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Original Article

# Effects of Electroacupuncture Combined with Chinese Herbal Medicine on Gut Microbiota and Metabolomics in Amyotrophic Lateral Sclerosis: A Prospective Study

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## Abstract

**Background and objectives:** Recent studies have highlighted a link between amyotrophic lateral sclerosis (ALS) and gut microbiota. This prospective study aimed to evaluate the effects of electroacupuncture combined with Chinese herbal medicine on gut microbiota and metabolomics in ALS patients.

**Methods:** Ten ALS patients were randomly assigned to either a treatment group (electroacupuncture with Chinese herbal medicine, n = 6) or a control group (waiting treatment, n = 4). Healthy controls (age- and sex-matched, n = 10) were also included. Data were collected after 12 sessions of electroacupuncture and follow-ups at three and six months. ALS functional rating scale scores were documented pre- and post-treatment. Stool samples were collected at two time points (T0 and T4 weeks) and analyzed, and metabolomic profiles from urine samples were analyzed post-treatment. Heatmap correlation analysis was used to explore relationships between microbiota, metabolomics, and clinical outcomes.

**Results:** Treatment with electroacupuncture reduced *Eisenbergiella* abundance in the treatment group. A significant positive correlation was found between *Lachnospiraceae* and ALS functional rating scale scores ($P < 0.005$ and $P < 0.001$, respectively). Differential expression of purine metabolism was observed in ALS patients ($P = 0.0017$).

**Conclusions:** Imbalances in the gut microbiome and metabolic disorders have been found among patients with ALS. These imbalances appear to be partially mitigated by treatment with electroacupuncture combined with Chinese herbal medicine. Our research suggests that *Eisenbergiella* might be a diagnostic biomarker and a potential therapeutic target for ALS.

## Introduction

Amyotrophic lateral sclerosis (ALS) is one of the World Health Organization's "five most difficult diseases," alongside cancer, AIDS, leukemia, and rheumatoid diseases. It is a neurodegenerative disorder characterized by the degeneration of both upper and lower motor neurons, leading to motor and non-motor symptoms, and often resulting in death from respiratory failure.[1] The annual incidence of ALS is approximately 1–2.6 cases per 100,000 people, with a prevalence rate of six cases per 100,000.[2] Ninety percent of ALS cases are sporadic, and the average age of onset is 58–60 years, with a typical survival time of three to four years.[3] The primary pathological feature is the progressive death of motor neurons. The underlying mechanisms are complex, and treatment options remain limited. Riluzole, the standard drug, extends survival by two to three months but is expensive and has side effects such as fatigue and nausea.[4]

Acupuncture, a non-drug intervention, shows promise for treating neurodegenerative diseases, including ALS. Both domestic and international studies have explored its potential,[5] and clinical research has rapidly developed since 2016. While the majority of studies are case reports, accounting for 59% of the total, with cohort studies making up the second largest proportion at 28%, clinical randomized controlled trials on acupuncture for ALS began in

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2016, with only four published to date, accounting for 13% of the research.[6] Acupuncture may enhance gut microbiota diversity and increase the content of beneficial microbiota, which could impact neuroinflammation and muscle strength in ALS patients.[7] Studies show that gut microbiota composition differs between ALS patients and healthy controls,[8] and acupuncture may improve swallowing and articulation, potentially delaying disease progression.[9,10]

Metabolomics, a technique for detecting dynamic responses to genetic or exogenous changes,[11] plays a critical role not only in the development of new diagnostic and therapeutic methods for human diseases but also in understanding the molecular mechanisms underlying those diseases.[12] Recently, an increasing number of studies have focused on the use of metabolomics in ALS.[13] It has been used to study ALS, including the connection between ALS and purines.[14] However, few studies have examined whether acupuncture could improve ALS by modulating metabolomics and gut microbiota. Gut-derived bacteria and toxins can affect the blood-brain barrier and contribute to neuroinflammation.[15] While previous studies have shown that acupuncture can improve the quality of life for ALS patients,[16] this study is the first to demonstrate that combining electroacupuncture with Chinese herbal therapy can improve the quality of life by regulating gut microbiota and metabolomics. This research will contribute to the design of larger prospective studies in the future.

## Materials and methods

### *Study cohort*

This prospective study analyzed data from ALS patients across various districts in Beijing. The study employed a placebo-controlled design with two parallel groups to investigate the effects of acupuncture combined with Chinese herbal medicine on the gut microbiome over a four-week intervention. Ten ALS patients voluntarily participated and were assigned to either the electroacupuncture group (n = 6) or the control group (waiting treatment group, n = 4). Informed consent was obtained from all participants before inclusion. Stool samples were collected at the beginning and end of the intervention for analysis. This study adhered to the Declaration of Helsinki and written informed consent for publication was obtained from all patients or their legal guardians. Given the rarity of ALS, recruitment was challenging, and the study included a small number of cases. Participants were recruited from the Beijing Askang Medical Technology Co., Ltd. patient platform from January to February 2023.

Inclusion criteria followed the 2019 Chinese Guidelines for the Diagnosis and Treatment of Amyotrophic Lateral Sclerosis. Patients were aged 30–70 years and had progressive ALS symptoms confirmed through clinical, neuro-electrophysiological, or pathological examinations. Patients who had not taken any hormonal drugs within two months and who signed the informed consent form were included.

Exclusion criteria included serious comorbidities (e.g., heart, brain, liver, kidney disease) or acupuncture treatment for ALS-related functions within the month prior to enrollment.

Ten healthy family members of ALS patients were recruited as matched controls. Healthy participants, aged 30–70, had no history of cardiovascular, hepatic, renal, neurological, or metabolic disorders, and no abnormalities in electrocardiogram, blood pressure, heart rate, respiratory status, or relevant laboratory tests. Demographic and medical history data were collected during the initial assessment.

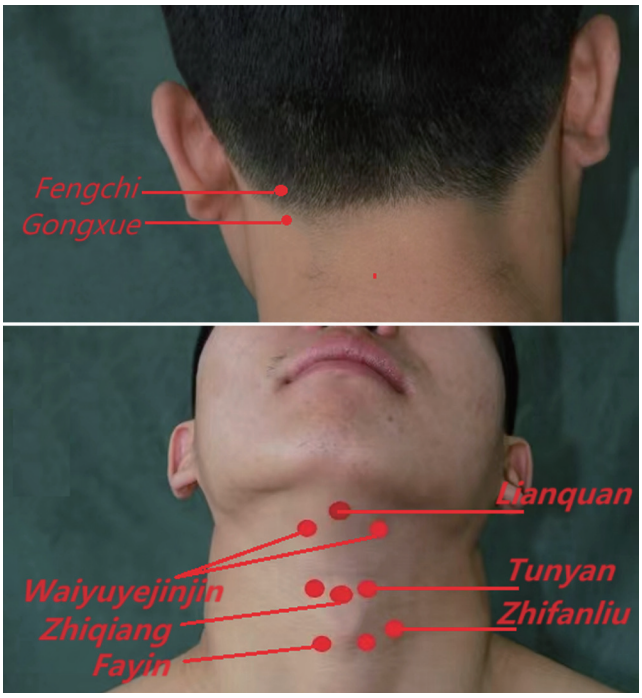


**Fig. 1. Acupuncture point diagram.**

Clinical efficacy was evaluated at five time points: before treatment; half a month, one month, three months, and six months after treatment. Participants were informed that participation was voluntary and that they could withdraw at any time. The study followed Good Clinical Practice guidelines and was approved by the Medical Ethical Review Committee of Capital Medical University (Z2022SY032) and registered in the Chinese Clinical Trial Registry (ChiCTR2400088244).

