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ORIGINAL

THE EFFECT OF TAI CHI FAN ON AUTISTIC CHILDREN AND CHANGES IN THEIR INTESTINAL MICROECOLOGY

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ABSTRACT

Background: The incidence of autism spectrum disorder (ASD) is still on the rise worldwide. Tai Chi fan is considered a non-pharmacological treatment with potential benefits for the treatment of ASD. However, there is currently insufficient evidence to support this claim. **Objective:** This study aims to determine the efficacy and safety of Tai Chi fan in improving ASD, and explore its possible impact on intestinal microecology. **Methods:** Based on a Randomized Controlled Trial (RCT) design and a gut microecology analysis program，this study recruited 40 children with ASD and randomly assigned them to either a Tai Chi fan group or a control group. The intervention consisted of eight weeks of Tai Chi fan exercise and rehabilitation as standard treatment, followed by a two-week follow-up. The primary outcome was autism severity, measured by the Autism Treatment Evaluation Checklist (ATEC) and the secondary outcome was the assessment of autism and its related symptoms using the Childhood Autism Rating Scale (CARS), Social Responsiveness Scale (SRS), and Autism Behavior Checklist (ABC). All above scales with higher scores indicating a greater degree of autism. The Test of Gross Motor Development, Third Edition (TGMD-3) was used to evaluate gross motor

development, with higher scores indicating better gross motor development. The study also collected 16s rDNA intestinal microecology samples to explore the association between the structure and function of gut microecology and the effect of Tai Chi fan exercise. Overall, the study aims to fully leverage the clinical and intestinal microecology data to assess the clinical effectiveness of Tai Chi fan exercise on autism spectrum disease. **Discussion:** We believe that the results of this study will enhance our comprehension of how Tai Chi fan exercise influences the clinical symptomatic manifestations linked with ASD and changes in intestinal micromorphology. These insights could potentially fortify the clinical evidence supporting the use of Tai Chi fan exercise in treating patients with ASD.

KEYWORDS: Autism Spectrum Disorder, Medicine, Chinese Traditional, Tai Ji, Gastrointestinal Microbiome

1. INTRODUCTION

Autism Spectrum Disorders (ASDs), also known as autism or autistic disorder, are a specific type of pervasive developmental disorder (McPartland & Volkmar, 2012). Epidemiological data show that roughly 1 in 100 children worldwide suffer from autism (Zeidan et al., 2022). Furthermore, the prevalence of autism worldwide has shown a significantly increasing trend (Lyall et al., 2017). Individuals with ASD present with various symptoms, including in social interaction, verbal and nonverbal communication, repetitive behaviors, and motor impairments (Fulceri et al., 2019; Geschwind, 2009). Moreover, those with autism exhibit higher rates of obesity and lower physical fitness compared to their typically developing peers (Báez-Sánchez & Bobko, 2021; Jones et al., 2017). Current clinical treatments for autism involve pharmacotherapy and rehabilitation training, supplemented by emerging therapies like art and animal-assisted therapy (de Pablo et al., 2023; Lanning, Baier, Ivey-Hatz, Krenek, & Tubbs, 2014; Phung & Goldberg, 2021). However, these therapies often require extensive one-on-one therapist support, resulting in high labor costs, and long-term medication use may produce adverse side effects, posing risks to children's physical well-being (Barnhill, Tami, Schutte, Hewitson, & Olive, 2016; Cheng, Rho, & Masino, 2017). Therefore, consequently, alternative intervention approaches that are cost-effective, easily implementable, and carry minimal side effects, such as sports exercise, warrant exploration for their therapeutic potential in autism treatment (Bahrami, Movahedi, Marandi, & Abedi, 2012; Bahrami, Movahedi, Marandi, & Sorensen, 2016; Bremer, Crozier, & Lloyd, 2016; Liu, Fedak, & Hamilton, 2016; MacDonald et al., 2012; Nazemzadegan, Babadi, Zeinali, & Kakavandi, 2016; Pan, 2010; Wang, Chen, Yoon, Klich, & Chen, 2022; Yilmaz, Yanardag, Birkan, & Bumin, 2004).

