Official Title: Effect of transcranial alternating current stimulation in patients with cerebellar ataxia (spinocerebellar ataxia type 3 and multiple system atrophy): a randomized proof-of-concept clinical trial.

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STUDY PROTOCOL FOR SCA3

1. Project title

Effect of transcranial alternating current stimulation in spinocerebellar ataxia type 3: a randomized proof-of-concept clinical trial.

2. Abstract of project

Background/Objective: Spinocerebellar ataxia type 3 (SCA3) is the most common SCA worldwide. Currently, no specific pharmacological and treatments can alter natural course of the disease. Clinical practice guidelines had confirmed the safety of transcranial alternating current stimulation (tACS) in treating neuropsychiatric diseases, but to our knowledge, no randomized clinical trials have investigated whether tACS is safety and efficacy during improve symptoms in SCA3.The objectives of this study are to identify the efficacy and safety of tACS during improving ataxia symptoms and non-ataxia symptoms in adult patients with SCA3.

Participants: Individual with SCA3

Design: A triple-blind, randomized sham-controlled trial with two groups. At least 80 subjects will be recruited and randomized into either active tACS group or sham tACS group.

Interventions: Cerebellar tACS (40 min, 2 mA, ramp-up and down periods of 10s each) will be delivered over 10 sessions, distributed in two groups of five consecutive days with a two-day break in between. The active tACS group will receive whole course current stimulation, while the control group will receive a daily 40–seconds current stimulation for two weeks.

Primary outcomes: The primary outcome was the proportion of participants whose Scale for the Assessment and Rating of Ataxia (SARA) score improved by at least 1.5 points compared with baseline (T0) on assessments immediately after treatment (T1), at 1-month (T2) and 3-month (T3) follow-up visits.

Secondary outcomes: Ataxia symptoms measured by SARA and The International Cooperative Ataxia Rating Scale (ICARS); health–related quality of life (HRQoL) measured by the EuroQol Five-dimensional questionnaire (EQ-5D); and objective quantitative outcome for gait measured by the wearable sensors.

Impact of the project: With the promising preliminary benefits of tACS on improving cerebellar motor functions and is safe and effective in treating neurological diseases, however,

the efficacy of tACS in cerebellar disorders with SCA3 has not been clinically investigated. Therefore, we performed a triple-blind, parallel-group, sham-controlled, randomized clinical trial to investigate the efficacy and safety of tACS in a large cohort of patients with SCA3. The primary outcome assessed ataxia severity, and secondary outcomes evaluated quality of life, and adverse events (AEs).

Introduction

Spinocerebellar ataxia type 3 (SCA3) is the most common autosomal dominantly inherited ataxia worldwide, also called Machado-Joseph disease (MJD), caused by an over-repetition of the trinucleotide CAG within the ATXN3 gene, which confers toxic properties to ataxin-3 (ATXN3) species[1]. It is a devastating neurodegenerative disorder that principally affects the deep cerebellar and pontine nuclei, basal ganglia, and spinal cord, and leading to severe disability and premature death. Clinical symptoms in SCA3 are not restricted to the deterioration in the motor domain, but non-motor symptoms (NMS), such as sleep disturbance, fatigue, neuropathy, cognitive decline, depression, and bladder disturbance. [2]. Currently, no specific pharmacological and treatments can alter natural course of the disease. Cerebellar transcranial alternating current stimulation (tACS) is an increasingly easy-used, safe, cheap, and noninvasive tool in neuroscience that is able to modulate cerebellar excitability[3-5]. Computational modeling studies have demonstrated the biophysical feasibility of modulating cerebellar structures using tACS with only negligible spreading effects to neighboring regions[6]. Initially, this technique has been successfully applied in healthy volunteers to investigate the neural correlates of motor learning and cognitive and emotional processes[7-17]. More recently, studies also started to explore its therapeutic potential in a variety of neurological conditions, such as Parkinson's disease [18,19], Alzheimer's disease[20,21], epilepsy[22], schizophrenia[23], depression[24], insomnia[25], and stroke[26]. No serious adverse events of cerebellar tACS have been reported in these and other studies and it is therefore considered a safe and tolerable method [27]. Possible side effects mainly include a transient optical illusion, itching, tingling, or mild burning sensation underneath the electrodes[27]. Inspired by these promising findings, we designed the clinically-oriented SCA3-tACS, randomized, parallel sham-controlled clinical trial to explore whether cerebellar tACS decreases ataxia severity and a variety of non-motor symptoms in a homogeneous cohort of SCA3 patients and if so,

what the duration of this beneficial effect is.

Methods

Ethics and dissemination

This study was carried out according to the Declaration of Helsinki and got the approval of the local ethics committee of The First Affiliated Hospital of Fujian Medical University, Fuzhou, China (MRCTA, ECFAH of FMU [2022]399) on Aug 5, 2022. The contacts of the ethics committee are: 0086–0591-87,981,028, fykyll@163.com, and No.20, Chazhong Road, Fuzhou, Fujian Province, China. Participants need to sign an informed consent form before this study and receive medical care after the study. The research results will be published through articles or conferences. Thus, this study will benefit the treatment of SCA3 patients and hopefully supply an effective non-pharmacological intervention soon.

Study participants

Participants were enrolled from the clinical Observation Cohort of the Organization in South-East China for Cerebellar Ataxia Research (OSCCAR) (NCT04010214) in the Department of Neurology, the First Affiliated Hospital of Fujian Medical University from August 2022 to March 2023. All eligible participants will get access to the specific treatment plans and they can register directly to attend the trial for randomization. All potential risks will be informed in the consent. The withdrawal reason will be carefully recorded if any participant quits halfway. To maximize trial compliance, potential risks, requirements, the study schedule, and benefits will be fully explained to all recruited participants.

Participants will be instructed to avoid any anti-rehabilitation treatment for SCA3 during the study. All participants will have freedom to quit at any time and choose other medical therapy strategies.

Inclusion criteria

We will include eligible participants: 1) detectable clinical symptoms in molecularly confirmed SCA3; 2) SARA score between 3–30 at a recent pre-study visit; 3) between 18–80 years of age; 4) informed consent was provided by the participant or their family members.

