Informed Consent Form

Title: Study Protocol for a Randomized Controlled of Fecal Microbiota Transplantation via Different Routes in Children with Moderate-to-Severe Autism Spectrum Disorder

Mach 19, 2025

Informed Consent Form

(Legal Guardian Version)

Dear Parent or Legal Guardian:

This informed consent form describes the research project titled "A Randomized Controlled Trial of Fecal Microbiota Transplantation via Different Routes in Children with Moderate-to-Severe Autism Spectrum Disorder" approved by the Shenzhen Science, Technology and Innovation Commission Natural Science Foundation. Your child is invited to participate in this clinical research. As researchers, we will explain the study to you and your child. For a minor to participate in this study, consent must be obtained from their parent(s) or legal guardian(s). This informed consent form provides you and your child with information to help decide whether to participate in this clinical research. Please read it carefully. If you have any questions, please direct them to the responsible investigator for this study.

Some parts of this document are required by regulations. This study has been approved by the Ethics Committee of Shenzhen Children's Hospital.

This study plans to enroll approximately 75 participants. The participating hospital is Shenzhen Children's Hospital.

What is "Giving Consent"?

Your child's participation in this study is voluntary, so you can decide whether you wish for your child to participate. If you agree for your child to participate, you must sign at the end of this document to indicate your consent for your child's participation. This process is called "giving consent."

Please complete the following steps before making your decision:

1. The researcher has introduced the study to you and your child;
2. You and your child understand the purpose and risks of this study;
3. You and your child are willing to comply with the relevant requirements of this study.

Whom should I or my child contact if we have any questions?

If you or your child have any questions or concerns about this study, you may consult the doctor responsible for this study: Dr. Dai Dongling, contact phone number: 18938690736.

If you or your child have any questions regarding your rights as a participant in this study, please contact the Medical Ethics Committee of Shenzhen Children's Hospital, contact phone

number: 0755-83008379.

Why is this research being conducted (Research Purpose)?

Research Background: Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder frequently accompanied by gastrointestinal (GI) symptoms. Recent advances highlight the role of gut microbiota dysbiosis and the microbiome-gut-brain axis (MGBA) in neurodevelopment. Evidence indicates that intestinal dysbiosis is a key susceptibility factor in ASD, potentially driving immune dysregulation, neuroinflammation, and altered brain function. Fecal Microbiota Transplantation (FMT) has emerged as a promising approach to restore intestinal microbial balance, showing potential in alleviating both core ASD symptoms and co-occurring GI disturbances in preliminary studies. However, the optimal delivery route for FMT in children with ASD is not established. This study aims to compare the efficacy and safety of two FMT delivery routes.

Research Objective: The primary objective is to compare the efficacy of FMT delivered via a nasojejunal tube (NJT) versus colonoscopy, both as add-on therapies to existing behavioral intervention, against a placebo control, in improving social interaction and communication in children with moderate-to-severe ASD. Secondary objectives include assessing effects on other behavioral symptoms, GI symptoms, investigating gut microbiota changes, and evaluating safety.

How will the study be conducted (Research Process)?

This is a single-center, randomized, double-dummy, triple-blind, placebo-controlled, three-arm parallel-group study. Participants will be randomly assigned to one of three groups:

Group 1 (FMT-NJ): Active FMT via nasojejunal tube + sham colonoscopy.

Group 2 (FMT-C): Active FMT via colonoscopy (with placement of a tube for subsequent infusions) + sham nasojejunal intubation.

Group 3 (Control): Placebo via nasojejunal tube + placebo via colonoscopy (sham procedures for both routes).

The FMT or placebo dosage is 5 ml/kg (maximum 100 ml), administered once every other day for a total of three sessions over 5 days.

Prior to the intervention, comprehensive screening will be performed, including medical history, physical examination, and laboratory tests (blood, urine, stool tests, ECG, etc.). A single,

rigorously screened healthy donor will provide the fecal material for the active FMT. The placebo is made from the same donor material but inactivated.