Tai Chi fan, has been suggested as a potential intervention for children with ASD due to its ability to regulate the body through physical movements,

inner thoughts, and breathing methods (Li et al., 2024; Zheng & Qu, 2020). The combination of physical exercise and music can increase children's participation and enjoyment in exercise interventions, leading to a more effective treatment. Furthermore, Tai Chi fan exercise can regulate qi activity, promote blood circulation, strengthen the muscles, and provide other health benefits (Song, Zhang, & Zhai, 2022). The "microbial-gut-brain axis" (Estes & McAllister, 2017) has emerged as a potential mechanism linking the gut microbiota with the neurological development of ASD, which indicates that the gut floracan produce two-way communication and interaction between the gastrointestinal tract and the central nervous system (Montiel-Castro, González-Cervantes, Bravo-Ruiseco, & Pacheco-López, 2013). Several studies indicate (De Angelis et al., 2013; Finegold, 2011; Finegold et al., 2010; Tomova et al., 2015) that the intestinal flora in individuals with autism differs from those in the general population of the same age.

Autistic patients tend to have a lower presence of Firmicutes and a higher amount of Bacteroidetes, Actinobacteria, and Proteobacteria in their gut. This abnormal gut bacterium affects ASD by producing abnormal metabolites such as higher levels of short-chain fatty acids (SCFAs), serotonin, and abnormal sulfur metabolism. The quantity and structure of this intestinal microecology showed a high correlation with autism. Research suggests that autism symptoms may be alleviated through improving gut function or fecal microbiota transplantation (Dinan & Cryan, 2017; Iglesias-Vázquez, Van Ginkel Riba, Arija, & Canals, 2020; Kang et al., 2019; Ristori et al., 2019; Strati et al., 2017). Alternatively, there is evidence that exercise can improve gut microecology (Chan et al., 2013; Du, Su, & Zhao, 2020), such as after prolonged exercise can increase in the levels of Prausnitzii and Lactobacillus, and obesity in human gut microecology of Blautia, Dialister, Roseburia gradually approaching the intestinal flora status of healthy people. Therefore, we propose a RCT using Tai Chi fan intervention for children with ASD. Our study aims to investigate the effects of Tai Chi fan exercise intervention on disease severity and related symptoms in children with ASD, as well as changes in their intestinal microecological characteristics.

2. Methods

2.1 Study design

The study was a prospective, randomized, single-blind, two-armed, parallel-group controlled trial. Due to the particularity of exercise intervention therapy, this study could not set up blind method for participants and intervention practitioners. Figure 1 shows a diagram with the different stages of the study. This protocol strictly complies with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines.

