STUDY PROTOCOL

Association Between Gut Microbiome Characteristics and Neurodevelopmental Functioning in Children With Autism Spectrum Disorder: A Multidomain Investigation of the Gut–Motor Axis

Acronym: GAIN-ASD

Protocol ID:	079/06/2026/ISRB/FSR/SIBMS
Document Date:	June 9, 2026
Study Type:	Observational – Cohort (Prospective)
Sponsor:	Saveetha University, Chennai, India
Principal Investigator:	Jeevarathinam Thirumalai
Affiliation:	Saveetha Institute of Basic Medical Sciences, Saveetha University, Chennai, Tamil Nadu – 602105, India
IRB Approval Number:	079/06/2026/ISRB/FSR/SIBMS
Study Start Date:	July 1, 2026 (Anticipated)
Primary Completion Date:	December 31, 2026 (Anticipated)
Document Date	June 08, 2026

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1. STUDY OVERVIEW AND IDENTIFICATION

Field	Information
Brief Title	Gut Microbiome Characteristics and Neurodevelopmental Functioning in Children With Autism Spectrum Disorder
Official Title	Association Between Gut Microbiome Characteristics and Neurodevelopmental Functioning in Children With Autism Spectrum Disorder: A Multidomain Investigation of the Gut–Motor Axis
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Study Type	Observational
Observational Model	Cohort
Time Perspective	Prospective
Protocol ID	079/06/2026/ISRB/FSR/SIBMS
Sponsor	Saveetha University, Chennai, Tamil Nadu, India
Principal Investigator	Jeevarathinam Thirumalai
Study Site	Saveetha Medical College and Hospital, Chennai, Tamil Nadu 602105
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IRB/ISRB Approval	Approved – 079/06/2026/ISRB/FSR/SIBMS, Saveetha Institute of Basic Medical Sciences
Enrollment (Anticipated)	600 participants (300 ASD, 300 Typically Developing)
Study Start Date	July 1, 2026 (Anticipated)
Primary Completion	December 31, 2026 (Anticipated)
Study Completion	December 31, 2026 (Anticipated)
Biospecimen Retention	Samples With DNA (Stool samples for microbiome analysis)

2. BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Autism Spectrum Disorder

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition characterized by persistent impairments in social communication and interaction, alongside restricted, repetitive patterns of behavior, interests, or activities (DSM-5). ASD manifests across a broad spectrum of severity and is associated with variable functional abilities spanning motor performance, sensory processing, cognitive functioning, behavioral regulation, and quality of life. The global prevalence of ASD is estimated at approximately 1 in 100 children, with significant societal and healthcare burden.

2.2 The Gut–Brain and Gut–Motor Axis in ASD

The gut–brain axis represents a bidirectional communication network integrating neural, hormonal, and immunological signals between the gastrointestinal system and the central nervous system. The resident gut

microbiome plays an essential modulatory role in these interactions, influencing neurotransmitter synthesis (e.g., serotonin, gamma-aminobutyric acid), immune homeostasis, and systemic inflammatory tone. Perturbations in gut microbial composition - referred to as dysbiosis - have been implicated in altered neurodevelopmental trajectories. An emerging extension, the gut–motor axis, posits direct or indirect relationships between gut microbial characteristics and motor functioning, highlighting the need for multidomain assessment frameworks.

2.3 Gut Microbiome Alterations in ASD

Multiple observational studies have identified significant differences in gut microbiome composition between children with ASD and typically developing peers. Commonly reported findings include reduced microbial diversity, altered Firmicutes-to-Bacteroidetes ratios, decreased abundance of butyrate-producing taxa (e.g., Faecalibacterium prausnitzii, Roseburia spp.), and increased abundance of Clostridium spp. and Desulfovibrio spp. Short-chain fatty acids, including butyrate and propionate, produced by gut microbiota have been proposed as key neuroactive metabolites influencing brain development and behavior.

2.4 Scientific Gaps and Justification

Existing research has predominantly focused on autism symptom severity, behavioral outcomes, and gastrointestinal complaints. Studies examining multi-domain neurodevelopmental functioning - including gross motor performance, sensory processing, sleep, and participation - within an integrated microbiome–rehabilitation framework are limited. No published study from an Indian pediatric ASD population has simultaneously characterized gut microbiome diversity and multidimensional neurodevelopmental functioning. The GAIN-ASD study addresses these gaps by conducting a prospective observational cohort investigation using validated, standardized assessment tools and 16S rRNA gene sequencing.