Exclusion Criteria:

Participants will be excluded who: 1) medical history of stroke, encephalitis, or epilepsy; 2) presence of metallic particles in the eye, medical pumps such as an implanted cardiac

pacemaker or neurostimulator, or surgical clips (above the shoulder line); 3) serious cognitive or behavioral disorders or mental illness; 4) had taken investigational products within 4 weeks prior enrollment into this study; 5) pregnant or breastfeeding.

This is a single-centre, triple-blind, randomised, sham-controlled intervention trial. A total of at least 80 recruited participants with SCA3 will be randomly and equally assigned to the active or sham treatment group. The randomization was created by a computer program in permuted blocks of four and managed by an offsite statistician who does not participate in this study. Every participant gets an unknown-beforehand number from a sealed opaque envelope, which determines the trial he/she will participate in. The envelopes for the two tACS plans (sham and active) have the same characteristics regarding size, color, appearance, weight, and odor, and different plans are numbered by the statistician. All participants were blinded to the specific assignment during the triple-blind treatment period until the end of the follow-up, except for any emergency when the blinding will be stopped. Participants will receive a 2-week intervention, followed by a 4-week follow-up (conducted at the 6th week, T2) and a 12-week follow-up (conducted at the 14th week, T3). The entire process will strictly follow the consolidated standards of reporting trials guidelines (Figure 1) and standard protocol items: recommendations for interventional trials checklist[28,29].

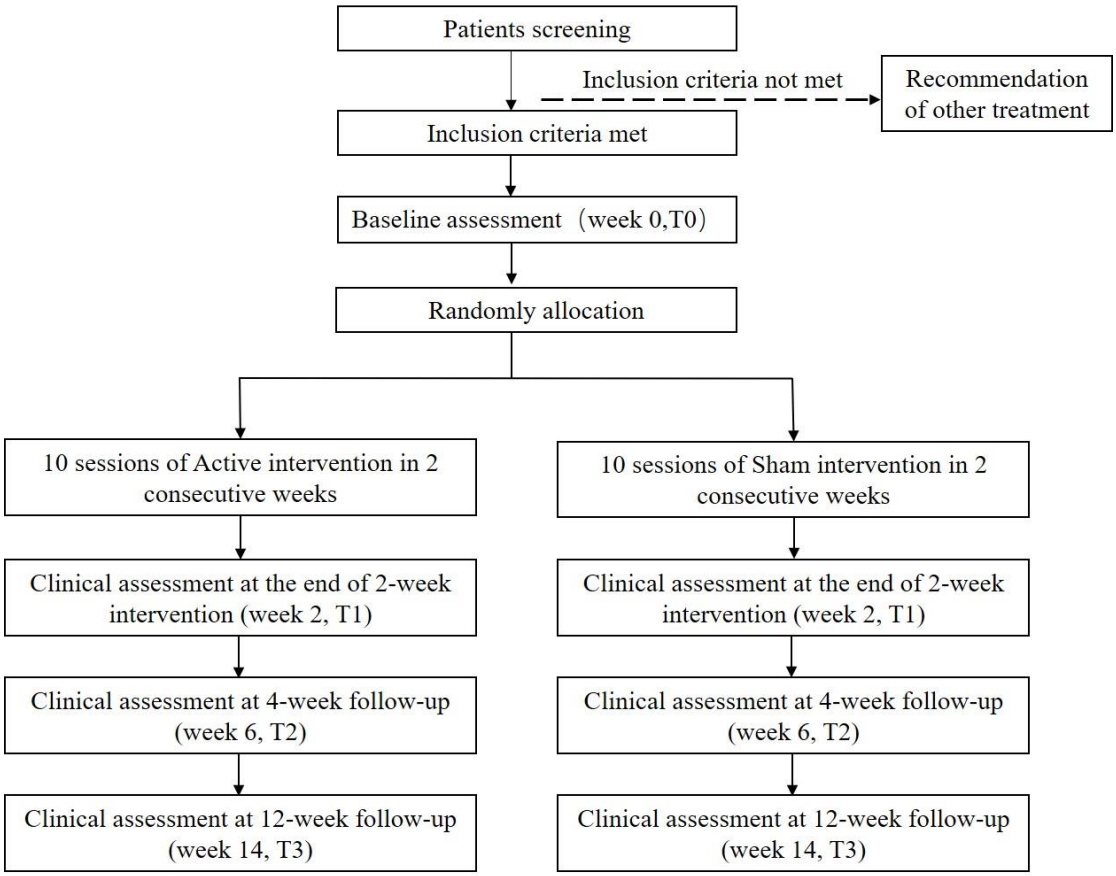

FIGURE 1 Design and flow of participants through the study.

Termination of the trial will be decided by the principal investigators, according to the following criteria: (1) lack of two consecutive tACS sessions, (2) severe adverse events (AEs), and (3) affected tACS-Assessment by treatment of other diseases. If a patient is lost to follow-up at T2 or T3, the data collected up to that point will still be used in the statistical analysis at T2/T3.

Sample size calculation

Positive effect is supposed to occur in both groups according to previous report[30]. The primary clinical remission criterion defined a ≥ 1.5 points SARA score decrease caused by 2 weeks of treatment. The SARA scores were expected to increase in 25% of placebo patients and 60% of the treated patients. 80% power was tested for 36 patients in each group, with a 35% δ between the groups, by using a 2-sided test and at the 5% level. Taking 10% attrition rate in consideration, 40 patients for each group will be needed to reach a minimum total sample size of 72. Based on these calculations, we estimate that at least 80 SCA3 participants will enroll in the study.