Patients received treatment at the Traditional Chinese Medicine outpatient department of Capital Medical University. Treatment was administered by Associate Professor Cui Hai, a Deputy Chief Physician with over 30 years of clinical experience in Chinese medicine. Acupuncture was performed using sterile steel needles from the Andi brand (0.40 × 40 mm, manufactured by Suzhou Medical Appliance in Jiangsu, China) and placebo needles (0.30 × 25 mm).

Acupuncture points included Fengchi and Gongxue on the neck, and Lianquan and Tunyan on the larynx, with a 0.25 mm × 40 mm needle to avoid deep insertions that may cause injury. The needle was quickly inserted, and the neck and lumbar spine points were directly stabbed for 15–25 mm. The tonifying and purging techniques, combined with twisting and lifting, were adopted. The degree of the patient's self-consciousness was assessed for local acid swelling. The same set of electrodes from the KW808-2 electric anesthesia instrument of the Great Wall brand was connected to the Fengchi and Gongxue acupoints on both sides of the spine (Fig. 1). The electrode connection aligned the current direction with that of the motor nerve conduction bundle. Electroacupuncture was applied at a 2 Hz frequency, with intensity adjusted according to patient tolerance. The therapy was administered on Tuesdays and Thursdays per week, and the electroacupuncture group also received Qidong Huoluo granules (Astragalus, Ophiopogon, etc.), a traditional Chinese medicine compound designed by our research group, with one dose per day.

### *Outcome measures*

#### Clinical outcome assessments

The ALS functional rating scale (ALSFRS) consisted of 12 items[17]: (1) Language; (2) Salivary secretion; (3) Swallowing; (4) Writing; (5) Cutting food and using utensils; (6) Dressing and health care; (7) Turning over and tidy the bedding on the bed; (8) Walking; (9) Climbing the stairs; (10) Difficulty breathing; (11) Sitting and breathing; and (12) Insufficient breathing. Each item was rated on a scale of 0–4, with total scores ranging from 0 (severely impaired) to 48 (normal). Pre- and post-treatment scores were assessed by trained specialists.

Manual muscle testing was performed using a modified classification,[18] The evaluation was as follows: Level 0: No muscle contraction observed (0 points); Level 1: Slight muscle contraction palpable, but no joint movement (1 point); Level 2: Joint could move to the maximum range of motion with the influence of gravity removed (2 points); Level 3: Can resist gravity, joint can move to the maximum range of motion (3 points); Level 4: Can resist moderate resistance while moving joint through the maximum range of motion (4 points); Level 5: Can resist sufficient resistance while moving joint through the maximum range of motion (5 points); An "S" or "SS" (S - spasticity) was added if spasticity is present, and "C" or "CC" (C - contracture) was added if there was contracture, indicating a special condition of the limb. Proximal and distal extremities were scored from 0 to 5 points for each part. The total rating ranged from 0 to 40, with higher scores indicating better muscle strength.

#### Sample collection and DNA extraction

Stool samples were collected at the Capital Medical University research center and frozen at −80°C within one hour. DNA was extracted using a QIAamp Fast DNA Stool Mini Kit (Qiagen, California, USA), and DNA concentration was measured with a NanoDrop 2000 (Thermo Scientific, USA).

#### DNA extraction for sequencing

DNA extraction for sequencing was performed using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). After DNA extraction from the experimental group, DNA concentration was measured with a TBS-380, and purity was assessed with NanoDrop 200. Integrity was evaluated via 1% agarose gel electrophoresis. DNA fragments were sheared to approximately 400 bp using a Covaris M220 (Gene Company Limited, China), and a PE library was constructed using NEXTFLEX Rapid DNA-Se (Bio Scientific, Austin, TX, USA). After Polymerase Chain Reaction (PCR) amplification, the PE150 sequencing platform was used to perform macro-factorial sequencing.

#### Microbial analysis

Raw sequencing data were processed using Fastp software for quality control. High-similarity reads were removed using BWA software, and MEGAHIT software assembled the sequences, selecting contigs ≥ 300 bp as the final assembly output. Open reading frame prediction of the assembled contigs was performed using Prodigal v2.6.3. The gene sequences were clustered using CD-HIT software to generate non-redundant gene sets. SOA Paligner software aligned high-quality reads to the gene sets at 95% identity for gene abundance quantification.

#### Non-targeted metabolomics analysis

Approximately 50 mg of each sample was mixed with 400 μL of methanol solution (containing 5 μg/mL L-2-chloro-phenylalanine as an internal standard. The mixture was swirled for 1 m, homogenized at 60 Hz for 3 m (twice), and then centrifuged at 13,000 rpm for 10 m at 4°C. The supernatant was collected. Quality control samples were created by mixing equal amounts of each sample. LC-MS analysis was conducted using Agilent 1290 Infinity ultra-high performance liquid chromatography, Agilent 6545 ultra high definition, and accurate-mass quadrupole time-of-flight spectrometry systems. The chromatographic column was Waters XSelect OR HSS T3 (2.5 μm, 100 × 2.1 mm), and the mobile phases were aqueous solution (0.1% formic acid) and B-acetonitrile (0.1% formic acid). Flow rate: 0.4 mL/m. Column temperature: 40°C. Sample size: 4 μL. Optimized chromatographic gradient: 0–3 m, 20% B; 3–9 m, 20–95% B; 9–13 m, 95% B; 13–13.1 m, 95–5% B; 13.1–16 m, 5% B. Mass spectrometry was conducted in both positive and negative ion modes, with a mass spectrum range of m/z 50–1,500.

#### Kyoto Encyclopedia of Genes and Genomes (KEGG) functional annotation

The KEGG database (http://www.genome.jp/kegg/) was a comprehensive resource for the systematic analysis of gene function, providing a vast knowledge base that integrated genomic and functional information. The KEGG GENES database provided gene and protein sequence details, while the KEGG PATHWAY database encompassed various pathways such as metabolic, synthetic, membrane transport, signaling, cell cycle, and disease-related pathways. The KEGG LIGAND database contained information on various chemical molecules, enzymes, and enzymatic reactions. The KEGG Module database comprised functional units curated by KEGG for genome annotation and biological interpretation. KEGG Orthology (KO) served as a phylogenetic classification system that grouped genes with similar sequences and functions, annotating known gene functions across species. BLASTP (BLAST Version 2.2.28+, http://blast.ncbi.nlm.nih.gov/Blast.cgi) was employed for non-redundant GENES set sequence comparison with the KEGG GENES database, with an e-value threshold of 1e-5. Functional annotation was performed using the KO-based Annotation System, and abundances in functional categories were calculated based on gene abundances associated with KO, Pathway, and Module categories.

## Results

### *Baseline characteristics*

Table 1 shows the baseline characteristics of the electroacupuncture group. Except for body mass index ($P = 0.01$), no significant differences were observed among the treatment groups in terms of demographic and clinical characteristics (all $P > 0.05$).

