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Review

Microbiome biomarkers in autism spectrum disorder: Toward prediction, diagnosis, and prognosis

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SUMMARY

Autism spectrum disorder (ASD) is a heterogeneous condition that lacks objective diagnostic biomarkers, often resulting in delayed intervention. Evidence increasingly links gut microbiota dysregulation to ASD pathophysiology via the microbiota-gut-brain axis, suggesting plausible translational applications. This review outlines mechanistic insights from preclinical and clinical studies to illustrate how microbial disturbances affect neurodevelopment. It examines the evolution of biomarker research from early 16S rRNA sequencing to advanced shotgun metagenomics incorporating functional integration, multi-omics, and genomic variants. Such advancements enhance diagnostic accuracy and generalizability. Although clinical causal evidence remains indirect, these microbial signatures show potential for early diagnosis, presymptomatic risk prediction, and tailored therapies. Key challenges include prospective validation in diverse cohorts, specificity testing against comorbidities, and addressing clinical heterogeneity. By summarizing methodological gaps and providing future guidance, this review aims to bridge mechanistic research and clinical practice to improve outcomes across the spectrum.

INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by deficits in social communication and interaction, alongside restricted and repetitive behaviors. Historically, ASD was considered a rare condition (<1%), whereas contemporary reports show that prevalence is in the range of 2%–3% across different regions of the world.[1] As an early-onset neurodevelopmental condition with multifaceted needs across the lifespan, ASD constitutes a major public health concern and burdens the healthcare, education, and social welfare systems. Early and accurate diagnosis is crucial for timely intervention to optimize outcome.[2] However, lack of objective biomarkers could make the diagnostic process for ASD challenging. The complexity of behavioral assessment that demands expertise also limits diagnostic efficiency, contributing to the common delay in diagnosis. This also precludes pre-symptomatic diagnosis, often leaving caregivers of children with elevated neurodevelopmental risk and concerns stressed with the clinical uncertainties.[3]

In recent years, evidence from preclinical animal models has linked gut microbiota to ASD pathogenesis through the microbiota-gut-brain axis (MGBA)[4] (Figure 1), a bidirectional communication network that links the gut to the central nervous system.[5] Supporting this postulation, individuals with ASD consistently exhibit altered gut microbiome community and its metabolites.[6,7] In addition to compositional differences, the functional characteristics of gut microbiota and its metabolic outputs are also distinctive in children with ASD when compared with typically developing (TD) children,[8,9] alluding to the possibility of harnessing the gut microbiome as a biomarker for ASD.[10]

The present review first summarizes the clinical studies and current mechanistic understanding of the MGBA in ASD. We then discussed the progression of translational research,