3. STUDY OBJECTIVES AND HYPOTHESES

3.1 Primary Objectives

- To characterize gut microbiome diversity and composition (alpha diversity, beta diversity, and relative abundance of bacterial taxa) in children with ASD compared to typically developing children.
- To examine the association between gut microbiome characteristics and autism severity as measured by the Indian Scale for Assessment of Autism (ISAA).

3.2 Secondary Objectives

- To investigate the association between gut microbiome characteristics and gross motor function (GMFM-88).
- To explore relationships between gut microbiome composition and sensory processing (SEQ-2.1), behavioral function (SDQ), sleep disturbance (PROMIS), and cognitive functioning (PROMIS Parent Proxy).

- To examine associations between gut microbiome characteristics and participation in daily activities (PPA) and health-related quality of life (PedsQL).
- To assess the role of dietary intake and anthropometric nutritional status as potential mediators or confounders.
- To characterize gastrointestinal symptom burden and its relationship with gut microbiome profiles in children with ASD.

3.3 Study Hypotheses

Primary Hypothesis: Children with ASD will exhibit reduced gut microbiome alpha diversity and distinct compositional profiles compared to typically developing children, and these microbiome characteristics will be significantly associated with autism severity scores on the ISAA.

Secondary Hypotheses: Gut microbiome diversity and specific bacterial taxa will be associated with gross motor function, sensory processing, behavioral difficulties, sleep quality, cognitive functioning, participation, and quality of life. Dietary intake patterns and nutritional status will moderate these microbiome–neurodevelopmental associations.

4. STUDY DESIGN

The GAIN-ASD study is a prospective observational cohort study comparing children with confirmed Autism Spectrum Disorder (ASD) to age- and sex-matched typically developing (TD) controls. Both cohorts will undergo a single-timepoint cross-sectional assessment at baseline comprising stool sample collection for gut microbiome analysis and multidomain neurodevelopmental evaluation. The study employs a non-probability convenience sampling method from participating centers at Saveetha Medical College and Hospital, Chennai, India.

Design Parameter	**Specification**
Study Type	Observational
Observational Model	Cohort (two groups)
Time Perspective	Prospective
Assessment Timepoints	Single baseline assessment
Setting	Tertiary care hospital and affiliated autism/rehabilitation centers
Sampling Method	Non-probability (convenience) sampling
Biospecimen	Stool samples with DNA retention for 16S rRNA sequencing
Total Sample Size	600 (ASD: n=300; Typically Developing: n=300)
Age Range	3–12 years
Study Duration	July 1, 2026 – December 31, 2026

5. STUDY POPULATION AND ELIGIBILITY

5.1 Cohort 1: Children With Autism Spectrum Disorder

The ASD cohort will comprise children aged 3 to 12 years with a confirmed clinical diagnosis of ASD receiving care at participating centers. A target enrollment of 300 children is planned.

Inclusion Criteria (ASD Cohort)

- Children aged 3 to 12 years (inclusive).
- Clinical diagnosis of ASD confirmed according to DSM-5 or ICD-11 criteria, documented from medical records or specialist assessment report.
- Stable clinical status at the time of enrollment, without acute intercurrent illness.
- Parent or legal guardian willing to provide written informed consent prior to participation.
- Child able to cooperate with stool sample collection and neurodevelopmental assessments, with caregiver assistance as appropriate.
- Parent or caregiver able and willing to complete standardized questionnaires and provide dietary and medical history.

Exclusion Criteria (ASD Cohort)

- Use of systemic antibiotics, probiotics, prebiotics, synbiotics, or bowel-cleansing agents within the 4–12 weeks preceding stool collection.
- Presence of acute gastrointestinal infection or febrile illness at the time of assessment.
- Known chronic gastrointestinal disorders independently altering gut microbiome composition (IBD, celiac disease, short bowel syndrome, chronic intestinal malabsorption).
- Major neurological, genetic, or metabolic disorders other than ASD that may independently affect neurodevelopmental functioning.
- Current use of medications known to significantly alter gut microbiota or bowel motility.
- Inability to provide a stool sample or to cooperate with any required study assessment.
- Refusal of informed consent by parent or legal guardian.