Participants will undergo the assigned procedures under sedation/anesthesia. Follow-up assessments will occur at multiple time points over 48 weeks, including behavioral evaluations (e.g., ADOS-2, CARS, SRS, ABC, CSHQ), GI symptom ratings (GSRS), collection of stool samples for microbiome analysis, and monitoring of safety and adverse events.

Are there other treatment options for my child (Alternative Treatments)?

Participation in this study may or may not improve your child's health condition. Your child can choose not to participate in this study and continue to receive the doctor's conventional treatment, using conventional medications (behavioral therapy, etc.).

What will my child need to do in the study (Participant Obligations)?

If you agree for your child to participate, your child will need to:

1. Complete baseline and follow-up questionnaires and behavioral assessments.
2. Undergo pre-procedure screening tests, which may include blood tests (approximately 8-10 ml per draw at specified times), urine tests, stool tests, ECG, etc.
3. Adhere to pre-FMT dietary and bowel preparation instructions.
4. Receive the three assigned intervention procedures (FMT or placebo/sham) during a hospitalization period.
5. Provide stool samples at multiple time points: before FMT, and at weeks 2, 6, 12, 24, and 48 after FMT.
6. Attend all scheduled follow-up visits at the hospital for assessments and safety monitoring.
7. Report any symptoms, illnesses, or changes in health to the research team promptly.
8. Maintain their usual stable behavioral intervention regimen and avoid major changes in diet or starting new probiotics/prebiotics/synbiotics during the study period unless discussed with the research doctor.

What risks and adverse reactions might my child experience by participating in this research (Risks and Discomforts of Participation)?

Risks related to procedures and FMT:

Sedation/Anesthesia Risks: As with any procedure requiring sedation, there are risks such as allergic reactions, breathing difficulties, or heart rhythm changes.

Procedure-related Risks:

Nasojejunal tube placement: May cause discomfort, sore throat, nosebleed, gagging, vomiting, or, rarely, injury to the nose, throat, or digestive tract.

Colonoscopy: May cause abdominal discomfort, bloating, cramping, or, very rarely, bleeding, perforation (tear), or infection.

FMT-related Risks: The transplanted microbiota may cause temporary symptoms such as diarrhea, constipation, bloating, belching, abdominal pain, nausea, vomiting, or mild fever. There is a potential risk, though believed to be low, of transmitting an infection from the donor material or causing an imbalance in your child's own gut microbiota that could lead to other health issues. Your child will be closely monitored for any such events. Specific but rare risks could include new infection, transient fever, or abdominal discomfort related to the tube placement.

All procedures will be performed by experienced medical staff. Any adverse events will be managed promptly, and compensation for trial-related injury will be provided as per regulations.

What are the potential benefits of this research for my child (Potential Benefits)?

Your child may or may not experience direct benefit from participating. Potential benefits could include improvement in ASD-related symptoms or gastrointestinal symptoms. The knowledge gained may help future children with ASD.

Will there be any remuneration for participating in this study?

No.

Do I need to pay any fees for participating in this study (Possible Additional Costs or Burden)?

Recipient: Participation in this project involves research procedures. The costs for the FMT/placebo preparations, the specific research-related procedures (e.g., sham procedures, research-specific infusions), and the research-related stool microbiome analyses will be covered by the study. However, the costs for routine medical care, hospital stays, standard laboratory tests, and medications that are part of standard clinical management will not be covered by the study and will be your responsibility. These are costs you would likely incur even if not participating in the study, so participation does not increase your extra expenses for standard care

Donor: For healthy donor children participating, all screening tests and procedures related to providing the fecal sample will be covered by the study funds.

What should I do if my child is injured during participation in the study (Medical Treatment and Compensation for Injuries)?

We will monitor your child closely. If your child sustains an injury or experiences an adverse event during the study, please contact your research doctor immediately. Your child will receive timely and necessary medical treatment. For any injury or damage to your legal rights and interests that has a confirmed causal relationship with this study or the trial intervention, this study will provide corresponding treatment, cover associated medical costs, and provide compensation in accordance with national laws and regulations.