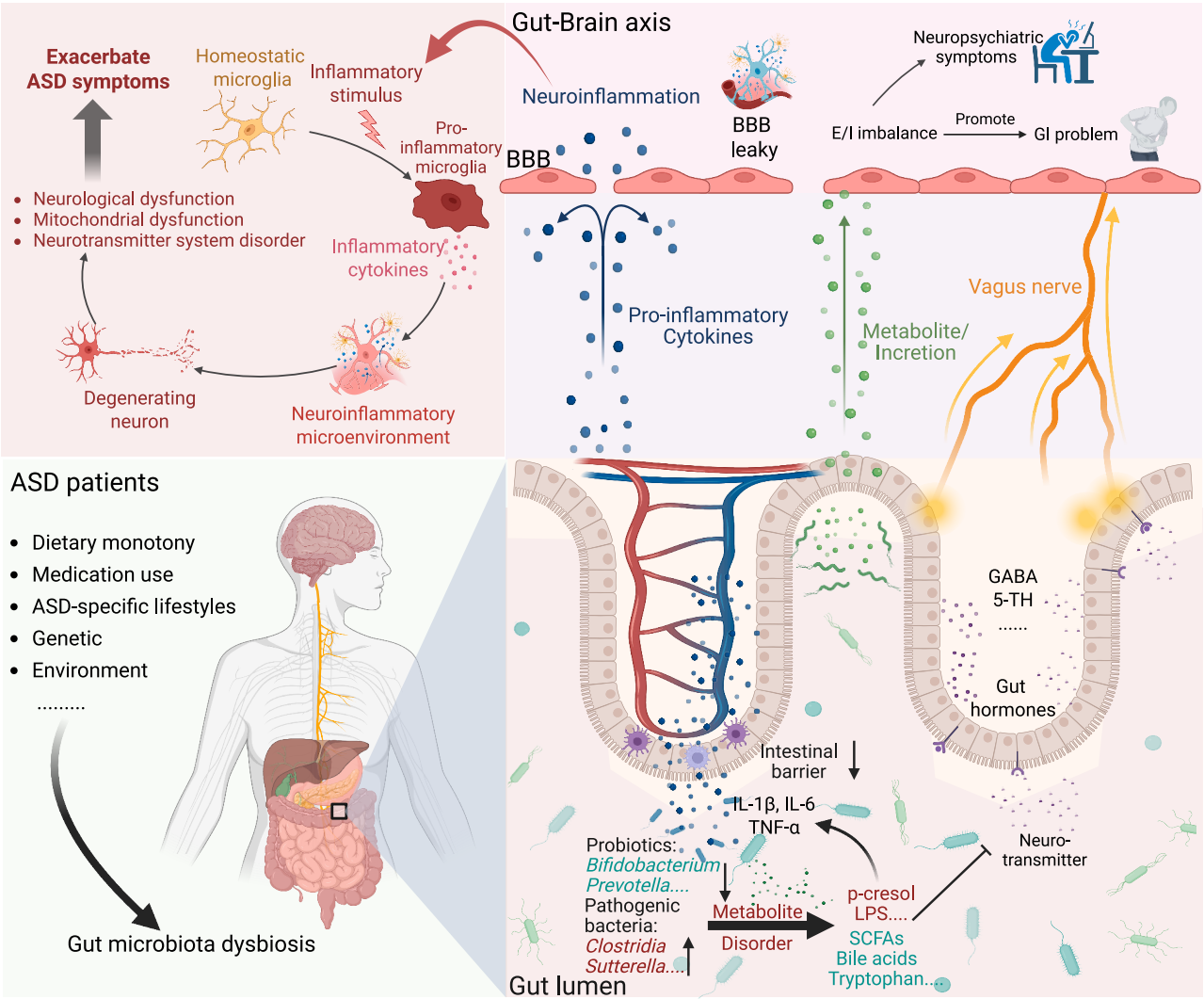

Figure 1. Hypothesized gut-brain axis communication mechanisms

ASD-related factors (e.g., diet, genetics, medication) lead to gut microbiota dysbiosis, with reduced beneficial bacteria and increased pathogens. Dysbiosis triggers metabolic changes (e.g., SCFAs, bile acids, neurotransmitters) and pro-inflammatory status in the gut. These signals propagate to the brain via a "leaky" blood-brain barrier or the vagus nerve, activating pro-inflammatory microglia and causing neuroinflammation. Neuroinflammation drives neuronal degeneration and neurotransmitter system dysfunction to exacerbate ASD symptoms. Conversely, neuropsychiatric symptoms from brain dysfunction reciprocally aggravate gastrointestinal issues to form a vicious cycle.

covering the advances in methodologies in developing gut microbiota biomarkers for ASD. Subsequently, we provide a critical appraisal to the gaps in existing empirical research, the potential applications across different clinical scenarios, as well as the hurdles in this developmental process, ultimately laying out a research roadmap and a recommendation guideline to further the progression in the field. While this article is not a systematic review, we did a comprehensive literature search to identify relevant research articles on the topic of using the gut microbiome as a diagnostic marker for ASD with the following main strategy: ("autism spectrum disorder" OR ASD) AND ("microbiota") AND ("biomarker" OR "AUC" OR "intervention" OR "mechanism"). In this review, we employ the term "ASD" in alignment with the diagnostic criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5), particularly as our discussion centers on biomarkers for diagnostic purposes. Our team recognizes that autism is not inherently a disorder and fully embraces the principles of neurodiversity,[11] with no intention of diminishing the value of different neurotypes.

CURRENT DIAGNOSTIC CHALLENGES OF ASD AND THE NEED FOR BIOMARKERS

Similar to other psychiatric disorders, ASD lacks objective biomarkers,[12] and the current diagnostic approach is entirely based on clinical observation and early developmental history according to the DSM-5 or the International Classification of Diseases

11th Revision criteria. While these criteria provide guidance on the assessment of symptomatology, the phenotypic heterogeneity, sex differences in clinical manifestations, presence of other co-occurring neuropsychiatric and physical symptoms, and variability of cognitive functions all pose significant challenges in the early and accurate identification of ASD.[13] For instance, diagnostic overshadowing is common among children with co-occurring anxiety and attention-deficit/hyperactivity disorder (ADHD).[14] The presentations of autistic symptoms among females could be qualitatively different from those in males.[15] Cognitive abilities may facilitate camouflaging to obscure the behavioral presentations of social difficulties.[16] Moreover, ASD exists on a continuum with typical development, such that symptoms and traits of ASD also exist in the general population.[17] Therefore, diagnosis often relies on skilled expertise and inevitably involves elements of subjective judgment. Even for skilled clinicians, diagnostic uncertainty is not uncommon, especially among toddlers against the background of the highly dynamic nature of child development.[18] As a result, while symptoms of ASD can emerge as early as 12 months of age, the average age of attaining a diagnosis is 4–5 years.[19] This reliance on expertise, especially in regions with limited resources, also creates a bottleneck for the child to receive a diagnostic label, which is often required by institutional bodies for the initiation of educational and social welfare support. The resultant delay in early intervention significantly impacts prognosis, as early intervention during the critical period of neuroplasticity in brain development may mitigate the subsequent risk of adverse functional and psychiatric outcomes.[2,20,21]

Furthermore, although this remains a subject of ongoing debate, the neurodevelopmental nature of ASD suggests that the onset of the condition predates observable symptom manifestation.[22] However, the reliance on behavioral observation precludes the possibility of predicting and identifying infants with ASD prior to symptom onset, which is especially relevant among children who are at higher risk, e.g., those with a family history of neurodevelopmental disorders.

To overcome these limitations, the scientific community has been actively exploring biomarkers to aid the diagnostic process, such as electroencephalogram, eye tracking, neuroimaging, and genetics.[23–26] Although signals from these modalities have shown consistent positive associations with ASD diagnosis, the effect sizes remain modest and generalizability is limited. On the other hand, the gut microbiota has gained significant attention as a potential biomarker of ASD with the evolving conceptualization of the MGBA and evidence from both preclinical and clinical research.[27] The dynamic nature of the gut microbiome contrasts with the static nature of genetic marker and could serve as a modifiable biosensor of ASD-related processes and developmental trajectories. Compared with neuroimaging approaches, which could be technically demanding in young children, harnessing the gut microbiota offers distinct advantages from the caregiver's perspective: it requires only a simple, non-invasive stool sample that can be collected at home without specialized equipment or clinical visits. This high accessibility makes it well suited in pediatric settings,[28] enabling scalability at the population level for broad translational applications.