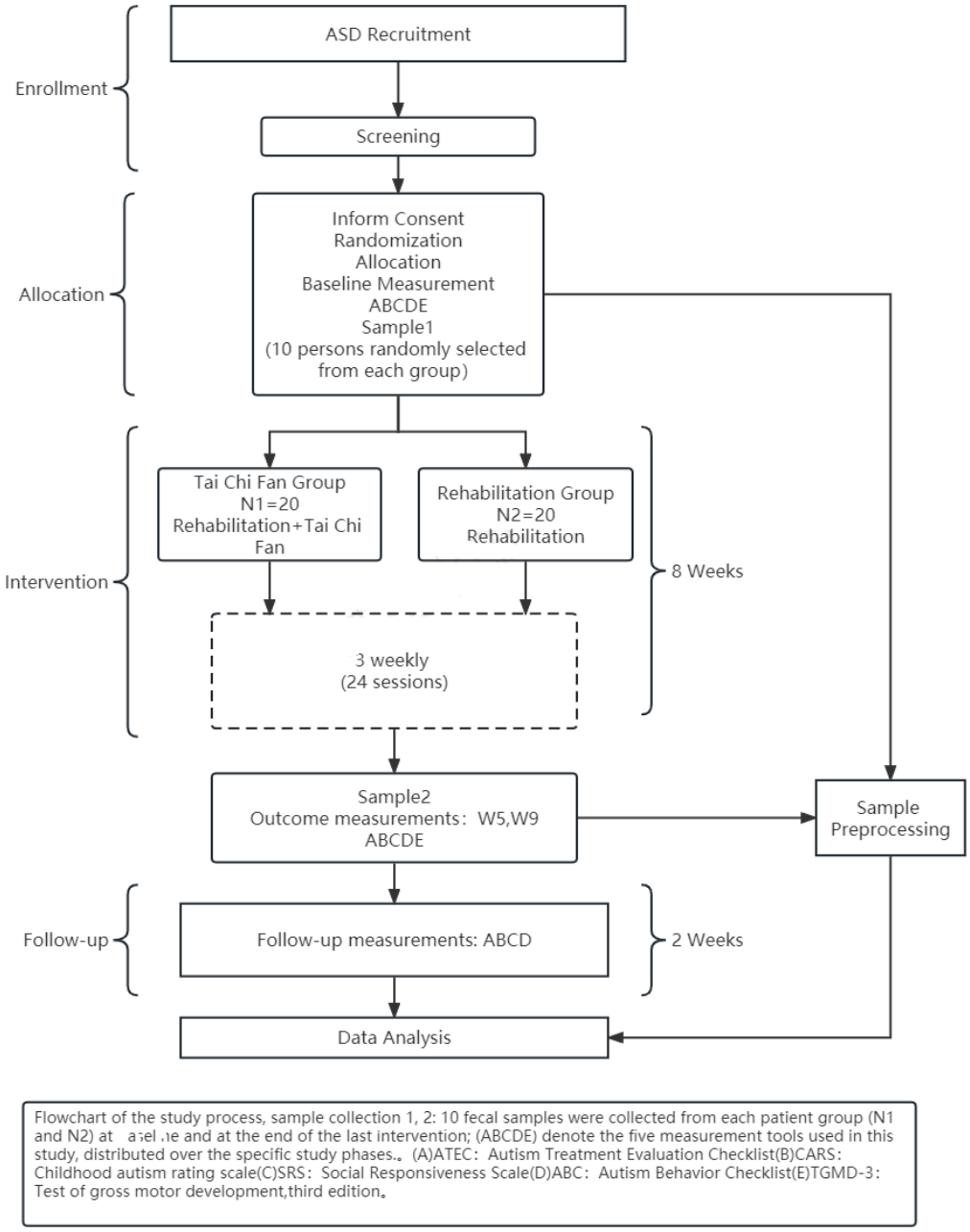

Figure 1: Flow chart of Study Process

2.2 Study population and sample recruitment

The study is scheduled to commence enrollment in May 2023 and is expected to be completed December 31, 2023. The study will recruit participants for intervention and assessment from Guangdong Province, China special education schools: The Zhanjiang Special Education School and the Wuchuan Special Education School. Participants will be recruited through on-site phone interviews conducted with teachers or guardians. Informed consent will be obtained from parents or legal guardians before enrollment. Participants should meet the inclusion and exclusion criteria before offering them the

opportunity to participate in the study.

Inclusion Criteria: 1. Meets the diagnosis of ASD in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) (see Supplementary Table 1 in Supplement 5); 2. Age between 6-17 years old, gender not limited；3. Children with mild to moderate autism diagnosed by Grade-A tertiary hospital；4.The children live a regular life；5.Subjects' parents or guardians understand the study and sign the informed consent.

Exclusion Criteria: 1. Patients with heart, liver, brain, kidneys, and hematopoietic system diseases； 2. Patients with other psychiatric system disorders or neurodevelopmental disorders；3. Physical disability impairments or bone and joint disorders that preclude exercise intervention；4.Have a regular exercise routine or have recently (4 weeks) received an exercise intervention similar to Tai Chi Fan； 5.Patients with gastrointestinal diseases, metabolic disorders, irritable bowel syndrome, food allergies, anemia, and epilepsy；6. Have a special diet: ketogenic diet, gluten-free diet, high-protein diet；7. Take antipsychotics.