Transcranial alternating current stimulation intervention

We applied 3 saline-soaked (0.9% NaCl) surface sponge electrodes (all rectangular-shaped in two sizes of 5×5 and 5×7 cm) to the scalp and used the battery-driven current stimulator (Neustim NSS18, Neuracle, Changzhou, China) to administer tACS. Five-digit codes were used to ensure blinding of the study coordinators with regard to the stimulation conditions. The Neustim NSS18 device does not display any information that would provide insights into whether active or sham stimulation is applied. The return electrode (5×7 cm) was placed 2 cm below the inion and the two active electrodes were over the bilateral buccinator muscles. After computational modeling of electric field distribution, we chose this CB-tACS particular montage with an extracephalic electrode, which can lead to significant entrainment of brain oscillations [6]. Electrodes are fixed with elastic gauze and coated with conductive gel to reduce contact resistance (<5 kΩ for all sessions).

The two stimulation electrodes each delivered an alternating sinusoidal current waveform with 2mA peak-to-peak amplitude at a frequency of 70 Hz. Each stimulation ramped up and down for 10 s respectively. For active 70Hz-tACS, the stimulation lasted for 40 mins. Sham stimulation was identical to active, except that stimulation only lasted for 40 seconds. All participants completed forty-minute daily sessions of 5 days/week for 2 consecutive weeks at approximately the same time of day (±90 min), combined with either Active-tACS or sham tACS. The treatment type (A-tACS vs. S-tACS) was encoded in the in-house software and was masked for both patients and the researchers. An independent technician to set the position of an internal switch on the active or sham controller. No one was aware of the switch position (T or B) was the sham position and treatment type (A-tACS vs. S-tACS) representative for sham, except for the independent technician. Researchers only needed to start stimulation according to the treatment type corresponding to the random number chosen by the participant, without knowing whether those specific numbers were assigned to A-tACS or S-tACS. The technician finally confirmed whether an A-tACS or S-tACS was delivered after each treatment was done.

Participants were asked to sit in a bright and quiet room during all stimulation period, with their eyes open but no speaking and no large movement. To examine differences between simulated perceptions, participants need to answer whether they feel they were treated with

real or sham stimulation, and whether they experienced tingling skin sensations or phosphenes/light flickers. Sensations were rated from 0 (no sensation) to 4 (very strong sensations).

Computational modelling of electric field distribution

Stimulation montage and modeling of electric field distribution were simulated using SimNIBS (version 3.2.4[31]). The same standard MNI152 head model was used and conductivities of various tissues were assigned according to Wagner and colleagues[32].

Procedures

The initial screening visit occurred at least 7 days before enrollment. Participants were interviewed to obtain demographic information and general clinical history by telephone or in person. After a screening visit to assess eligibility, participants were invited to attend in-person visits at T0, T1, T2, and T3. Treatment fidelity was monitored by the principal investigator and the lead clinician in charge of the study through periodic observations of assessment and treatment sessions.

The following procedures were repeated at different timepoints (T0, T1, T2, T3) over the study period.

Assessment of symptom of ataxia

SARA is a validated clinical scale to measure the severity of ataxia comprising eight items assessing the gait, posture, speech, and limb kinetic functions. Higher SARA scores indicate worse performance, and the scores range from 0 (no ataxia) to 40 (most severe ataxia). SARA scores were obtained by experienced, blinded investigators, and the assessments were video recorded. To also used the International Cooperative Ataxia Rating Scale (ICARS), which widely used to measure ataxia severity. The ICARS comprises 19 items from four subscales, including 7 posture and gait disturbances, 7 kinetic functions, 3 oculomotor disorders, and 2 speech disorders. Higher ICARS scores indicate worse performance, and the total score of all subscores ranges from 0 to 100.

Assessment of life ability and quality of life

Health-related quality of life (HRQoL) was measured using the European Five Dimensions Questionnaire (EQ-5D-3L), which includes the EQ-5D descriptive system, the European Five Dimensions Questionnaire Visual Analogue Scale (EQ-VAS), and the Utility Index. The EQ-

5D-3L covers five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, for which a three-level response (no, moderate, or extreme problems) is given. The EQ-5D-3L describes a total of 243 (=35) health states, wherein 11,111 represents the best health state and 33,333 represents the worst health state.

Quantitative evaluation indices

Gait variability

Gait variability (GV) was defined as the percent range of variance (regularity and consistency) of a step cycle that is directly linked to dynamic postural control.[34] GV may reflect subtle changes in neurodegenerative diseases, has been observed in pre-symptomatic SCA, is correlated with clinical disease severity scores, increases with progression of ataxia, and is associated with fall risk.[35-38] Participants were instructed to walk in a comfortable and natural state on an overground 22-m distance in a well-lit flat room without any obstacles. Participants walked from 1 meter before the walkway and through 1 meter past the walkway to prevent acceleration and deceleration effects contributing to assessment of gait performance. Participant instructions were as follows: “When I say go, walk across the mat and then continue walking until I tell you to stop. Walk at your normal speed, as if you were walking down the street to go to the store.” Gait parameters were measured using two nine-dimensional (three-axis accelerometers + three-axis gyroscopes + three-axis magnetometers) Inertial Measurement Unit (BWT901CL, WIT-MOTION, Shenzhen, China), as well as two flexible pressure sensor insoles. Use Visual Studio software (Visual Studio 2017, Microsoft, the USA) was used to synchronize timestamp and task data and to process the raw data. Participants completed the walk three times at their selected speed and the mean and standard deviation of gait parameters, including stride time variability, double support time variability, toe-out angle variability, and toe-off angle variability were determined. A detailed description of the technical devices is provided elsewhere. [39]

Adverse Events

Participants were assessed at the end of each treatment session for adverse events (AEs), vital signs, and discomfort ratings (for potential scalp sensations associated with CB-tACS) using the commonly used questionnaire in CB-tACS trials, with telephone call every 2 weeks to record AEs during follow-up. Participants were instructed to report any additional adverse

events experienced during or after treatment that were not included in the questionnaire.

Outcomes

Primary outcome measurement

Efficacy outcome. The primary outcome measurement was the proportion of participates with clinical remission at the completion of treatment (defined as the reduction of SARA score at least 1.5 point from baseline).

