BMC Medicine

RESEARCH ARTICLE

Open Access

Effect of fecal microbiota transplantation on patients with sporadic amyotrophic lateral sclerosis: a randomized, double-blind, placebo-controlled trial

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Abstract

Background Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder marked by the progressive loss of motor neurons. Recent insights into ALS pathogenesis underscore the pivotal role of the gut microbiome, prompting an investigation into the potential therapeutic impact of fecal microbiota transplantation (FMT) on sporadic ALS patients.

Methods Conducted as a double-blind, placebo-controlled, parallel-group, randomized clinical trial, the study enrolled 27 participants from October 2022 to April 2023. The participants were followed up for 6 months from February 2023 to October 2023, during in-person visits at baseline, week 15, week 23, and week 35. The participants, evenly randomized, received either healthy donor FMT (FMT, $n=14$) or a mixture of 0.9% saline and food coloring (E150c) as sham transplantation (placebo, $n=13$). The primary outcome measured the change in the ALS Functional Rating Scale-Revised (ALSFRS-R) total score from baseline to week 35. Secondary outcomes included changes in gastrointestinal and respiratory functions, muscle strength, autonomic function, cognition, quality of life, intestinal microbiome composition, and plasm neurofilament light chain protein (NFL). Efficacy and safety outcomes were assessed in the intention-to-treat population.

Results A total of 27 randomized patients (47% women; mean age, 67.2 years), 24 participants completed the entire study. Notably, ALSFRS-R score changes exhibited no significant differences between FMT (6.1 [SD, 3.11]) and placebo (6.41[SD, 2.73]) groups from baseline to week 35. Secondary efficacy outcomes, encompassing respiratory function, muscle strength, autonomic function, cognition, quality of life, and plasm NFL, showed no significant differences. Nevertheless, the FMT group exhibited improvements in constipation, depression, and anxiety symptoms. FMT induced a shift in gut microbiome community composition, marked by increased abundance of *Bifidobacterium*, which persisted until week 15 (95% CI, 0.04 to 0.28; $p=0.01$). Gastrointestinal adverse events were the primary manifestations of FMT-related side effects.

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Conclusions In this clinical trial involving 27 sporadic ALS patients, FMT did not significantly slow the decline in ALSFRS-R score. Larger multicenter trials are needed to confirm the efficacy of FMT in sporadic ALS patients and to explore the underlying biological mechanisms.

Trial registration Chinese Clinical Trial Registry Identifier: ChiCTR 2200064504.

Keywords Amyotrophic lateral sclerosis, Fecal microbiota transplantation, Randomized controlled trial

Background

Amyotrophic lateral sclerosis (ALS) stands as a devastating and currently incurable neurodegenerative disorder affecting both upper and lower motor neurons, inevitably culminating in respiratory insufficiency and fatality within a relatively short timeframe of 2–5 years after disease onset [1, 2]. Despite ongoing researches, the prevailing ALS treatments predominantly focus on symptom management [3], with only riluzole [4] and edaravone [5] exhibiting modest efficacy. Approximately 90% of ALS cases are sporadic, suggesting environmental factors may impact disease risk, onset, and progression [6]. Emerging evidence suggests a link between alterations in gut microbiota composition and gastrointestinal disturbances in various neurodegenerative diseases, ALS included [7–9]. Notably, microbiome dysbiosis has been associated with elevated pro-inflammatory cytokines and activated microglia in the spinal cord and brain, indicating a potential neuroimmune-mediated pathway [10, 11].

Accumulating research suggests that ALS is a multisystem disorder primarily characterized by motor deficits, alongside prevalent and notable non-motor symptoms [12, 13]. Neuropsychiatric symptoms, cognitive and behavioral changes, pain, disrupted sleep, fatigue, and problematic saliva are increasingly acknowledged [14]. Cognitive impairment affects up to half of ALS patients. Depression and anxiety affect around 44% and 33% of ALS patients, respectively [15, 16]. Additionally, constipation is a common gastrointestinal symptom in ALS [17]. Furthermore, depression adversely impacts the quality of life and is linked to a poorer prognosis [18]. Assessing these extra-motor features is crucial for comprehensively understanding the impact of potential treatments on all aspects of ALS symptoms.

Fecal microbiota transplantation (FMT) has emerged as a promising method for restoring intestinal microbial ecology [19], widely recognized for its efficacy in treating recurrent *Clostridium difficile* infection [20]. FMT has shown positive effects in human and animal models of various neurological disorders, including multiple sclerosis, Parkinson's disease, and Alzheimer's disease [21–24]. However, evidence supporting the administration of FMT in patients with ALS is limited to case reports [25, 26]and clinical trials targeting switching the immune system by restoring T-regulatory lymphocytes (Treg) number [27, 28]. This brings attention to the uncertainty regarding whether FMT treatment can lead to sustained microbiota engraftment and improved clinical outcomes in sporadic ALS. Therefore, this exploratory randomized controlled FMT trial in sporadic ALS patients aimed to investigate the effects of sequential treatments involving either healthy donor FMT or placebo control on the progression of the disease.

Methods

Study design and recruitment strategy

We conducted a randomized, double-blind, placebo-controlled study involving sporadic ALS patients recruited from The First Affiliated Hospital of Zhengzhou University in China. The study spanned from October 2022 to April 2023, with a follow-up period of 6 months from February 2023 to October 2023. Our study consisted of 27 sporadic ALS patients, aged 18 to 65 years, diagnosed with probable or defined ALS according to the Revised El Escorial Criteria [29], and with disease onset within 2 years. Additionally, participants had a forced vital capacity percentage (FVC%) greater than 70%. Exclusion criteria included having a first-degree relative or more than one relative with ALS, a diagnosis of major depression or psychosis based on DSM-V criteria, acute infection or inflammatory conditions within the preceding 4 weeks, history of abdominal surgery, autoimmune or chronic inflammatory conditions (such as rheumatoid arthritis, chronic or active hepatitis B or C, human immunodeficiency virus, pancreatitis, advanced non-alcoholic steatohepatitis, or liver cirrhosis), probiotic or antibiotic use in the past 3 months, active malignancy, pregnancy, and drug abuse. All participants provided written informed consent upon entry into the study.

This study received approval from the Institutional Ethics Committees of the First Affiliated Hospital of Zhengzhou University (2021-KY-0385–002), and the clinical trial was registered in the Chinese Clinical Trial Registry. The complete protocol and study design are available in Additional file 2 [7–9, 27, 30–44]. All our analyses were prespecified, in accordance with the study protocol and analysis plan. Patients who were already receiving riluzole could continue their medication, provided that the regimen remained unchanged. However, initiation of

riluzole during FMT treatment (0–11 weeks) and follow-up (11–35 weeks) was prohibited.

At the screening visit, all patients underwent a comprehensive clinical and neurological evaluation. This evaluation included the assessment of the ALSFRS-R score, the forced expiratory volume in 1 s to forced vital capacity ratio (FEV1/FVC), medical history, and other relevant factors. The participants' perception and acceptability of FMT were also evaluated. Additionally, venous blood samples were collected for a complete blood examination, hepatic and renal function tests, and C-reactive protein assessment. Stool samples were collected for routine stool microscopy and culture, as well as for the *Clostridium difficile* toxin assay.