5.2 Cohort 2: Typically Developing Children

The TD cohort will consist of 300 age- and sex-matched children without a diagnosis of ASD or other neurodevelopmental disorder. TD children will be matched to the ASD cohort on age (±6 months) and sex. The same exclusion criteria regarding gastrointestinal health and medication use will apply.

6. RECRUITMENT AND ENROLLMENT

Participants will be recruited through Saveetha Medical College and Hospital outpatient departments, including pediatric rehabilitation, developmental pediatrics, child neurology, child psychiatry, and affiliated autism care centers. Recruitment will also utilize community outreach through autism support groups and school-based programs. Written informed consent will be obtained from parents or legal guardians prior to any study procedure.

Enrollment will proceed sequentially until the target sample size is achieved. Subgroup recruitment targets will be monitored for age strata (3–5 years, 6–8 years, 9–12 years) and sex distribution. An anticipated enrollment period of

six months (July–December 2026) is planned. Enrollment progress will be monitored by the Data Monitoring Committee.

7. PROCEDURES AND ASSESSMENTS

7.1 Gut Microbiome Assessment

Stool samples will be collected from participants at study enrollment (baseline). Participants and caregivers will be provided with standardized stool collection kits and written instructions. Samples will be collected by the caregiver at home or at the study site and transported to the laboratory within the specified timeframe. Microbial DNA will be extracted using validated commercial extraction protocols. Gut microbiome characterization will be performed using 16S rRNA gene amplicon sequencing (V3-V4 hypervariable region). Bioinformatic analysis will be conducted using QIIME2 or DADA2 pipelines for quality control, denoising, ASV generation, taxonomic classification against SILVA or Greengenes2, and diversity analyses.

Primary microbiome outcome variables:

- Alpha diversity: Shannon Diversity Index, Simpson Diversity Index, Chao1 Richness Index, Observed ASVs.
- Beta diversity: Bray-Curtis dissimilarity, UniFrac distances (weighted and unweighted), visualized by PCoA.
- Relative abundance: Phylum-, family-, genus-, and species-level taxa; Firmicutes/Bacteroidetes ratio.
- Biospecimens stored at −80°C and used solely for research purposes.

7.2 Neurodevelopmental Assessments

Assessment Tool	Domain	Respondent	Outcome Type
Indian Scale for Assessment of Autism (ISAA)	Autism Severity	Clinician/Caregiver	Primary
Gross Motor Function Measure-88 (GMFM-88)	Gross Motor Function	Clinician (direct)	Secondary
GI Symptom Questionnaire + Bristol Stool Scale	GI Symptoms	Caregiver-reported	Secondary
Strengths and Difficulties Questionnaire (SDQ)	Behavioral Function	Caregiver-reported	Secondary
PROMIS Sleep Disturbance Scale	Sleep Quality	Caregiver-reported	Secondary
Participation Questionnaire for Preschoolers with ASD (PPA)	Participation	Caregiver-reported	Secondary
Sensory Experiences Questionnaire (SEQ-2.1)	Sensory Processing	Caregiver-reported	Secondary
PROMIS Parent Proxy Cognitive Function	Cognitive Function	Caregiver-reported	Secondary
Pediatric Quality of Life Inventory	Quality of Life	Caregiver/Child-	Secondary

(PedsQL)		reported	

7.3 Dietary Assessment

Dietary intake will be assessed using parent proxy-reported 24-hour dietary recalls collected on 2–3 non-consecutive days, including at least one weekend day, using the multiple-pass recall method. Information on energy intake, macronutrients, dietary fiber, and key food groups will be recorded. A supplementary food frequency questionnaire will assess habitual consumption of fruits, vegetables, whole grains, fermented foods, sugar-sweetened beverages, and ultra-processed foods. Composite healthy and unhealthy diet scores will be derived.

7.4 Anthropometric and Nutritional Assessment

Standardized anthropometric measurements - height (cm), weight (kg), and derived BMI (kg/m²) - will be obtained using calibrated instruments. Age- and sex-specific z-scores for WAZ, HAZ, BAZ, and WHZ will be calculated using WHO Child Growth Standards and WHO Growth Reference (5–19 years). Nutritional status will be categorized as undernutrition, normal, overweight, or obese according to WHO criteria.