The secondary outcome measures were proportion of patients with clinical remission at T2 and T3, and the changes at different timepoints (T1, T2, T3) from T0, compared to sham stimulation, including SARA, ICARS, EQ-5D, and gait variability. The safety outcomes included vital signs (blood pressure, body temperature, heart rate, pulse), adverse effects linked to tACS.

Data management And Quality Control

To ensure the rigor of this study, the whole process will be carried out strictly according to Guidelines for Good Clinical Practice of the International Conference on Harmonisation (ICH). Data will be collected from T0, T1, T2, and T3, respectively. All investigators, trial supervisors, and raters involved in the study must be trained before they participate in communicating and instructing participants, assessing, collecting data, and completing the CRF. Double entries of data into the software Yidu Research platform (Yiducloud (Beijing) Technology Ltd.) will be finished by two independent investigators, and these electronic data will be stored on a secure university server with regularly back-up and password protection. Yidu Research platform has an authority management mechanism associated with personnel and roles and a strict data audit mechanism to fully protect the security of data utilisation. Double-check will be performed to correct inconsistent entries or typos. The details of the quit participants, including reasons, date, AEs, and duration of the treatment, will be recorded. The final trial dataset, including the intent-to-treat and per-protocol dataset will be analyzed by an independent statistician.

Data analysis

Baseline characteristics will be summarized and presented using appropriate descriptive statistics. Normality of the variables will be assessed by using the skewness statistic and normal probability plot. Baseline characteristics between two groups will be compared using

independent–t test for continuous variables and the χ^2 analysis or Fisher's Exact test for categorical variables. Generalized estimating equations (GEE) [40] analysis will be adopted to adjust for any significant differences in baseline characteristics. Baseline characteristics of the participants who completed the study will be compared with those who did not finish the study. All primary and secondary outcomes will be compared between groups on the basis of intention–to–treat (ITT) principle. GEE models will be used to assess differential change of each primary and secondary outcomes across different time–points between two groups and within group from baseline. IBM SPSS 26.0 software will be used for all statistical analysis. Two–tailed test will be used and statistical significance will be set at a p–value < 0.05.

Intention-to-treat (ITT) refers to all eligible patients who were randomized to treatment, include drop-out cases.

Per-protocol (PP) refers to the set of cases that have completed the overall study protocol, a subset of the FAS in which each subject in the dataset is a valid case or sample with good adherence, no protocol violations, and complete baseline values for key indicators.

Missing data

Due to the long duration of intervention and the long follow-up period, so there may be a lot of missing data. If missing data were found, the percentage of missing data will be reported, the potential patterns of missing data should be examined, and appropriate method should be used for multiple imputation of missing data. The multiple imputation method will be preferred for analyzing the missing data, and the complete-case data should be reported in the manuscript as sensitivity analysis.

Analysis of primary outcome

The Type I error rate (α-level) used in the assessment of χ^2 analysis or Fisher's Exact test for the primary efficacy outcome is 5%. The assessment of significance for the A-tACS versus S-tACS contrasts will use χ^2 analysis or Fisher's Exact test, based on ITT, PP, and sensitivity analysis (multiple assumptions for the missing value).

Analysis of secondary outcomes

The Type I error rate (α-level) used in the assessment of pair-wise treatment comparisons for the primary efficacy outcome is 5%. All secondary outcomes will be compared between groups on the basis of intention–to–treat (ITT) principle. GEE models will be used to assess

differential change of each secondary outcomes across different time–points between two groups and within group from baseline.

ALFF, ReHo, and FC Analysis

Statistical analysis of MRI data was performed using Matlab2013b, SPM12 (https://www.filion.ucl.ac.uk/spm/) (Sidhu et al. 2016). The Flexible factorial of second-order analysis was used to design the statistical matrix and to set the three factors of subjects, groups, and time. Groups and time were fixed factors, and the serial number of subjects was a random factor. Then, the interaction matrix of groups × time to analysis was set to determine the intergroup interaction effect. The resulting statistical map was set at $p<0.05$ (AlphaSim correction) with a combined voxel $p<0.001$. Use RestPlus toolbox (https://github.com/topics/restplus, version 1.2) was used to extract the values of the above abnormal brain regions, and Bonferroni tests were performed for all results with significant differences to verify between-group differences. Statistical significance was set at $p<0.05$. Image presentation used the xjview toolbox in Matlab 2013b.

Analysis of intra-network functional connectivity

A one-sample Student's t-test was applied to the spatial maps of all participants for each IC using the SPM12 toolbox, and regions surviving multiple comparison corrections with voxel-level family-wise error (FWE) correction ($p<0.05$) were saved as the specific group-level mask for each group. These four masks for each IC were then integrated into a union mask, which was used for the group comparison. A single model using a flexible-factorial design (2×2) was performed to obtain the ICs showing significant between-group differences. Regions showing significant differences (voxel-level FWE correction, $p<0.05$) were saved as the mask for subsequent post-hoc analyses, and the significance level was set at $p<0.0125$ (0.05/4) for Bonferroni correction across 4 tests.

Software and significant level of the statistical analyses

IBM SPSS 26.0 (IBM, Armonk, NY, USA) software and R, version 4.2.1 software (The R Project for Statistical Computing, www.r-project.org) will be used for all statistical analysis. Statistical significance was defined as $P<0.05$ with 2-sided testing.

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STUDY PROTOCOL FOR MSA

1. Project title

Safety and efficacy of transcranial alternating current stimulation in multiple system atrophy–Cerebellar Type: a randomized proof-of-concept clinical trial.

2. Abstract of project

Background/Objective: Multiple system atrophy (MSA) is a rare, progressive neurodegenerative disorder characterized by a variable combination of autonomic failure, parkinsonism, cerebellar ataxia, and pyramidal signs. The etiology of MSA is unknown and there is no effective treatment available. Depending on the predominant motor symptom, MSA can be classified as MSA with predominant parkinsonism (MSA-P) or MSA with predominant cerebellar ataxia (MSA-C). In MSA-C, cerebellar dysfunction manifests as gait ataxia, limb ataxia, ataxic dysarthria, and eye movement abnormalities such as dysmetria, saccadic intrusion, and ocular dysmetria. Currently, no specific pharmacological and treatments can alter natural course of the disease. As well, we found that there are not enough clinical trials involving MSA-C and these studies usually had small samples. Clinical practice guidelines had confirmed the safety of transcranial alternating current stimulation (tACS) in treating neuropsychiatric diseases, but to our knowledge, no randomized clinical trials have investigated whether tACS is safety and efficacy during improve symptoms in MSA-C. The objectives of this study are to identify the efficacy and safety of tACS during improving ataxia symptoms and non-ataxia symptoms in adult patients with MSA-C.