Donor screening and FMT preparation

Donor screening procedures, as previously detailed [44], involved the recruitment of healthy donors through advertising, employing stringent selection criteria, and conducting thorough screening investigations (Additional file 2) [44]. A total of 213 potential donors were prescreened through questionnaires, with 144 proceeding to clinical and laboratory screening. Ultimately, 15 donor volunteers met the rigorous criteria, establishing them as healthy donors. Throughout the donation process, an independent panel of clinical experts meticulously assessed standardized and controlled procedures. Fresh stool samples from healthy donors, stored in sterile containers, were promptly transported to sterile operation laboratories within 2 h of collection and then stored at −80 °C until frozen or utilized for FMT. Frozen stool from healthy donors was amalgamated from 3–5 donors at various collection time points, resulting in distinct batches. Each stool batch provided FMT treatment for 1 to 3 patients. Approximately 50 g of frozen pooled stool underwent mixing with 200 ml of normal saline and subsequent filtration. The resulting fecal suspension was blended with edible glycerol, achieving a final glycerol concentration of 10%. This mixture was then stored in 200 ml sterile containers and immediately frozen at −80 °C. The placebo suspension was made by mixing normal saline with 0.5% food coloring (E150c) and glycerol (10%). It had a comparable appearance and color and was organoleptically indistinguishable from fecal suspension.

Randomization and masking

Eligible patients were randomly assigned to receive either FMT or placebo in a 1:1 ratio, facilitated by an online random number generator (http://www.random.org). The randomization process was carried out by an individual not otherwise involved in the trial to ensure impartiality. Both patients and investigators remained unaware of the treatment allocation throughout the study. The primary and secondary outcomes were evaluated by neurologists blinded to the treatment (QYZ, DXL, RZ, and PC), All neurologists were prohibited from sharing any information that could have led to the identification of the patients. To maintain masking, both healthy donor fecal suspensions and placebo suspensions were presented in sterile containers that were identical in appearance and packaging. Follow-up visits were exclusively conducted by neurologists and staff members who were blinded to the study assignment. The randomization record was meticulously maintained in a separate document, and other study data were kept blinded to the administered treatment. This rigorous approach to randomization and masking ensured the integrity and objectivity of the study outcomes.

Interventions

To achieve the initial depletion of the native intestinal microbiota prior to transplantation, eligible patients underwent a prescribed regimen. This involved the oral administration of ciprofloxacin (500 mg, twice daily) (Jingxin Pharma, China) and oral metronidazole (500 mg, three times daily) (Shuanghe Pharma, China) for 7 days. Bowel preparation was accomplished using polyethylene glycol electrolyte solution, and patients observed an overnight fast (8 h) prior to scheduled colonoscopies. Screening colonoscopies were then performed, during which the transendoscopic enteral tubing (TET) tube was securely affixed to the ileocecal junction of the colon. Subsequently, a single 200 mL suspension, either healthy donor stool suspension or placebo suspension, was infused into the ileocecal junction through the TET tube at 5 mL/min under waking state. This procedure was repeated daily for 7 consecutive days, constituting one transplantation cycle. A total of three treatment cycles were conducted at 4-week intervals, as illustrated in Additional file 1: Fig. S1. Following each transplantation, patients were instructed to maintain a prone position for 2 h to facilitate the colonization of fecal microbiota in the colon. This comprehensive intervention protocol ensured the standardized implementation of FMT and allowed for a systematic assessment of its impact on the study participants.

Outcomes

The primary outcome was to evaluate the impact of FMT on ALSFRS-R score, a comprehensive measure of disease progression. ALSFRS-R score, ranging from 0 to 48, with higher values indicating better function, was assessed from baseline to week 35. This scale comprises 12 subscales across four domains: bulbar, fine motor, gross motor, and respiratory, reflecting the clinical progression

of the disease. The primary treatment comparisons were conducted between the FMT and placebo groups.

Secondary outcomes encompassed changes in ALSFRS-R scores at weeks 15 and 23. Other changes from baseline to weeks 15, 23, and 35 were assessed by Modified Norris Scale scores (limb, bulbar, and total), Milano-Torino (MiToS) functional staging, ALS Assessment Questionnaire (ALSAQ-40) scores, grip and pinch strength, and FVC%. Gastrointestinal function changes were evaluated through the Gastrointestinal Symptom Rating Scale (GSRS), Constipation Scoring System (CSS), and Patient Assessment of Constipation Quality of Life (PAC-QoL) scores. Cognitive alterations were gauged by the Mini-Mental State Examination (MMSE), fatigue severity by the Fatigue Severity Scale (FSS), and depressive/anxiety symptoms by Hamilton Depression Rating Scale (HAMD) and Hamilton Anxiety Rating Scale (HAMA) scores at weeks 15, 23, and 35. Autonomic dysfunction symptoms were explored with the Composite Autonomic Symptom Score (COMPASS 31) during the same intervals. Additional outcomes included changes in neurofilament light chain protein (NFL) plasm levels, time to non-invasive ventilation (NIV), and shifts in microbiota composition assessed through 16S ribosomal RNA gene sequencing. Assessments were conducted at four pivotal visits: on-site visits at baseline, week 15, week 23, and week 35.

Safety considerations involved monitoring adverse events throughout the FMT treatment phase (0–11 weeks) and up to 12 weeks post the final FMT treatment cycle. This comprehensive approach allowed us to thoroughly evaluate the potential impact of FMT on multiple facets of ALS progression and associated physiological parameters.

Microbiota analysis

Stool samples were systematically collected from all patients at baseline, week 15, week 23, and week 35. The extraction of microbial DNA from these samples followed the cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) method, strictly adhering to the manufacturer's guidelines. Subsequent to extraction, DNA concentration and purity were meticulously assessed on 1% agarose gels. A 16S rRNA gene fragment comprising V3 and V4 hypervariable regions (V3 forward primer, 5′-TACGGRAGGCAGCAG-3′; V4 reverse primer, 5′-CTACCNGGGTATCTAAT-3′) was amplified using an optimized and standardized 16S amplicon-library preparation protocol. The resulting library was subjected to sequencing on an Illumina NovaSeq platform, generating 250 bp paired-end reads. Denoising was conducted utilizing either the DADA2 or deblur module within the QIIME2 software, yielding initial Amplicon Sequence Variants (ASVs). ASVs with an abundance of less than 5 were subsequently filtered out. Taxonomic classifications, spanning from kingdom to genus level, were assigned to the representative sequences of each sample using the q2-feature-classifier plugin in QIIME2, relying on the Silva Database (http://www.arb-silva.de). Microbial alpha diversity (including observed ASVs, Shannon, and Simpson indices) and beta diversity were computed using QIIME2. To discern differences in microbial beta diversity between samples, Bray–Curtis distance matrices were calculated utilizing the vegan package in R. Principal Coordinate Analysis (PCoA) was then applied to these matrices, and the results were visually represented through the ggplot2 package in R. The assessment of significant variations in beta diversity was conducted employing permutational multivariate analysis of variance (PERMANOVA) with the adonis function within the vegan package in R.

