetic activity and thereby affecting gut motility. Despite this, there is currently no established and validated clinical treatment using VNS for functional GI conditions encompassing IBS-C, in response to the limitations of invasive VNS, a noninvasive alternative known as taVNS has emerged. This method is dependent on the anatomical fact that the auricular concha is richly innervated by sensory vagus nerves (14). However, the efficacy of taVNS in managing IBS-C and the underlying

mechanisms involving multiomics are not well understood and have been scarcely explored.

Given the implication of high sympathetic tone and an impaired vagal pathway in the pathophysiology of IBS (15), it is reasonable to propose that taVNS might be a beneficial treatment for patients with IBS-C. A recent study demonstrated enhancements in anorectal physiological function and autonomic nervous system activity among patients with IBS-C after taVNS intervention (16). Despite these promising findings, there is a dearth of evidence in the literature on the taVNS implications on multiomics in patients with IBS-C. The primary objectives of this preliminary study were (i) to assess the effects of taVNS on constipation and abdominal pain symptoms in patients with IBS-C; (ii) to evaluate the influence of taVNS on autonomic functions, rectal sensation, and acetylcholine (Ach) levels; and (iii) to explore potential mechanisms involving gut microbiota, short-chain fatty acids (SCFAs), and gut microbiota-derived tryptophan metabolites.

METHODS

Patients

Outpatients who fulfilled the Rome IV diagnostic criteria for IBS-C were identified and enrolled in this study between September 2023 and May 2024. The inclusion criteria were as follows: individuals aged 18–70 years diagnosed with IBS-C according to the Rome IV criteria (17). The exclusion criteria were as follows: (i) patients with severe cardiovascular and respiratory ailments, diabetes, nephropathy, and different chronic illnesses linked to GI problems, including inflammatory bowel disease, ulcers, and cancer; (ii) individuals who were pregnant or planning to become pregnant; (iii) individuals with a history of alcohol or drug abuse; (iv) individuals who had undergone abdominal surgery within the past 6 months; (v) individuals who had consumed probiotics or antibiotics during the investigation period or within 2 weeks before enrollment; (vi) individuals taking medications affecting mental status (antidepressants, sedatives, and hypnotics); and (vii) individuals with severe psychological disorders. Notably, a 2-week washout period for probiotics or antibiotics was selected to minimize the effect on intestinal flora (18).

This study was approved by the Ethics Committee of the First Affiliated Hospital of USTC (2023-KY296) and registered in the Chinese Clinical Trial Registry (ChiCTR2400085832). All patients were required to provide written informed consent.

Sample size calculation

Furthermore, the sample size was calculated using G*power analysis, with the visual analog scale (VAS) score as the primary outcome. The mean VAS pain score for the control group was 3.1, with an SD of 2.2. The treatment group exhibited a mean VAS pain score of 1.1, with an SD of 1.1 (16). Considering a 20% dropout rate, to achieve a statistical power of 80% in a 1-tailed *t* test for the variation between the 2 groups, a sample size of 22 patients (11 patients per group) was necessary, with an α level of 5%. The sample size was determined using the TrialSize package within R software (version 3.1.0, http://www.R-project.org).

Transcutaneous auricular vagus nerve stimulation

Unilaterally positioned electrodes were placed at the auricular concha. Pulses were sent through the electrodes using a watch-size digital stimulator (SNM-FDC01; Ningbo Maida Medical Device, Ningbo, China) (see Supplementary Figure 1a, Supplementary Digital Content 1, http://links.lww.com/AJG/D501). taVNS or sham-taVNS therapy was administered once daily (3 PM) for 4 weeks. The duration of each treatment session was 30 minutes. The stimulation settings were established with a 2-second duration for the active phase and a 3-second duration for the resting phase. The electrical pulses possessed a period of 0.5 milliseconds, rate of 25 Hz, and amplitude that ranged from 0 to 2 mA, which was the highest amount that the patient could tolerate. These parameters were selected based on prior research on auricular VNS, which demonstrated both prokinetic and analgesic effects (16,19,20). The taVNS intervention was administered with a pair of electrodes at the auricular cymba concha and cavity of concha location, whereas sham-taVNS was conducted at sham points located at the earlobe and antihelix (see Supplementary Figure 1b, Supplementary Digital Content 1, http://links.lww.com/AJG/D501) with identical parameters to taVNS (21).

Experimental protocol

This clinical trial was conducted with a randomized, controlled, and single-blind design. Upon obtaining written informed consent, eligible patients were allocated to their respective groups based on the concealed randomization sequence. The patients were unaware of the specific treatment being administered. Of 52 eligible patients, 8 were excluded and 2 declined to participate, leaving 42 patients who were randomly classified in a 1:1 ratio to either the taVNS or sham-taVNS group using a central randomization system.

The taVNS and sham-taVNS groups included 21 patients each, with 1 patient lost to follow-up in each group (dropout rate of 4.76% for each group). Patients were asked to avoid using laxatives and/or linaclotide during the trial unless IBS-C-related symptoms were intolerable because this medication might affect the gut microbiota and metabolites. Finally, 35 patients completed the study per standard protocol, with 5 patients using laxatives and/or linaclotide during the study. The intention-to-treat analysis ($n = 20$ in both groups) was used in this study.

The standardized IBS Symptom Severity System (IBS-SSS) score, IBS Quality of Life (IBS-QOL) questionnaire, VAS score, weekly frequency of spontaneous bowel movements (SBMs), weekly frequency of complete spontaneous bowel movements (CSBMs), Bristol Stool Form Scale (BSFS) score, Hamilton Anxiety Scale (HAMA) score, and Hamilton Depression Scale (HAMD) score were recorded for each patient 4 weeks before and after treatment. Anorectal functional and heart rate variability (HRV) tests were conducted for all patients at baseline and after 4-week taVNS. Paired fecal samples ($n = 11$) were collected from taVNS group patients who completed the study per protocol before and after treatment for determining gut microbiota and metabolism, whereas blood samples were collected from the selected 11 patients in the taVNS group and 11 patients in the sham group. In this trial, the VAS score was selected as the primary outcome, and the other outcomes, including IBS-SSS, IBS-QOL, SBMs, CSBMs, BSFS, HAMA, HAMD, HRV, serum Ach content, and gut microbiota were considered secondary outcomes. According to the criteria established by the FDA, patients were classified as responders if the primary outcome improved by $\geq$30% from the baseline, as measured by the VAS score (22). The flowchart is illustrated in Figure 1. There were no statistically significant differences among the patients in age, sex, body mass index, and duration (Table 1). Detailed demographic data of the patients are provided in Supplementary Table S1 (see Supplementary Digital Content 1, http://links.lww.com/AJG/D501).

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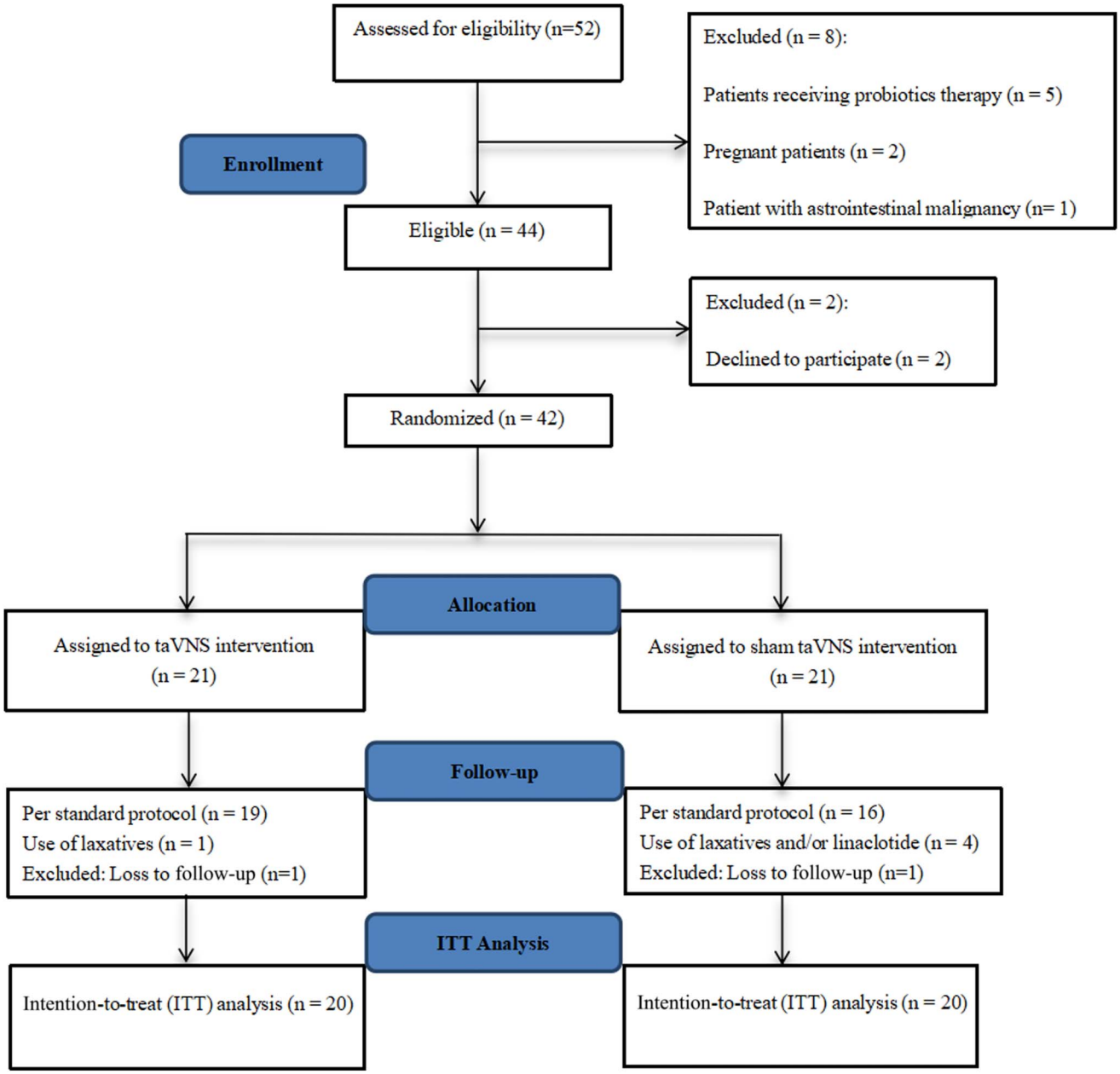