Participants: Individual with MSA-C

Design: A triple-blind, randomized sham-controlled trial with two groups. At least 84 subjects will be recruited and randomized into either active tACS group or sham tACS group.

Interventions: Cerebellar tACS (40 min, 2 mA, ramp-up and down periods of 10s each) will be delivered over 10 sessions, distributed in two groups of five consecutive days with a two-day break in between. The active tACS group will receive whole course current stimulation, while the control group will receive a daily 40–seconds current stimulation for two weeks.

Primary outcomes: The primary outcome was the difference in total Unified Multiple System Atrophy Rating Scale (UMSARS) score between the two groups being treated for 2 weeks (T1).

Secondary outcomes: The difference in total UMSARS score between the two groups at 1-month follow-up visits (T2) and 3-month follow-up visits (T3). Ataxia symptoms measured by SARA and The International Cooperative Ataxia Rating Scale (ICARS); health–related quality of life (HRQoL) measured by the the Multiple System Atrophy Quality of Life questionnaires (MSA-QoL) ; and objective quantitative outcome for gait measured by the wearable sensors.

Impact of the project: With the promising preliminary benefits of tACS on improving cerebellar motor functions and is safe and effective in treating neurological diseases, however, the efficacy of tACS in cerebellar disorders with MSA-C has not been clinically investigated. Therefore, we performed a triple-blind, parallel-group, sham-controlled, randomized clinical trial to investigate the efficacy and safety of tACS in a large cohort of patients with MSA-C. The primary outcome assessed ataxia severity, and secondary outcomes evaluated quality of life, and adverse events (AEs).

Introduction

Multiple-system atrophy with predominant cerebellar ataxia (MSA-C) is a sporadic, adult-onset, and rapidly progressive fatal neurodegenerative disease that presents with cerebellar ataxia and other associated symptoms of undetermined etiology with a mean life expectancy of 6–9 years after diagnosis, and increases functional disability, the likelihood of falls and the social economic burden of patients [[1, 2]. Cerebellar ataxia, the main symptom of MSA-C, is the most disabling symptom because there is no satisfactory symptomatic treatment available; no curative or neuroprotective treatments for the disease have been identified to date [3]. Therefore, various pharmacological and non-pharmacological trials have been attempted, and non-invasive brain stimulation has been regarded as a possible treatment candidate for cerebellar ataxia.

Cerebellar transcranial alternating current stimulation (tACS) is an increasingly easy-used, safe, cheap, and noninvasive tool in neuroscience that is able to modulate cerebellar excitability[3-5]. Computational modeling studies have demonstrated the biophysical feasibility of modulating cerebellar structures using tACS with only negligible spreading effects to neighboring regions[6]. Initially, this technique has been successfully applied in healthy volunteers to investigate the neural correlates of motor learning and cognitive and emotional

processes[7-17]. More recently, studies also started to explore its therapeutic potential in a variety of neurological conditions, such as Parkinson's disease [18,19], Alzheimer's disease[20,21], epilepsy[22], schizophrenia[23], depression[24], insomnia[25], and stroke[26]. No serious adverse events of cerebellar tACS have been reported in these and other studies and it is therefore considered a safe and tolerable method [27]. Possible side effects mainly include a transient optical illusion, itching, tingling, or mild burning sensation underneath the electrodes[27]. Inspired by these promising findings, we designed the clinically-oriented MSA-tACS, randomized, parallel sham-controlled clinical trial to explore whether cerebellar tACS decreases ataxia severity and a variety of non-motor symptoms in a homogeneous cohort of MSA-C patients and if so, what the duration of this beneficial effect is.

Methods

Ethics and dissemination

This study was carried out according to the Declaration of Helsinki and got the approval of the local ethics committee of The First Affiliated Hospital of Fujian Medical University, Fuzhou, China (MRCTA, ECFAH of FMU [2022]399) on Aug 5, 2022. The contacts of the ethics committee are: 0086–0591-87,981,028, fykyll@163.com, and No.20, Chazhong Road, Fuzhou, Fujian Province, China. Participants need to sign an informed consent form before this study and receive medical care after the study. The research results will be published through articles or conferences. Thus, this study will benefit the treatment of MSA-C patients and hopefully supply an effective non-pharmacological intervention soon.

Study participants

Participants were enrolled from the clinical Observation Cohort of the Organization in South-East China for Cerebellar Ataxia Research (OSCCAR) (NCT04010214) in the Department of Neurology, the First Affiliated Hospital of Fujian Medical University from August 2022 to March 2024. All eligible participants will get access to the specific treatment plans and they can register directly to attend the trial for randomization. All potential risks will be informed in the consent. The withdrawal reason will be carefully recorded if any participant quits halfway. To maximize trial compliance, potential risks, requirements, the study schedule, and benefits will be fully explained to all recruited participants.

Participants will be instructed to avoid any anti-rehabilitation treatment for MSA-C during the

study. All participants will have freedom to quit at any time and choose other medical therapy strategies.

Inclusion criteria

We will include eligible participants: 1) 30–80 years old; 2) Clinically diagnose or probable MSA-C according to the latest MSA diagnostic criteria; 3) <8 years MSA-C medical history; 4) Independent walk (or with assistance); 5) >3 years life expectancy; 6) Contraceptive measures for women of childbearing age; 7) informed consent was provided by the participant or their family members.