Plasm collection and NFL assessment

Blood samples were collected from all patients at baseline, week 15, week 23, and week 35, centrifuged at 2000 g for 10 min, and plasm extracted within 2 h of collection. Plasm aliquots were stored at − 80 °C until further use. Plasm NFL levels were measured by enzyme-linked immunosorbent assay using a commercially available kit according to the manufacturer's instructions (E-EL-H6203, Elabscience). The lower and upper limit of quantification of the assay is 3.13 and 200 pg/mL. Intra-assay and inter-assay reproducibility were assessed by analyzing plasm sample triplicates in five separate runs. All coefficients of variation of concentrations of duplicate determinations were less than 20%.

Statistical analysis

Our sample size estimation was based on the assumption that FMT for ALS would yield a discernible difference of 3.9 points in the change of the ALSFRS-R total score from baseline to week 35 between the two study groups. With an assumed standard deviation (SD) of 4.1 points, a total sample size of 38 (19 in each group) was deemed sufficient to achieve 80% power, employing a two-sample *t*-test (two-sided, alpha = 0.05), and accounting for up to a 10% potential loss to follow-up.

Primary and secondary efficacy analyses used all available data for all participants in the modified intention-to-treat (ITT) population, including participants who discontinued the study but remained in the study. Missing data for the continuous variables were imputed using the last observation carried forward method. The primary and secondary outcomes were compared between treatment groups using a mixed-effects repeated-measures model. This model incorporated treatment and

period as fixed effects, with the patient as a random effect. Additionally, covariates included age, sex, BMI, disease duration, ALSFRS-R slope at baseline (reflecting the rate of change in the ALSFRS-R total score from symptom onset to baseline), and riluzole treatment.

The baseline progression rate may impact intervention response, as suggested by previous studies [5, 45]. Consequently, the ALSFRS-R slope at baseline was evaluated after data lock as a covariate to adjust the model. Adjusted mean treatment effects, accompanied by corresponding 95% confidence intervals (CI) and *p*-values, were reported. The normal distribution of continuous variables was assessed using the Kolmogorov–Smirnov test. Between-group comparisons of continuous variables, presented as mean (SD) or median (interquartile ranges, IQRs) as appropriate. At baseline, differences in demographic data between the FMT and placebo groups were evaluated by unpaired *t*-test and Mann Whitney *U* test for normally and non-normally distributed continuous data, respectively. Categorical data were evaluated through the χ^2 test and Fisher's exact test. Safety analyses were performed encompassing all patients who underwent at least one cycle of FMT treatment using χ^2 test. The time to NIV was analyzed post hoc using a Cox proportional-hazards model. Statistical analyses were conducted using SPSS version 21.0 (IBM Corporation) and GraphPad Prism 8.0 (GraphPad Software, Inc.). A significance level of $p<0.05$ was applied. The lme4 and emmeans packages were used to conduct the mixed models analyses in R version 4.2.1. This comprehensive approach ensured robust statistical evaluation and interpretation of the FMT intervention's impact on ALS progression.

Results

From October 2022 to April 2023, a total of 49 ALS patients underwent screening for eligibility. Of these, 27 met the eligibility criteria and were subsequently randomly assigned to receive either FMT ($n=14$) or placebo ($n=13$). The first patient was enrolled on October 17, 2022, and the last patient was recruited on February 5, 2023. The 6-month follow-up was successfully concluded in October 2023. The participants followed up for 6 months from February 6, 2023, to October 30, 2023. Unfortunately, two patients in the FMT group (one lost to follow-up before week 23, and one died due to rapid disease progression before week 23) and one patient (died due to rapid disease progression before week 23) in the placebo group were lost to follow-up, resulting 24 participants who completed the week 35 assessment (Fig. 1). It is noteworthy that recruitment had to be prematurely halted due to funding constraints, leading to an incomplete attainment of the prespecified sample size.

The clinical trial comprised 27 patients, with 15 males (55.6%) and 12 females (44.4%), and a mean (SD) age of 50.1 (8.5) years. All enrolled participants successfully adhered to the study protocol. The baseline characteristics of the patients, as detailed in Table 1, were well-balanced between the FMT and placebo groups. In the intention-to-treat analysis, encompassing all 27 patients, no significant imbalances were observed in baseline demographics and clinical data between the two treatment groups.

Primary outcome

Throughout the 35-week duration of the study, Fig. 2 illustrates the variations in total ALSFRS-R scores for each patient, categorized by treatment group. From baseline to week 35 (or discontinuation), the mean ALSFRS-R score exhibited a reduction in the FMT group (6.1 [SD, 3.11]) in comparison to the placebo group (6.41 [SD, 2.73]) at week 35. However, the adjusted mean between-group difference was 0.34 (95% CI, −0.91 to 1.60; $p=0.60$; as detailed in Table 2).

Secondary outcomes

In examining secondary efficacy endpoints, there were no notable differences between FMT and placebo across various parameters, except for gastrointestinal function and mood scores. Changes from baseline to weeks 15, 23, and 35 in secondary efficacy outcomes for the ITT population are summarized in Table 2. The FMT and placebo groups did not significantly differ in the decrease of ALSFRS-R total score during treatment (week 15: difference, 0.61 [95% CI, −0.15 to 1.37], $p=0.27$; week 23: difference, 0.60 [95% CI, −0.42 to 1.62], $p=0.60$) (Fig. 2). Moreover, there were no significant differences observed in the impact of FMT on the average changes in Modified Norris Scale scores when monitoring the progression of the disease. Quality of life, as assessed by the ALSAQ-40 score, showed no difference between patients who received FMT and those who received a placebo (Table 2).

A significant decrease in outcome measures at week 35 (respiratory measures FVC%) indicated a worsened condition in both groups. Additionally, a significant difference in overall gastrointestinal function was observed in the final analysis. The mean changes from baseline in the scores on CSS reached statistical significance between the two groups at weeks 23 and 35 (week 23: difference, −3.54 [95% CI, −6.28 to −0.80], $p=0.017$; week 35, difference, −3.81 [95% CI, −6.57 to −1.05], $p=0.01$). The mean decreases in PAC-QoL scores from baseline at all evaluation points in the FMT group were significantly greater than those in the placebo group (Table 2). The mean changes in the GSRS scores from baseline were