8. OUTCOME MEASURES

8.1 Primary Outcome Measures

Outcome 1: ISAA Total Score - Autism severity assessed by the Indian Scale for Assessment of Autism (ISAA). Total score used to determine symptom severity and examine associations with gut microbiome characteristics. [Time Frame: Baseline]

Outcome 2: Gut Microbiome Diversity and Composition - Assessed from stool samples using 16S rRNA gene sequencing. Primary microbiome outcomes: alpha diversity (Shannon, Simpson, Chao1), beta diversity measures, and relative abundance of bacterial taxa. [Time Frame: Baseline – single stool sample at enrollment]

8.2 Secondary Outcome Measures

Outcome 3: GMFM-88 Total Score - Gross motor function and dimension scores; association with gut microbiome. [Baseline]

Outcome 4: GI Symptom Severity Score - Constipation, diarrhea, abdominal pain, bloating, Bristol Stool Scale. [Baseline]

Outcome 5: SDQ Total Difficulties Score - Behavioral and emotional functioning; higher = greater difficulties. [Baseline]

Outcome 6: PROMIS Sleep Disturbance Scale - Sleep quality, difficulties, satisfaction; higher = greater disturbance. [Baseline]

Outcome 7: PPA Score - Participation in home, preschool, community, play, social activities. [Baseline]

Outcome 8: SEQ-2.1 Total Score - Sensory hyperreactivity, hyporeactivity, sensory seeking behaviors. [Baseline]

Outcome 9: PROMIS Parent Proxy Cognitive Function - Attention, memory, concentration, learning; higher = better function. [Baseline]

Outcome 10: Dietary Intake Score - Energy, macronutrients, fiber, dietary pattern scores (covariate). [Baseline]

Outcome 11: PedsQL Total Score - Physical, emotional, social, school functioning; higher = better QoL. [Baseline]

Outcome 12: Anthropometric Nutritional Status - WAZ, HAZ, BAZ, WHZ z-scores; WHO nutritional status categories. [Baseline]

Outcome 13: BMI-for-Age Z-Score - BMI (kg/m²) age-sex z-score by WHO reference; nutritional status indicator. [Baseline]

9. STATISTICAL ANALYSIS PLAN

9.1 Sample Size Justification

A total sample size of 600 participants (300 ASD, 300 TD) is planned. This provides adequate statistical power (≥80%) at α=0.05 to detect small-to-medium effect sizes (Cohen's $d \geq 0.25$) in alpha diversity comparisons and correlation analyses between microbiome indices and neurodevelopmental outcome scores. An additional 15% enrollment buffer has been incorporated to account for anticipated attrition.

9.2 Statistical Methods

- Descriptive statistics: Continuous variables summarized as mean ± SD or median (IQR); categorical variables as frequencies and percentages.
- Group comparisons (ASD vs. TD): Independent samples t-test or Mann-Whitney U test for continuous variables; chi-square or Fisher's exact test for categorical variables.
- Alpha diversity: Shannon, Simpson, Chao1 indices compared using Mann-Whitney U test with Benjamini-Hochberg FDR correction.
- Beta diversity: PERMANOVA (adonis2) to test group differences; ANOSIM for complementary analysis; PCoA plots for visualization.
- Differential abundance: LEfSe, DESeq2, or MaAsLin2 to identify differentially abundant taxa between ASD and TD cohorts, with FDR correction.
- Association analyses: Spearman or Pearson correlations between microbiome indices and outcome scores. Multivariable linear regression incorporating age, sex, BMI-for-age z-score, antibiotic history, and dietary pattern scores as covariates.
- Mediation analysis: Dietary intake and nutritional status examined as potential mediators using structural equation modeling or Baron-Kenny approach.

- Missing data: Multiple imputation by chained equations (MICE) applied if missingness >5% for any key variable.
- Software: R (version ≥4.3) using phyloseq, vegan, DESeq2, MaAsLin2, ggplot2, and mice. Significance threshold: $\alpha = 0.05$ (two-tailed) with FDR correction.