Exclusion Criteria:

Participants will be excluded who: 1) medical history of stroke, encephalitis, or epilepsy; 2) presence of metallic particles in the eye, medical pumps such as an implanted cardiac pacemaker or neurostimulator, or surgical clips (above the shoulder line); 3) serious cognitive or behavioral disorders or mental illness; 4) had taken investigational products within 4 weeks prior enrollment into this study; 5) pregnant or breastfeeding.

This is a single-center, triple-blind, randomized, sham-controlled intervention trial. A total of at least 84 recruited participants with MSA-C will be randomly and equally assigned to the active or sham treatment group. The randomization was created by a computer program in permuted blocks of four and managed by an offsite statistician who does not participate in this study. Every participant gets an unknown-beforehand number from a sealed opaque envelope, which determines the trial he/she will participate in. The envelopes for the two tACS plans (sham and active) have the same characteristics regarding size, color, appearance, weight, and odor, and different plans are numbered by the statistician. All participants were blinded to the specific assignment during the triple-blind treatment period until the end of the follow-up, except for any emergency when the blinding will be stopped. Participants will receive a 2-week intervention, followed by a 4-week follow-up (conducted at the 6th week, T2) and a 12-week follow-up (conducted at the 14th week, T3). The entire process will strictly follow the consolidated standards of reporting trials guidelines (Figure 1) and standard protocol items: recommendations for interventional trials checklist[28,29].

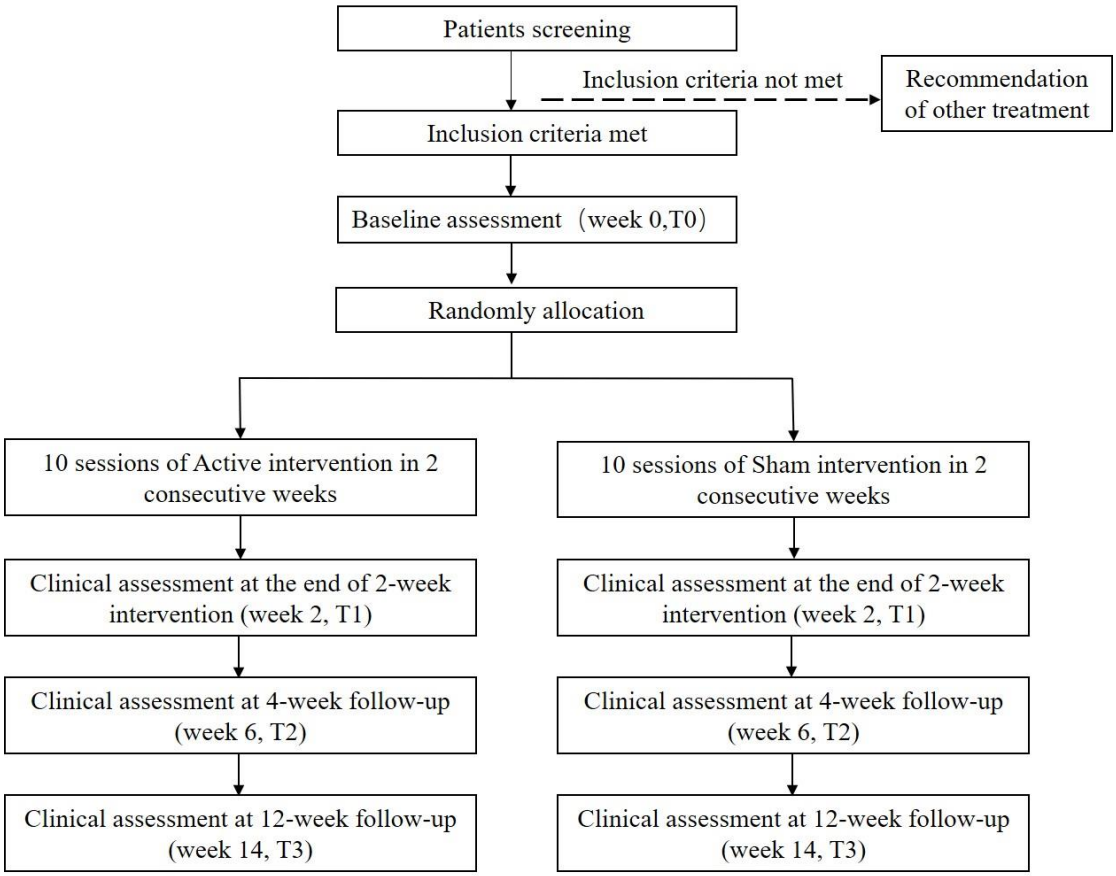

FIGURE 1 Design and flow of participants through the study.

Termination of the trial will be decided by the principal investigators, according to the following criteria: (1) lack of two consecutive tACS sessions, (2) severe adverse events (AEs), and (3) affected tACS-Assessment by treatment of other diseases. If a patient is lost to follow-up at T2 or T3, the data collected up to that point will still be used in the statistical analysis at T2/T3.

Sample size calculation

Our sample size was calculated as previously described (PMID: 31278067). This study is expected to produce α of 0.05 at 80% power for a treatment effect of 50% on the annual progression of the movement examination score on the Unified Multiple System Atrophy Rating Scale (UMSARS); this contains 4 points from the active group and 8 points from the placebo group, which is above the minimal clinically detectable significant level of 3.8 points (PMID: 27474888). Therefore, we conducted this study to determine whether active tACS treatment would have a positive effect on MSA-C patients. The target total patients was 84, with an expected attrition rate of 10%.

Transcranial alternating current stimulation intervention

We applied 3 saline-soaked (0.9% NaCl) surface sponge electrodes (all rectangular-shaped in two sizes of 5×5 and 5×7 cm) to the scalp and used the battery-driven current stimulator (Neustim NSS18, Neuracle, Changzhou, China) to administer tACS. Five-digit codes were used to ensure blinding of the study coordinators with regard to the stimulation conditions. The Neustim NSS18 device does not display any information that would provide insights into whether active or sham stimulation is applied. The return electrode (5×7 cm) was placed 2 cm below the inion and the two active electrodes were over the bilateral buccinator muscles. After computational modeling of electric field distribution, we chose this CB-tACS particular montage with an extracephalic electrode, which can lead to significant entrainment of brain oscillations [6]. Electrodes are fixed with elastic gauze and coated with conductive gel to reduce contact resistance (<5 kΩ for all sessions).