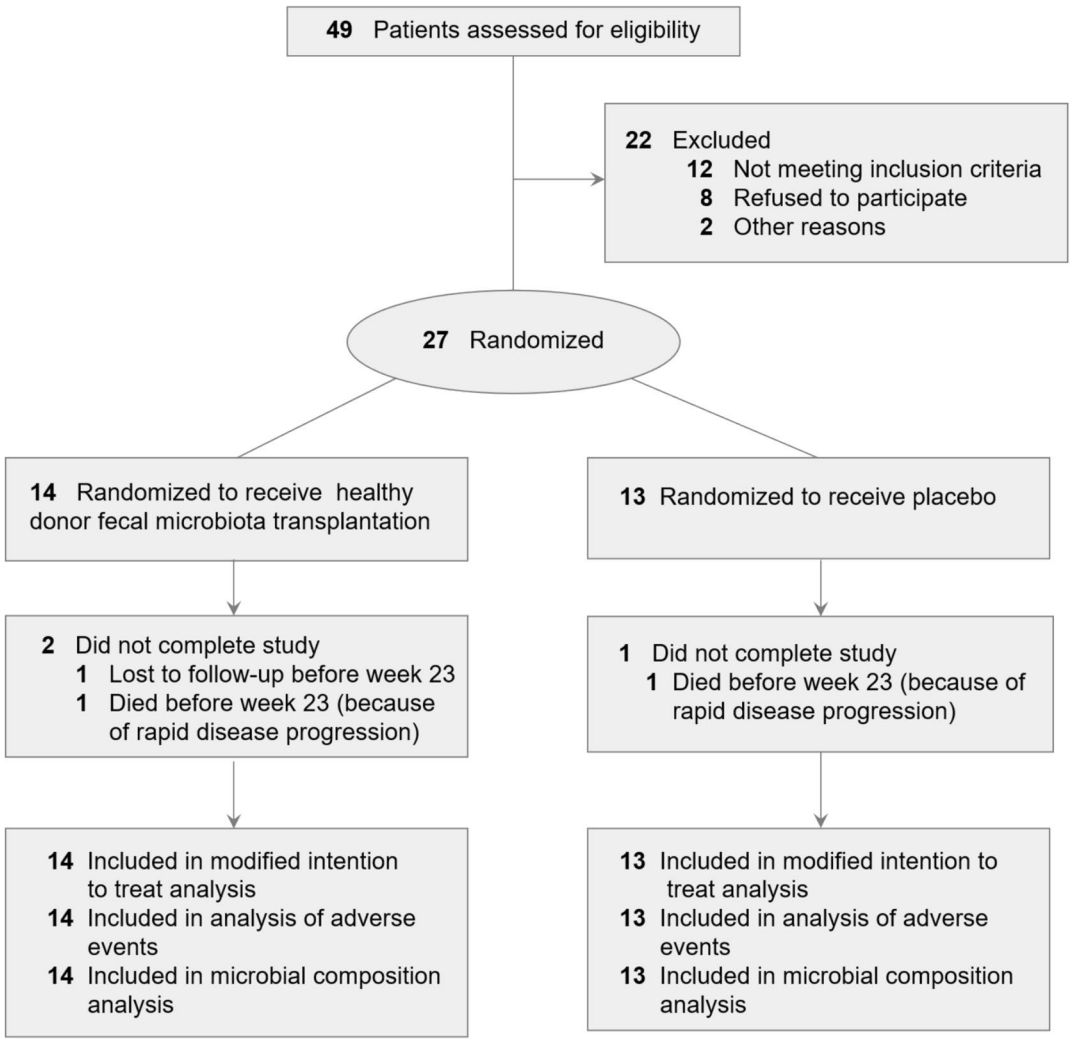

Fig. 1 Trial profile

significantly different between the FMT and placebo groups only at week 23 (difference, −1.61 [95% CI, −2.40 to −0.83], $p=0.0002$) (Table 2).

In terms of mood scores, the mean decreases in HAMA and HAMD scores from baseline to weeks 15, 23, and 35 in the FMT group were consistently greater than those in the placebo group (Table 2). The mean changes in pinch strength from baseline were significantly different between the FMT and placebo groups only at week 35 (difference, 0.15 [95% CI, 0.04 to 0.26], $p=0.015$). However, no difference was observed in FSS, MMSE, grip strength, or COMPASS 31 at the end of the 3-cycle FMT treatment in patients given FMT compared with placebo (Table 2). Mean plasm NFL levels were similar between the two groups. In exploratory analyses, we performed a post hoc analysis to determine if FMT improved respiratory failure. There was no difference in the time to NIV between the FMT and placebo groups ($p=0.81$, Additional file 1: Fig. S2) using the Kaplan–Meier method and the Cox proportional-hazards model. After adjusting for age, sex, BMI, disease duration, riluzole use, and ALSFRS-R slope at baseline, the difference in time to NIV from randomization remained unchanged.

Microbial composition analysis

Profiling of the stool microbiome using 16S rRNA gene sequencing revealed differences in community composition between healthy donors and individuals with sporadic ALS. The assessment of microbial communities' alpha diversity, employing metrics such as the Shannon index, observed ASVs, and Simpson index, provided insights into both the richness and evenness of the microbiota. Notably, no discernible differences in the α-diversity index were observed between ALS patients and their healthy donors (Fig. 3A and Additional file 1: Table S1). Examining the phylum level, *Firmicutes* and

Table 1 Baseline characteristics of the participants

Characteristics	FMT group ($n=14$)	Placebo group ($n=13$)	p value
Age (years)	50.93 (9.53)	49.31 (14.71)	0.63
Sex			
Female, *n* (%)	6 (42.9)	6 (46.1)	0.86
Male, *n* (%)	8 (57.1)	7 (53.9)	0.86
Height (m)	1.69 (0.08)	1.67 (0.51)	0.55
Wight (kg)	64.00 (11.30)	59.77 (16.65)	0.26
BMI (kg/m^2)	22.42 (2.70)	21.55 (6.45)	0.43
Onset			
Bulbar, *n* (%)	3 (21.4)	2 (15.4)	0.69
Spinal, *n* (%)	11 (78.6)	11 (84.6)	0.69
Duration of disease (months)	16.93 (4.18)	15.31 (6.42)	0.40
ALSFRS-R total score	30.86 (8.22)	31.77 (10.34)	0.77
ALSFRS-R slope at baseline	0.99 (0.41)	1.01 (0.30)	0.84
Modified Norris Scale score	64.64 (19.17)	71.62 (22.09)	0.29
ALSAQ-40 score	108.29 (34.08)	116.92 (40.88)	0.54
MiToS score[a]	1.00 (1.00, 2.00)	1.00 (1.00, 1.00)	0.79
FSS score	27.43 (10.52)	31.69 (11.96)	0.32
GSRS score	3.57 (1.83)	3.62 (1.82)	0.95
CSS score[a]	3.0 (1.0, 8.0)	5.0 (2.0, 8.0)	0.55
PAC-QOL score[a]	43.0 (36.0, 48.0)	52 (44.0, 56.0)	0.40
MMSE score	27.93 (1.64)	27.69 (8.48)	0.73
HAMD score	10.71 (9.27)	8.15 (6.11)	0.42
HAMA score	5.50 (3.35)	4.00 (2.69)	0.24
COMPASS 31 score	16.64 (9.68)	20.31 (11.02)	0.40
FVC (%)	81.17 (4.82)	80.53 (24.35)	0.75
Grip strength (kg)	20.81 (7.54)	19.58 (10.31)	0.18
Pinch strength (kg)	1.50 (0.62)	1.68 (0.72)	0.49
Plasm NFL (pg/ml)	89.01 (77.01)	95.54 (73.74)	0.83
Riluzole use, *n* (%)	10 (71.40)	9 (69.20)	0.90