ASD GUT MICROBIOTA DYSBIOSIS AND GUT-BRAIN AXIS MECHANISMS

Compositional and taxonomic alterations of the gut microbiota in ASD

The earliest clinical evidence supporting the involvement of the gut microbiome in ASD dates back to case reports documenting abnormal gut permeability,[29] symptomatic relief following vancomycin treatment,[30] and elevated levels of *Clostridium* species in children with ASD.[31] Over the past decade, advances in high-throughput sequencing technologies have enabled comprehensive profiling of the gut microbial community, leading to a surge of research that has consistently demonstrated altered gut microbiome composition (β-diversity) in children with ASD.[6,7] Early efforts in this field primarily focused on identifying differentially abundant taxes between children with ASD and controls. These studies commonly reported enrichment of potentially pathogenic bacteria, including species within the class Clostridia (e.g., *Clostridium perfringens*),[32–34] as well as the genera *Desulfovibrio*[35,36] and *Sutterella*,[37,38] which are implicated in the production of neurotoxic metabolites and induction of gut mucosal inflammation.[35,36,39–42] Concurrently, reductions in beneficial bacteria, such as the genera *Bifidobacterium* and *Prevotella*, have been observed in ASD.[43,44] These taxa promote intestinal homeostasis through anti-inflammatory effects, regulation of vitamin B metabolism, and competitive inhibition of pathogens.[45–49] Although a general pattern of imbalance between pathogenic and beneficial bacteria emerges across studies, inconsistence persists for numerous specific taxa.[7] There have also been contradictory findings of enriched beneficial bacterial genera such as *Lactobacillus* and *Bacteroides* in ASD,[33,50] which could be attributable to the methodological heterogeneity and the numerous confounders of gut microbiome composition such as age, sex, diet, socioeconomical status, and geographical regions.

Microbial metabolic outputs that inform gut-brain pathomechanism in ASD

Shifts in gut microbiota composition can profoundly alter microbial metabolic pathways that influence human physiology. The functional redundancy within the microbiome, wherein diverse taxa can converge on overlapping metabolic roles, suggests that shifting focus from taxonomic profiles to functional outputs could offer a complementary perspective on pathomechanistic contributions to ASD, circumventing the pitfalls of taxon-centric interpretations and potentially reconciling inconsistencies.[51] Functional analysis and metabolomic studies have identified several key microbial metabolic alterations in ASD. These include alterations in short-chain fatty acids (SCFAs),[52–54] elevated levels of the neurotoxic metabolite *p*-cresol,[9,55] dysregulated inflammatory pathways,[56,57] and perturbations in neurotransmitter metabolism, such as the tryptophan-kynurenine pathway,[58–60] glutamate,[32] and γ-aminobutyric acid (GABA).[61] A recent meta-omics analysis further revealed a linkage between inflammation, sphingolipids, and steroid hormone dysregulation, suggesting that these metabolic changes may act in concert to affect the MGBA in ASD.[62]

These microbial metabolic outputs are implicated in ASD pathophysiology. For instance, SCFAs serve as key mediators

of gut-brain signaling[63] and may link to mitochondrial dysfunction and epigenetic dysregulation in ASD.[64] On the other hand, *p*-cresol, a phenolic compound derived from microbial tyrosine metabolism, exhibits neurotoxic effects that disrupt neuronal development.[65] Furthermore, these microbial metabolic perturbations coincide with some of the proposed mechanisms in ASD, including the linkage between pro-inflammatory state, kynurenine-driven excitotoxicity, and excitatory-inhibitory (E/I) imbalance.[66–68] Collectively, these findings position the gut microbiome as a plausible mediator of these metabolic disturbances to the pathophysiology in ASD.

Fine-grained mechanistic insights from preclinical animal models

While clinical studies provide compelling associations between gut dysbiosis and ASD pathophysiology along the MGBA, establishing causality in humans remains challenging due to ethical and methodological constraints. Preclinical animal models, on the other hand, offer relatively robust evidence of the microbiome's causal role in ASD-like behaviors. For instance, fecal microbiota transplant (FMT) from children with ASD to germ-free mice induces social deficits and repetitive behaviors.[4] Reciprocally, FMT from neurotypical donors to established ASD mouse models, such as valproic acid (VPA)-induced or BTBR mice, alleviates the behavioral phenotypes.[69,70]

Mechanistic insights from animal models not only align with clinical observations of metabolic, immune, and neural alterations but also elucidate their interrelatedness. Early work demonstrated that intraventricular propionic acid infusion in rats elicits ASD-like repetitive behaviors, implicating SCFAs in pathogenesis.[71] Probiotic interventions in VPA-exposed rats ameliorate social deficits by restoring fecal SCFAs.[72] Downstream, SCFA effects interface with inflammation: acetate supplementation in *Shank3*-knockout mice rescues social deficits and prefrontal cortex transcriptomic signatures related to synaptic plasticity and immune regulation.[73] These results corroborate the roles of SCFA signaling in immune and inflammatory response.[74]