2.3 Sample size calculation

The primary outcome measure for the efficacy of the treatment was the severity of autism, as measured by the ATEC. Based on the experience of previous admissions, exercise therapy was used as an intervention and evaluated at the end of the 12th week of treatment. The results indicated that the intervention group showed a significant reduction in ATEC scores of 11.14±13.49 compared to the control group, who received the original rehabilitation training teaching mode from the rehabilitation center, which showed a reduction of 4.72±12.45. The current study utilized a test level of α=0.05 (bilateral), a test efficacy (1-β) =0.80, and a test effectiveness (1-β) =0.80. The PASS 11(Power Analysis and Sample Size) was used to determine the required sample size, and based on the 1:1 distribution of the number of people between the two groups, it was calculated that 16 people were needed in each group. Considering the loss rate of 20%, 20 people were needed in each group, and a total of 40 people were needed to be enrolled.

2.4 Randomization procedure

After signing the informed consent (see the operative document in Supplement 3), and completing the baseline assessment, the trial staff will inform all eligible subjects (N=40) that they have an equal chance of being assigned to either the exercise therapy intervention group or the maintenance of the rehabilitation base control group. The randomization procedure will be conducted using the intelligent centralized randomization system developed by the Big Data Laboratory of South China Research Center of Acupuncture and

Moxibustion, Guangzhou University of Traditional Chinese Medicine. This system will generate the random assignment sequence and assign each participant to either the exercise intervention group (N=20) or the control group (N=20) in a 1:1 ratio.

2.5 Blinding

Due to the specificity of the exercise intervention methodology, this study does not employ blinding of the subject children, guardians, and intervention implementation researchers, who were centrally trained in the operation prior to study implementation to ensure uniformity in the teaching movements. The efficacy evaluators, data managers, and analysts were unaware of the subgroups and did not participate in the program's design and specific clinical implementation. In addition, if families of participating children happen to meet other families of participating subjects, they will be asked to refrain from discussing their experiences during the training period. If a participant in the study reports a severe adverse event during the intervention period, the researcher will unblind the patients immediately and review their medical records. Otherwise, the researcher will treat the patient as an instance of drop-out. The investigator and statistician will jointly complete a review of the records and data before unblinding occurs.

2.6 Relevant concomitant care permitted or prohibited during the trial

Participants will not be allowed to participate in any additional sports intervention programs or engage in regular exercise outside of the program during the study period. Children and their families will also be informed about the importance of maintaining their lifestyle and dietary habits, and avoiding major changes as much as possible.

2.7 Informed consent

The informed consent form will be provided in paper form, and the patient's family will retain one copy, while one copy will be kept by the research team. The informed consent form will include information on the study's background, purpose, intervention methods, assessment indicators, potential benefits, and the possibility of adverse events. Furthermore, it will also mention the collection of biological samples, such as fecal matter, from the children to analyze intestinal flora data.

2.8 Intervention

Tai Chi fan action：The Tai Chi fan movement derived from the type 36 standard Tai Chi fan movement stipulated by the General Administration of Sport of China, and the movement procedure of the exercise intervention group consisted of 6 hand movements and two steps. The basic movements of the

fan include Fan Opening, Fan Closing and Overlapping, Fan Thrust, Fan Hooking Parry, Fan Uppercut, Fan Pointing, Bow Stance, and Empty Stance.