### *Clinical outcome*

#### ALSFRS after electroacupuncture treatment

After four weeks of treatment, Figure 2 shows a significant improvement in the ALSFRS for the electroacupuncture combined with the Chinese herbal therapy group compared to the waiting acupuncture group. Statistical analysis revealed a significant difference in ALSFRS scores between pre- and post-treatment ($P = 0.0045$). Clinical assessments were conducted at five time points: pre-treatment, half a month of treatment, at the end of treatment, three months post-treatment, and six months post-treatment (Fig.

**Table 1. Comparison of baseline data among the electroacupuncture, waiting treatment, and healthy control groups**

| Category | Electroacupuncture combined Chinese herbal group (n = 6) | Waiting acupuncture group (n = 4) | Healthy controls (n = 10) | *P*-value |
|---|---|---|---|---|
| Age (year) | 53.00 ± 11.59 | 49.00 ± 9.90 | 56.80± 14.82 | 0.597 |
| Sex, n, female | 3 (50.0%) | 1 (25.0%) | 6 (60.0%) | 0.289 |
| Years of education (year) | 11.83 ± 3.66 | 13.25 ± 3.40 | 13.00±2.83 | 0.728 |
| BMI (kg/m2), mean ± SD | 20.24 ± 3.43 | 23.36 ± 1.28 | 23.85±0.94 | 0.01 |
| Bristol scores of stools, mean ± SD | 4.17 ± 0.41 | 4.00 ± 0.82 | 4.40 ± 0.97 | 0.682 |
| Duration of the disease (month) | 105.67 ± 99.87 | 90.50 ± 35.23 | / | 0.781 |
| Expected percentage improvement in acupuncture(%) | 53.33 ± 15.06 | 68.75 ± 6.29 | / | 0.093 |
| Speech | 2.50 ± 0.84 | 3.00 ± 0.82 | / | 0.378 |
| Salivation | 3.17 ± 0.75 | 2.75 ±0.50 | / | 0.363 |
| Ingurgitation | 3.00 ± 0.89 | 2.50 ± 0.58 | / | 0.356 |
| Writing | 2.50 ± 1.22 | 2.50 ± 0.58 | / | 0.100 |
| Cut food | 3.67 ± 0.52 | 3.50 ±1.00 | / | 0.735 |
| Handling equipment | 4.00 ± 0.00 | 3.25 ± 0.96 | / | 0.083 |
| Clothing and hygiene | 2.17 ± 0.98 | 2.50 ± 0.58 | / | 0.562 |
| Turn over in bed and make the bedding | 2.83 ± 1.17 | 2.50 ± 0.58 | / | 0.616 |
| Walking | 2.50 ± 1.05 | 1.50 ± 1.73 | / | 0.283 |
| Climb stairs | 1.83 ± 1.33 | 1.50 ± 1.73 | / | 0.738 |
| Respiratory rate | 3.50 ± 1.22 | 3.00 ± 0.00 | / | 0.447 |
| Respiratory function | 3.50 ± 1.22 | 3.00 ± 0.00 | / | 0.447 |
| The deltoid | 3.33 ± 1.86 | 2.50 ± 1.29 | / | 0.462 |
| The triceps | 3.33 ± 1.86 | 2.50 ± 1.29 | / | 0.462 |
| The biceps | 3.33 ± 1.86 | 2.50 ± 1.29 | / | 0.462 |
| Extensor carpi | 3.33 ± 1.63 | 2.50 ± 1.29 | / | 0.419 |
| Carpal flexor | 3.00 ± 1.55 | 2.50 ± 1.29 | / | 0.610 |
| Iliopsoas muscle | 3.50 ± 1.38 | 2.50 ± 1.29 | / | 0.283 |
| The quadriceps | 4.00 ± 1.26 | 2.50 ± 1.29 | / | 0.106 |
| Biceps femoris | 4.00 ± 1.26 | 2.50 ± 1.29 | / | 0.106 |
| Tibialis anterior muscle | 4.00 ± 1.26 | 2.50 ± 1.29 | / | 0.106 |
| The gastrocnemius | 4.00 ± 1.26 | 2.50 ± 1.29 | / | 0.106 |

BMI, body mass index, was calculated as weight in kilograms divided by height in meters squared. SD, standard deviation. Elevated values signify a superior status. Statistical analysis used non-parametric testing.

2a). This suggests that electroacupuncture combined with Chinese herbal therapy may improve ALS patient outcomes.

### Manual muscle testing score in ALS participants

After four weeks of electroacupuncture treatment (Table 2), there was no significant improvement in the manual muscle testing scores for the electroacupuncture combined with the Chinese herbal group ($P > 0.05$). This suggests that electroacupuncture combined with Chinese herbal medicine could improve some, but not all, in patients with ALS.

No adverse events were reported, and the incidence of adverse events did not differ significantly between the treatment (electroacupuncture and Chinese herbal) and control groups. Additionally, there was no significant bleeding observed in the combined treatment group.

### *Changes in fecal microbial diversity and metabolic signatures in ALS*

Figure 3a shows that the Sob index at the genus level revealed no significant difference in alpha diversity of the gut microbiota among ALS groups. The composition analysis chart of gut bacterial group management in the ALS participant groups illustrates the first principal component on the horizontal axis (contributing 17.44%) and the second principal component on the vertical axis (contributing 11.24%). In the abscissa plot, the first principal component accounted for 19.3%, and the second principal component accounted

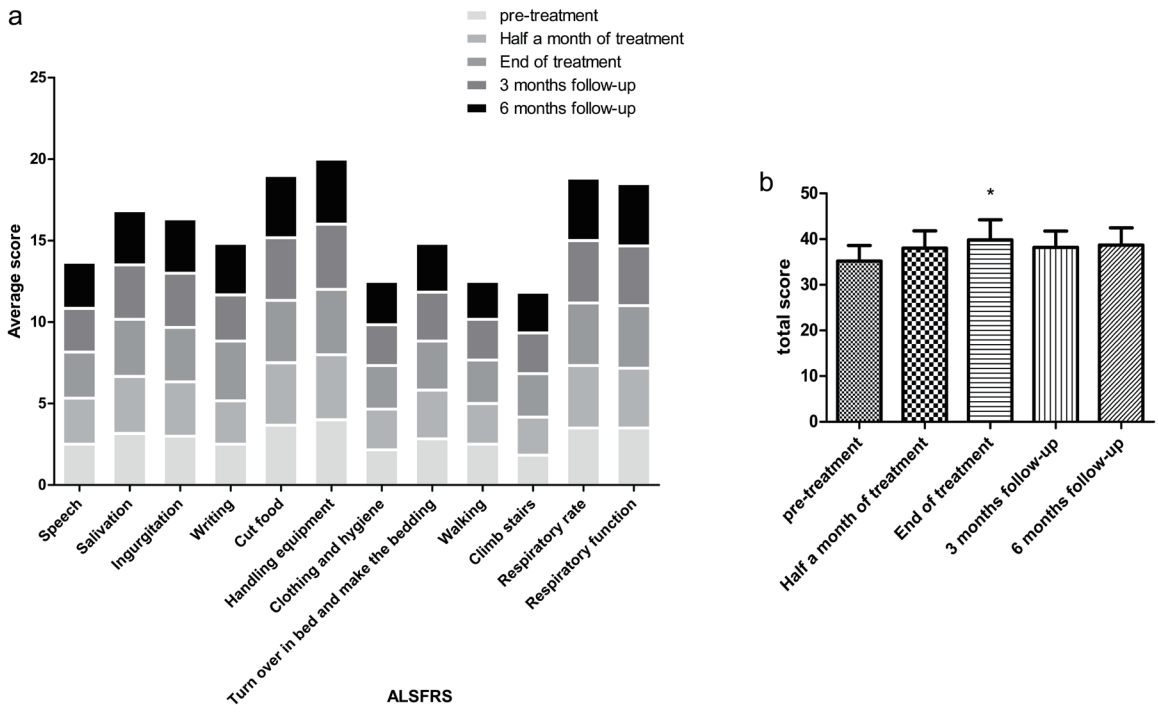


**Fig. 2. Amyotrophic lateral sclerosis (ALS) Functional rating scale after four weeks of treatment and follow-up at three and six months.** (a) Five time points for ALS functional rating scale (ALSFRS). (b) Total score at each time point.