Immune dysregulation induced by the microbiome intersects with E/I imbalance, a hallmark of ASD,[67] in preclinical models. For instance, multi-strain probiotics in VPA-exposed rats enhance sociability, while suppressing serum pro-inflammatory cytokines (e.g., IL-6 and TNF-α).[75] Extending this linkage, *Lactobacillus murinus* in BTBR mice drives brain infiltration and activation of IFN-γ-producing $CD4^+$ and $CD8^+$ T cells, elevating fecal and cerebral glutamate levels, while suppressing GABA. This perturbation skews the glutamate/GABA ratio, disrupts E/I homeostasis, and intensifies repetitive and social deficits. Notably, FMT from healthy donors or protective probiotics reverses these immune-neural cascades and behavioral impairments.[76]

At the neurotransmitter and synaptic levels, ASD-FMT in germ-free mice dysregulates serotonergic components in the gut-brain axis: colonic tryptophan hydroxylase (TPH1) upregulation with serotonin transporter (SERT) and 5-HT1A receptor downregulation, contrasted by prefrontal TPH2 and SERT elevation, alongside perturbed tryptophan metabolites.[60] Therapeutic probiotics *Lactobacillus reuteri* restores social motivation in multiple ASD mouse models via vagus nerve-dependent oxytocin-dopaminergic reward pathway,[77] while *Lactiplantibacillus plantarum* PS128 reinstates hippocampal and prefrontal dendritic arborization, spine density, and glutamate receptor expression in VPA mice.[78] Complementing these, microbial *p*-cresol, elevated in human ASD, induces social avoidance and stereotypies in mice by dampening ventral tegmental dopamine neuron excitability in the social reward circuit.[79]

Collectively, these preclinical data reinforce clinical observations, while revealing the MGBA's intricate mechanisms in ASD. Whether these represent parallel or converging pathways (e.g., via neuroinflammation) remains unresolved, as does their interaction with the genetic component given ASD's high heritability.[80] Although mechanistic understanding is incomplete, this empirical support bolsters the gut microbiome's promise as a biomarker for ASD.

Current challenges and opportunities of translational applications

Converging lines of evidence from human association studies and preclinical animal models suggest considerable translational potential for the MGBA as biomarker for ASD. Within the framework of MGBA lies substantial opportunity, as the microbiome extends beyond a purely diagnostic role by serving as a modifiable therapeutic target. Preclinical models demonstrate that gut microbiome modulation can ameliorate ASD-like behaviors even in genetic models of ASD mice, suggesting that such therapies may produce changes that override genetic predispositions.[76,77] Despite the preliminary nature of existing human clinical trials,[81] the available arsenal of microbiome modulation strategies—including probiotics, prebiotics, synbiotics, and FMT,[82] opens avenues for mechanism-informed interventions. Given this dual diagnostic and therapeutic potential, the following section reviews the existing literature on microbial biomarker studies in ASD, focusing on reported discriminatory accuracies, key taxonomic and functional features, and prospects for clinical translation.

Microbial biomarkers for ASD prediction and diagnosis

As discussed, symptoms of ASD emerge around 12 months of age, and yet formal diagnosis often occurs later at 4–5 years. This creates a window of opportunity for gut microbiome-based biomarkers to serve two different purposes: (1) early prediction of ASD risk during the pre-symptomatic or early symptomatic toddler stage, when the clinical presentation is evolving and diagnostic uncertainty is common, and (2) identification of children who have already met the criteria for ASD. The two objectives necessitate distinct methodological approaches. Predictive biomarkers require prospective longitudinal designs, ideally initiating from birth or early infancy. However, given the low prevalence of ASD in the general population, tracking of clinical outcomes in high-risk cohorts (i.e., younger siblings of children with ASD)[83] is an approach often adopted. In contrast, diagnostic biomarkers can be evaluated through cross-sectional case-control studies comparing children with established ASD with neurotypical controls or siblings.

Predictive marker for ASD: Preliminary data from longitudinal cohorts

To date, the majority of microbiome biomarker research in ASD has pursued the diagnostic approach, employing case-control designs to assess discriminatory performance (reviewed in the following section). There is a paucity of studies that have examined the predictive microbiome markers for ASD, due to substantial logistical and methodological demand, such that no validated predictive models have emerged from gut microbiome research. The limited longitudinal studies available are largely exploratory.

Two recent prospective studies provide illustrative exploratory evidence. Zuffa et al.[84] followed 35 infants, including 19 with high-risk vs. 16 with low-risk ASD from 5 to 36 months. At 5 months, high-risk infants displayed reduced *Bifidobacterium*, increased *Clostridium* and *Klebsiella*, and lower fecal GABA levels. At 36 months, the high-risk group had higher Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) scores, although no participants received formal ASD diagnoses. Laue et al.[85] profiled 304 infants in a general-population cohort using shotgun metagenomics and metabolomics from 6 weeks to 2 years. Early-life microbial genes involved in SCFA synthesis, along with fecal butyrate concentrations, were associated with higher degree of autism traits, quantified by the Social Responsiveness Scale (SRS-2). Complementing smaller exploratory studies, a large prospective Swedish birth cohort found that gut microbiome profiles at 1 year of age were associated with subsequent diagnoses of neurodevelopmental disorders, including ASD.[86]

Overall, these studies provide preliminary evidence that early-life gut microbial composition, functionality, and metabolites (e.g., SCFAs and their metabolic pathways) may be associated with emerging ASD-related traits. These data call for larger cohorts with standardized diagnostic endpoints to investigate the predictive ability of the gut microbiome for diagnostic outcomes. Another fundamental limitation is our incomplete understanding of normative gut microbiome maturation during early life and its association with neurodevelopment.[87] Without robust benchmarks for typical microbial succession and its interplay with neurodevelopment, it remains challenging to discern whether observed deviations in high-risk ASD cohorts could reflect causal mechanisms.