Figure 1. Flowchart of study participation.

Questionnaires

The IBS-SSS questionnaire is a validated tool used internationally to evaluate overall IBS-like symptoms (23). It comprises 5 subscores, with each subscore rated on a 100-point Likert scale with the maximum total score of 500 points, 0 indicating no symptoms and 500 indicating extremely severe symptoms.

Patients rated their abdominal pain using VAS score, ranging from 0 (no pain) to 10 (most severe pain) (22,24). The HAMA and HAMD were used to assess the mental state of patients. The HAMA is a 5-point Likert scale ranging from 0 (asymptomatic) to 4 (extremely severe), encompassing 14 items related to anxiety symptoms (25). In addition, the HAMD includes 17 items that were grouped into 5 structural factors, with a 5-point Likert scale ranging from grade 0 (no symptoms) to grade 4 (extremely severe symptoms) (26).

The IBS-QOL measure is a validated tool specifically designed to evaluate the impact of IBS on patients' QOL. It comprises 34 IBS-specific items rated on a 5-point Likert scale, with a higher score indicating greater impairment of QOL. The IBS-QOL includes 8 subscales assessing various dimensions (27).

The BSFS is a widely used tool to assess bowel habits, with scores ranging from 1 to 7 corresponding to stool types 1 to 7 (from hardest to softest). A lower BSFS score indicates more severe constipation (28).

Measurements

High anorectal resolution manometry. The high anorectal resolution manometry (MedKinetic, Ningbo, China) test was performed on all patients using a water-perfused anorectal manometric catheter. Eight pressure sensors were affixed to the

Table 1. Demographic characteristics and IBS-related symptoms at baseline

	taVNS (n = 20)	sham-taVNS (n = 20)	t/χ^2	*P* value
Age (yr)	48.10 ± 11.54	48.75 ± 12.23	−0.173	0.864
Sex (female) (n)	15	15	0.000	1.000
BMI (kg/m^2)	21.63 ± 2.64	22.30 ± 1.36	−1.001	0.323
Duration (mo)	66.20 ± 17.79	63.90 ± 16.56	0.323	0.748
IBS symptoms				
IBS-SSS total score	272.00 ± 35.03	295.50 ± 66.69	−1.395	0.171
Abdominal pain intensity	58.00 ± 14.36	61.00 ± 17.74	−0.588	0.560
Abdominal pain frequency	58.00 ± 12.81	60.00 ± 17.17	−0.418	0.679
Abdominal distension	57.00 ± 11.74	64.00 ± 15.36	−1.619	0.114
Dissatisfaction of bowel habit	47.00 ± 10.03	53.50 ± 17.25	−1.100	0.278
Interference on life in general	52.00 ± 10.05	57.00 ± 16.25	−1.170	0.249
VAS score	5.45 ± 1.23	5.05 ± 1.15	1.062	0.295
HRV				
LF/(HF + LF)	0.57 ± 0.05	0.55 ± 0.04	−0.909	0.369
HF/(HF + LF)	0.43 ± 0.05	0.45 ± 0.04	0.909	0.369
LF/HF	1.33 ± 0.27	1.26 ± 0.23	0.967	0.340
Rectal sensitivity				
RAIR (mL)	21.75 ± 3.35	20.50 ± 5.10	0.915	0.366
First sensation (mL)	27.00 ± 6.57	25.50 ± 5.83	0.764	0.450
Desire of defecation (mL)	53.25 ± 6.34	50.75 ± 9.90	0.951	0.348
Maximum tolerable volume (mL)	238.00 ± 37.08	221.00 ± 35.53	1.481	0.147
Mental health				
HAMA	20.45 ± 4.66	19.50 ± 5.97	0.561	0.578
HAMD	18.75 ± 6.03	18.45 ± 4.22	0.182	0.856
Bowel habit				
BSFS score	1.75 ± 0.44	1.65 ± 0.59	0.607	0.547
SBM frequencies (per week)	4.00 ± 1.30	3.50 ± 1.28	1.228	0.227
CSBM frequencies (per week)	2.55 ± 1.10	2.30 ± 0.98	0.760	0.452

BMI, body mass index; BSFS, Bristol Stool Form Scale; CSBM, complete SBM; HAMA, Hamilton Anxiety Scale; HAMD, Hamilton Depression Scale; HF, high frequency; HRV, heart rate variability; IBS, irritable bowel syndrome ; IBS-SSS, IBS Symptom Severity System; LF, low frequency; RAIR, rectoanal inhibitory reflex; SBM, spontaneous bowel movement; VAS, visual analog scale.

catheter at intervals of 1 cm (29). Before anorectal manometric evaluations, glycerine enemas were administered for bowel preparation. A sophisticated pressure transduction technique was used by the catheter that allowed each pressure sensor to measure pressure throughout a 2.5 mm length in 12 radially scattered sectors. After inserting a catheter into the rectum, the minimum volume required to trigger the rectoanal inhibitory reflexes (RAIRs) was measured by gradually inflating a rectal balloon in decrements of 10 mL. The process began at 10 mL and continued until the relaxation of the anal sphincter was detected at a lower volume of distension. The evaluation of rectal sensation included gradually inflating the rectal balloon in increments of 10 mL until the patient first reported feeling. Subsequently, the balloon was inflated in 10 mL increments to elicit sensations of maximum urge to defecate and urge to defecate. Patients subjectively reported these sensations, and the threshold volumes required to induce them were recorded.

Evaluation of autonomic functions. The autonomic functions were estimated by analyzing the spectrum characteristics of HRV obtained from electrocardiogram data. Patients were directed to engage in a period of relaxation for 10 minutes, followed by assuming a reclined position and having their HRV measured for a period of 30 minutes. The HRV signal was extracted from the electrocardiogram by recognizing R peaks and computing RR intervals with the aid of earlier developed and validated specialized software. In addition, the power values of various frequency sub-bands and the HRV signal's overall power spectrum were computed. Sympathetic activity accounts for the predominant electrical activity estimated in the low-frequency (LF) range (0.04–0.15 Hz). Conversely, the power detected in the high-frequency (HF) range (0.15–0.50 Hz) is indicative solely of parasympathetic or vagal activity. In this investigation, sympathetic activity was quantified as LF/(HF + LF), whereas vagal activity was quantified as

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HF/(HF + LF) (30). Meanwhile, the LF/HF ratio is an important indicator of the vagal/sympathetic balance (31).

Blood Ach and NO assay. Baseline and post-trial blood samples were collected from 11 patients from each group. The samples were allowed to coagulate naturally for approximately 10 minutes and were then centrifuged immediately. For each patient, 2 mL of blood was transferred to a tube containing a blood clotting agent and centrifuged at 2,500 rpm for 10 minutes. Serum from the procoagulant tube was collected (approximately 0.5 mL) and stored at −80 °C for further analysis within 6 months. The serum Ach and nitric oxide (NO) levels were measured using enzyme-linked immunosorbent assay kits (Nanjing Jiancheng, Nanjing, China). The batch numbers of the Ach and NO products were 20240415 and 20240410, respectively.

Safety assessment. All patients who participated in this trial were included for safety assessment. During the study period, the adverse events were defined as ear skin rash, dizziness, palpitation, and intolerable diarrhea. In addition, the blood biochemical indicators were examined before and after treatment. All of the above were recorded in detail for safety findings.