Data are mean (SD) or *n* (%) unless otherwise stated

BMI body mass index, *ALSFRS-R* ALS Functional Rating Scale-Revised, *ALSAQ-40* the 40-item ALS Assessment Questionnaire, *MiToS* Milano-Torino functional staging, *FSS* Fatigue Severity Scale, *GSRS* Gastrointestinal Symptom Rating Scale, *CSS* Constipation Scoring System, *PAC-QoL* Patient Assessment of Constipation Quality of Life, *MMSE* Mini-Mental State Examination, *HAMD* Hamilton Depression Scale, *HAMA* Hamilton Anxiety Scale, *COMPASS 31* Composite Autonomic Symptom Score, *FVC%* force vital capacity percentage, *NFL* neurofilament light chain protein

[a] Data was given as median and IQR after normality was evaluated using the Shapiro–Wilk test. *IQR*, interquartile range

Actinobacteriota emerged as the most prevalent phyla in ALS patients (Fig. 3C). At the genus level, healthy donors exhibited an elevated abundance of *Bifidobacterium* (Fig. 3D).

Post FMT, individuals in both the FMT and placebo groups displayed no significant alterations in stool microbiota alpha diversity from baseline to week 35 (Fig. 3A and Additional file 1: Table S1). Despite baseline similarities in stool microbiota compositions between the groups, a noteworthy shift in microbial beta diversity emerged in the FMT group at week 15 (4 weeks post-FMT), a difference not sustained in subsequent time points (PERMANOVA, $p=0.001$) (Fig. 3B). Focusing on the top 10 genera, the FMT intervention induced detectable changes in several genera at week 15, as highlighted in Additional file 1: Table S2. Notably, the relative abundances of *Bifidobacterium* increased following FMT compared to the placebo group. Moreover, these significant shifts persisted up to week 23, a phenomenon observed exclusively in patients from the FMT group (Fig. 3B). Importantly, no disparities in stool microbial diversity or composition were noted at week 35 between the FMT and placebo groups.

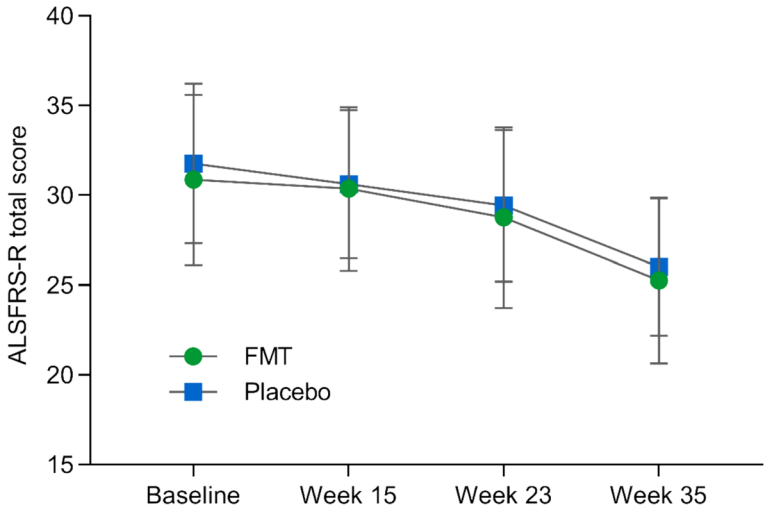

Fig. 2 Primary efficacy outcomes. The graph illustrates the mean Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) total scores from baseline to week 35. The standard deviation is represented by the error bars. The interventions are denoted as follows: fecal microbiota transplantation (FMT) and placebo

Adverse events

Adverse events were systematically evaluated in all patients ($n=27$) who underwent at least one cycle of FMT treatment (Table 3). Notably, no serious adverse events were documented throughout the study duration. The predominant minor adverse events reported encompassed gastrointestinal complaints, specifically diarrhea ($n=3$ in the FMT group and $n=3$ in the placebo group), abdominal pain ($n=1$ in the FMT group and $n=2$ in the placebo group), and abdominal bloating ($n=1$ in the FMT group and $n=1$ in the placebo group). Importantly, these gastrointestinal disturbances were characterized as mild, transient, predominantly occurring during the initial FMT cycle, and spontaneously resolving within 72 h.

Discussion

This randomized clinical trial investigated the safety and efficacy of healthy donor FMT as a treatment for sporadic ALS. However, recruitment was terminated early before the prespecified sample size was reached due to funding constraints, restricting the statistical power of FMT outcomes. Upon modifying the covariates after data lock, both primary and secondary outcomes were assessed as post hoc analyses. Consequently, all findings from these analyses should be interpreted as hypothesis-generating rather than providing evidence of efficacy. This study found that FMT did not demonstrate effectiveness in altering disease progression, as assessed by primary and secondary outcomes, including total ALSFRS-R score and Modified Norris Scale scores [46, 47]. Although the sample size was insufficient to draw definitive conclusions regarding the efficacy of FMT in patients with sporadic ALS, this study provided valuable pilot data. Notably, the study established the safety of long-term delivery of mixed fecal suspension from allogeneic donors via TET, with no serious adverse events reported, and a similar overall incidence of adverse events between FMT and placebo groups.

An increasing number of studies have reported the use of FMT in the treatment of neurodegenerative diseases [48–50], demonstrating the improvement of motor symptoms. However, no clinical trials have been reported so far on the use of FMT for the treatment of ALS. Therefore, our study is the first to provide data from a randomized controlled trial of FMT in ALS patients. Meanwhile, we have been following the ongoing RCT research conducted by the Italian team to assess if we can achieve similar outcomes [27, 28]. To date, only two case studies targeting slow decline in the ALSFRS-R score have been reported [25, 26]. Although there was no significant improvement observed in the primary outcome or measures of respiratory and motor function. A noteworthy positive effect was detected in chronic constipation measures (CSS, PAC-QoL) and mood disorders (HAMA, HAMD) in the FMT group. Additionally, FMT is relatively safe and low-cost, suggesting that further clinical research on FMT for ALS may hold value.

Sporadic ALS patients often have gastrointestinal dysfunction during the disease course, and up to half of patients suffer from constipation [17, 51]. Constipation is associated with gut dysbiosis [52, 53], and FMT also significantly decreased PAC-QoL score in Parkinson's disease and progressive supranuclear palsy-Richardson's syndrome patients [24, 50]. Moreover, the study indicated