The two stimulation electrodes each delivered an alternating sinusoidal current waveform with 2mA peak-to-peak amplitude at a frequency of 70 Hz. Each stimulation ramped up and down for 10 s respectively. For active 70Hz-tACS, the stimulation lasted for 40 mins. Sham stimulation was identical to active, except that stimulation only lasted for 40 seconds. All participants completed forty-minute daily sessions of 5 days/week for 2 consecutive weeks at approximately the same time of day (±90 min), combined with either Active-tACS or sham tACS. The treatment type (A-tACS vs. S-tACS) was encoded in the in-house software and was masked for both patients and the researchers. An independent technician to set the position of an internal switch on the active or sham controller. No one was aware of the switch position (T or B) was the sham position and treatment type (A-tACS vs. S-tACS) representative for sham, except for the independent technician. Researchers only needed to start stimulation according to the treatment type corresponding to the random number chosen by the participant, without knowing whether those specific numbers were assigned to A-tACS or S-tACS. The technician finally confirmed whether an A-tACS or S-tACS was delivered after each treatment was done.

Participants were asked to sit in a bright and quiet room during all stimulation period, with their eyes open but no speaking and no large movement. To examine differences between simulated perceptions, participants need to answer whether they feel they were treated with

real or sham stimulation, and whether they experienced tingling skin sensations or phosphenes/light flickers. Sensations were rated from 0 (no sensation) to 4 (very strong sensations).

Computational modelling of electric field distribution

Stimulation montage and modeling of electric field distribution were simulated using SimNIBS (version 3.2.4[31]). The same standard MNI152 head model was used and conductivities of various tissues were assigned according to Wagner and colleagues[32].

Procedures

The initial screening visit occurred at least 7 days before enrollment. Participants were interviewed to obtain demographic information and general clinical history by telephone or in person. After a screening visit to assess eligibility, participants were invited to attend in-person visits at T0, T1, T2, and T3. Treatment fidelity was monitored by the principal investigator and the lead clinician in charge of the study through periodic observations of assessment and treatment sessions.

The following procedures were repeated at different timepoints (T0, T1, T2, T3) over the study period.

The primary outcome was the change from baseline to week 2 in the Unified Multiple System Atrophy Rating Scale (UMSARS) total score. Higher UMSARS scores denote poorer health.

Severity of neurological deficits

UMSARS is a clinician-scored rating scale to assess the severity of symptoms for patients with MSA. Disease severity was rated with part I (activities of daily living, 12 items), part II (motor examination, 14 items), part III (autonomic examination), and part IV (global disability) of the Unified Multiple System Atrophy Rating Scale (UMSARS). The total UMSARS score is the sum of parts I and II. UMSARS has also been demonstrated to be reliable and valid. Higher UMSARS scores denote poorer health.

Assessment of symptom of ataxia

SARA is a validated clinical scale to measure the severity of ataxia comprising eight items assessing the gait, posture, speech, and limb kinetic functions. Higher SARA scores indicate worse performance, and the scores range from 0 (no ataxia) to 40 (most severe ataxia). SARA scores were obtained by experienced, blinded investigators, and the assessments were video

recorded. To also used the International Cooperative Ataxia Rating Scale (ICARS), which widely used to measure ataxia severity. The ICARS comprises 19 items from four subscales, including 7 posture and gait disturbances, 7 kinetic functions, 3 oculomotor disorders, and 2 speech disorders. Higher ICARS scores indicate worse performance, and the total score of all subscores ranges from 0 to 100.

Assessment of life ability and quality of life

Health-related quality of life (HRQoL) was measured using the MSA-QoL questionnaire, developed in 2008. The MSA-QoL is composed of 40-item questionnaire that asks patients to rate their level of difficulty with specific mental or physical domains, as well as a question asking patients to mark overall life satisfaction on a scale from 0 to 100. The questionnaire was developed from physician and patient reports and psychometric data analysis and has been demonstrated to be reliable and valid. Items include questions about mobility, coordination, ability to complete self-care, other activities of daily living, symptoms of dysautonomia, sleep quality, cognitive function, and social/emotional impacts of the disease. In total, there are 14 motor items, 12 nonmotor items, and 14 emotional/social items. Higher total scores on the MSA-QoL indicate higher levels of impairment, and higher scores on the life satisfaction item correspond with higher life satisfaction. The original version was translated to adapt to the Chinese-speaking audience. Since 2008, patients have been asked to complete the MSA-QoL questionnaire during each annual consultation.

Quantitative evaluation indices

Gait variability

Gait variability (GV) was defined as the percent range of variance (regularity and consistency) of a step cycle that is directly linked to dynamic postural control.[34] GV may reflect subtle changes in neurodegenerative diseases, has been observed in pre-symptomatic SCA, is correlated with clinical disease severity scores, increases with progression of ataxia, and is associated with fall risk.[35-38] Participants were instructed to walk in a comfortable and natural state on an overground 22-m distance in a well-lit flat room without any obstacles. Participants walked from 1 meter before the walkway and through 1 meter past the walkway to prevent acceleration and deceleration effects contributing to assessment of gait performance. Participant instructions were as follows: “When I say go, walk across the mat

and then continue walking until I tell you to stop. Walk at your normal speed, as if you were walking down the street to go to the store." Gait parameters were measured using two nine-dimensional (three-axis accelerometers + three-axis gyroscopes + three-axis magnetometers) Inertial Measurement Unit (BWT901CL, WIT-MOTION, Shenzhen, China), as well as two flexible pressure sensor insoles. Use Visual Studio software (Visual Studio 2017, Microsoft, the USA) was used to synchronize timestamp and task data and to process the raw data. Participants completed the walk three times at their selected speed and the mean and standard deviation of gait parameters, including stride time variability, double support time variability, toe-out angle variability, and toe-off angle variability were determined. A detailed description of the technical devices is provided elsewhere. [39]

Adverse Events

Participants were assessed at the end of each treatment session for adverse events (AEs), vital signs, and discomfort ratings (for potential scalp sensations associated with CB-tACS) using the commonly used questionnaire in CB-tACS trials, with telephone call every 2 weeks to record AEs during follow-up. Participants were instructed to report any additional adverse events experienced during or after treatment that were not included in the questionnaire.

