STUDY PROTOCOL

Official Title: Brain-Gut Axis Regulatory Mechanism of Anxiety and Depression in Irritable Bowel Syndrome: A Multimodal 7T MRI and Multi-omics Observational Study

NCT Number: [To be assigned after ClinicalTrials.gov registration]

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Sponsor: Air Force Medical University, Second Affiliated Hospital (Tangdu Hospital)

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Study Phase: Observational Study

TABLE OF CONTENTS

Protocol Summary

Background and Rationale

Study Objectives

Study Design and Methods

Study Population

Sample Size Justification

Study Procedures and Assessments

Data Management and Statistical Analysis

Risk Minimization and Safety Monitoring

Ethics and Regulatory Considerations

References

1. PROTOCOL SUMMARY

Title: Brain-Gut Axis Regulatory Mechanism of Anxiety and Depression in Irritable Bowel Syndrome

Study Design: Prospective, observational, case-control study

Study Duration: April 2025 – April 2026

Study Sites: Air Force Medical University, Second Affiliated Hospital (Tangdu Hospital)

Population: 100 IBS patients and 100 healthy controls (HC), aged 18–60 years

Primary Objective: To investigate the brain-gut axis regulatory mechanisms underlying anxiety and depression in IBS using 7T multimodal MRI, metagenomics, and metabolomics.

Key Assessments:

7T MRI: Structural (3D-T1WI), functional (resting-state fMRI), diffusion (DWI/DTI), and metabolic (ASL) imaging

Clinical: Rome IV criteria, symptom scores, Bristol Stool Scale

Psychological: Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI), Pittsburgh Sleep Quality Index (PSQI), cognitive assessments

Biological: Fecal metagenomic sequencing, serum and fecal metabolomics

2. BACKGROUND AND RATIONALE

2.1 Study Background

Irritable Bowel Syndrome (IBS) is a common functional gastrointestinal disorder characterized by recurrent abdominal pain and altered bowel habits, with a global prevalence of approximately 4.8%. IBS significantly impairs quality of life and imposes substantial economic burden—estimated annual direct and indirect costs in China exceed 123 billion RMB.

Anxiety and depression are frequent comorbidities in IBS. Approximately 50% of IBS patients meet diagnostic criteria for mood disorders, and in two-thirds of comorbid cases, IBS diagnosis precedes the onset of mood disorders. This temporal sequence suggests that gut-brain axis (GBA) dysfunction plays a central role in the pathophysiology of both IBS and its psychiatric comorbidities.

The gut-brain axis is a bidirectional communication network integrating endocrine, immune, neural, and microbial signals. Gut microbiota influence host physiology through metabolites such as short-chain fatty acids (SCFAs), bile acids, and tryptophan derivatives. IBS patients exhibit

characteristic microbiome alterations, including increased Firmicutes, decreased Bacteroidetes, and reduced abundance of beneficial genera such as Bifidobacterium and Faecalibacterium. These alterations may regulate intestinal permeability, visceral sensitivity, and neuroinflammation through metabolites (e.g., histamine, bile acids), thereby triggering anxiety and depression.

However, current studies are limited by sampling techniques and in vivo brain functional research methodologies, making it difficult to elucidate the molecular mechanisms of anxiety and depression in IBS. Ultra-high-field 7T MRI offers submillimeter resolution and multimodal imaging capabilities (structural, functional, and metabolic), enabling precise capture of brain functional network reorganization. Combined with metagenomics and metabolomics, this study aims to decode the gut microbiota–metabolite–brain functional network associations in IBS patients, providing evidence for targeted therapy and rapid diagnosis.

2.2 Rationale

Biological Basis of IBS-Anxiety/Depression Comorbidity

Gut microbiota influence the central nervous system directly or indirectly through metabolites such as secondary bile acids and tryptamine. For example, tryptamine activates 5-HT receptors to regulate intestinal secretion, while secondary bile acids (e.g., α-MCA, HDCA) inhibit TH17 cell differentiation via TGR5 receptors, attenuating intestinal inflammation. Key brain regions in IBS patients (e.g., amygdala, anterior cingulate cortex) show reduced gray matter volume and altered resting-state functional connectivity, overlapping with neural circuits implicated in anxiety and depression. Genome-wide association studies have identified shared genetic risk loci between IBS and anxiety/depression (e.g., 5-HT transporter gene polymorphisms), suggesting common pathophysiological mechanisms.