Table 2 Primary and secondary endpoints

Outcome	Estimates and standard errors		Adjusted mean difference (95% CI)	*p* value
	FMT group ($n=14$)	Placebo group ($n=13$)		
Primary outcome				
Mean change from baseline to week 35				
ALSFRS-R total score	−6.10 (0.83)	−6.41 (0.76)	0.34 (−0.91,1.60)	0.60
Secondary Outcomes				
Mean change from baseline to week 15				
ALSFRS-R total score	−0.71 (0.61)	−1.32 (0.60)	0.61 (−0.15,1.37)	0.27
Modified Norris Scale score	−3.03 (1.22)	−3.43 (1.22)	0.40 (−2.75, 3.55)	0.80
ALSAQ-40 score	1.72 (2.54)	2.84 (2.78)	−1.12 (−7.08, 4.83)	0.79
MiToS score	0.01 (0.09)	−0.01 (0.08)	0.02 (−0.21, 0.26)	0.84
FSS score	−1.53 (0.77)	−0.68 (0.69)	−0.85 (−2.54, 0.84)	0.34
GSRS score	0.21 (0.42)	0.07 (0.44)	0.14 (−0.63, 0.90)	0.73
CSS score	3.20 (1.00)	4.98 (1.10)	−1.78 (−4.48, 0.92)	0.21
PAC-QOL score	−15.52 (2.68)	−1.17 (1.88)	−14.35 (−20.41, −8.34)	0.0002
MMSE score	0.01 (0.19)	−0.15 (0.21)	0.17 (−0.28, 0.62)	0.47
HAMD score	−3.39 (1.25)	0.90 (1.28)	−4.28 (−7.00, −1.55)	0.0041
HAMA score	−2.23 (0.63)	0.72 (0.53)	−2.95 (−4.55, −1.35)	0.002
COMPASS 31 score	−2.27 (0.97)	−2.24 (0.92)	−0.04 (−2.09, 2.01)	0.97
FVC (%)	1.65 (1.28)	−1.23 (1.22)	2.89 (−0.15, 4.45)	0.08
Grip strength (kg)	−0.95 (0.39)	−1.57 (0.37)	0.62 (−0.37, 1.61)	0.24
Pinch strength (kg)	−0.01 (0.04)	−0.07 (0.04)	0.06 (−0.03, 0.16)	0.21
Plasm NFL (pg/ml)	−55.45 (39.6)	28.60 (33.7)	−84.04 (−165.18, −2.90)	0.06
Mean change from baseline to week 23				
ALSFRS-R total score	−2.50 (0.70)	−3.07 (0.64)	0.60 (−0.42,1.62)	0.60
Modified Norris Scale score	−5.56 (1.19)	−7.67 (1.26)	2.11 (−1.23, 5.44)	0.25
ALSAQ-40 score	9.19 (5.09)	10.20 (4.97)	−1.01 (−8.49, 6.48)	0.82
MiToS score	0.16 (0.11)	0.00 (0.09)	0.16 (−0.11, 0.43)	0.25
FSS score	−0.03 (0.68)	1.01 (0.64)	−1.20 (−2.73, 0.35)	0.15
GSRS score	−1.00 (0.42)	0.62 (0.43)	−1.61 (−2.40, −0.83)	0.0002
CSS score	2.71 (1.00)	6.25 (1.10)	−3.54 (−6.28, −0.80)	0.017
PAC-QOL score	−15.66 (2.46)	−1.01 (2.01)	−14.65 (−20.54, −8.75)	0.0002
MMSE score	0.50 (0.19)	−0.49 (0.23)	0.54 (0.03, 1.06)	0.05
HAMD score	−4.09 (1.44)	2.23 (1.42)	−6.32 (−9.52, −3.11)	0.0006
HAMA score	−1.96 (0.63)	2.62 (0.55)	−4.59 (−6.23, −2.94)	< .0001
COMPASS 31 score	−0.29 (0.81)	−0.01 (0.7)	−0.27 (−2.23, 1.68)	0.79
FVC (%)	−2.47 (1.26)	−3.75 (1.26)	1.28 (−1.90, 4.45)	0.44
Grip strength (kg)	−1.34 (0.46)	−2.58 (0.41)	1.24 (0.08, 2.39)	0.05
Pinch strength (kg)	−0.11 (0.04)	−0.18 (0.04)	0.08 (−0.03, 0.18)	0.17
Plasm NFL (pg/ml)	−4.50 (29.40)	14.26 (23.10)	−18.75 (−87.15, 49.65)	0.60
Mean change from baseline to week 35				
Modified Norris Scale score	−11.31 (1.19)	−14.42 (1.26)	3.11 (−0.23, 6.44)	0.08
ALSAQ-40 score	22.95 (5.83)	22.69 (5.63)	0.26 (−7.24, 7.77)	0.95
MiToS score	0.30 (0.12)	0.25 (0.10)	0.05 (−0.24, 0.35)	0.73
FSS score	2.10 (0.64)	3.40 (0.63)	−1.23 (−2.97, 0.50)	0.17
GSRS score	0.46 (0.47)	0.97 (0.49)	−0.51 (−1.36, 0.34)	0.24
CSS score	2.70 (1.02)	6.51 (1.11)	−3.81 (−6.57, −1.05)	0.01
PAC-QOL score	−13.83 (2.35)	1.89 (1.99)	−15.73 (−21.51, −9.94)	0.0001
MMSE score	−0.07 (0.20)	−0.52 (0.26)	0.44 (−0.12, 1.01)	0.13
HAMD score	−2.43 (1.25)	4.32 (1.27)	−6.75 (−9.51, −3.99)	0.0001

Table 2 (continued)

Outcome	Estimates and standard errors		Adjusted mean difference (95% CI)	*p* value
	FMT group ($n=14$)	Placebo group ($n=13$)		
HAMA score	−3.00 (0.79)	3.99 (0.66)	−4.29 (−6.32, −2.27)	0.0005
COMPASS 31 score	1.44 (1.04)	0.25 (0.96)	1.19 (−1.21, 3.59)	0.34
FVC (%)	−8.22 (1.12)	−9.48 (1.26)	1.26 (−0.70, 4.22)	0.42
Grip strength (kg)	−3.00 (0.53)	−3.79 (0.46)	0.79 (−0.51, 2.09)	0.25
Pinch strength (kg)	−0.24 (0.05)	−0.38 (0.04)	0.15 (0.04, 0.26)	0.015
Plasm NFL (pg/ml)	−8.53 (43.10)	13.05 (35.40)	−21.58 (−113.7, 70.54)	0.65

ALSFRS-R scores 0–48 (best). Modified Norris Scale scores 0–102 (best). ALSAQ-40 score 200–40 (best). MiToS scores 5–0 (best). FSS scores 63–9 (best). GSRS scores 45–0 (best). CSS scores 30–0 (best). PAC-QoL scores 140–28 (best). MMSE scores 0–30 (best). HAMD scores 81–0 (best). HAMA scores 56–0 (best). COMPASS 31 scores 100–0 (best). *ALSFRS-R* ALS Functional Rating Scale-Revised, *ALSAQ-40* the 40 item ALS Assessment Questionnaire, *MiToS* Milano-Torino functional staging, *FSS* Fatigue Severity Scale, *GSRS* Gastrointestinal Symptom Rating Scale, *CSS* Constipation Scoring System, *PAC-QOL* Patient Assessment of Constipation Quality of Life, *MMSE* Mini-Mental State Examination, *HAMD* Hamilton Depression Scale, *HAMA* Hamilton Anxiety Scale, *COMPASS 31* Composite Autonomic Symptom Score, *FVC%* force vital capacity percentage, *NFL* neurofilament light chain protein. The primary and secondary outcomes between treatment groups were assessed on an intention-to-treat basis using a mixed-model, repeated-measures analysis