Outcomes

Primary outcome measurement

The primary outcome was the change from baseline to week 2 in the Unified Multiple System Atrophy Rating Scale (UMSARS) total score (sum of parts I and II). PMID: 15452868. Higher UMSARS scores denote poorer health.

The secondary outcomes included the change in UMSARS part II (UMSARS II; motor examination scores, on which scores range from 0 to 56), the changes at different timepoints (T1, T2, T3) from T0, compared to sham stimulation, including SARA, ICARS, MSA-QoL, and gait variability. The safety outcomes included vital signs (blood pressure, body temperature, heart rate, pulse), adverse effects linked to tACS.

Data management And Quality Control

To ensure the rigor of this study, the whole process will be carried out strictly according to Guidelines for Good Clinical Practice of the International Conference on Harmonisation (ICH). Data will be collected from T0, T1, T2, and T3, respectively. All investigators, trial

supervisors, and raters involved in the study must be trained before they participate in communicating and instructing participants, assessing, collecting data, and completing the CRF. Double entries of data into the software Yidu Research platform (Yiducloud (Beijing) Technology Ltd.) will be finished by two independent investigators, and these electronic data will be stored on a secure university server with regularly back-up and password protection. Yidu Research platform has an authority management mechanism associated with personnel and roles and a strict data audit mechanism to fully protect the security of data utilisation. Double-check will be performed to correct inconsistent entries or typos. The details of the quit participants, including reasons, date, AEs, and duration of the treatment, will be recorded. The final trial dataset, including the intent-to-treat and per-protocol dataset will be analyzed by an independent statistician.

Data analysis

Baseline characteristics will be summarized and presented using appropriate descriptive statistics. Normality of the variables will be assessed by using the skewness statistic and normal probability plot. Baseline characteristics between two groups will be compared using independent–t test for continuous variables and the χ^2 analysis or Fisher's Exact test for categorical variables. Generalized estimating equations (GEE) [40] analysis will be adopted to adjust for any significant differences in baseline characteristics. Baseline characteristics of the participants who completed the study will be compared with those who did not finish the study. All primary and secondary outcomes will be compared between groups on the basis of intention–to–treat (ITT) principle. GEE models will be used to assess differential change of each primary and secondary outcomes across different time–points between two groups and within group from baseline. IBM SPSS 26.0 software will be used for all statistical analysis. Two–tailed test will be used and statistical significance will be set at a p–value < 0.05.

Intention-to-treat (ITT) refers to all eligible patients who were randomized to treatment, include drop-out cases.

Per-protocol (PP) refers to the set of cases that have completed the overall study protocol, a subset of the FAS in which each subject in the dataset is a valid case or sample with good adherence, no protocol violations, and complete baseline values for key indicators.

Missing data

Due to the long duration of intervention and the long follow-up period, so there may be a lot of missing data. If missing data were found, the percentage of missing data will be reported, the potential patterns of missing data should be examined, and appropriate method should be used for multiple imputation of missing data. The multiple imputation method will be preferred for analyzing the missing data, and the complete-case data should be reported in the manuscript as sensitivity analysis.

Analysis of primary outcome

The Type I error rate (α-level) used in the assessment of χ^2 analysis or Fisher's Exact test for the primary efficacy outcome is 5%. The assessment of significance for the A-tACS versus S-tACS contrasts will use χ^2 analysis or Fisher's Exact test, based on ITT, PP, and sensitivity analysis (multiple assumptions for the missing value).

Analysis of secondary outcomes

The Type I error rate (α-level) used in the assessment of pair-wise treatment comparisons for the primary efficacy outcome is 5%. All secondary outcomes will be compared between groups on the basis of intention–to–treat (ITT) principle. GEE models will be used to assess differential change of each secondary outcomes across different time–points between two groups and within group from baseline.

ALFF, ReHo, and FC Analysis

Statistical analysis of MRI data was performed using Matlab2013b, SPM12 (https://www.filion.ucl.ac.uk/spm/) (Sidhu et al. 2016). The Flexible factorial of second-order analysis was used to design the statistical matrix and to set the three factors of subjects, groups, and time. Groups and time were fixed factors, and the serial number of subjects was a random factor. Then, the interaction matrix of groups × time to analysis was set to determine the intergroup interaction effect. The resulting statistical map was set at $p<0.05$ (AlphaSim correction) with a combined voxel $p<0.001$. Use RestPlus toolbox (https://github.com/topics/restplus, version 1.2) was used to extract the values of the above abnormal brain regions, and Bonferroni tests were performed for all results with significant differences to verify between-group differences. Statistical significance was set at $p<0.05$. Image presentation used the xjview toolbox in Matlab 2013b.

Analysis of intra-network functional connectivity

A one-sample Student's t-test was applied to the spatial maps of all participants for each IC using the SPM12 toolbox, and regions surviving multiple comparison corrections with voxel-level family-wise error (FWE) correction ($p<0.05$) were saved as the specific group-level mask for each group. These four masks for each IC were then integrated into a union mask, which was used for the group comparison. A single model using a flexible-factorial design (2×2) was performed to obtain the ICs showing significant between-group differences. Regions showing significant differences (voxel-level FWE correction, $p<0.05$) were saved as the mask for subsequent post-hoc analyses, and the significance level was set at $p<0.0125$ (0.05/4) for Bonferroni correction across 4 tests.

Software and significant level of the statistical analyses

IBM SPSS 26.0 (IBM, Armonk, NY, USA) software and R, version 4.2.1 software (The R Project for Statistical Computing, www.r-project.org) will be used for all statistical analysis. Statistical significance was defined as $P<0.05$ with 2-sided testing.