Evolution of gut microbiota-based diagnostic biomarkers for ASD

In contrast to predictive models, which target early risk identification, the majority of studies in the field have focused on diagnostic biomarkers for ASD. This progression mirrors advances in our understanding of the gut microbiome's role in ASD, evolving from early analyses of diversity indices and taxonomic composition, primarily with 16S rRNA sequencing, to more mechanistically informative functional pathway analyses enabled by high-resolution shotgun metagenomics. Subsequent developments have incorporated multi-omics integration and multi-kingdom and microbial genomics variants perspectives, alongside increasingly sophisticated machine learning frameworks.

Below, we summarize key research efforts that have evaluated the diagnostic utility of gut microbiota biomarkers in ASD (Figure 2). Beginning with simple associative findings, the field has progressed toward robust, integrated models that enhance diagnostic accuracy, while providing deeper insights into gut-brain axis mechanisms—narrowing the gap between correlative observations and clinically actionable tools.

Taxonomic classifier: From single- to multi-cohort validation

Early investigations into gut microbiome differences in ASD used single-cohort designs to identify taxonomic differences. These early studies were constrained by small sample sizes and lack of external validation, resulting in inconsistent signatures that lacked generalizability. Kang et al.[43] pioneered this line of inquiry by pyrosequencing the 16S rRNA gene in fecal samples from 20 children with ASD and 20 neurotypical controls. With a multi-genus logistic regression and random forest classifiers, cross-validated areas under the curve (AUCs) of 0.839 and 0.837 were obtained. Kong et al.,[88] in 20 ASD and 19 matched controls, derived individual taxonomic features with AUCs up to 0.724. Dan et al.[89] applied random forest to 24 differential genera in a larger cohort of 143 ASD and 143 controls and reached an AUC of 0.931. Finally, a shotgun metagenomics study by Ye et al. in 71 ASD and 11 controls selected a 10-species panel (e.g., *Prevotella buccae, Bifidobacterium longum, Clostridium ramosum, Streptococcus thermophilus*) via random forest, reporting an AUC of 0.947.[90]

Despite internal AUCs exceeding 0.80, these early taxonomic-only models suffered from risks of overfitting, irreproducible markers, and lack of external validation. Hence, researchers subsequently adopted multi-cohort approaches to harmonize confounding factors such as geographical and demographic characteristics, and to derive more generalizable results with external validation. Key examples include Xu et al.,[91] who meta-analyzed 10 cohorts of 16S datasets (569 individuals with ASD and 450 controls) to produce a 12-genus panel achieving validation AUC of 0.761. Wan et al.[92] used ultra-deep metagenomics for species markers, yielding discovery and validation AUCs of 0.826 and 0.762, respectively; Wang et al. tested *Eubacterium* sp. *CAG 248*, *Prevotella copri*, *Bifidobacterium pseudocatenulatum*, and 64 other species markers across cohorts from China and Moscow. Despite substantial dietary differences, this taxonomic panel maintained strong diagnostic accuracy (AUC = 0.984 and 0.81, respectively), and a combined model yielded an AUC of 0.86.[93]

Although multi-cohort approaches filtered noise and boosted credibility, AUC performance rarely exceeded 0.80 in external sets. Furthermore, with signatures predominantly composed of taxonomic features, the underlying mechanistic insight is limited.

Advancing to functional pathway analysis and multi-omics integration

A major progression in microbial biomarker research for ASD has been the incorporation of metabolic pathways through metagenomic and multi-omics approaches. Purely taxonomic models are constrained by considerable inter-individual and inter-cohort variability in microbial composition. Functional redundancy explains why this taxonomic variability can coexist with more consistent functional profiles across individuals and populations.