potential positive effects on psychological health, as evidenced by changes in HAMD and HAMA scores. This aligns with emerging evidence of the gut-brain axis's role in anxiety and depression [54, 55]. 16S rDNA sequencing highlighted the distinct beta diversity variations between patients with ALS and healthy donors. Specifically, the *Firmicutes* and *Actinobacteriota* phylum were predominant in patients with ALS. After FMT, there was a significant increase in the abundance of the *Bifidobacterium* genus. This rise in *Bifidobacterium* abundance has also been documented in a case study exploring FMT for respiratory failure in ALS [25]. Healthy donor stools are abundant in genera of *Bifidobacterium*, which have the capacity to produce and deliver neuroactive substances such as gamma-aminobutyric acid [56]. Interestingly, Tian et al. found that *Bifidobacterium* is a promising candidate psychobiotic that attenuates depression and associated gastrointestinal disorders [57]. Thus, FMT intervention increased the bacteria *Bifidobacterium* could relieve constipation and psychiatric symptoms. For these reasons, we believe that although the generalizability of our results is imperfect, it should be meaningful for patients.

While acknowledging imperfect generalizability, the study underscores the significance of these findings for ALS patients. Importantly, it contributes robust clinical evidence in the nascent field of FMT for neurodegenerative diseases beyond *Clostridioides difficile* infections, building on reports of potential benefits in Parkinson's disease, multiple sclerosis, and Alzheimer's disease [24, 58, 59]. The use of mixed fecal suspension from multiple donors in this trial aimed to ensure infusion supply and minimize the risk of therapeutically ineffective donor stool. The administration of antibiotics before FMT was not randomized in our study, precluding a definitive determination of its impact on FMT outcomes. Notably, a prior randomized trial demonstrated that utilizing an antibiotic combination comprising amoxicillin, metronidazole, and tetracycline in patients with ulcerative colitis resulted in the depletion of antibody titers to *Fusobacterium* spp. [60]. Subsequent findings indicated that this pre-FMT antibiotic regimen effectively depleted dysbiotic microbiota and potentially established an ecological niche conducive to the engraftment of donor microbiota [61]. In our study, antibiotics were administered in both the FMT and placebo groups. Consequently, any clinical effects observed can be attributed to FMT rather than antibiotic therapy alone. This approach ensures that the impact of FMT on study outcomes remains the focus, allowing for a clearer understanding of the potential therapeutic effects of FMT in the absence of confounding variables associated with antibiotic treatment.

Limitations

Several limitations were present in this trial. Firstly, the sample size was relatively small, and recruitment ceased before reaching the intended target of 38 patients, thereby restricting the statistical power of FMT outcomes. This trial, being exploratory, aimed to offer preliminary data on the feasibility of utilizing maintenance FMT for individuals with sporadic ALS. Environmental and social factors may influence the effectiveness and outcomes of clinical trials, highlighting the need for larger, multi-center studies to assess the efficacy of FMT in ALS. Secondly, the study was not designed to assess the impact of riluzole treatment. Although nearly all patients had received riluzole before enrollment, with consistent dosage and frequency, the influence of this medication on functional scales needs careful consideration. The susceptibility of functional scales to

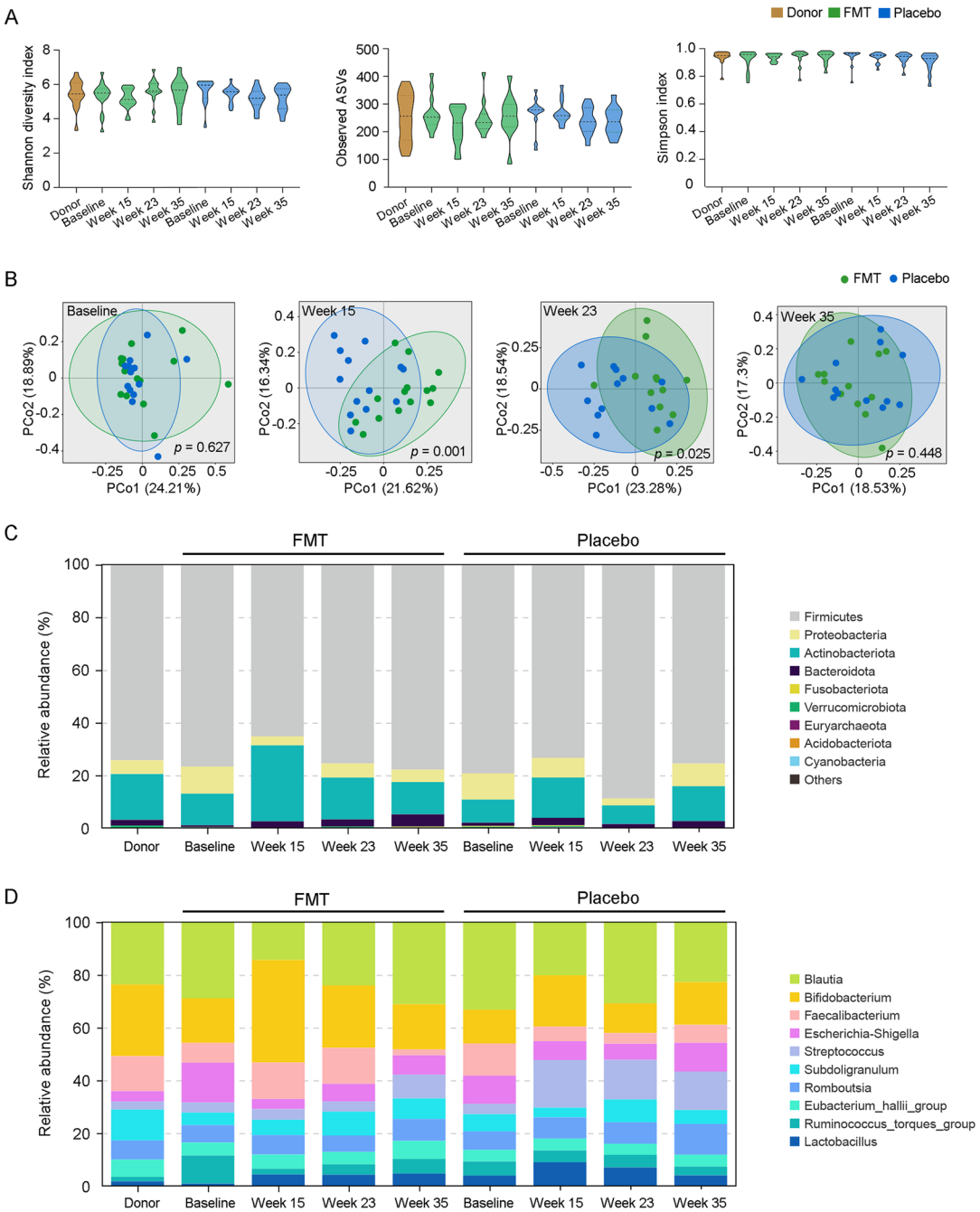

Fig. 3 Changes in fecal microbiota for participants after FMT. **A** Comparative analysis of α-diversity in fecal microbiota, assessed using 16S rDNA amplicon sequencing, among donor, healthy donor fecal microbiota transplantation (FMT), and placebo groups. The data are presented as median values with minimum and maximum ranges. **B** Principal Coordinates Analysis (PCoA) plot based on Bray–Curtis dissimilarities at the genus level, depicting the fecal microbiota differences between FMT and placebo groups at baseline, week 15, week 23, and week 35. Ellipses represent 95% confidence intervals. **C** Bar plots illustrating the phylum-level composition in fecal samples for the donor, FMT, and placebo groups. **D** Bar plots illustrating the genus-level composition in fecal samples for the donor, FMT, and placebo groups. FMT denotes donor fecal microbiota transplantation, while placebo signifies sham transplantation. These analyses provide a comprehensive insight into the dynamic changes in fecal microbiota composition following FMT across different experimental groups