10. DATA MANAGEMENT AND QUALITY ASSURANCE

Data collection will be performed using structured data collection forms (paper-based and/or electronic). All data will be entered into a secure, password-protected institutional database with role-based access controls. Data validation checks will be implemented to minimize entry errors. Source data verification will be conducted by the principal investigator. Data will be de-identified using unique participant codes; the linking key will be stored separately in a secure restricted-access file. Electronic data will be stored on institutional secure servers with regular encrypted backups. The Data Monitoring Committee (DMC) will conduct periodic reviews of data quality, protocol compliance, and participant safety.

11. ETHICAL CONSIDERATIONS

The study has been reviewed and approved by the Institutional Scientific Review Board (ISRB) of Saveetha Institute of Basic Medical Sciences, Saveetha University (Approval No: 079/06/2026/ISRB/FSR/SIBMS). The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, ICMR National Ethical Guidelines for Biomedical and Health Research, and applicable institutional policies. Written informed consent will be obtained from the parent or legal guardian of each participant prior to enrollment. Assent will be sought from children with sufficient developmental capacity. Participation is entirely voluntary; withdrawal at any time will not affect clinical care. Risks are limited to minor discomfort associated with stool sample collection.

12. CONFIDENTIALITY AND DATA PROTECTION

All participant information will be kept strictly confidential. Participants will be assigned unique de-identified codes; no names or personal identifiers will appear in the research database, analysis files, publications, or reports. Data will be accessible only to the research team on a need-to-know basis. Published reports will present group-level aggregate data only. Individual participant data (IPD) sharing will be considered post-publication subject to institutional ethics approval, data-sharing agreements, and applicable regulatory requirements.

13. DISSEMINATION PLAN

Study findings will be disseminated through peer-reviewed publication in an indexed international journal and presentation at relevant scientific conferences in pediatric rehabilitation, neurodevelopmental research, and gut microbiome science. The ClinicalTrials.gov registry record will be updated with results following completion of the study. The findings are intended to contribute to the evidence base on microbiome–neurodevelopment interactions in ASD and inform translational and rehabilitation research directions.

14. FUNDING AND CONFLICTS OF INTEREST

This study is sponsored by Saveetha University. The funding source has no role in study design, data collection, analysis, interpretation, or publication decisions. The principal investigator declares no conflicts of interest related to this study. No pharmaceutical or commercial industry funding is received for this study.

15. REFERENCES

1. Baranek GT, Watson LR, Boyd BA, et al. Hyporesponsiveness to social and nonsocial sensory stimuli in children with autism, children with developmental delays, and typically developing children. Development and Psychopathology. 2013;25(2):307-320. PMID: 23627949

2. Kang DW, Adams JB, Gregory AC, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. Microbiome. 2017;5(1):10. PMID: 28122648

3. Vuong HE, Hsiao EY. Emerging roles for the gut microbiome in autism spectrum disorder. Biological Psychiatry. 2017;81(5):411-423. PMID: 27773355

4. Cryan JF, O'Riordan KJ, Cowan CS, et al. The microbiota-gut-brain axis. Physiological Reviews. 2019;99(4):1877-2013. PMID: 31460832

5. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). Washington, DC: APA; 2013.

6. QIIME 2 Development Team. QIIME 2: Reproducible, interactive, scalable and extensible microbiome data science. PeerJ. 2018;2:e27295v2.

7. McMurdie PJ, Holmes S. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE. 2013;8(4):e61217.

ClinicalTrials.gov Informed Consent Form

INFORMED CONSENT FORM

Association Between Gut Microbiome Characteristics and Neurodevelopmental Functioning in Children With Autism Spectrum Disorder: A Multidomain Investigation of the Gut–Motor Axis

Study Acronym: GAIN-ASD

Protocol ID:	079/06/2026/ISRB/FSR/SIBMS
Document Date:	June 9, 2026
Consent Type:	Parent/Guardian Consent for Child Participant (Ages 3–12 years)
Sponsor:	Saveetha University, Chennai, India
Principal Investigator:	Jeevarathinam Thirumalai
Contact:	+91 6384577805 | jeevarathinamhope@gmail.com
Study Site:	Saveetha Medical College and Hospital, Chennai – 602105
IRB Approval:	079/06/2026/ISRB/FSR/SIBMS – Institutional Scientific Review Board

This Informed Consent Form will be made publicly available on ClinicalTrials.gov following study recruitment closure and PRS review. No participant identifiable information is included. A signed version of this form will be retained at the study site and will not be uploaded.