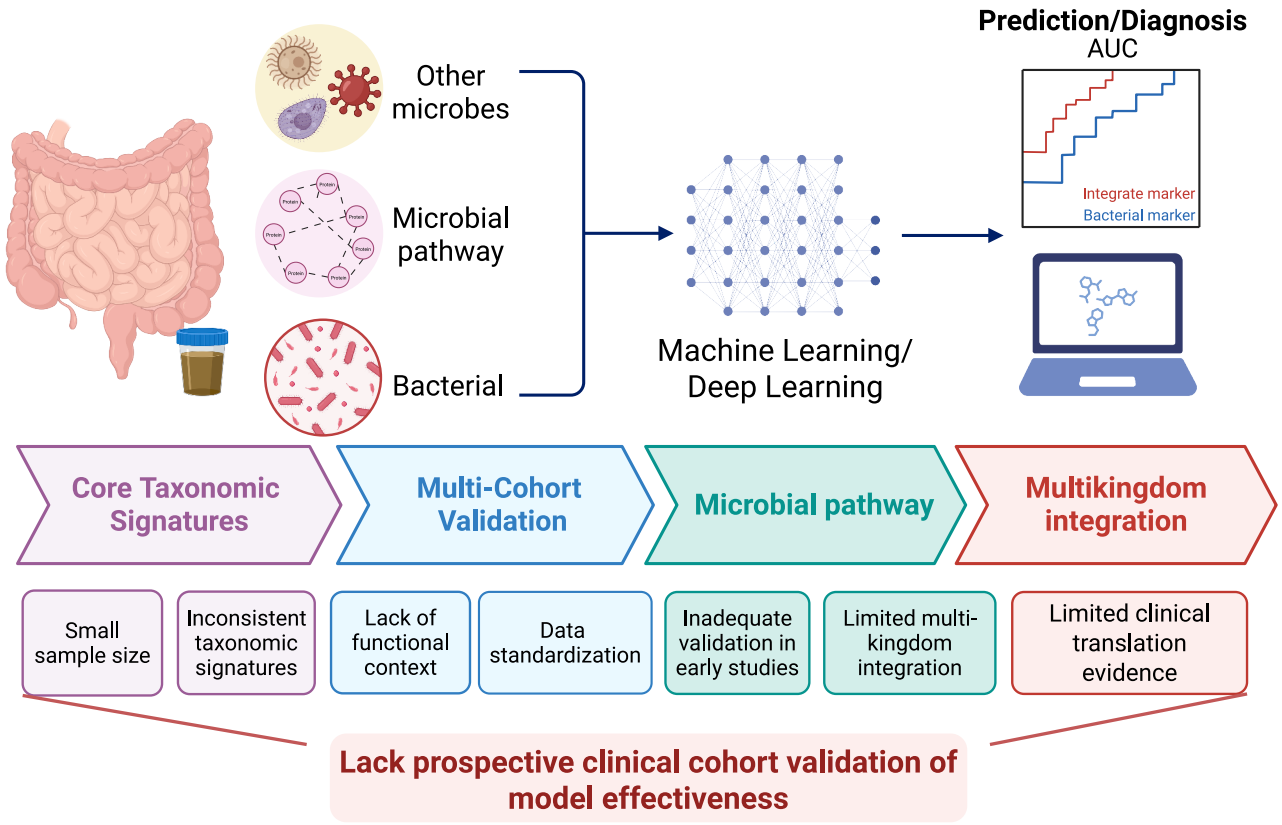

Figure 2. Evolution of gut microbiota-based biomarkers for ASD prediction and diagnosis

Microbial biomarker development for ASD has advanced through progressive stages of increasing complexity and robustness: from early core taxonomic signatures in small, single-center cohorts, to multi-cohort validation for enhanced generalizability, then to functional pathway analysis and multi-omics integration for greater stability and mechanistic insight, and most recently to multi-kingdom approaches encompassing bacteria, archaea, fungi, viruses, and shared metabolic pathways. These diverse inputs are analyzed via machine learning and deep learning to yield diagnostic models. Despite progress, persistent limitations at each stage highlight the key unmet need: large-scale prospective clinical cohort validation of real-world model effectiveness.

By prioritizing these shared functional outputs over variable taxa, pathway-based and multi-omics strategies may yield more reproducible and generalizable biomarkers. They also provide clearer links between microbial functions and ASD pathophysiology through the gut-brain axis, turning correlative taxonomic findings into mechanistically meaningful tools.[94]

Initial studies demonstrated the potential of pathway-based approaches but were again constrained by small cohorts and lack of external validation. Wu et al.[95] analyzed 169 children with ASD and 128 controls, identifying six differentially abundant pathways (including oxidative phosphorylation) and developing a random forest classifier with an AUC of 0.827. Similarly, Zhang et al.[96] used a quasi-paired cohort (39 ASD, 40 TD individuals) to uncover deficiencies in detoxification pathways, notably the γ-glutamyl cycle (PWY_4041) and methylphosphonate degradation (PWY0-1533), yielding a diagnostic AUC of 0.88.

Larger multi-cohort investigations subsequently strengthened evidence for functional integration. Lou et al.[58] examined 773 ASD and 429 TD individuals, showing that combining two operational taxonomic units (*Veillonella* and Enterobacteriaceae) with 17 metabolic functions produced an AUC of 0.86 in the discovery cohort and 0.67–0.82 across three independent validation cohorts—highlighting improved robustness over taxonomy-alone models. Wan et al.[28] further advanced this by integrating five bacterial taxa with functional annotations from 44 metagenome-assembled genomes, achieving AUCs of 0.886 (discovery) and 0.734 (validation), and demonstrated stability across age, gender, and comorbidities.

Multi-omics studies have extended these gains by linking microbial functions to host physiology. Morton et al.[97] integrated metagenomic, metatranscriptomic, and other omics data from 528 ASD-control pairs, associating species such as *Prevotella copri*, *Bifidobacterium longum*, and *Bifidobacterium callitrichidarum* with immune and metabolic dysregulation. A microbiome-derived classifier achieved an overall AUC of 0.78 across cohorts (exceeding 0.87 in six studies).

Multi-kingdom integration: An advanced paradigm in biomarker development

Although metabolic pathway integration has substantially enhanced biomarker stability and interpretability, a bacteria-centric view has largely overlooked the synergistic contributions of other members in the gut ecosystem, including archaea, fungi, and viruses.[98] For fungi, Strati et al.[33] and Zou et al.[99] reported

higher abundance of *Candida* species in children with ASD, which may contribute to gut dysbiosis through immune dysregulation and potential disruption of gut barrier integrity. On the other hand, Wan et al.[100] reported enrichment of certain bacteriophages (e.g., *Clostridium*, *Bacillus*, and Enterobacteria phages) in ASD, while Shahin et al.[101] noted expansion of Caudoviricetes bacteriophages, which can modulate bacterial abundance through lysis or prophage induction, thereby disrupting gut homeostasis and altering neuroactive metabolite production. These studies, although smaller in scale and did not aim to develop a diagnostic marker for ASD, highlight the importance of considering the whole gut microbiome community in relation to potential pathomechanisms within the MGBA for developing diagnostic biomarkers.