Even after signing this informed consent form, you still retain all your legal rights.

Will my child's information be kept confidential (Confidentiality)?

We will make every effort within the limits of the law to protect your and your child's personal privacy. Any public reports concerning the results of this research will not disclose any of your or your child's personal information. The case materials of this study may be reviewed by the investigator, the Ethics Committee of Shenzhen Children's Hospital and the National Medical Products Administration.

Your child's medical information and medical records will be managed using codes and will not contain any personally identifiable information (name, date of birth, school, home address, phone number, medical record number, social security card number, etc.). Research data will be stored in written and electronic archives for 10 years after the study concludes. The code linking this data to your and your child's private information will be kept in a secure location at the investigator's institution (department) with dual control, stored at Shenzhen Children's Hospital. Your child's feces specimens will be sent to the Shenzhen Institute of Advanced Technology for research. Any remaining specimens will not be used for secondary purposes.

During the study, we will promptly contact you regarding any significant new developments or medical information related to your child's health, such as recommending some examinations for your child to determine these new pieces of information. I will also promptly inform you of any new information that may affect your choice to continue your child's participation in the study.

Is my child required to participate in this study (Voluntary Participation/Withdrawal from the Study)?

Participation in the study depends entirely on your and your child's voluntary choice. You and

your child may refuse to participate in this study or withdraw at any time during the research process. This will not affect your relationship with the doctor or result in any loss of medical care or other benefits for your child.

If your child requires other treatment, or if your child does not comply with the research plan, or if trial-related injury occurs, or for any other reason, the research physician may terminate your child's continued participation in this study.

How will my child's biological samples and medical information be handled (Future Use of Samples/Information)?

Besides this study, your child's biological specimens and research data might be used again in future research. This information and these samples will be assigned codes (not containing private information linkable to you or your child personally), ensuring that anyone accessing these samples and information cannot identify you or your child personally. We will similarly make every effort within the limits of the law to keep your and your child's personal privacy information confidential. You may also declare refusal for your child's biological specimens and research data to be used in research other than this current study.

Informed Consent Form (Signature Page)

I have read this informed consent form and have discussed this study with the doctor and asked questions. The doctor has explained to me in detail the study's purpose, process, potential risks, and benefits, and has answered all my questions. I understand that participation in this study is voluntary.

I confirm that I have had sufficient time to consider this, including the potential risks arising from participation. I can consult the doctor for more information at any time, can withdraw from this study at any time without discrimination or retaliation, and that medical treatment and benefits will not be affected by withdrawal from the study. I also understand that if withdrawing midway, especially if my child withdraws from the study for treatment reasons, promptly informing the doctor of any changes in condition and completing corresponding physical and laboratory examinations will be beneficial for my child and the entire study. If any other treatment is needed for my child due to changes in condition, I will seek the doctor's opinion beforehand or truthfully inform the doctor afterwards.

I and my child voluntarily participate in this study. I and my child agree to allow the investigator, sponsor, health administration/supervision departments/ drug and food supervision and administration departments, and the Ethics Committee to review my child's research materials. I and my child also agree to the future use of the data for other similar research topics.

I will receive a signed and dated original copy of the informed consent form.

Participating Child's Name:

Legal Guardian Signature: Relationship to Child: Contact Phone Number:

Legal Guardian Signature: Relationship to Child: Contact Phone Number:

Date:Y/M/D _____/_____/_____

I (signature) _____Agree□ or Refuse□ that the researcher, after removing my and my child's personal information, may use my child's biological specimens and related data again in subsequent research related to this project.

Participating Child's Name:

Legal Guardian Signature: Relationship to Child: Contact Phone Number:

Legal Guardian Signature: Relationship to Child: Contact Phone Number:

Date:Y/M/D_____/____/____

Investigator's Statement:

I confirm that I have explained the details of this study to the participant's guardian, including their rights and potential benefits and risks, and have given them a signed original copy of the informed consent form.

Informed Consent Discussion Doctor (Investigator) Signature:_____

Signature Date: Y/M/D_____/_____/_____ Contact Phone Number:_____