16S rRNA sequencing to analyze fecal microbiota. Gut microbiota for taVNS group patients with IBS-C before and after treatment were assessed using the approach used in our earlier investigation (32). The specimens were kept at −80 °C. The DNA extraction was applied from the specimens and then diluted to a concentration of 1 μg/μL using sterile water to measure the DNA concentration and purity. The primer sequences sed for amplification of the 16S rRNA gene's V3-V4 regions were as follows: 805R (5′-GACTACHVGGGTATCTAATCC-3′) and 341F (5′-CCTACGGGNGGCWGCAG-3′), with each primer including a unique barcode. PCR reactions were performed and then purified using a gel extraction kit (Qiagen, Hilden, Germany), following the combination of equal density ratios. Afterward, libraries for sequencing were created and then sequenced using an Illumina NovaSeq platform, resulting in the production of 250-bp paired-end reads. The paired-end readings were linked to the corresponding specimens and combined using FLASH (vI.2.7). The raw tags were subjected to quality filtering in QIIME (v1.9.1) to get tags of high quality and cleanliness. For eliminating chimeric sequences, IDs were aligned with the SILVA database using the UCHIME approach. On the basis of a similarity threshold exceeding 97%, operational taxonomic units were assigned using UPARSE software version 7.0.1001. The Mothur approach was used in conjunction with the SILVA database to assign taxonomic information to every sequence of the specimen. The DEseq2 program was deployed to detect differentially abundant operational taxonomic units across groups. A significance criterion of $P < 0.05$ and $|\log2(fc)| > 1$ were used.

Measurement of fecal SCFAs. Our prior developed gas chromatography-mass spectrometry (GC-MS) approach was used to test fecal SCFA levels (32). The freeze-dried fecal specimens were reconstituted in a 0.5% phosphoric acid solution with a volume of 5 mL; subsequently, to dilute 60 μL of the resulting supernatant, 240 μL of this solution was deployed. The mixture was subjected to sonication for a duration of 5 minutes and was then centrifuged at a relative centrifugal force of 3,000*g* for a period of 10 minutes. SCFAs were extracted from the specimens using a Geno/Grinder 2010 equipped with 300 μL of butanol (SPEX, Metuchen, NJ). Before undertaking GC-MS study, isotopically labeled internal standards were ascertained using a Pegasus GC-TOFMS system (Leco, St. Joseph, MI) and an Agilent 7890A gas chromatograph equipped with a MultiPurpose Sampler MPS (GERSTEL, Mülheim a der Ruhr, Germany). The separation procedure was executed with a flow rate of 1 mL/min of helium carrier gas. A volume of 1 L of the administered specimen was used in a split mode with a 1:10 ratio. At first, the oven was heated to 70 °C and kept at that temperature for 1 minute. The temperature was first set at 70 °C for 1 minute and then steadily raised, eventually stabilizing at 240 °C, which was maintained for 2 minutes. The entire duration of the oven's operation was 15.8 minutes. Data collection was conducted using a full scan mode, which covered a mass range from m/z 40 to 550. The ionization process used electron impact at an energy level of 70 eV.

Tryptophan metabolism analysis. Tryptophan metabolism analysis was applied using ultra-high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS/MS) (33). The fecal samples of the patient were processed by treating them with an extract solution. The solution was generated by combining methanol, acetonitrile, and water in a ratio of 2:2:1, which was precooled at −40 °C. In addition, 0.1% formic acid and a number of isotopically labeled internal standards were incorporated into the solution. The fecal specimens were mixed well with this extract solution. After centrifugation, the resulting supernatant (400 μL) was evaporated using N2 gas and subsequently reconstituted with 100 μL of 0.1% formic acid. Further centrifugation was conducted to prepare the samples for analysis. The compounds of interest were isolated by means of UHPLC using an ExionLC System (SCIEX). Using a SCIEX 6500 QTRAP + triple quadrupole mass spectrometer with an IonDrive Turbo V electrospray ionization interface, the analysis was conducted. Curtain gas pressure of 40 psi, IonSpray voltage of ± 4500 V, temperature of 500 °C, ion source gas 1 pressure of 30 psi, and ion source gas 2 pressure of 30 psi were the precise parameter configurations used. The quantitative analysis of the target compounds and acquisition of mass spectrum data were conducted using SCIEX Analyst Workstation software (version 1.6.3) and SCIEX MultiQuant software (version 3.0.3). The efforts to analyze the metabolism of tryptophan were supported by Shanghai Biotree Biotech.

Statistical analyses

Statistical analyses were conducted using Statistical Package for Social Sciences (version 22.0) and GraphPad Prism (version 8.0) software. Descriptive statistics for quantitative variables are reported as mean with corresponding SD. The symbol Δ denotes the change from baseline to post-treatment. Data with normally distributed variables were compared using a 2-tailed *t* test, and Mann-Whitney *U* test was used for data with non-normally distributed variables (as determined using Shapiro-Wilk test). Changes in the data before and after treatment were assessed using paired *t* tests, while differences between groups were examined via independent *t* tests. Categorical variables are presented as rates and analyzed using the χ^2 test, while Fisher exact test was used when frequencies were <5 in a category. Spearman correlation was applied to investigate the relationship between gut microbiota, metabolism, and symptom severity (VAS score). The outcomes were also analyzed using a mixed model. The mixed model was implemented using the lme4 package in R, and the *P* values were calculated using Satterthwaite's degrees-of-freedom approach. A $P < 0.05$ indicated a statistical significance.

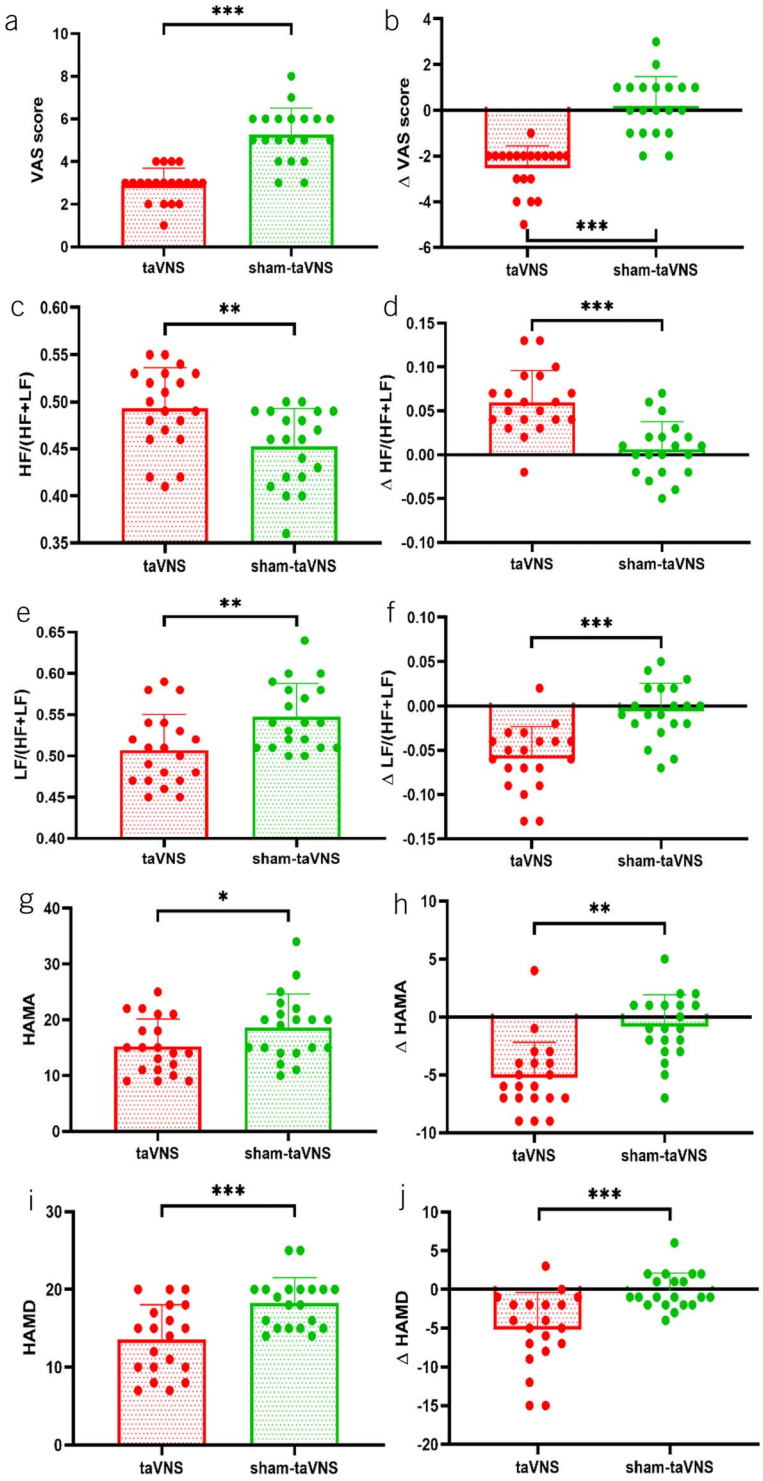

Figure 2. Comparison between taVNS and sham taVNS on VAS scores, heart rate variability, and mental health. (**a**) The VAS in taVNS group's score was significantly lower than that in the sham-taVNS group at week 4. (**b**) The taVNS group's Δ VAS scores was significantly less than that for the sham-taVNS group. (**c**) The HF/(HF + LF) in taVNS group's score was significantly higher than that in the sham-taVNS group at week 4. (**d**) The taVNS group's Δ HF/(HF + LF) was significantly more than that for the sham-taVNS group. (**e**) The LF/(HF + LF) in taVNS group's score was significantly lower than that in the sham-taVNS group at week 4. (**f**) The taVNS group's Δ LF/(HF + LF) was significantly less than that for the sham-taVNS group. (**g**, **i**) The HAMA scores and HAMD scores in taVNS group's score were significantly lower than that in the sham-taVNS group at week 4, respectively. (**h**, **j**) The taVNS group's Δ HAMA scores and HAMD scores were significantly less than that for the sham-taVNS group, respectively. Independent sample *t* test is used for **a**, **c**, **e**, **g**, **i**. Mann-Whitney *U* test is used for **b**, **d**, **f**, **h**, **j**. Error bars depict SD; Δ indicates postprotocol minus baseline changes. Mean ± SD is presented. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. HAMA, Hamilton Anxiety Scale; HAMD, Hamilton Depression Scale; HF, high frequency; LF, low frequency; taVNS, transcutaneous auricular vagal nerve stimulation; VAS, visual analog scale.