for 11.2% (Fig. 3b). Comparing the distances of each point, patients with ALS clustered in the Principal Coordinate Analysis diagram, indicating substantial differences in the gut bacteria of patients with ALS before and after treatment. Typing analysis and stability between patients with ALS and healthy controls were evaluated. The average variation degree was 0.473 in healthy controls, while the average variation degree in ALS patients was 0.548, indicating that the colony stability of the healthy population was better than that of the patient group. From the typing analysis, the difference between the healthy group and the patient group was obvious (Fig. 3b).

### *Electroacupuncture combined with Chinese herbal medicine partially ameliorates gut dysbiosis*

After treatment, no significant differences were observed at the phylum level in *Firmicutes* ($P > 0.05$). However, significant changes were found at the family level in *Lachnospiraceae* ($P = 0.02032$), *Ruminococcaceae* ($P = 0.03393$), and *Monoglobaceae* ($P = 0.03439$) (all $P < 0.05$) (Fig. 4a). Additionally, the amount of *Eisenbergiella* ($P = 0.003473$) in the electroacupuncture combined with Chinese herbal group was significantly decreased at the genus level. Compared with the pre-treatment group, the number of *Ruminococcus* increased after electroacupuncture combined with Chinese herbal treatment ($P = 0.03167$). In LEfSE Bar analyses, *Ruminococcus* and *Monoglobus* were enriched in healthy controls, whereas *Blautia* and *Lachnospiraceae* were more abundant in ALS patients (Fig. 4b).

**Table 2. Comparison of MMT between the electroacupuncture combined Chinese herbal group and the waiting treatment group**

| Variables | Electroacupuncture combined Chinese herbal group | Waiting treatment group | *P* |
|---|---|---|---|
| MMT (the deltoid) | 4.00 ± 1.10 | 2.50 ± 1.29 | 0.083 |

MMT, manual muscle test.

### *Relationships between gut microbe and ALS clinical indicators*

At the genus level, there was a positive correlation between *Ruminococcus* and ALSFRS of major clinical indicators (ingurgitation, writing, handling equipment, clothing and hygiene, bedding, walking, climbing stairs, and breathing). In other words, the higher the abundance of *Ruminococcus*, the more conducive it was to improving the efficacy of ALSFRS scores for major clinical indicators. There was also a positive correlation between the beneficial bacteria *Lachnospiraceae* and the clinical indicators ALSFRS, indicating that the higher the abundance of *Lachnospiraceae*, the more conducive it was to improving the efficacy of the main clinical indicators in ALSFRS scores (Fig. 5).

### *Comparison of metabolites in ALS patients and healthy subjects*

Figure 6a shows that adenosine 3′,5′-diphosphate, 4,11-dichloro-5,12-dihydroquino[2,3-b]acridine-7,1,xi-8-acetonyldihydrosanguinarine, Zonisamide, and Dacarbazine levels in patients were significantly higher than those in healthy controls, suggesting that purine metabolites in the body may increase.

Additionally, a metabolite linear correlation analysis between ALS patients and healthy controls was performed using Pearson correlation in Figure 6b, revealing that adenosine 3′,5′-diphosphate had a positive correlation with 4,11−dichloro−5,12−dihydroquino[2,3−b]acridine−7,14−dione and cyclobrassinin, but a negative relationship with hamamelitannin (all $P < 0.05$).