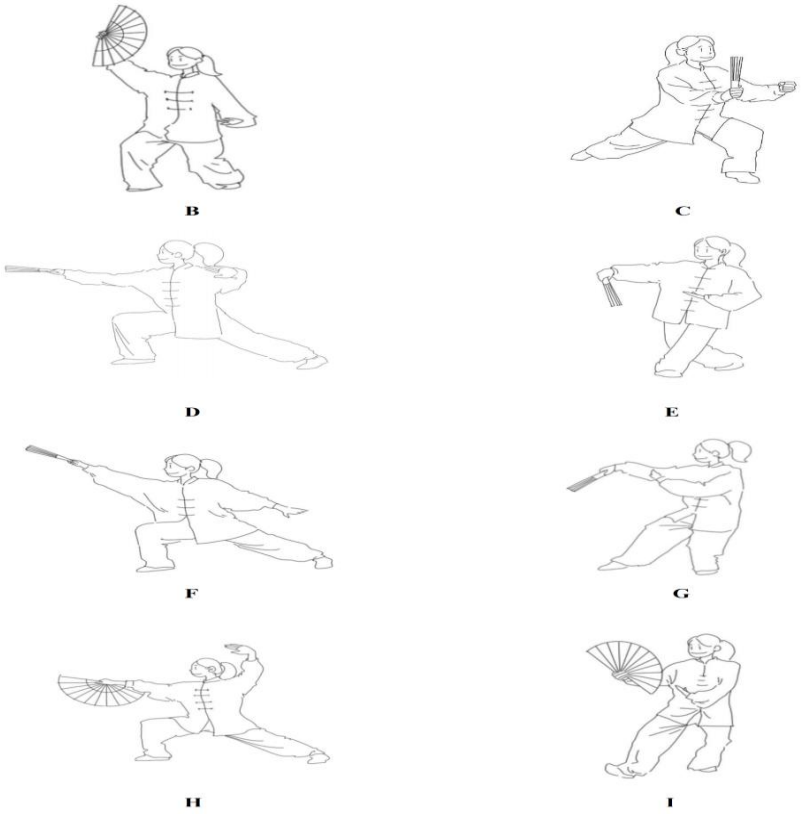

Figure 2: Tai Chi fan action

Tai Chi Fan Equipment: The length of the fan is approximately the root of the fan held in the sportsperson's left hand, with the head of the fan facing upwards and the head of the fan not lower than the athlete's elbow. The curved edge of the upper part of the fan should not be higher than 1.5cm above the top of the fan bone.

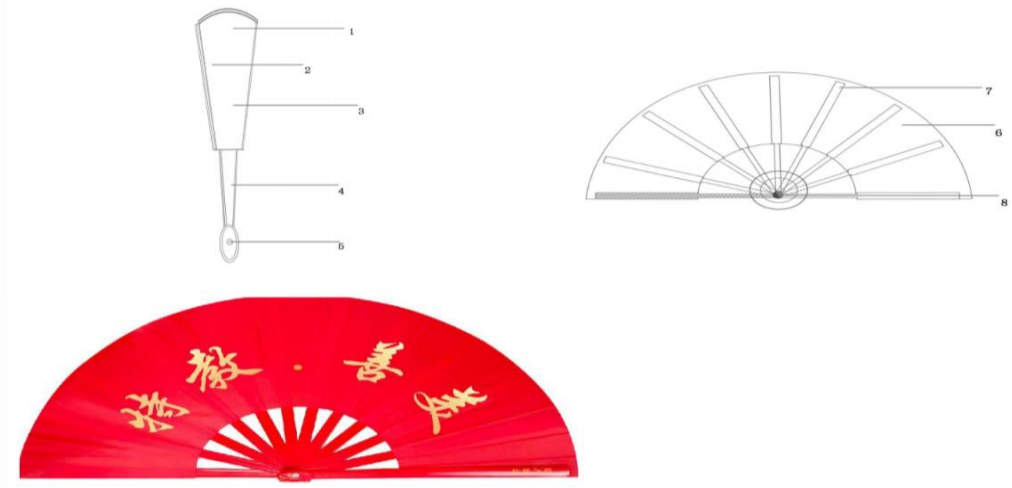

Figure 3: Tai Chi Fan Equipment

Intervention group：The exercise intervention group will receive Tai Chi Fan training for eight weeks. During this time, they will attend three 60-minute sessions each week. Each session will consist of a 10-minute warm-up, 40 minutes of Tai Chi Fan exercise instruction and practice, and a 10-minute cool-down period to allow the child's body to gradually relax. Each Tai Chi Fan training session will be led and monitored by a professional Tai Chi Fan instructor who will carefully guide the exercises and correct inappropriate movements.

Control group: Children in the control group will continue with their original rehabilitation program without additional changes. Assessments will be conducted to measure the relevant indicators during the specified period.