Technical Advantages of 7T MRI

7T MRI 3D-T1-weighted imaging can identify neuronal microstructural changes at submillimeter resolution. Diffusion-weighted imaging (DWI) reduces fiber tracking error rates by 40% compared to 3T, enabling clearer visualization of fine fiber projections. Resting-state fMRI reveals functional connectivity abnormalities, while arterial spin labeling (ASL) assesses cerebral hemodynamics. Integration of these neuroimaging modalities with metabolomics data enables construction of a comprehensive brain-gut axis regulatory atlas.

3. STUDY OBJECTIVES

Primary Objective:

To investigate the brain-gut axis regulatory mechanisms of anxiety and depression in IBS by integrating 7T multimodal MRI, fecal metagenomics, and metabolomics.

Secondary Objectives:

To identify key gut microbiota and metabolite signatures associated with brain functional reorganization in IBS patients with anxiety/depression.

To construct a brain-gut axis regulatory atlas using 7T MRI multimodal imaging.

To discover potential probiotic/prebiotic targets and rapid diagnostic biomarkers for IBS with psychiatric comorbidities.

Scientific Hypothesis:

IBS-related anxiety and depression are mediated by specific gut microbiota–metabolite profiles that modulate brain functional networks via the gut-brain axis, and these alterations can be captured by 7T multimodal MRI and multi-omics approaches.

4. STUDY DESIGN AND METHODS

4.1 Study Design

This is a prospective, observational, case-control study conducted at Air Force Medical University, Second Affiliated Hospital.

4.2 Study Population

Inclusion Criteria – IBS Group:

Right-handed, aged 18–60 years, education ≥6 years

Meets Rome IV diagnostic criteria for IBS:

Symptom onset ≥6 months prior to diagnosis

Recurrent abdominal pain ≥3 days/month in the last 3 months, plus ≥2 of:

a. Improvement with defecation

b. Onset associated with change in stool frequency

c. Onset associated with change in stool form (appearance)

Supporting symptoms: abnormal stool frequency (<3/week or >3/day), abnormal stool form (Bristol Types 1–2 or 6–7), straining, urgency, incomplete evacuation, mucus in stool, or bloating

Exclusion Criteria – IBS Group:

History of anti-anxiety/depressant medication (SSRIs/SNRIs, Chinese patent medicines, etc.)

within the past month

History of antibiotics or probiotics use within the past month

Inflammatory bowel disease (IBD), intestinal obstruction, other gastrointestinal diseases, or gastrointestinal surgery

Current or past psychiatric or neurological disorders (e.g., psychosis, brain tumors, consciousness disorders)

Severe hepatic/renal insufficiency, cardiovascular/cerebrovascular disease, or malignancy

Contraindications to 7.0T MRI:

Ferromagnetic implants (cardiac pacemaker, defibrillator, neurostimulator, aneurysm clip, cochlear implant, or any metallic foreign body)

Non-ferromagnetic implants (e.g., titanium alloy, orthopedic implants), intrauterine devices, non-removable dentures (including implants)

Metal foreign bodies in eyes or body (metal fragments, shrapnel, metal shavings), history of welding work or metallic foreign body injury

Tattoos or permanent makeup (eyebrows, lips) within the last month

Claustrophobia

Fever

Inclusion Criteria – Healthy Control (HC) Group:

Right-handed, aged 18–60 years, education ≥6 years

All physiological indices within normal range, no disease manifestations

No family history of psychiatric or neurological disorders

5. SAMPLE SIZE JUSTIFICATION

Based on prior literature and preliminary data, sample size is calculated using the formula for comparing means between two independent samples:

$n = [2 \times (Z\alpha/2 + Z\beta)^2 \times \sigma^2] / \delta^2$

Parameters:

Significance level: $\alpha = 0.05$ (two-sided)

Power: $1-\beta = 80\%$

Effect size: $\delta = 0.5$

Standard deviation: $\sigma = 1.0$

Calculation: Minimum 64 subjects per group. Considering a 20% dropout rate, the final target is 100 IBS patients and 100 healthy controls.