### *KEGG pathway analysis of gut microbiota and metabolomics*

KEGG function prediction showed that the top 15 metabolic path-

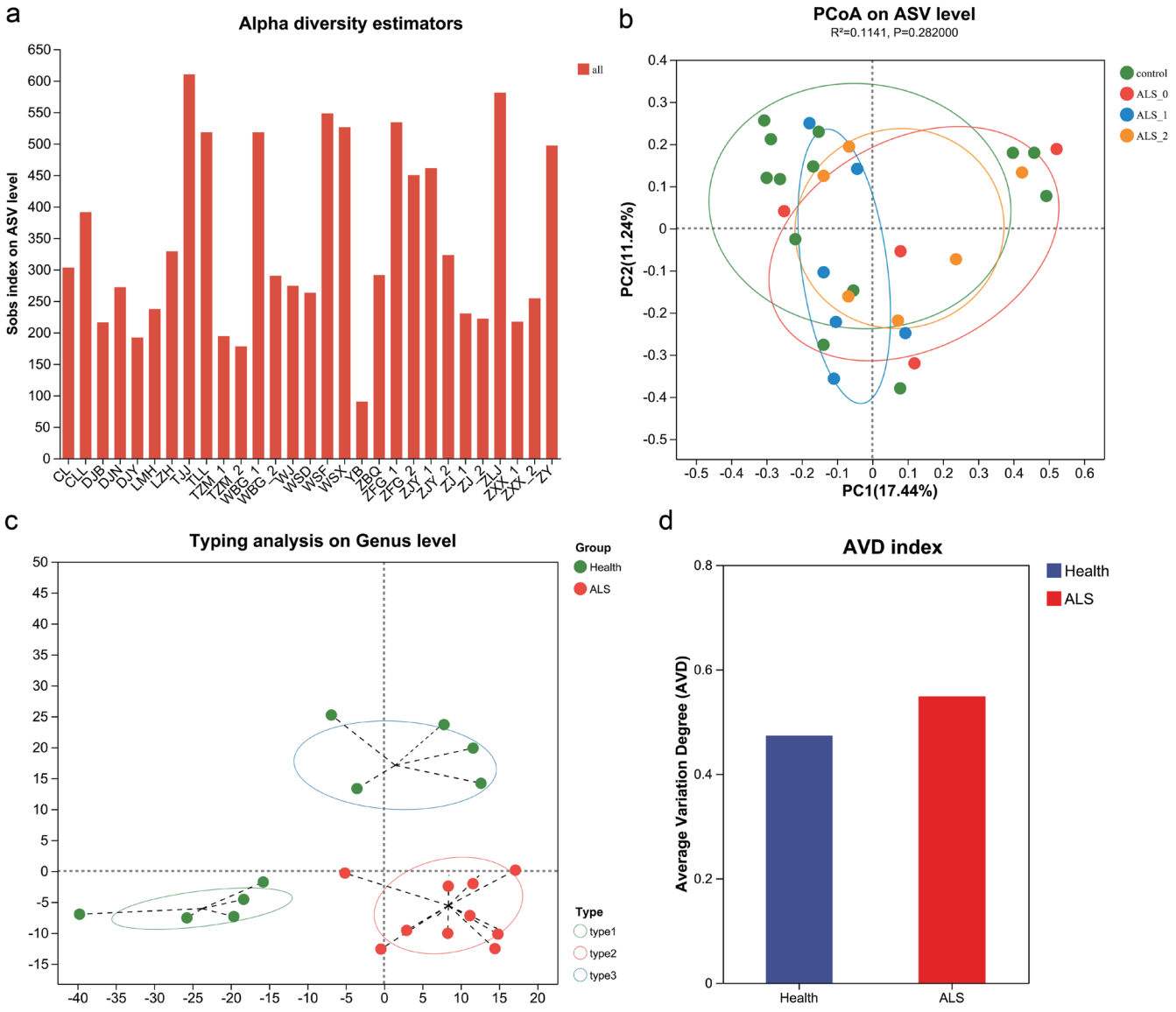


**Fig. 3. Sample basic information.** (a) Alpha diversity using Sob index; (b) Beta diversity in the electroacupunctrue group pre- and post-treatment in principal coordinates analysis (PCoA) at genus level. Colony typing analysis and stability in amyotrophic lateral sclerosis (ALS) patients and healthy controls. (c) Typing at genus level; (d) Average variation degree (AVD) (Healthy group n = 10, ALS group n = 10). ASV, amplicon sequence variant; PC, principal coordinates.

ways in ALS patients were K00799 (glutathione S-transferase), K02350 (DNA polymerase zeta), K03164 (DNA topoisomerase II), K03509 (DNA polymerase eta), K04371 (mitogen-activated protein kinase), K04728 (serine-protein kinase ATM), K06276 (3-phosphoinositide-dependent protein kinase-1), K08734 (DNA mismatch repair protein MLH1), K08735 (DNA mismatch repair protein MSH2), K08736 (DNA mismatch repair protein MSH3), K08738 (cytochrome c), K10847 (DNA-repair protein complementing XP-A cells), K10849 (DNA excision repair protein ERCC-1), K14686 (solute carrier family 31), and K17686 (P-type Cu+ transporter). The gene abundance after electroacupuncture combined with Chinese herbal treatment was higher than that after the treatment, and the gene abundance in K00799 (glutathione S-transferase), K03164 (DNA topoisomerase II), and K08738 (cytochrome c) was higher post-treatment compared to pre-treatment. This indicates that electroacupuncture combined with Chinese herbal treatment could partly improve gene abundance more effectively than the waiting treatment group. From the perspective of gene function prediction, it was confirmed that electroacupuncture combined with Chinese herbal treatment could better regulate the intestinal microflora of ALS patients on certain metabolic pathways (Fig. 7a). Figure 7b demonstrated that purine metabolism was found to be differentially expressed among ALS patients ($P$ = 0.0017), and abnormal expression of enzymes involved in the metabolic pathway was also confirmed.

## Discussion

In this study, the results indicated that combining electroacupuncture with Chinese herbal medicine was more effective than no

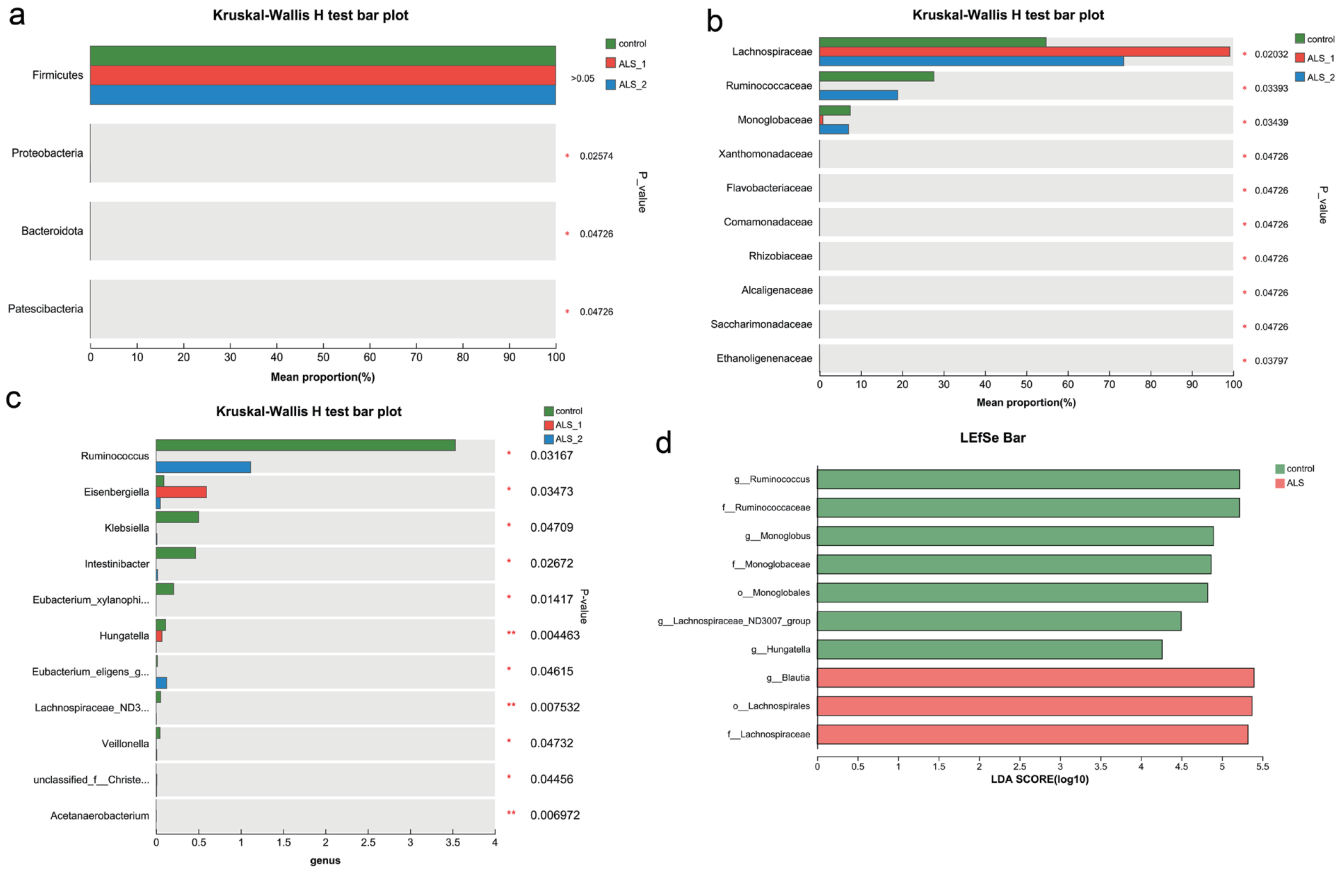


**Fig. 4. Barplots in different level abundance analysis.** Barplots showing genus-level abundance of enriched genera among the pre-treatment group (n = 6), post-treatment group (n = 6), and healthy controls (n = 10) at the phylum (a) and family (b) levels. Barplots of genus-level abundance of enriched genera among the pre-treatment group (n = 6), post-treatment group (n = 6), and healthy controls (n = 10). (c) Barplots illustrate the abundance of enriched genera at the genus level; (d) LEfSe Bar differences between amyotrophic lateral sclerosis (ALS) patients and healthy controls). ALS_1 refers to the pre-treatment group, and ALS_2 refers to the post-treatment group.

treatment for patients with ALS over a four-week period. Muscle atrophy and weakness, as the initial phases of ALS, could represent a window of opportunity for intervention during the early and middle stages of the disease.[19] This study was designed as a prospective study to evaluate the effectiveness and mechanisms of combined acupuncture and herbal medicine therapy for ALS, compared to a non-treatment group. This clinical trial is the first study to explore the impact of combining electroacupuncture and herbal medicine on gut microbiota and metabolomics in ALS patients.