Outcomes and measurements：The case report form（CRF）form will gather information on the child and their family or guardian, along with data on the child's demographics, primary and secondary outcomes, and gut flora. Primary and secondary outcome indicators will be measured and assessed at baseline, week 9, and week 11 of the follow-up period, and gut flora will be measured and assessed at baseline and week 9.

Table 1(a): Assessment schedule

	SCREEN	BASELINE ASSESSMENT AND ALLOCATION	INTERVENTION		FOLLOW-UP
TIMEPOINT(DAY)	-5～-1	-1～0	28[a]	56[a]	70[a]
SCREEN ELIGIBILITY	×				
INFORM CONSENT	×				
ALLOCATION		×			
DEMOGRAPHIC		×			
VITAL SIGNS		×			
PREVIOUS HISTORY		×			
DAILY REHABILITATION		×			
DAILY DIET AND EXCRETION		×			
DAILY EXERCISE		×			
ATEC		×	×	×	×
CARS		×	×	×	×
SRS		×	×	×	×
ABC		×	×	×	×
TGMD-3		×	×	×	

Table 1(b): Assessment schedule

	SCREEN	BASELINE AND ALLOCATION	ASSESSMENT	INTERVENTION	FOLLOW-UP
INTESTINAL FLORA		×		×	
RANDOMIZATION		×			
ADVERSE EVENT[B]			×	×	×
CM[B]			×	×	×

ATEC：Autism Treatment Evaluation Checklist；CARS：Childhood autism rating scale；SRS：Social Responsiveness Scale；ABC：Autism Behavior Checklist；TGMD-3：Test of gross motor development, third edition; CM: Concomitant Medication; a indicates±5 days；b Indicates recording where necessary

3. Primary Outcome：

Autism Treatment Evaluation Checklist (ATEC): The ATEC is designed to assess changes in the severity of ASD in children aged 2 to 12. This questionnaire, filled out by a guardian or teacher, evaluates the effectiveness of treatment. The ATEC is divided into four sections: Speech/Language/Communication (14 items), Sociability (20 items), Sensory/Cognitive Awareness (18 items), and Health/Physical/Behavioral (25 items). Each section's scores are added together to give a total score between 0 and 179. The initial assessment's total score, referred to as the "baseline total score," categorizes severity into mild (20-49), moderate (50-79), and severe (>80). The ATEC's purpose is to track individual progress post-intervention by comparing baseline scores with subsequent scores. It's important to note that the ATEC is not diagnostic; it solely measures changes in severity, aiding in monitoring treatment effects.

4. Secondary Outcomes：

① Childhood autism rating scale (CARS):The CARS scale is a standardized diagnostic tool created by E. Schopler, R.J. Reichler, and B.R. Renner in 1980 for infants and toddlers over years old, with a maximum total score of 60. Scores less than 30 indicate non-autistic conditions. Severe autism is characterized by a total score equal to or greater than 36, with at least five items scoring more than 3 points. Mild to moderate autism is indicated if the total score ranges between 30 and 36, with fewer than five items scoring below 3 points. The purpose of this scale is to distinguish children with autism from children with other mental disorders and to assess the degree of impairment in children with autism.

②Social Responsiveness Scale (SRS): The Social Responsiveness

Scale (SRS) was developed by Constantino and Gruber in 2005 for children aged 4-18. It quantifies social skills to evaluate children's social competence and can be used for ASD screening and diagnosis. The SRS is divided into five subscales with a total of 65 items: social perception, social cognition, social communication, social motivation, and autistic behavioral style. The score is based on the total score of the SRS, which ranges from 0-195, with higher scores indicating more severe social problems. The SRS focuses on social and communication skills to evaluate a child's social situation, with less emphasis on stereotyped behaviors. Mild social difficulties are indicated by a score of 60-75, moderate social difficulties by a score of 75-90, and severe social difficulties by a score of 90 or higher.

③Autism Behavior Checklist (ABC): The ABC was developed by Krug et al.in 1978 for the screening of children with autism. It consists of 57 items scored on a scale of 1 to 4, depending on their relevance to autism in five domains: sensory behaviors, interactional associative behaviors, somatic and object use, verbal behaviors, and social and self-care. The total score ranges from 0 to 158, with higher scores indicating higher levels of autism-like behaviors. This scale is intended for children aged 2 to 14 and should be completed by parents or teachers.