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Table 2. IBS-SSS subscore and total score in the 2 groups at week 4

	taVNS (n = 20)	sham-taVNS (n = 20)	*t*	*P* value
Abdominal pain intensity	43.00 ± 11.74	58.00 ± 8.94	−4.544	<0.001
Abdominal pain frequency	42.00 ± 14.36	53.00 ± 13.42	−2.503	0.017
Abdominal distension	36.00 ± 15.36	60.00 ± 14.51	−5.080	<0.001
Dissatisfaction of bowel habit	38.00 ± 15.76	60.00 ± 12.98	−4.819	<0.001
Interference on life in general	41.00 ± 17.74	63.50 ± 13.48	−4.515	<0.001
IBS-SSS total score	200.00 ± 53.31	294.50 ± 35.76	−6.583	<0.001

IBS-SSS, Irritable Bowel Syndrome Symptom Severity System; taVNS, transcutaneous auricular vagal nerve stimulation.

RESULTS

Participants

Of the 52 eligible patients, 8 were excluded (based on the exclusion criteria) and 2 refused to participate, leaving 42 patients who were randomly classified in a 1:1 ratio to either the taVNS or sham-taVNS group (21 patients in each group) using a central randomization system, with 1 patient lost to follow-up in each group (aged 31 and 42 years, both female). Of the 8 excluded patients, 5 (aged 25–51 years, 3 women and 2 men) used probiotics, 2 women (aged 28 and 31 years) were pregnant, and 1 man (aged 52 years) had colorectal cancer. The 20 patients in the taVNS group (average age = 48.10 ± 11.54 years) included 5 men and 15 women with an average IBS-C duration of 66.20 ± 17.79 months. The 20 patients in the sham group (average age = 48.75 ± 12.23 years) included 5 men and 15 women with an average IBS-C duration of 63.90 ± 16.56 months. The flowchart of IBS patient recruitment is depicted in Figure 1. No significant differences were observed in the demographic parameters between the 2 groups (Table 1). Detailed demographic data of the patients are provided in Supplementary Table S1 (Supplementary Digital Content 1, http://links.lww.com/AJG/D501).

Baseline data

The IBS-SSS total score (272.00 ± 35.03 vs 295.50 ± 66.69, $P = 0.171$) was comparable between the patients with IBS in the 2 groups. Furthermore, no significant difference was observed between the taVNS and sham-taVNS groups in the IBS-SSS subscores. No significant changes were observed in autonomic functioning between the taVNS and sham-taVNS groups regarding HF/(HF + LF) ($P = 0.369$) and LF/(HF + LF) ($P = 0.369$). Moreover, there was no significant difference in the VAS scores between the 2 groups ($P = 0.295$). Regarding rectal sensitivity, there was no significant difference in the RAIR between the 2 groups ($P = 0.366$), initial feeling ($P = 0.450$), urge to defecate ($P = 0.348$), and maximum tolerated volume ($P = 0.147$). There was no significant difference in bowel habits between the 2 groups, as indicated by the BSFS ($P = 0.547$), SBM ($P = 0.227$), and CSBM ($P = 0.452$). Furthermore, no significant difference was observed in the HAMA and HAMD scores between the 2 groups in the first stage ($P > 0.050$). The results are presented in Table 1.

Primary outcome

Effects of taVNS on VAS score. At the baseline, there is an absence of significant difference in the VAS score ($P = 0.295$) between the taVNS and sham-taVNS groups. At week 4, the VAS score was significantly lower in the taVNS group compared with the sham-taVNS group (2.90 ± 0.79 vs 5.25 ± 1.25, $P < 0.001$) (Figure 2a). In addition, the change in VAS scores (Δ VAS) was significantly greater in the taVNS group than the sham-taVNS group (−2.55 ± 1.00 vs 0.20 ± 1.28, $P < 0.001$) (Figure 2b). It is noteworthy that after 4-week treatment, the responder rate in the taVNS group was 85.00% (17/20) compared with 10.00% (2/20) in the sham-taVNS group ($\chi^2 = 22.556$, $P < 0.001$). Meanwhile, the taVNS significantly reduced the VAS score than the sham-taVNS at week 4 when using the mixed-effects model (−2.341, 95% confidence interval [CI] −3.037 to −1.663; $P < 0.001$; see Supplementary Table S2, Supplementary Digital Content 1, http://links.lww.com/AJG/D501).

Secondary outcomes

Effects of taVNS on IBS overall symptoms. There was no significant variation in the scores of IBS-SSS and its subscores (all $P > 0.050$) at baseline between the group receiving taVNS and the group receiving sham-taVNS. However, at week 4, the IBS-SSS score at week 4 was significantly lower in the taVNS group compared with the sham-taVNS group (200.00 ± 53.31 vs 294.50 ± 35.76, $P < 0.001$) (see Supplementary Figure S2a, Supplementary Digital Content 1, http://links.lww.com/AJG/D501). Moreover, the change in IBS-SSS scores (Δ IBS-SSS) was significantly greater in the taVNS group than in the sham-taVNS group (−72.00 ± 75.44 vs −1.00 ± 59.11, $P = 0.001$) (see Supplementary Figure S2b, Supplementary Digital Content 1, http://links.lww.com/AJG/D501). Similarly, at baseline between the 2

Table 3. Adverse events between taVNS and sham-taVNS groups

	Ear skin rash	Dizziness	Palpitation	Diarrhea	Total
taVNS (n = 20)	1 (5.00%)	0 (0.00%)	1 (5.00%)	1 (5.00%)	3 (15.00%)
sham-taVNS (n = 20)	1 (5.00%)	1 (5.00%)	0 (0.00%)	0 (0.00%)	2 (10.00%)
P value (Fisher exact test)	1.000	1.000	1.000	1.000	1.000

taVNS, transcutaneous auricular vagal nerve stimulation.

groups, no significant disparity in IBS-SSS subscale ratings was found (all $P > 0.050$). Notably, all subscale scores in the taVNS group were significantly declined at week 4 compared with the sham-taVNS group (all $P < 0.050$). The findings are summarized in Table 2. Meanwhile, the IBS-SSS score exhibited a statistically significant difference between the 2 groups from baseline to week 4 (−93.248, 95% CI −122.243 to −64.272; $P < 0.001$).

Effects of taVNS on bowel habit. The changes in bowel habits for patients with IBS-C were also investigated. The taVNS group has significant improvements contrasted with the sham-taVNS group at week 4. The BSFS scores improved (3.00 ± 0.79 vs 1.80 ± 0.62, $P < 0.001$), as did weekly SBM frequencies (6.05 ± 1.36 vs 3.85 ± 1.09 times, $P < 0.001$) and weekly CSBM frequencies (4.00 ± 1.30 vs 2.80 ± 1.15 times, $P = 0.004$). Furthermore, the changes in the taVNS group were significantly greater than in the sham-taVNS group: Δ BSFS score (1.25 ± 0.79 vs 0.15 ± 0.59, $P < 0.001$), Δ weekly SBM frequencies (2.05 ± 1.32 vs 0.35 ± 1.14, $P < 0.001$), and Δ weekly CSBM frequencies (1.45 ± 0.51 vs 0.50 ± 0.69, $P < 0.001$) (see Supplementary Figure S3a–f, Supplementary Digital Content 1, http://links.lww.com/AJG/D501). Moreover, the mixed-effects model revealed a significant difference between the 2 groups from baseline to week 4 in CSBMs (1.186, 95% CI 0.499–1.873; $P = 0.001$), SBMs (2.205, 95% CI 1.464–2.946; $P < 0.001$), and BSFS (1.207, 95% CI 0.827–1.586; $P < 0.001$).