6. STUDY PROCEDURES AND ASSESSMENTS

6.1 Case Report Form (CRF) Modules

Module 1: Demographics

Age, sex, education, height, weight, BMI, lifestyle (smoking/alcohol history)

Module 2: Clinical Assessment

Rome IV criteria score, abdominal pain frequency/intensity, Bristol Stool Scale

Module 3: Psychological Evaluation

Auditory Verbal Learning Test, Color Test, Montreal Cognitive Assessment (MoCA), Memory Span Test, Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI), Pittsburgh Sleep Quality Index (PSQI)

Module 4: Imaging Data

7T MRI: structural, functional, and metabolic imaging

Module 5: Biospecimen

Fecal metagenomic sequencing; serum and fecal metabolomics

Module 6: Follow-up

Symptom remission time, medication use, adverse events

6.2 Follow-up Schedule

Baseline (Enrollment):

Complete clinical assessment, psychological scales, MRI scan, fecal/blood sample collection

3-Month Follow-up (IBS Group Only):

Repeat anxiety/depression scales

Repeat fecal/blood sample collection

Assess symptom relief (abdominal pain frequency, stool characteristics)

6-Month Follow-up (Optional):

Long-term efficacy tracking

Repeat imaging data collection (selected patients)

Follow-up Workflow:

Subjects sign informed consent and enter baseline assessment

Research nurses remind follow-up appointments via telephone/outpatient visits

Complete CRF at each follow-up and upload to Electronic Data Capture (EDC) system

Data manager reviews and locks data

7. DATA MANAGEMENT AND STATISTICAL ANALYSIS

7.1 Data Management

Platform: REDCap electronic data capture system

Entry: Double independent data entry with verification

Cleaning: Exclude variables with >20% missing values; use multiple imputation for missing data

7.2 Statistical Analysis

Group comparisons: Independent t-test (normal distribution) or Mann-Whitney U test (non-normal)

Correlation analysis: Pearson or Spearman correlation coefficients

Multi-omics integration: Sparse Canonical Correlation Analysis (sCCA), Principal Component Analysis (PCA)

Machine learning: Random Forest for key biomarker selection; ROC curve for diagnostic efficacy evaluation

Covariate adjustment: Age, sex, BMI, and other confounders in multivariate regression models

8. BIAS CONTROL AND QUALITY MANAGEMENT

8.1 Bias Control

Selection bias: Strict adherence to inclusion/exclusion criteria; consecutive enrollment

Measurement bias: Double-blind psychiatric evaluation; automated imaging analysis algorithms

Confounding bias: Multivariate regression adjustment for age, sex, BMI

Reporting bias: Protocol pre-registration on ClinicalTrials.gov; results reported per STROBE guidelines

8.2 Quality Management

Standard Operating Procedures (SOP): Develop SOPs for sample collection, MRI scanning, and data analysis

Training: Quarterly GCP, data management, and ethics training for research staff

Internal Audit: Semi-annual random inspection of 10% of CRFs against source data

External Oversight: Annual quality audit by third-party CRO

9. SAFETY MONITORING

9.1 Adverse Event (AE) Monitoring

Record MRI-related discomfort (e.g., claustrophobia) and biospecimen collection risks (e.g., vasovagal response)

Serious Adverse Events (SAE): Report to Ethics Committee within 24 hours; suspend study and initiate emergency protocol if necessary

9.2 Safety Reporting

Quarterly summary of adverse event incidence rates

Assessment of causality with study procedures

10. ETHICS AND REGULATORY CONSIDERATIONS

10.1 Ethics Review

This protocol and the informed consent form must be submitted to the Ethics Committee of the Second Affiliated Hospital of Air Force Medical University (Tangdu Hospital) for written approval before study initiation. The principal investigator must submit annual progress reports to the Ethics Committee and notify the committee in writing upon study completion or termination. Any protocol amendments require Ethics Committee approval prior to implementation, except for changes necessary to eliminate immediate hazards to subjects.

10.2 Informed Consent

Subjects will be invited to participate in this clinical research study, which requires collection and use of imaging data, serum, and fecal samples. Participation is voluntary. This study has been reviewed and approved by the Institutional Ethics Committee.

Risks and Discomforts:

This study involves ultra-high-field 7.0T MRI scanning, the most advanced submillimeter clinical MRI technology. Unlike X-ray and CT, MRI uses no ionizing radiation and can be repeated safely. No studies have demonstrated harm from MRI scanning.

Benefits:

Participants will receive systematic fecal and blood testing, comprehensive cognitive assessment, and ultra-high-field brain MRI. Any medical questions will be addressed with free consultation.