In our recent study, we applied a metagenomic sequencing method to a distinct ALS cohort, alongside a study of a subset of participants that analyzed pre- and post-treatment effects of electroacupuncture combined with Chinese herbal therapy. This approach revealed that ALS was correlated with significant changes in the composition and functionality of gut microbiota, and that one month of electroacupuncture treatment partially ameliorated microbial dysbiosis. The gut microbiome of ALS patients exhibited a marked alteration in overall microbial diversity. Although no significant variations in alpha diversity were observed either within or between individuals in the microbiome study, significant differences in beta diversity were found, indicating a distinct shift from the pre-treatment state. As a result, our findings may provide a basis for optimizing future studies with larger cohorts to ensure sufficient statistical power to capture microbial diversity.

Interestingly, ALS-enriched genera were notably unique compared to those in the normal human gut. It is well accepted that potentially pathogenic bacteria, typically present in low quantities, can thrive and contribute to autoimmunity or inflammation under inflammatory conditions. The increase in pathogenic bacteria may directly contribute to the muscle atrophy observed in patients,[20] which was also reflected in the lower body mass index of the patient group compared to healthy controls in this study's baseline data. A rare genus within the *Proteobacteria* family showed the most significant correlation with ALS patients, particularly demonstrating an increased abundance of *Eisenbergiella* in ALS patients. However, the predominant genera present in ALS patient samples after four weeks of acupuncture treatment were from the *Firmicutes* family, suggesting that the gut microbiota profile could be utilized to differentiate pre- and post-treatment in ALS patients.

Based on our study, age is a significant factor influencing the severity of muscle atrophy. In elderly individuals, research has shown that the decrease in butyrate levels is associated with a decline in *Ruminococcus*, *Eubacterium*, and *Acetanaerobacterium*, along with an increase in *Intestinibacter*.[21] Obesity-mediated gastrointestinal-microbiome alterations are thought to impact local and low-grade systemic inflammation in aging.[22] Our observations align with a previous study that found a positive association between *Lachnospiraceae* abundance and all ALSFRS scores,

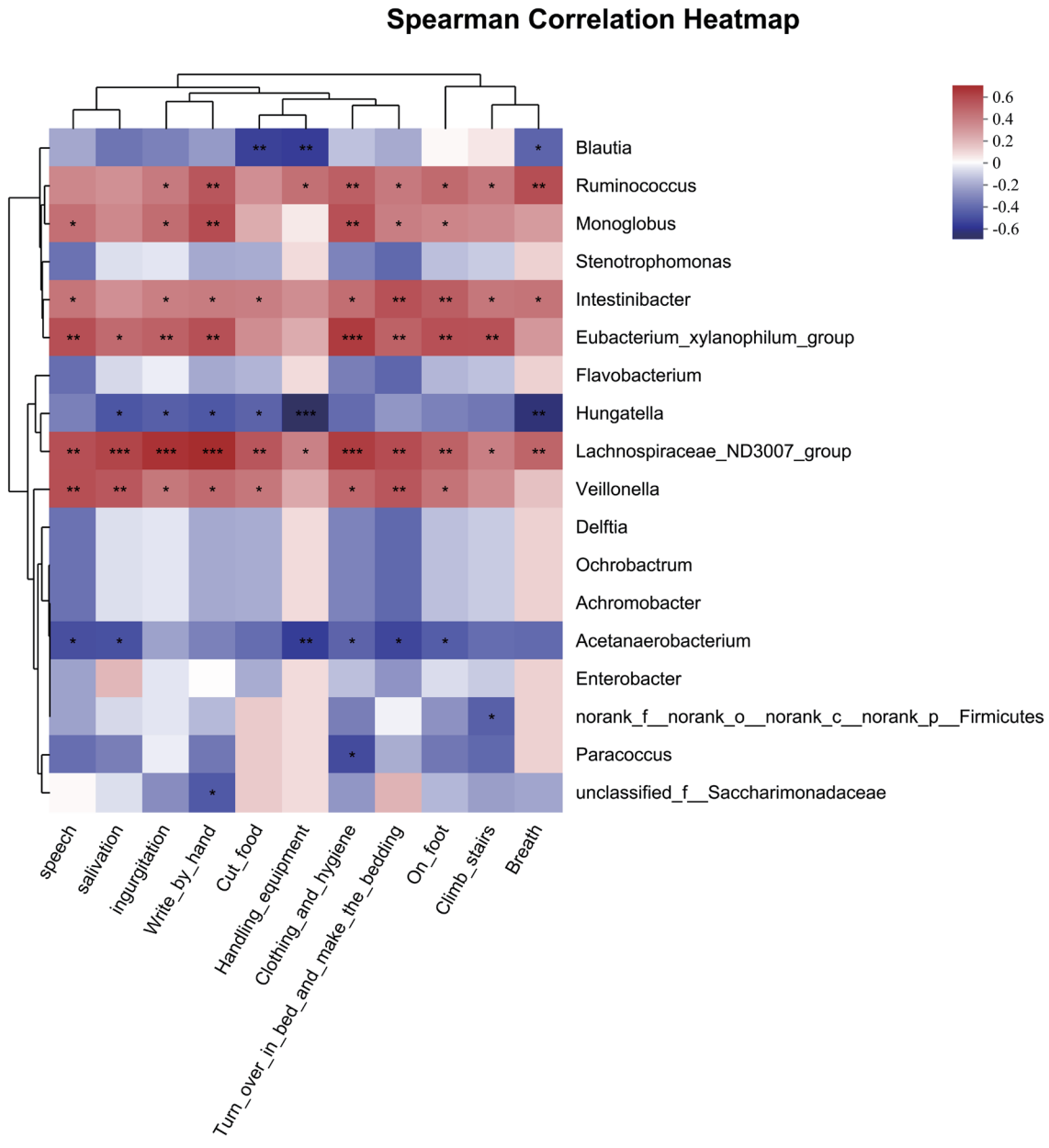


**Fig. 5. Correlation heatmap analysis at the genus level.**

including language, salivary secretion, swallowing, writing, cutting food, using utensils, dressing, and healthcare, turning over and tidying bedding, walking, climbing stairs, breathing, and sitting in ALS patients. This suggests that *Lachnospiraceae* may play a role in muscle atrophy and weakness.[23] Several anaerobic intestinal microbiota, including species in *Ruminococcus*, *Bacteroides*, *Blautia*, *Bifidobacterium*, *Faecalibacterium*, and *Collinsella*, are known to synthesize short-chain fatty acids (SCFAs) through the fermentation of dietary fibers.[24] SCFAs are recognized for their positive effects on health, particularly due to their anti-inflammatory properties, and reduced SCFA production by microbiota leads to increased luminal oxygen concentration in mice, resulting in the proliferation of facultative anaerobes.[25] Acupuncture is regarded as a safe and effective method for enhancing muscle strength.[26] We hypothesize that improvements in the combination of acupuncture and herbal medicine may be achieved by modulating the gut microbiome structure, particularly by decreasing the biomarker flora of sarcopenia *Eisenbergiella* abundance, thereby achieving rapid alleviation of the signs and symptoms associated with ALS.[27] SCFA-producing bacteria with elevated fecal SCFA concentrations can enhance the absorption of energy from fibers, suppress opportunistic pathogens, and protect the host from inflammatory and colonic diseases.[28]