Effects of taVNS on autonomic functions. At baseline, no distinctions existed in the HF/(HF + LF) ($P = 0.369$), LF/(HF + LF) ($P = 0.369$), and LF/HF ($P = 0.340$) ratios between the taVNS and sham-taVNS groups. Nevertheless, at week 4, the HF/(HF + LF) ratio manifested a significant elevation in the taVNS group contrasted with the baseline (0.49 ± 0.04 vs 0.43 ± 0.05, $P < 0.001$) and sham-taVNS group (0.49 ± 0.04 vs 0.45 ± 0.04, $P = 0.005$). Similarly, the LF/(HF + LF) ratio manifested a significant decrease in the taVNS group at week 4 compared with the baseline group (0.51 ± 0.04 vs 0.57 ± 0.05, $P < 0.001$), and a significant distinction between the 2 groups at week 4 was observed (0.51 ± 0.04 vs 0.55 ± 0.04, $P = 0.005$) (Figure 2c–f). Moreover, at week 4, the LF/HF ratio significantly decreased in the taVNS group compared with the baseline group (1.05 ± 0.19 vs 1.33 ± 0.27, $P < 0.001$), and a significant difference was observed between the 2 groups at week 4 (1.05 ± 0.19 vs 1.22 ± 0.21, $P = 0.008$). The Δ HF/(HF + LF) of the taVNS group manifested a significant rise (0.06 ± 0.04 vs 0.01 ± 0.03, $P < 0.001$), whereas the Δ LF/(HF + LF) (−0.06 ± 0.04 vs −0.01 ± 0.03, $P < 0.001$) and Δ LF/HF (−0.29 ± 0.18 vs −0.03 ± 0.15, $P < 0.001$) were significantly diminished contrasted with the sham-taVNS group (see Supplementary Figure S4a and b, Supplementary Digital Content 1, http://links.lww.com/AJG/D501). Meanwhile, the mixed-effects model revealed a significant difference between the 2 groups from baseline to week 4 in HF/(HF + LF) (0.040, 95% CI 0.013–0.066; $P = 0.005$), LF/(HF + LF) (−0.040, 95% CI −0.066 to −0.013; $P = 0.005$), and LF/HF (−0.178, 95% CI −0.316 to −0.040; $P = 0.012$).

Effects of taVNS on mental health. No significant variations in HAMA and HAMD scores at baseline between the taVNS and sham-taVNS groups were found (both $P > 0.050$). However, at week 4 in the taVNS group, both the HAMA score (15.20 ± 4.96 vs 20.45 ± 4.66, $P < 0.001$) and HAMD score (13.55 ± 4.49 vs 18.75 ± 6.03, $P < 0.001$) were significantly diminished in contrast with baseline. In addition, the HAMD score was significantly lower in the taVNS group compared with the sham-taVNS group at week 4 (13.55 ± 4.49 vs 18.25 ± 3.26, $P < 0.001$) (Figure 2g and i). Notably, the change in HAMA scores (Δ HAMA) was significantly greater in the taVNS group compared with the sham-taVNS group (−5.25 ± 3.06 vs −0.85 ± 2.78, $P < 0.001$), as was the change in HAMD scores (Δ HAMD) (−5.20 ± 4.82 vs −0.20 ± 2.31, $P < 0.001$) (Figure 2h and j). Meanwhile, the mixed-effects model revealed that taVNS significantly reduced the HAMA (−3.566, 95% CI −6.903 to −0.230; $P = 0.037$) and HAMD (−4.739, 95% CI −7.518 to −1.960; $P = 0.001$) scores compared with the sham-taVNS at week 4.

Effects of taVNS on QOL. At baseline, no significant distinction in the IBS-QOL scores was found between the 2 groups ($P > 0.050$). However, at week 4, the IBS-QOL scores were significantly higher in the taVNS group compared with baseline (72.60 ± 23.28 vs 111.00 ± 25.53, $P < 0.001$). In addition, the IBS-QOL scores at week 4 were significantly higher in the taVNS group compared with the sham-taVNS group (72.60 ± 23.28 vs 96.85 ± 22.42, $P = 0.002$) (see Supplementary Figure S5a, Supplementary Digital Content 1, http://links.lww.com/AJG/D501). The change in IBS-QOL scores (Δ IBS-QOL) was significantly greater in the taVNS group compared with the sham group (−38.40 ± 23.57 vs −9.15 ± 23.61, $P = 0.001$) (see Supplementary Figure S5b, Supplementary Digital Content 1, http://links.lww.com/AJG/D501). The mixed-effects model revealed a significant difference in the IBS-QOL score between the 2 groups from baseline to week 4 (−23.524, 95% CI −38.762 to −9.286; $P = 0.002$).

Effect of taVNS on rectal sensation. At baseline, there were no significant differences in threshold volumes for RAIR, first sensation, desire to defecate, and maximum tolerable volume of patients between the 2 groups ($P = 0.366$, $P = 0.450$, $P = 0.348$, and $P = 0.147$, respectively). However, at week 4, the RAIR was significantly lower in the taVNS group compared with baseline (17.75 ± 4.13 vs 21.75 ± 3.35 mL, $P = 0.002$). By contrast, the threshold volumes for first sensation (31.50 ± 6.30 vs 27.00 ± 6.57 mL, $P = 0.033$) and desire to defecate (59.00 ± 8.68 vs 53.25 ± 6.34 mL, $P = 0.022$) were significantly higher in the taVNS group at week 4 compared with baseline.

Meanwhile, RAIR (17.75 ± 4.13 vs 20.50 ± 4.26 mL, $P = 0.045$) was significantly lower in the taVNS group than in the sham-taVNS group at week 4. Similarly, the threshold volumes for first sensation (31.50 ± 6.30 vs 26.25 ± 5.59 mL, $P = 0.008$) and desire to defecate (59.00 ± 8.68 vs 51.25 ± 6.86 mL, $P = 0.003$) were significantly higher in the taVNS group compared with the sham-taVNS group at week 4. In addition, the taVNS group's Δ RAIR (−4.00 ± 5.03 vs 0.00 ± 3.97 mL, $P = 0.017$) was significantly lower than that for the sham-taVNS group, while the taVNS group's Δ first sensation (4.50 ± 5.10 vs 0.75 ± 5.20 mL, $P = 0.033$) and desire to defecate (5.75 ± 7.66 vs 0.50 ± 7.24 mL, $P = 0.030$) were significantly higher than those of the sham-taVNS group (see Supplementary Figure S6a–h, Supplementary Digital Content 1, http://links.lww.com/AJG/D501). The mixed-effects model revealed a significant difference between the 2 groups in RAIR (−2.797, 95% CI −5.392 to −0.203; $P = 0.035$), first sensation (5.093, 95% CI 1.497–8.688; $P = 0.006$), and desire to defecate (7.523, 95% CI 2.788–12.257; $P = 0.002$) from baseline to week 4.

Adverse events during taVNS/sham-taVNS. In this study, the taVNS treatment was well tolerated. The adverse events included ear skin rash (n = 1 in each group), dizziness (n = 1 in the sham-taVNS group), palpitation (n = 1 in the taVNS group), and intolerable diarrhea (n = 1 in the taVNS group). These events

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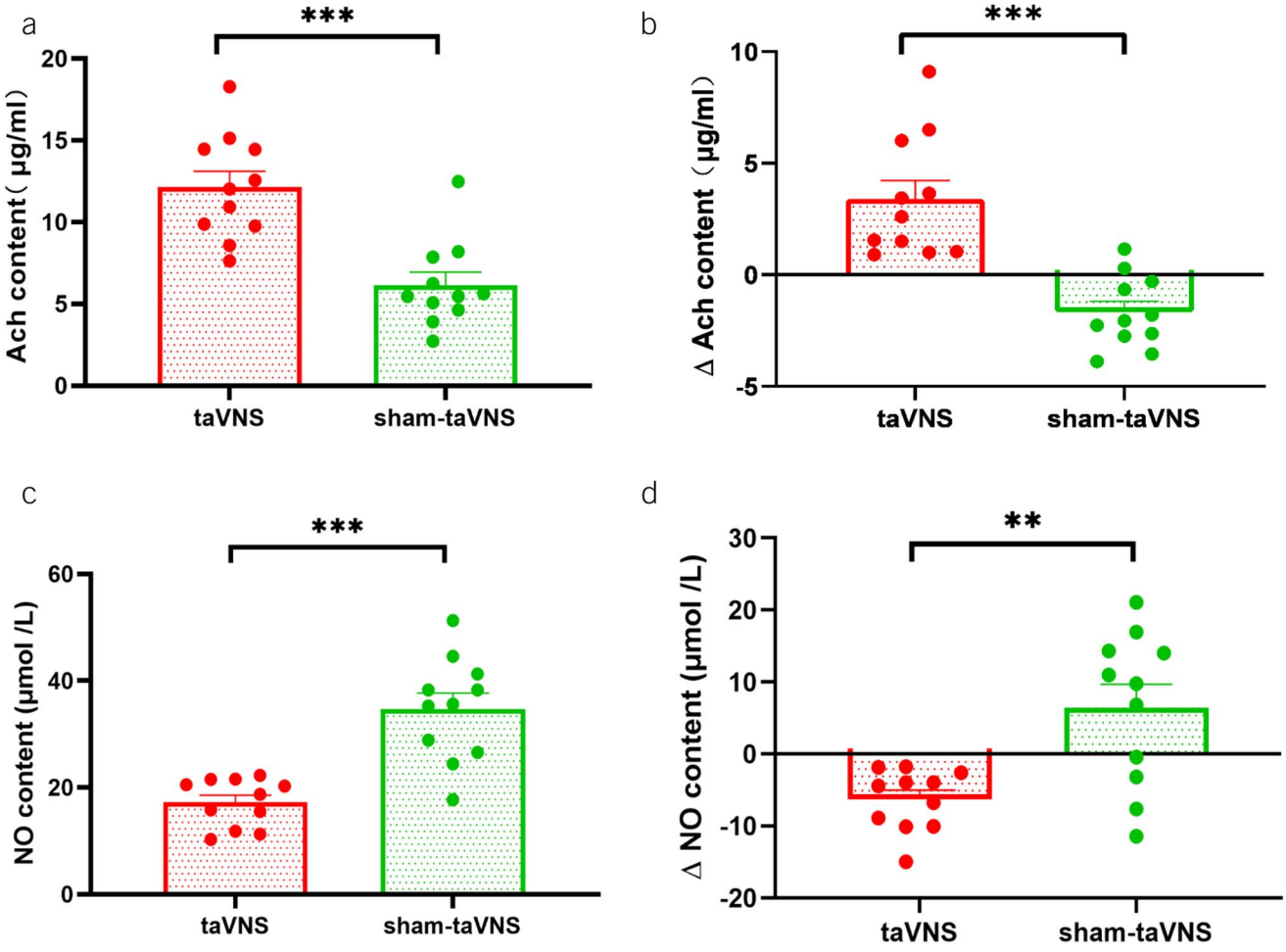