④Test of gross motor development, third edition(TGMD-3): The TGMD-3 is a test designed to assess children's gross motor development. It consists of two primary sections: Translation movement ability（TMA）and Object Manipulation Ability（OMA）. The TMA contains six movements, which measures the fluid, coordinated movement of the child's body needed to move the child from one direction to another. The OMA consists of 7 movements and measures children's object manipulation skills in throwing, striking, and catching a ball. Each movement is scored between 3 and 5, contributing to a total score of 100 points.

4.1 Intestinal flora

For the study of intestinal flora, stool samples were collected from each group before and after the intervention. Ten individuals were randomly selected from each group for this process. Guardians were instructed to collect the stool samples in separate bags to avoid mixing with urine. After collection, the samples were immediately sent to the laboratory and stored at -80°C. The microbial community in the gut flora was analyzed using 16S rDNA sequencing. The study focused on examining the community's abundance and diversity, including conducting α and β diversity analyses. (The details were provided in Section 2, Supplement 1.)

4.2 Definition and Management of Adverse Events

In this trial, an adverse event refers to any negative event experienced

by the subject. This can include events unrelated to the intervention approach used. The investigator must record all adverse events observed during the trial, whether reported by the guardian or the child, regardless of whether the event occurred in the intervention group or whether or not it was related to the intervention protocol. Additionally, any new illnesses or worsening of pre-existing symptoms should also be reported by the researchers during the intervention. However, poorly effective exercise interventions are not considered adverse events.

Treatment of adverse events: In the event of any negative occurrences, the trial staff will document various details such as the intensity, timing, duration, treatment measures, and results in the adverse event report form. After considering all factors, they will then assess the relevance of these events to the exercise intervention. Additionally, the investigator will take appropriate measures to ensure the patient's safety and decide whether to proceed or terminate based on the situation. Any cases that are terminated due to adverse events will be thoroughly investigated and documented by the researcher.

5. Statistical analysis plan

(1) Analysis of baseline characteristics: The original data were recorded using Microsoft Excel, and demographic characteristics were compared at baseline using SPSS25.0(Statistical Product and Service Solutions). Continuous data were tested for normality using the Shapiro-Wilk test. Data conforming to normal distribution were described using mean ± standard deviation ($\bar{x}$ ± S), while non-normal data were described using median (interquartile range, IQR). Statistical analyses were performed using *t*-tests for normal data and non-parametric tests for non-normal data. Categorical variables were described using frequency percentages (n%), unordered categorical variables using the chi-square test, ordered categorical variables using the Mann-Whitney U test, and hierarchical variables using the Wilcoxon rank sum test.

(2) Primary outcome: The primary outcome indicator, ATEC scores, were analyzed using the Full Analysis Set（FAS）dataset. Missing data were filled in using an appropriate method (Last observation carried forward). Statistical analyses were performed using independent samples *t*-tests for comparisons between the two groups and paired samples *t*-tests for comparisons within groups before and after the intervention. Analyses were conducted to determine whether the intervention group had a superior treatment effect and a meaningful reduction in autism when additional exercise was added to the control group's rehabilitation training. Repeated measures ANOVA was used as needed to analyze differences between groups at different intervention time points. Sensitivity analyses were performed using the Per Protocol （PP）dataset as necessary.

(3) Secondary Outcome: Secondary outcome indicators (CARS, ABC, SRS, and TGMD-3 scores) were analyzed using PP sets. Data were analyzed using *t*-tests or non-parametric tests. To observe the therapeutic effect of motor intervention on other symptoms of children with autism, such as social responses, behavioral patterns, and gross motor development, in addition to its effect on the degree of autism.

(4) Intestinal microflora: Gut flora analysis involved sequencing of sample gut flora, ASV clustering, α and β diversity analysis, differential microbial analysis, and Pearson linear correlation analysis to analyze the relationship between changes in gut flora indicators pre- and post-intervention and changes in autism-related indicators in both groups.