Privacy and Confidentiality:

All personal information and study data will be kept strictly confidential. Medical records will be

stored in locked cabinets accessible only to research personnel. Regulatory authorities and Ethics Committee members may review records as required by law. Published results will not disclose any individual identifying information.

Compensation:

If privacy breaches or study-related injuries occur, compensation will be provided from study funds.

Voluntary Participation:

Participation is voluntary. Subjects may withdraw at any time without penalty or loss of medical benefits. The investigator may discontinue a subject's participation for non-compliance, study-related injury, or other reasons.

Contact Information:

For questions regarding this study or participant rights, contact the Ethics Committee of the Fourth Military Medical University Tangdu Hospital at: 029-84717761.

11. REFERENCES

[1] Ford AC, Sperber AD, Corsetti M, Camilleri M. Irritable bowel syndrome. Lancet. 2020;396(10263):1675-1688.

[2] Zamani M, Alizadeh-Tabari S, Zamani V. Systematic review with meta-analysis: the prevalence of anxiety and depression in patients with irritable bowel syndrome. Aliment Pharmacol Ther. 2019;50(2):132-143.

[3] Mayer EA. Gut feelings: the emerging biology of gut-brain communication. Nat Rev Neurosci. 2011;12(8):453-466.

[4] Margolis KG, Cryan JF, Mayer EA. The Microbiota-Gut-Brain Axis: From Motility to Mood. Gastroenterology. 2021;160(5):1486-1501.

[5] Pittayanon R, Lau JT, Yuan Y, et al. Gut Microbiota in Patients With Irritable Bowel Syndrome-A Systematic Review. Gastroenterology. 2019;157(1):97-108.

[6] Liu HN, Wu H, Chen YZ, et al. Altered molecular signature of intestinal microbiota in irritable bowel syndrome patients compared with healthy controls: A systematic review and meta-analysis. Dig Liver Dis. 2017;49(4):331-337.

[7] De Palma G, Shimbori C, Reed DE, et al. Histamine production by the gut microbiota induces visceral hyperalgesia through histamine 4 receptor signaling in mice. Sci Transl Med.

2022;14(655):eabj1895.

[8] Bhattarai Y, Williams BB, Battaglioli EJ, et al. Gut Microbiota-Produced Tryptamine Activates an Epithelial G-Protein-Coupled Receptor to Increase Colonic Secretion. Cell Host Microbe. 2018;23(6):775-785.e5.

[9] Mayer EA, Labus J, Aziz Q, et al. Role of brain imaging in disorders of brain-gut interaction: a Rome Working Team Report. Gut. 2019;68(9):1701-1715.

[10] Edwinson AL, Yang L, Peters S, et al. Gut microbial β-glucuronidases regulate host luminal proteases and are depleted in irritable bowel syndrome. Nat Microbiol. 2022;7(5):680-694.

[11] Zhang L, Wang HL, Zhang YF, et al. Stress triggers irritable bowel syndrome with diarrhea through a spermidine-mediated decline in type I interferon. Cell Metab. 2025;37(1):87-103.e10.

[12] Li Z, Ma Q, Deng Y, et al. Irritable Bowel Syndrome Is Associated With Brain Health by Neuroimaging, Behavioral, Biochemical, and Genetic Analyses. Biol Psychiatry. 2024;95(12):1122-1132.

[13] Eijsbouts C, Zheng T, Kennedy NA, et al. Genome-wide analysis of 53,400 people with irritable bowel syndrome highlights shared genetic pathways with mood and anxiety disorders. Nat Genet. 2021;53(11):1543-1552.

[14] Mayer EA, Ryu HJ, Bhatt RR. The neurobiology of irritable bowel syndrome. Mol Psychiatry. 2023;28(4):1451-1465.

[15] Labus JS, Hollister EB, Jacobs J, et al. Differences in gut microbial composition correlate with regional brain volumes in irritable bowel syndrome. Microbiome. 2017;5(1):49.

APPENDICES

Appendix A: Case Report Form (CRF)

Appendix B: Informed Consent Form

Appendix C: MRI Safety Screening Form

Appendix D: Standard Operating Procedures (SOPs)

Protocol Version: 1.0

Version Date: June 27, 2025

Study Title (Chinese): 肠易激综合征下焦虑抑郁的脑肠轴调控机制研究

Study Title (English): Research on the Brain-Gut Axis Regulatory Mechanism of Anxiety and Depression in Irritable Bowel Syndrome