Figure 3. Comparison between taVNS and sham taVNS on Ach and NO. (**a**) The Ach content in the taVNS group was significantly higher than that in the sham-taVNS group at week 4. (**b**) The taVNS group's Δ Ach content was significantly more than that for the sham-taVNS group. (**c**) The NO content in the taVNS group was significantly lower than that in the sham-taVNS group at week 4. (**d**) The taVNS group's Δ NO content was significantly less than that for the sham-taVNS group. Independent sample *t* test is used for **a**, **c**. Mann-Whitney *U* test is used for **b**, **d**. Error bars depict SD; Δ indicates postprotocol minus baseline changes. Mean ± SD is presented. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Ach, acetylcholine; NO, nitric oxide; taVNS, transcutaneous auricular vagal nerve stimulation.

occurred during the first taVNS treatment but were tolerable and relieved with subsequent continuous treatment. There was no significant difference in the adverse events between the 2 groups (3 vs 2, $P = 1.000$; Table 3). No abnormal laboratory results were observed before or after treatment.

taVNS increased Ach and decreased NO in blood. The levels of Ach and NO in serum were determined using ELISA. Ach content in the taVNS group at week 4 was markedly higher than that at baseline (12.15 ± 3.21 vs 8.75 ± 1.61 μg/mL, $P = 0.005$), whereas NO content in the taVNS group at week 4 was markedly lower than that at baseline (17.23 ± 4.51 vs 23.55 ± 6.60 μmol/L, $P = 0.016$). The serum levels of Ach in the taVNS group were significantly greater than in the sham-taVNS ($P < 0.001$) group at week 4, whereas the serum levels of NO in the taVNS were significantly lower than in the sham-taVNS ($P = 0.007$) group at week 4. In addition, the taVNS group's Δ Ach content ($P < 0.001$) was significantly higher than that of the sham-taVNS group, whereas Δ NO content ($P = 0.034$) in the taVNS group was significantly lower than that of the sham-taVNS group (Figure 3a–d).

Gut microbiota analysis at baseline and after treatment. Unique intestinal bacterial genera and shared core in the taVNS group before and after treatment were compared and shown in a Venn diagram (Figure 4a). Our study confirmed that 1,471 and 1,533 bacteria were unique to patients in the taVNS group after treatment and at baseline, respectively. However, no significant difference was noted regarding α-diversity measured by Chaol index, Shannon index, and Simpson index or β-diversity measured by principal component analysis, principal coordinate analysis, and nonmetric multidimensional scaling analysis in the taVNS group after treatment compared with baseline (see Supplementary Figure S7, Supplementary Digital Content 1, http://links.lww.com/AJG/D501).

The relative abundance of gut bacteria at the phylum and genus levels in the taVNS group before and after treatment is shown (Figure 4b). There were significant differences in the phyla *Campylobacterota* ($P = 0.008$) and *Patescibacteria* ($P = 0.023$) before and after treatment (Figure 4c). Notably, taVNS led to a significantly improved level of the genus *Bifidobacterium* ($P = 0.038$) (Figure 4d).

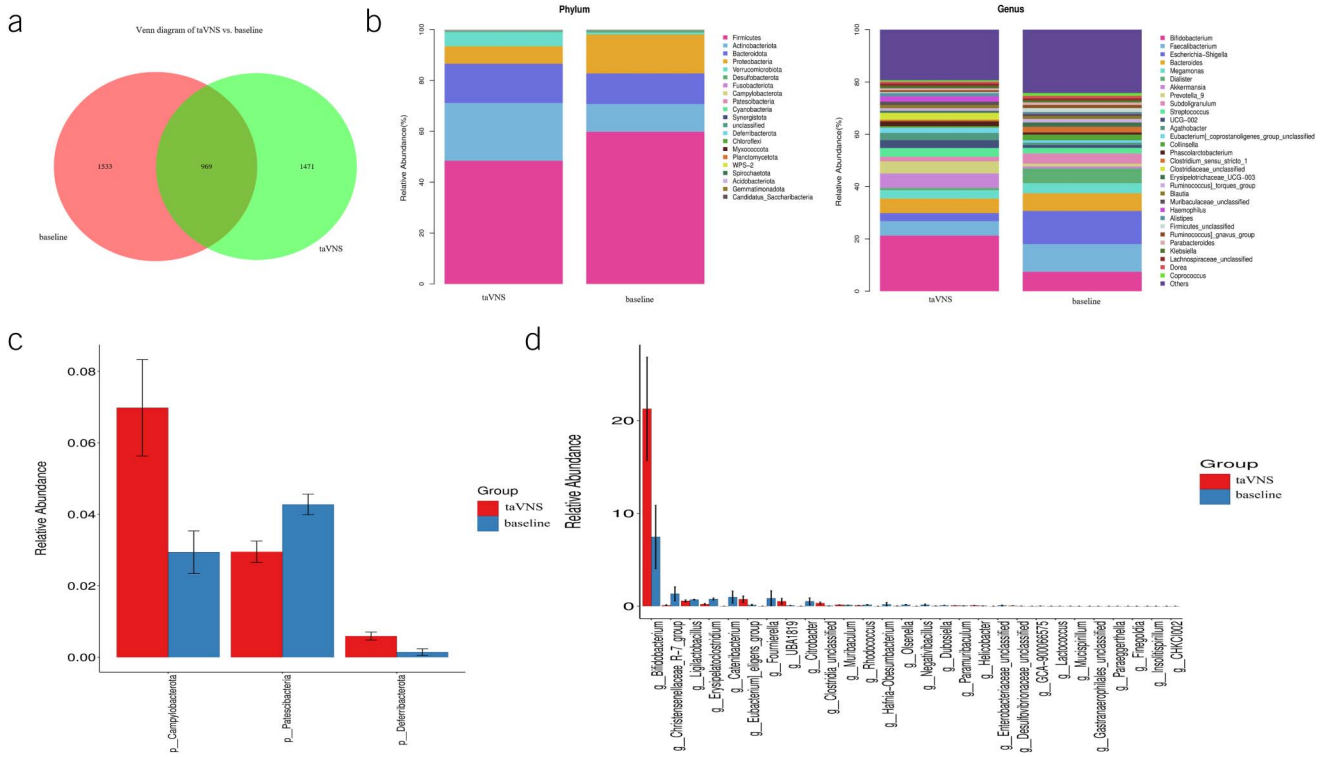

Figure 4. Gut microbiota in taVNS group patients before and after treatment (n = 11). (**a**) Venn diagram showing common taxa. (**b**) Gut microbiota compositions at the phylum and genus levels. Bar graphs of the differentially enriched bacteria are shown at the (**c**) phylum level and (**d**) genus level. taVNS, transcutaneous auricular vagal nerve stimulation.

Levels of SCFAs before and after treatment. We found significantly higher levels of acetic acid ($P = 0.003$), butyric acid ($P = 0.011$), and propionic acid ($P = 0.005$) in patients with IBS-C after taVNS at week 4 compared with baseline. However, there were no differences in other SCFAs between pre-taVNS treatment and post-taVNS treatment (Figure 5).