A landmark study by Su et al. markedly advanced the field by developing a 31-marker diagnostic panel encompassing bacteria, archaea, fungi, viruses, microbial genes, and metabolic pathways.[61] Using a discovery cohort (709 ASD and 374 neurotypical) and a balanced 1:1 paired subcohort, the team developed a 31-marker panel that included *Streptococcus thermophilus* (bacteria), *Natrinema pellirubrum* (archaea), *Candida albicans* (fungi), a *Faecalibacterium phage* (virus), and depleted metabolic pathways in ASD, such as the GABA shunt and ubiquinol-7 biosynthesis.

The model demonstrated consistent diagnostic performance across cohorts, with AUCs of 0.91 (discovery), 0.87 (independent hospital cohort), 0.89 (community cohort), and 0.78 (international public dataset). Classification was prominently driven by depleted biosynthesis pathways for ubiquinol-7 (an antioxidant coenzyme) and thiamine diphosphate, implicating oxidative stress and metabolic dysregulation in ASD pathogenesis. Notably, single-kingdom models exhibited inferior performance (AUCs 0.68–0.87), confirming the diagnostic advantage conferred by non-bacterial kingdoms and functional elements. By embracing broader microbial interactions, multi-kingdom and multi-omics integration not only elevates biomarker reliability and cross-cohort generalizability but also deepens the understanding of gut-brain axis mechanisms, offering promising avenues for precision diagnostics and targeted interventions in ASD.

Strain-level variation and microbial genomic heterogeneity

Further advancing the field, recent studies have begun to explore strain-level microbial genomic variation, including single-nucleotide variants (SNVs), insertions/deletions (InDels), and structural variants (SVs), which can confer functional heterogeneity within the same species to influence host-microbe interactions.[102] A recent investigation identified a distinct layer of ASD-associated microbial variants (1,369 SNVs, 233 InDels, and 195 SVs) that were not detectable through conventional taxonomic profiling,[103] which were linked to altered metabolic outputs and host responses. When incorporated into a 20-feature panel (combining species, genes, and genomic variants), this approach achieved superior diagnostic performance (AUC = 0.961 in the discovery cohort, median AUC = 0.78 in external datasets) compared with models using species, genes, and pathways alone, highlighting strain-level resolution as also a promising next frontier for enhancing biomarker precision and mechanistic insight.

Limitation of existing studies that hinders translational application

Existing microbiota-based biomarker studies for ASD, while promising, are constrained by methodological limitations. First, the cross-sectional design across the majority of the reviewed studies precludes establishment of causality, rendering it unclear whether microbial alterations contribute to ASD pathophysiology or emerge as consequences of ASD-associated behaviors such as restrictive eating.[104] Establishing the causal role of the altered gut microbiome is crucial to transcend correlative biomarkers into predictive and treatment indicators.[105]

Second, other than the variation in stool sample processing, sequencing platform, and bioinformatic analysis pipeline, heterogeneous findings may arise from inadequate control for major confounders known to shape gut microbiota composition. Among the mentioned studies, while the majority of studies consider the effect of sex and age, only a limited number of studies considered socioeconomic status.[28,61] Dietary intake, which is especially relevant for children with ASD given the common restrictive eating pattern,[106] while captured in a few studies, varies in the instruments used and data processing: some employed standardized instruments such as food-frequency questionnaires or 3-day dietary recall,[28,61,92] while others adopted in-house surveys on special diet.[43,88] Gastrointestinal symptoms, which are common in ASD and independently alter microbiota,[107] were evaluated in some[43,61,88,89,92,96] but absent in others, while one study only included ASD children with gastrointestinal symptoms.[103] Although most studies consider the use of antibiotics and probiotics, use of psychiatric medications (e.g., antipsychotics, ADHD medications, and antidepressants), although known to impact the gut microbiome[105] and frequently used in children with ASD, was only documented or excluded in only a subset.[28,61,96]

Third, while ASD diagnosis was generally robust in most studies using standardized research instruments of Autism Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic Observation Schedule, Second Edition (ADOS-2), or by clinicians using the DSM-5 criteria, assessment of control children tends to be minimally reported and inconsistent. Without rigorous control evaluation, subclinical ASD traits or undiagnosed conditions could contaminate the "neurotypical" group, thereby reducing specificity and inflating false-negatives. Methods for screening and ruling out ASD (e.g., SRS-2 or AQ-Child) were explicitly described in some studies,[61,92] performed via clinical evaluation in others,[88,96] but were minimal or absent in many.[43,58,89,90] Furthermore, common childhood psychiatric conditions such as ADHD and anxiety[108] may overshadow autistic features,[14] yet comprehensive evaluation of multiple psychiatric conditions by specialists is often not feasible in sizable cohorts. This situation highlights the need for validated screening instruments with high negative predictive value when aiming for clean case-control designs.