(5) Persistence analysis of the efficacy: The PP set was used to analyze differences in primary outcome indicators between groups at two weeks of follow-up using independent samples *t*-tests or nonparametric tests, and differences in primary outcome indicators between groups at different time points in the follow-up process were analyzed using repeated-measures ANOVA as needed.

(6) Exploratory Data Analysis: ① Subgroup analysis: Subgroup analyses were conducted based on gender, age, height and weight, and baseline autism level of participating children to assess the impact of these variables on autism treatment outcomes. ② Sensitivity analysis: The PP dataset will also be analyzed to validate the findings obtained from the FAS dataset. If the results of both analyses align, it indicates that the findings are more reliable and less influenced by any missing data. Another sensitivity analysis involves excluding individual cases with high heterogeneity prior to conducting the analysis.

(7) Safety analysis: The safety of the intervention method was analyzed using Safety Set（SS）. The incidence, type of event, severity, duration, and regression of adverse events in the trial were recorded, and the relationship between adverse events and treatment was analyzed, including cases withdrawn due to adverse events.

All the above tests were performed using a two-sided test with a test level of $\alpha = 0.05$; $p < 0.05$ was statistically significant, and the confidence interval was set at 95% (95% CI).

5.1 Confidentiality

Personal information collected from children and their guardians will be used only for research purposes and analyzed in a way that protects individual privacy. All information related to individual privacy will not be disclosed or released.

6. Discussion

1. Expected outcomes: We anticipate that our clinical study will demonstrate the effectiveness of Tai Chi fan exercise, a traditional sport, in reducing autism severity in children. We also expect to observe changes in gut microecology before and after the intervention and investigate potential correlations between symptom improvement and changes in gut microecology in children with autism who practice Tai Chi fan exercise.

2. Guidance for clinical practice: This study aims to identify key factors influencing the clinical response to sports in individuals with ASD. We also aim to uncover the microbe-host mechanism behind the response to traditional sports in ASD, using an analysis of intestinal microecology and clinical information. These findings will contribute to a better understanding of how sports can benefit children with ASD.

3. Differences and similarities: While many previous studies have supported the impact of exercise on children with autism, to our knowledge, there have been no clinical studies on combining traditional Chinese physical activity and enterotomies in children with autism. This clinical trial study uses a prospective, randomized controlled design, and sequencing technology to analyze the pathogenesis and therapeutic effects of autistic children comprehensively, exploring the role of traditional Chinese sports Tai Chi fan in improving the intestinal flora of children with autism.

4. Advantages of this study: Tai Chi fan exercise is a moderate-intensity exercise considered safe and therapeutic. Given the limitations of capital, technology, site, personnel, and other conditions, the current use of routine autism rehabilitation treatment is limited worldwide. However, Tai Chi fan sports are more accessible in terms of site, equipment, and cost. The sports equipment used is a Tai Chi fan, which is cheap and has high safety.

5. Possible challenges and response: There are some challenges with this clinical trial study, such as the inability to blind subject children due to the specificity of the intervention method, which may increase the likelihood that control children and their families will seek the same exercise intervention. We addressed this by informing families in the control group that the program would continue after the 8-week exercise program and allowing children in the control group to participate in the Tai Chi Fan program. There may also be difficulties with data collection; we plan to use school teachers' observations combined with parents' reports to make data collection more complete and honest.

In conclusion, this research aims to examine how practicing Tai Chi fan can impact symptoms related to autism and gut flora in individuals with ASD. The findings may demonstrate that a Tai Chi fan exercise program can benefit individuals with ASD. This study provides important insights into the

effectiveness of Tai Chi fan as a treatment for autism and highlights the need for further research on exercise rehabilitation for individuals with ASD. The study received ethical approval from the Ethics Review Committee of Panyu Hospital of Traditional Chinese Medicine, Guangzhou City, Guangdong Province, China (Project No. 2023023), and the trial was registered in the China Clinical Trial Registry as ChiCTR2300070927.

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6.2 Acknowledgments

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6.3 Conflict of interest

The authors declared that their study was conducted without any business or financial affiliations that could create a conflict of interest.

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