Levels of tryptophan metabolites before and after treatment. Using UHPLC-MS/MS, we found that 3-hydroxyanthranilic acid ($P = 0.007$), anthranilic acid ($P = 0.026$), and L-tryptophan ($P = 0.002$) were markedly less abundant in patients with IBS-C after taVNS therapy at week 4 compared with baseline (Figure 6).

Correlation analysis between pain score and multiomics data

Correlation between abdominal pain score (VAS score) and gut microbiota and metabolic profiles was tested using Spearman analysis. The outcomes revealed significantly positive correlations between abdominal pain score (VAS score) and Bifidobacterium ($P = 0.005$), Paramuribaculum ($P = 0.038$), Mucispirillum ($P = 0.022$), 3-hydroxyanthranilic acid ($P < 0.001$), acetic acid ($P = 0.022$), propionic acid ($P = 0.016$), and butyric acid ($P = 0.033$) in the taVNS group before and after treatment (see Supplementary Figure S8, Supplementary Digital Content 1, http://links.lww.com/AJG/D501).

DISCUSSION

In this clinical trial, we found that a 4-week taVNS intervention significantly ameliorated IBS-C's primary symptoms associated with constipation by improving CSBMs/week and BSFS score and abdominal pain by reducing VAS scores. This led to an overall alleviation of IBS-C symptoms and an improvement in QOL. In addition, taVNS treatment demonstrated beneficial effects on the patient's mental health by reducing levels of anxiety and depression. Moreover, the results of the high anorectal resolution manometry assessments indicated that taVNS improved rectal sensation in patients with IBS-C, including improvements in the threshold volume for initial sensation, urge to defecate, and RAIR. Mechanistically, taVNS elevated serum levels of Ach and reduced NO, while also enhancing vagal activity as determined by spectral analysis of HRV. Taken together, these results suggest that taVNS could be a therapeutic option for patients with IBS-C.

In this study, we observed significant improvement in major symptoms of constipation and abdominal pain through the non-invasive application of taVNS. After 4-week taVNS therapy, there was an overall improvement in IBS symptoms, including reductions in the severity and frequency of abdominal pain, abdominal bloating, satisfaction with defecation, and QOL, as evidenced by decreases in the IBS-SSS and IBS-QOL scores. This improvement in IBS symptoms likely contributed to the observed alleviation of anxiety and depression, as indicated by reductions in the HAMA and HAMD scores, consistent with prior research (34). Previous randomized clinical trials have demonstrated the effectiveness of taVNS in alleviating abdominal pain in patients with functional abdominal pain disorders and IBS in adolescents (35,36).

Ach plays a crucial role in peristalsis and the acceleration of GI smooth muscle contraction, with its levels linked to vagus nerve activity. Gastric vagal efferent fibers connect with most post-ganglionic neurons of the myenteric plexus along the GI tract (37), forming cholinergic pathways that influence GI motility. Muscarinic Ach receptors M2 and M3 are primary subtypes expressed in alimentary smooth muscle cells and are responsible

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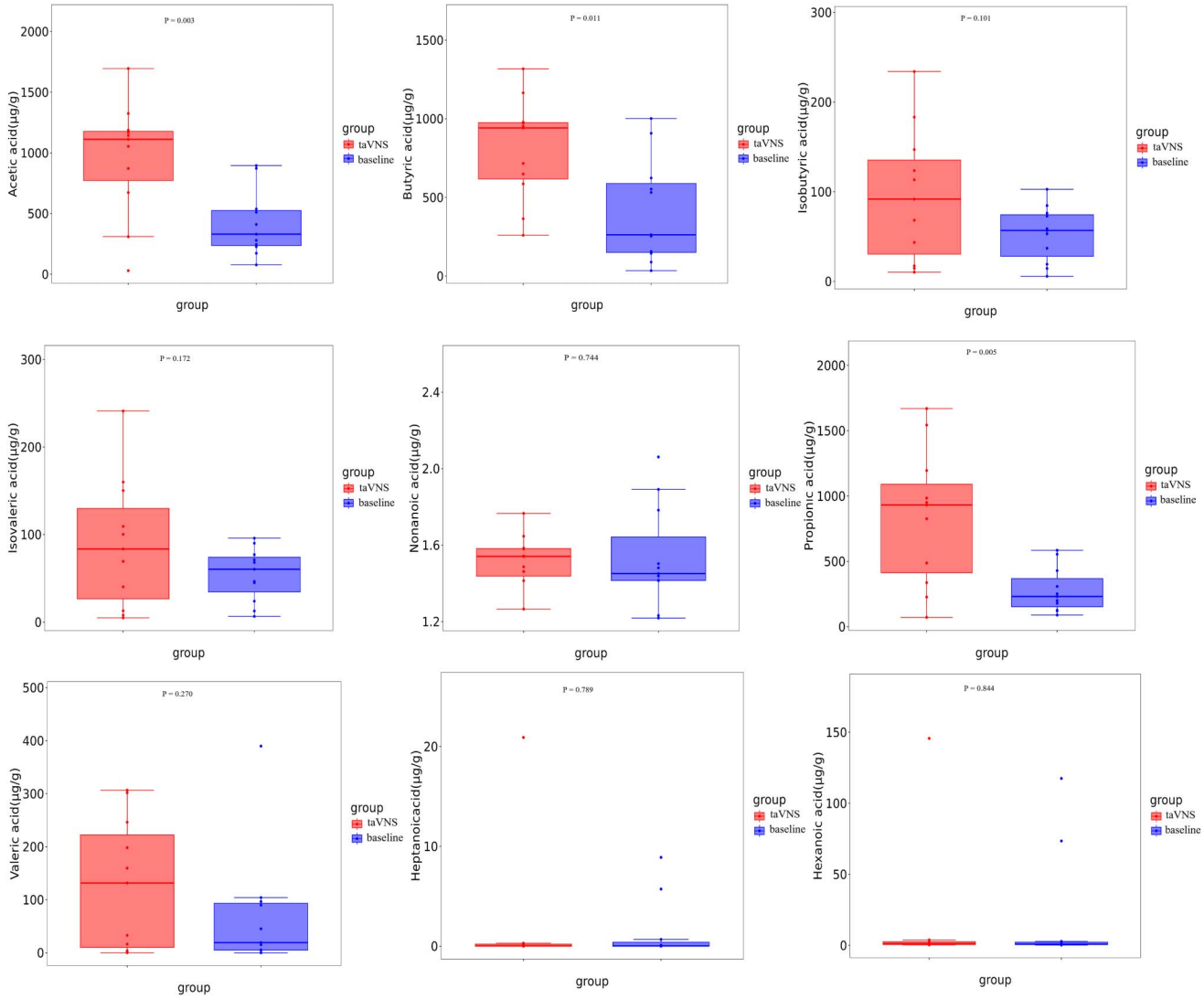

Figure 5. Differences in SCFAs in taVNS group patients before and after treatment. GC-MS analysis showed significantly higher acetic acid ($P = 0.003$), butyric acid ($P = 0.011$), and propionic acid ($P = 0.005$) levels in patients received taVNS. Parametric or nonparametric tests for paired samples were used. GC-MS, gas chromatography-mass spectrometry; SCFA, short-chain fatty acid; taVNS, transcutaneous auricular vagal nerve stimulation.

for Ach-induced intestinal contractions (38,39). Animal studies using M2 or M3 receptor knockout mice have shown that these receptors trigger contraction of the ileum by different mechanisms, whereas in wild-type mice, a synergistic pathway involving both subtypes is activated (40). Meanwhile, Liang et al (41) demonstrated that electric acupuncture effectively alleviated constipation in model mice by reducing NO release. Our study revealed a significant increase in serum Ach content after taVNS treatment, supporting the involvement of taVNS in the vagal-cholinergic pathway. Conversely, NO, considered an inhibitory neurotransmitter in the enteric nervous system, was significantly decreased after taVNS intervention. Collectively, changes in Ach and NO levels in blood may contribute to the beneficial effects of taVNS in patients with IBS-C.

VHS and delayed gut transit are recognized as the main underlying processes in IBS-C (42). Our previous research demonstrated that deep, slow breathing significantly enhanced the threshold volume for initial sensation, urge to defecate, and maximum tolerable volume in patients with IBS-C, potentially through the augmentation of vagal activity (29). In this trial, we found that taVNS had a similar effect on improving rectal sensation in patients with IBS-C, akin to prior studies using transcutaneous electrical acustimulation. Huang et al (42) reported that transcutaneous electrical acustimulation raised the threshold for rectal feeling, including the need to have a bowel movement and maximal tolerance, in patients with IBS-C. Our study revealed that taVNS hindered both the minimum volume of rectal distention needed to trigger the RAIR and the threshold volume for the first feeling of rectal distention and the desire to defecate. The enhancement in rectal sensation caused by taVNS may have had a role in reducing stomach pain complaints. The enhanced rectal sensation seen with taVNS may be ascribed to the likely stimulation of the vagus nerve in the rectum and/or a putative pathway that involves the transmission of signals from the vagus nerve to the sacral nerves.