INFORMATION FOR PARENTS/LEGAL GUARDIANS

You are being invited to allow your child to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please read this information carefully and take time to ask questions. You do not have to agree to your child participating. If you decide to take part, you are free to withdraw at any time without giving a reason, and without affecting the medical care your child receives.

1. WHAT IS THE PURPOSE OF THIS STUDY?

We are inviting children with Autism Spectrum Disorder (ASD) and children with typical development to participate in a research study called the GAIN-ASD study (Gut and Neurodevelopmental Interactions in Autism Spectrum Disorder).

The purpose of this study is to investigate whether there is a relationship between the types of bacteria and microorganisms living in a child's intestines (the "gut microbiome") and how the child develops and functions in everyday life. Recent research suggests that the gut microbiome may play a role in brain development and function. We want to study this relationship in children with ASD and in typically developing children to learn more about this connection.

This study will not offer any new treatment or change your child's current clinical care.

2. WHY HAS MY CHILD BEEN INVITED?

Your child has been invited because they are between 3 and 12 years of age and have a confirmed diagnosis of Autism Spectrum Disorder (or, for the comparison group, because they do not have a neurodevelopmental diagnosis and are a similar age and sex to a child with ASD in our study). We are aiming to recruit a total of 600 children - 300 children with ASD and 300 typically developing children - from Saveetha Medical College and Hospital and affiliated centers.

3. DOES MY CHILD HAVE TO TAKE PART?

No. Taking part is entirely voluntary. You are free to decide not to allow your child to participate. If you decide to participate and then change your mind, you can withdraw your child at any time, without giving a reason. Your decision will not affect your child's medical care, the services they receive, or your relationship with Saveetha Medical College and Hospital or any participating center.

4. WHAT WILL HAPPEN IF MY CHILD TAKES PART?

If you agree to let your child take part, the following will occur during a single study visit (or as coordinated with your existing appointments):

4.1 Stool Sample Collection

You will be provided with a stool collection kit with written instructions. You will be asked to collect a small stool sample from your child at home or at the clinic using the kit. The kit contains a clean collection container and detailed instructions. This sample will be used to analyze the types of bacteria and microorganisms living in your child's gut (microbiome analysis). The sample will be stored in our laboratory and DNA from the sample will be analyzed. No other tests will be performed on the sample without your separate consent.

4.2 Neurodevelopmental Assessment

Your child will undergo several developmental assessments at the study site. These are non-invasive and are similar to routine developmental checks. They include observation and assessment of motor skills (how your child moves and balances) and a brief structured assessment of autism symptoms. Some assessments involve your child performing simple physical tasks with guidance from a trained assessor. The assessments are child-friendly and will be stopped if your child is distressed.

4.3 Questionnaires Completed by You

You will be asked to complete several questionnaires about your child covering: sensory experiences; behavior and emotions; sleep; participation in daily activities; quality of life; and gastrointestinal symptoms. These questionnaires take approximately 45–60 minutes in total to complete. Assistance will be provided if needed.

4.4 Dietary Assessment

You will be asked to complete a 24-hour dietary recall for your child on 2–3 days (including at least one weekend day), describing everything your child ate and drank. A brief food frequency questionnaire about typical eating habits will also be completed.

4.5 Measurements

We will measure your child's height and weight using standard equipment. These measurements are used to calculate nutritional status according to international standards.

5. WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

- Stool collection: May be unfamiliar but causes no physical harm. Instructions provided to minimize inconvenience.
- Developmental assessments: May be tiring for younger children. Assessments will be stopped or paused if your child shows signs of distress. Breaks will be offered.
- Questionnaires: Some questions relate to your child's behavior and health, which may be sensitive. You may skip any question you are not comfortable answering.
- Time commitment: Estimated total participation time is approximately 2–3 hours, which may be spread across one or two visits.

6. WHAT ARE THE POSSIBLE BENEFITS?

There are no direct clinical benefits to your child from participating in this study. However, your child will receive a comprehensive neurodevelopmental assessment, and you will receive a brief summary report of the assessment findings for your reference. Participation contributes to advancing scientific knowledge about ASD and the gut microbiome, which may benefit children with ASD in the future.