Metabolomic results showed that purine metabolism was the most significantly different between patients and healthy subjects. Uric acid serves as the terminal metabolite in human purine metabolism,[29] and abnormal uric acid levels in patients may be an important factor triggering oxidative stress, which leads to ALS.[30] Oxidative stress can cause gene mutations in superoxide dismutase

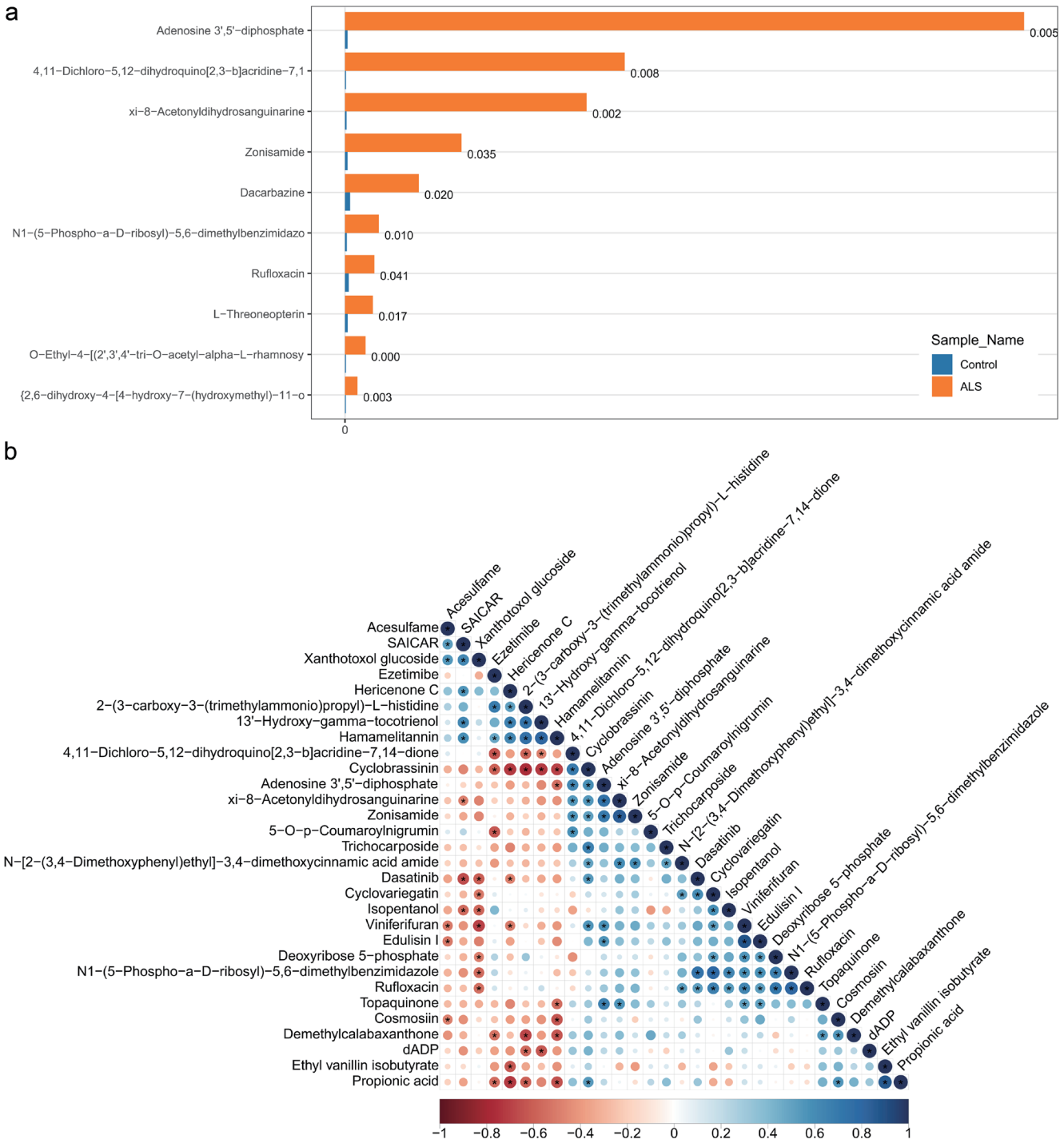


**Fig. 6. Metabolites analysis in ALS patients.** (a) Differences in metabolites between amyotrophic lateral sclerosis patients (n = 10) and healthy controls (n = 10). (b) Metabolite correlation analysis between amyotrophic lateral sclerosis (ALS) patients and healthy controls.

1, which encodes antioxidant defense proteins. By reducing nicotinamide adenine dinucleotide phosphate oxidase, peroxide production increases, antioxidant consumption in the body rises, uric acid levels decrease, and motor neuron damage leads to ALS. *In vitro* and *in vivo* studies have shown that elevated uric acid levels protect neurons against oxidative stress.[31] Therefore, increasing uric acid levels through acupuncture and Chinese herbal medicine may reduce oxidative stress and slow the rate of disease progression.[32]

The Fengchi point is located in the nuchal area under the occipital bone in the depression between the sternocleidomastoid and trapezius muscles. Modern medicine explains this from the perspective of nerve and blood vessel flow, suggesting that the lower part of