It is commonly acknowledged that GI motility is influenced by the activation of the vagus nerve and/or the suppression of the sympathetic nervous system, with dysfunction in autonomic neurological processes being a key factor in the manifestation of

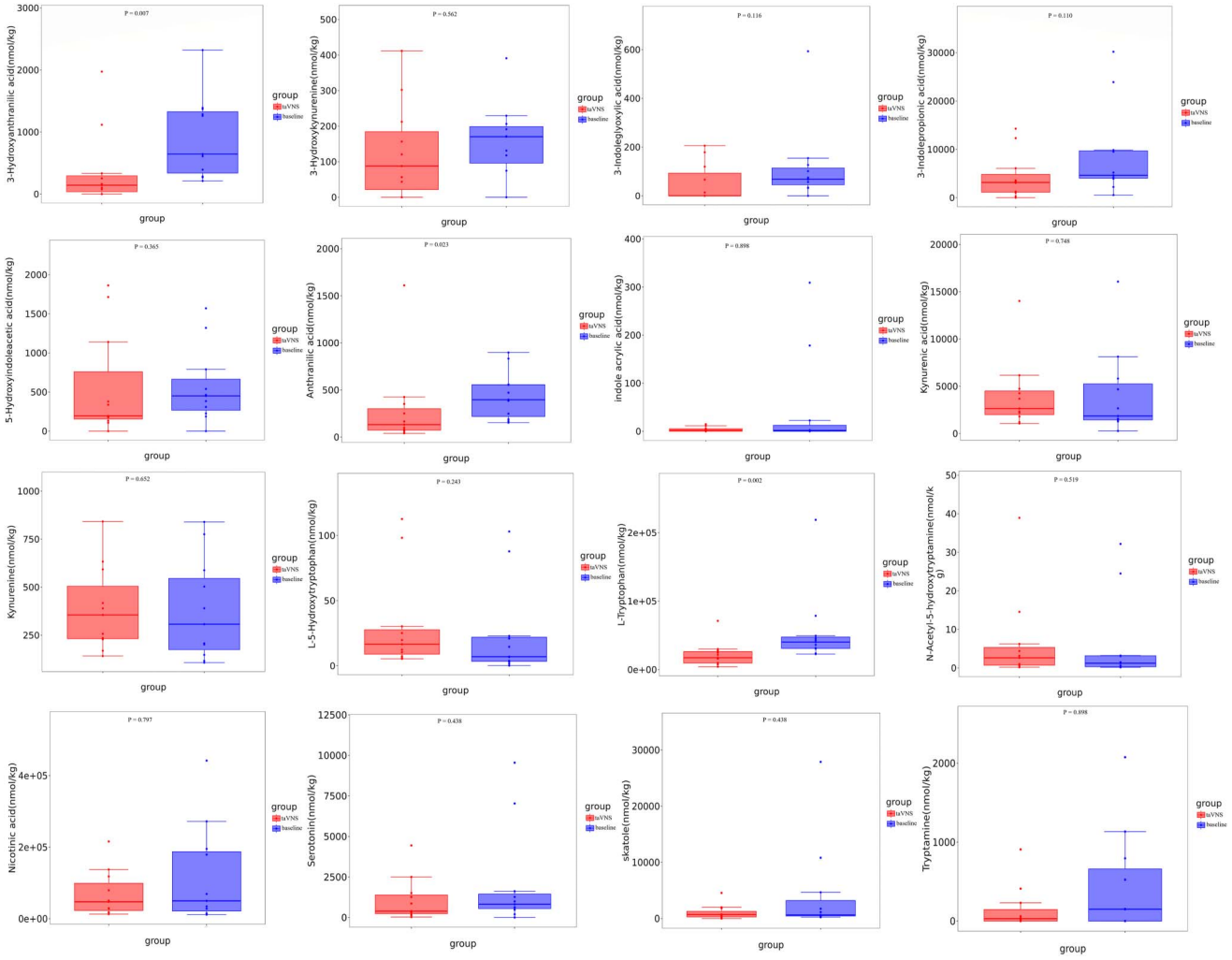

Figure 6. Differences in tryptophan metabolites in taVNS group patients before and after treatment. UHPLC-MS/MS analysis showed significantly lower 3-hydroxyanthranilic acid, anthranilic acid, and L-tryptophan levels in patients received taVNS. Parametric or nonparametric tests for paired samples were used. taVNS, transcutaneous auricular vagal nerve stimulation; UHPLC-MS/MS, ultra-high-performance liquid chromatography coupled with mass spectrometry.

compromised GI motility (43). Prior clinical study has shown that taVNS decreases sympathetic nervous system function (44). VHS is a key factor in chronic abdominal pain associated with functional GI disorders, and vagus nerve modulation has been shown to have an antinociceptive effect within the viscera (45,46). Through spectral analysis of HRV, we observed that taVNS increased vagal activity while decreasing sympathetic activity. These intriguing results underscore the involvement of autonomic mechanisms in the alleviation of IBS-C symptoms with taVNS. Although the precise neural pathway was not explored in this study, prior clinical research (47) indicated that taVNS increased the gut-brain axis through a nucleus of the solitary tract-midbrain pathway. Therefore, it is possible that taVNS enhances vagal activity, leading to improvements in colon motility and VHS, ultimately reducing key symptoms of constipation and abdominal pain.

The novelty of our findings lies in the possible implications of taVNS on the gut microbiome and metabolic profiles. The brain-gut-microbiome axis is involved in understanding the IBS pathophysiology. A previous study revealed a link between a reduction in *Bifidobacterium* and the dysfunction of the gut barrier (48). In addition, animal research demonstrated that moxibustion therapy led to an increase in the relative abundance of *Lactobacillus* and *Clostridium XIVa* while decreasing levels of *Prevotella*, *Bacteroides*, and *Clostridium XI* (49). Pu et al (50) manifested that depression-like symptoms and systemic inflammation caused by transferring depression-related bacteria by fecal microbiota transplantation may be avoided by subdiaphragmatic vagotomy. This research highlights the importance of the gut-microbiota-brain axis, which is reliant on the vagus nerve, in this process. Our investigation revealed that taVNS had a substantial positive impact on the abundance of the species Bifidobacterium, which aligns with the outcomes of our prior preclinical investigation (11). This investigation is the first clinical investigation to demonstrate the taVNS implications on gut microbiota in patients with IBS-C. We found that taVNS significantly improved the SCFAs levels (e.g., acetic, butyric and propionic acids) in patients with IBS-C. In addition, taVNS led to decreased levels of tryptophan-derived metabolites, including 3-hydroxyanthranilic acid and L-tryptophan, in patients with IBS-C. Collectively, these findings suggest that gut microbiota and their derived SCFAs and tryptophan-derived metabolites could be contributed in the positive consequences of taVNS in patients with IBS-C.

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This study has several limitations, including (i) it was a single-blind trial conducted at a single center with a small sample size. Therefore, double-blind or triple-blind designs (blinding of patients, researchers, and statistical analysts) are required in the future; (ii) in the intention-to-treat analysis, subsequent data should be imputed and included in the final analysis for patients who were lost to follow-up. However, it is difficult to predict the outcomes after treatment or imputation of data based on baseline measurements. Therefore, these data were excluded; (iii) patients were not classified into normal or slow colon transit categories; (iv) no long-term follow-up observations were made; (v) only single-arm multiomics data (pre-taVNS and post-taVNS) were analyzed. Therefore, further research is warranted.

In summary, this clinical study suggests that noninvasive taVNS can improve both constipation and abdominal pain, as well as anxiety and depression, in patients with IBS-C. The alleviation of IBS-C symptoms by taVNS may be attributed to its integrative effects on rectal functions mediated through vagal, cholinergic, and multiomics mechanisms.

CONFLICTS OF INTEREST

Guarantor of the article: Yue Yu, MD, PhD.

Specific author contributions: Y.Y. and K.H.: designed the study. Y.Y.: received funding for the study. J.L., C.L., M.Y., M.Z., B.W., and J.T.: carried out the study. Y.Y. and J.L.: administered the project. J.L. and C.L.: analyzed the data. Y.Y.: verified the data. J.L. and K.H.: drafted the manuscript. All authors provided a critical review and approved the final manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Potential competing interests: None to report.

Trial registration: http://www.chictr.org.cn (ChiCTR2400085832).

Data transparency statement: The data for this study are available to other researchers for scientific research purposes on request and after the proposed analysis plan has been approved. For data access, please contact the corresponding author. The raw sequence data have been deposited in the SRA database under accession number PRJNA1108941.

Study Highlights

WHAT IS KNOWN

- ✓ The dysfunction of the vagal pathway plays a role in the pathophysiology of irritable bowel syndrome (IBS).
- ✓ The efficacy of vagus nerve stimulation for IBS management has been studied.

WHAT IS NEW HERE

- ✓ Transcutaneous auricular vagus nerve stimulation (taVNS) ameliorated constipation-predominant IBS-related symptoms and rectal functions.
- ✓ taVNS activated vagus nerve activity and cholinergic pathway.
- ✓ taVNS improved gut microbiome and metabonomic profiles.

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