7. WILL MY CHILD'S INFORMATION BE KEPT CONFIDENTIAL?

Yes. All information collected about your child will be kept strictly confidential. Your child will be assigned a unique research code number. Their name and personal identifying information will not be used in any research database, analysis, publication, or public document. Data will be stored securely on institutional servers with access limited to the research team. Research findings will be published as group-level summaries only; no individual can be identified.

As part of ClinicalTrials.gov registration requirements, this consent form (without any signed copies or participant identifiers) will be made publicly available on the ClinicalTrials.gov website after the study recruitment period ends. Signed consent forms will be retained securely at the study site and will not be uploaded or shared publicly.

8. WILL ANY BIOLOGICAL SAMPLES BE STORED?

Stool samples and extracted microbial DNA will be retained in the laboratory under appropriate storage conditions (−80°C) for the duration of the study and as permitted by institutional ethical guidelines. Samples will be used solely for the research objectives described in this consent form. No sample will be used for any other purpose without separate ethical approval and your written consent.

9. WHAT HAPPENS WITH THE RESULTS?

Results of this study will be analyzed and reported as group-level findings. We plan to publish results in a peer-reviewed scientific journal and present findings at scientific conferences. Individual results will not be reported back to participants unless they have direct clinical relevance and are confirmed by standard clinical testing. Study results will also be reported on ClinicalTrials.gov following study completion.

10. WHO HAS REVIEWED AND APPROVED THIS STUDY?

This study has been independently reviewed and approved by the Institutional Scientific Review Board (ISRB) of Saveetha Institute of Basic Medical Sciences, Saveetha University (Approval No: 079/06/2026/ISRB/FSR/SIBMS). This study is registered on ClinicalTrials.gov, the U.S. National Library of Medicine's clinical trial registry.

11. WHO CAN I CONTACT FOR MORE INFORMATION?

If you have any questions about the study, please contact the Principal Investigator:

Name:	Jeevarathinam Thirumalai
Institution:	Saveetha Institute of Basic Medical Sciences, Saveetha University
Address:	Chennai, Tamil Nadu – 602105, India
Phone:	+91 6384577805
Email:	jeevarathinamhope@gmail.com
IRB Contact:	bms.simats@saveetha.com | +91 73586 95525

PARENT/GUARDIAN CONSENT DECLARATION

I have read and understood the information provided in this Informed Consent Form (Version 1.0, dated June 9, 2026) about the GAIN-ASD study (Protocol ID: 079/06/2026/ISRB/FSR/SIBMS). I have had sufficient time to consider whether to allow my child to participate. I have had the opportunity to ask questions and received satisfactory answers. I understand that my child's participation is voluntary and that I may withdraw my child at any time without consequence to their medical care.

I agree to allow my child to participate in this study, including stool sample collection for gut microbiome analysis, neurodevelopmental assessments, completion of questionnaires, dietary recall, and anthropometric measurements as described in this form.

PARTICIPANT INFORMATION	
Child's Name:	_____
Child's Date of Birth:	_____ Age: _____
Child's Sex:	_____
Diagnosis (if ASD):	_____
PARENT/GUARDIAN INFORMATION	
Name of Parent/Guardian:	_____
Relationship to Child:	_____
CONSENT	
Signature of Parent/Guardian:	_____
Date:	_____
WITNESS (if applicable)	
Name of Witness:	_____
Signature of Witness:	_____
Date:	_____
INVESTIGATOR/DELEGATE	
Name:	_____
Signature:	_____
Date:	_____

CHILD ASSENT (Where Developmentally Appropriate)

For children with sufficient developmental capacity, assent will be sought. The assessor will explain the study in age-appropriate language and record whether the child agreed to participate.

Child agreed to participate:	☐ Yes ☐ No ☐ Not applicable (assent not sought – developmental level)
Assessor Name:	_____
Assessor Signature:	_____
Date:	_____

Document Information: GAIN-ASD Informed Consent Form | Version 1.0 | June 9, 2026 | NCT Number: Pending Assignment | Principal Investigator: Jeevarathinam Thirumalai | Saveetha University | IRB: 079/06/2026/ISRB/FSR/SIBMS

Note: Signed copies of this form will be retained securely at the study site and will not be uploaded to ClinicalTrials.gov.