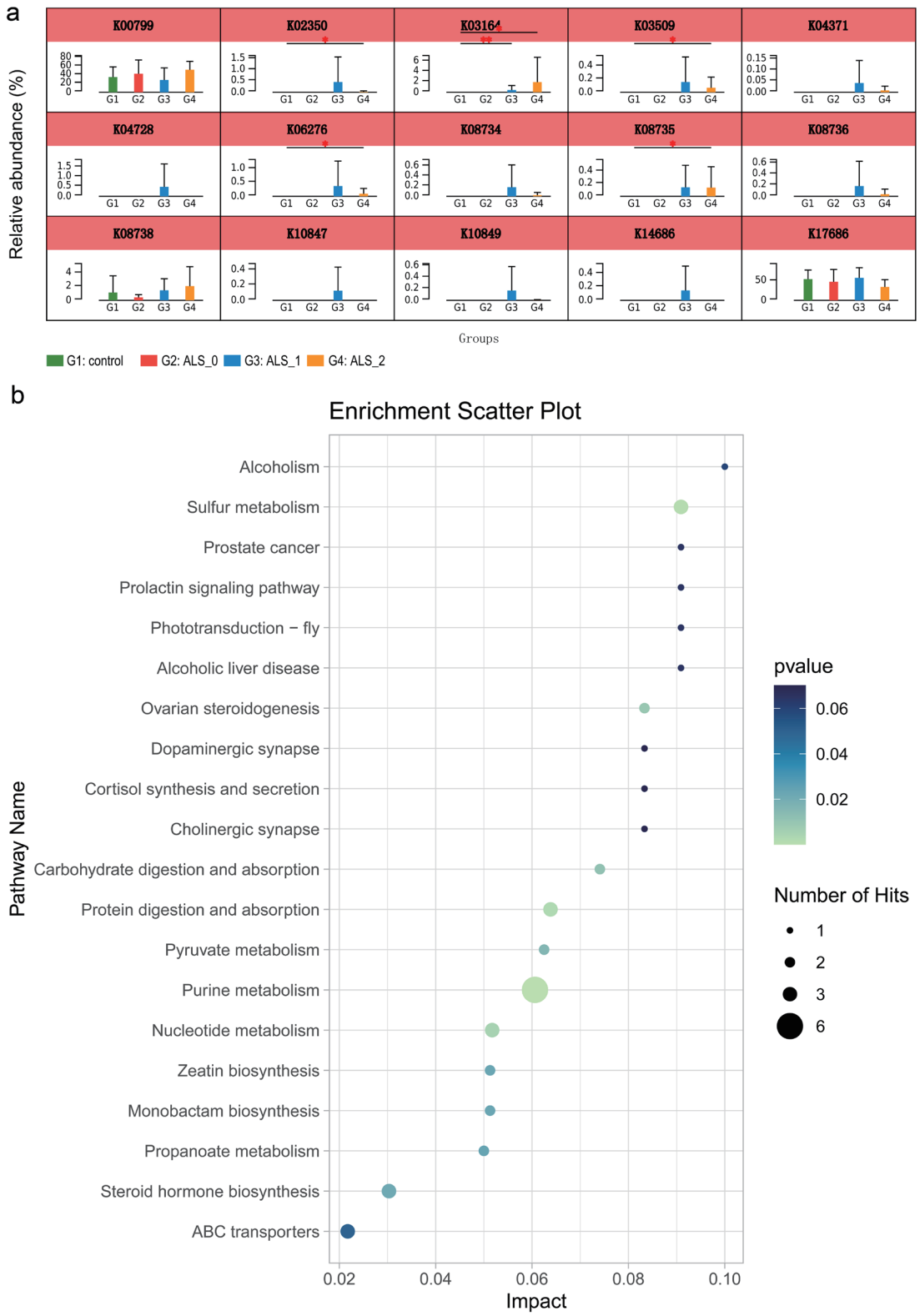


**Fig. 7. KEGG pathway analysis in ALS patients.** (a) Map of differences between metabolic pathways. (G1, healthy controls; G2, waiting treatment group; G3, pre-treatment group; G4, post-treatment group; ALS_0 refers to the waiting treatment group; ALS_1 refers to the pre-treatment group,and ALS_2 refers to the post-treatment group). (b) Enrichment analysis of ALS differential metabolites in the KEGG pathway. ALS, amyotrophic lateral sclerosis; KEGG, Kyoto Encyclopedia of Genes and Genomes.

the Fengchi point is near the vertebral artery and vertebral vein. Acupuncture at Fengchi could promote the establishment of collateral circulation at the bleeding focus and improve cerebral blood flow. A change to improve the microcirculation promotes the recovery of pharyngeal function.[10] Through stimulation of the glossopharyngeal nerve, the sublingual spirit sutra, the three-prong spirit sutra, and the mind-wandering spirit sutra, the stimulation is transmitted to the supreme motor spirit sutra, and then the spirit sutra impulse is conveyed to the affected organ. The great brain dermis can be used to regulate the cortical brainstem bundle.[9] Therefore, acupuncture may be beneficial in improving swallowing function.

The advantage of our study is that we evaluated ALS patients across multiple levels, including manual muscle testing, acupuncture reliability prediction, metabolomics, and the gut microbiome. This comprehensive evaluation was employed to detect potential biomarkers involved in the impact of acupuncture on ALS. Furthermore, the follow-up time in this study was longer than in other studies on acupuncture therapy for ALS.[33] Clinical evaluation was conducted at five different time points: before treatment, half a month after treatment, one month after treatment, three months after treatment, and six months after treatment, allowing us to better assess the long-term effects of acupuncture and Chinese medicine combined therapy on patients. Our investigations aimed to offer a thorough understanding of the potential roles of the gut microbiome and metabolomics in ALS; however, several constraints remain to be addressed in future research.

The present analysis of the effects of electroacupuncture combined with Chinese herbal medicine on ALS patients offers valuable insights but is not without its limitations. Our review of historical data revealed that the treatment combination, as originally reported, demonstrated improvements in certain clinical outcomes compared to the waiting-list control group. However, several factors should be considered when interpreting these findings. A primary limitation of this prospective approach is the potential for selection bias, as the original study may not have randomized participants in a manner that accounts for all confounding variables. Additionally, information bias could have influenced the accuracy of the data, particularly if the medical records were not uniformly maintained or if there were inconsistencies in how the outcomes were documented.[33] Furthermore, the generalizability of our findings is limited by the small sample size of the original study, which may not be representative of the broader ALS patient population. The conclusion is based on findings from a very small sample size. The lack of a contemporaneous control group that also received standard-of-care treatment, beyond the waiting list, further limits the conclusions that can be drawn regarding the comparative effectiveness of the treatment combination. Another consideration is the temporal nature of the data, as the original study was conducted in a different era of ALS management, possibly before recent advancements in care. This could affect the applicability of our findings to current clinical practice. Despite these limitations, our analysis suggests that the combination of electroacupuncture and Chinese herbal medicine may have contributed to the observed improvements in the gut microbiota and metabolomic profiles of ALS patients. The reduction in pathogenic bacteria and the positive correlation between beneficial bacteria and clinical indices highlight potential therapeutic avenues worthy of further investigation. Future research should address the limitations identified in this prospective analysis by employing randomized controlled trial designs with larger and more diverse cohorts. Additionally, the integration of modern diagnostic and monitoring techniques could provide a more comprehensive understanding of the impact of such interventions in ALS patients.

## Conclusions

Electroacupuncture combined with Chinese herbs may improve the degree of muscle atrophy in ALS patients by decreasing pathogenic bacteria, such as *Eisenbergiella*. Additionally, purine metabolism was found to be differentially expressed in these patients.

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## Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, or publication of this article.

## Author contributions

Conceptualization (TQW), formal analysis (XDY, TYL), supervision (HC), and writing – review and editing (YWZ, RQZ). All authors read and approved the final manuscript for submission.

## Ethical statement

This study adhered to the Declaration of Helsinki and written informed consent for publication was obtained from all patients or their legal guardians. The study was approved by the Medical Ethical Review Committee of Capital Medical University (Z2022SY032) and was registered in the Chinese Clinical Trial Registry (ChiCTR2400088244). The patient in Figure 1 consented to publication.

## Data sharing statement

The data used to support the findings of this study are available from the author (Tianqi Wang) upon request. The SRA of the study records can be accessed via the following link after the indicated release date: https://www.ncbi.nlm.nih.gov/sra/PRJNA1041708.

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