Table 3 Adverse events

	Adverse events		*p* valve
	FMT group (*n* = 14)	Placebo group (*n* = 13)	
Any	6	8	0.33
Dizziness	0	0	-
Abdominal pain	1	2	0.50
Abdominal bloating	1	1	0.96
Diarrhea	3	3	0.92
Nausea	0	0	-
Vomiting	0	0	-
Infection	0	0	-
Fever	0	0	-
Bacteraemia	0	0	-
Respiratory difficulties	0	0	-
Proctalgia	1	2	0.50
Rash	0	0	-
Dyspnea	0	0	-
Difficulty sleeping	0	0	-
Palpitations	0	0	-
Fatigue	0	0	-
Weight loss	0	0	-

Data are the number of patients

medication effects requires careful interpretation of our findings regarding the potential influence of medication on patient outcomes. Thirdly, the administration of antibiotics to both treatment groups before FMT raises uncertainty about the added benefit of this complementary microbial manipulation in enhancing the clinical efficacy of FMT in ALS. Fourthly, 16S rDNA sequencing rather than shotgun metagenomic sequencing posed limitations on the depth of microbiota analysis. The absence of multi-omics analysis further hindered a comprehensive evaluation of the complex functional implications associated with microbial modulation. Fifthly, while healthy donors are deemed eligible at the time of donation, long-term follow-up is essential to mitigate the risk of future chronic diseases that may pose potential hazards to participants. Sixthly, the randomization process did not specifically account for ALS criteria such as pre-baseline disease progression, which could introduce bias in patient allocation. However, no significant imbalances were noted in baseline demographics and clinical data between the two treatment groups. Consequently, we believe that despite the imperfect generalizability of our results, they remain acceptable. Finally, given the trial's focus on enrolling patients in the early stages of ALS with moderate progression, the applicability of FMT to individuals with severe bulbar dysfunction remains unclear. Additionally, the utilization of a multi-donor approach precluded the identification of specific microbial effects attributable to individual donors. These limitations collectively underscore the need for further research and consideration when interpreting the results of this study.

Conclusions

The results of this preliminary study involving patients with sporadic ALS provide data on adverse events and changes in the total ALSFRS-R score following the administration of FMT from healthy donors. Larger randomized controlled trials are needed to further confirm the safety and efficacy of FMT in treating ALS patients. However, noteworthy improvements were observed in nonmotor function, concomitant with alterations in the microbiota community, characterized by an increase in *Bifidobacterium*. This underscores the need for further exploration of the nuanced relationship between FMT, microbiota dynamics, and nonmotor function in the context of ALS management.

Abbreviations

ALS	Amyotrophic lateral sclerosis
ALSAQ-40	40-Item Amyotrophic Lateral Sclerosis Assessment Questionnaire
ALSFRS-R	ALS Functional Rating Scale-Revised
ASVs	Amplicon sequence variants
BMI	Body mass index
CI	Confidence interval
COMPASS 31	Composite Autonomic Symptom Score 31
CSS	Constipation Scoring System
CTAB	Cetyltrimethylammonium bromide
FEV1/FVC	Forced expiratory volume in 1 s to forced vital capacity ratio
FMT	Fecal microbiota transplantation
FSS	Fatigue Severity Scale
FVC%	Forced vital capacity percentage
GSRS	Gastrointestinal Symptom Rating Scale
HAMA	Hamilton Anxiety Rating Scale
HAMD	Hamilton Depression Rating Scale
IQRs	Interquartile ranges
ITT	Intention-to-treat
MiToS	Milano-Torino functional staging
MMSE	Mini-Mental State Examination
NFL	Neurofilament light chain protein
NIV	Non-invasive ventilation
PAC-QoL	Patient Assessment of Constipation Quality of Life
PCoA	Principal Coordinate Analysis
PERMANOVA	Permutational multivariate analysis of variance
RCT	Randomized controlled trial
SD	Standard deviation
SDS	Sodium dodecyl sulfate
TET	Transendoscopic enteral tubing
Treg	T-regulatory lymphocytes

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-024-03781-6.

Additional file 1: Table S1. α-diversity index at baseline, week 15, week 23, and week 35. Table S2 Mean Changes in Relative Abundance of Genera after FMT. Fig S1. The Study Design of FMT Procedures. Fig. S2 NIV-free survival.

Additional file 2. Protocol.

Acknowledgements
We are grateful to all patients, their families, the investigators, the site staff, and the trial teams.

Authors' contributions
XJW, XBD, and MMM conceived and designed the study; QYZ, DXL, RZ, and PC acquired the data; RYF, AW, and HZW analyzed and interpreted the data; JQW, HZW, and QYZ accessed and were responsible for the raw data associated with the study; RYF, DXL, and RZ performed the statistical analysis; RYF and XJW drafted the manuscript; XJW, XBD, MMM, and JFT critically revised the important intellectual content of the manuscript; XJW took the decision to submit the manuscript for publication. All authors read and approved the final manuscript.

Funding
This work was supported by the National Natural Science Foundation of China (No. 82122022 and 82171248), National Natural Science Foundation of China Youth Science Fund (No. 82201407), National Key Research and Development Program of China (No. 2021YFC2501200 and No. 2021YFC2502100), Natural Science Foundation of Henan Province for Distinguished Young Scholars (No. 222300420017), Henan Province Young and Middle-Aged Health Science and Technology Innovation (No. YXKC2022009 and YXKC2020033), Funding for Scientific Research and Innovation Team of The First Affiliated Hospital of Zhengzhou University (No. QNCXTD2023008 and No. ZYCXTD2023003), and Clinical Medicine First-Class Discipline Talent Cultivation Program of Zhengzhou University (No. 32410700TP010).

Data availability
The data collected in this study, including de-identified participant data and the data dictionary, are available to researchers through the corresponding author Prof. Xuejing Wang upon reasonable request. These data will be available for 3 years after publication. Data requests require a methodologically sound proposal as well as a data access agreement and approval by the local ethics committee.

Declarations

Ethics approval and consent to participate
Ethical approval was obtained from the Institutional Ethics Committees of the First Affiliated Hospital of Zhengzhou University (2021-KY-0385–002). Participants gave informed consent to participate before being enrolled.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 17 January 2024 Accepted: 18 November 2024
Published online: 02 December 2024

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