Finally, model validation strategies across the reviewed studies further underscore important limitations. While internal validation approaches such as cross-validation or train-test splits were commonly employed,[89–93,96] external validation on independent cohorts has only emerged in more recent work. Su et al.[61] and Chen et al.[103] evaluated performance across

multiple independent in-house and public datasets, Wan et al.[28] and Lou et al.[58] tested on a separate validation cohorts, Wu et al.[95] included newly collected external samples, and Morton et al.[97] leveraged multi-cohort meta-analysis. The sole reliance on internal validation carries a substantial risk of overfitting and overestimation of diagnostic performance. When combined with marked heterogeneity in methodological approaches, this dependence severely limits the generalizability of reported microbial signatures and impedes the development of field-wide consensus.

Collectively, these gaps highlight the pressing need for longitudinal studies and greater external validation through open data sharing and coordinated meta-analyses, alongside improved harmonization of study methodologies, including rigorous confounder adjustment and thorough clinical evaluation of controls, to enhance cross-study comparability in future research.

Challenges in real-world applicability and clinical heterogeneity of autism

Amid these limitations that require ongoing effort to address, translating biomarker models into clinical practice faces additional practical hurdles. These include the complexities of clinical diagnosis, the heterogeneous nature of ASD, and the setting in which testing would be implemented.

The primary challenge stems from the spectrum nature of ASD and its substantial overlap with other conditions. ASD is not a binary condition but exists on a continuum, with varying severity and subthreshold traits (e.g., broader autism phenotype).[109] Children with developmental concerns also often present with multiple co-occurring neurodevelopmental features or physical illnesses to complicate differential diagnosis. In contrast, most biomarker studies employ clean case-control designs that compare children with formal ASD diagnoses against neurotypical controls, potentially inflating diagnostic performance by exaggerating differences between clearly demarcated groups.

This case-control framework may limit specificity of gut microbiome biomarkers in real-world settings. Even if microbiota alterations contribute to ASD pathophysiology, it remains unclear whether current biomarkers can distinguish ASD from related conditions along the continuum or from disorders with overlapping gut dysbiosis profiles, such as ADHD or eczema.[110,111] Although Su et al.[61] provided evidence of disease specificity—demonstrating reduced discriminative performance of their 31-marker multi-kingdom panel when applied to independent pediatric cohorts with ADHD (n = 118; AUC = 0.51) or atopic dermatitis (n = 78; AUC = 0.58), systematic and direct comparison between ASD, ADHD, other neurodevelopmental conditions, and subclinical ASD is largely absent. This gap leaves the full specificity and real-world discriminative power of current microbiota-based markers incompletely characterized for routine clinical practice. In this regard, the use of siblings as controls has dual merits: they tackle multiple shared environmental and genetic factors that confound the microbiome,[112] while also having a higher prevalence of other neurodevelopmental conditions and autistic traits than the general population.[113] This approach can isolate ASD-specific signals and allow comparison along the spectrum of autistic phenotypes and related neurodevelopmental conditions. The spectrum nature of ASD extends beyond its continuum with neurotypical development to profound intra-diagnostic heterogeneity.[114] Cross-sectionally, this manifests in varying severity across core symptom domains, frequent co-occurring conditions (e.g., ADHD, anxiety, depression, and sleep disturbances),[115] and broad differences in cognitive abilities.[116] Longitudinally, developmental trajectories and prognosis diverge substantially, with some individuals achieving symptom remission, while others facing persistent, significant impairments.[117] This heterogeneity raises fundamental questions about underlying biological drivers of diverse clinical outcomes. In neuroimaging and genetics, subclassification of ASD cohorts has uncovered subgroup-specific signals tied to distinct phenotypes.[118,119] In contrast, microbiome research relies predominantly on study designs that treat ASD as a largely homogeneous entity and is yet to explore the dimensionality of ASD meaningfully. Such approaches obscure potential links between microbiota profiles and specific symptom domains, comorbidities, and prognosis.

As discussed, the modifiable nature of the gut microbiome further positions it as a potential therapeutic target. However, limited understanding of how microbiota alterations interface with specific clinical phenotypes—or which ASD features are most strongly associated with such changes—hampers progress toward targeted interventions. The *Lancet* Commission on autism emphasizes the need for interventions tailored to individual profiles of need.[120] This knowledge gap likely contributes to the inconsistent efficacy observed in previous microbiota modulation therapies,[81] although symptom-targeted probiotic formulations for ASD have only recently begun to emerge.[121] Hence, elucidating microbiome-phenotype relationships is essential for developing prognostic tools and treatment-selection biomarkers that enable personalized support.

Beyond scientific implications, ASD heterogeneity intersects with the neurodiversity paradigm, which frames autistic differences as natural variation rather than solely deficits and disability. A purely medicalized biomarker risks reinforcing pathologization and stigmatization. Yet failing to identify individuals with substantial needs could delay access to interventions that optimize long-term outcomes.[121] Biomarkers may thus be more valuable if they predict specific risks of actual medical needs (e.g., co-occurring psychopathology) rather than providing a simple binary classification. At minimum, deployment of a biomarker for ASD must balance diagnostic utility with ethical considerations around labeling and societal perception.

The intended use of a microbiota-based test further influences its clinical utility. Diagnostic performance metrics (e.g., sensitivity, specificity, and AUC) must be interpreted in the context of disease prevalence and testing thresholds. Given ASD's relatively low population prevalence,[1] universal screening with even highly specific tests could yield excessive false-positives and substantially reduce the accuracy of the test. This may also arouse unnecessary anxiety among caregivers and place extra burden on diagnostic services for confirmatory assessment. Such tools may be better positioned as adjunctive aids in high-risk populations (e.g., children with developmental concerns or

Table 1. Recommendations for future gut microbiota biomarker studies in ASD

Research domain	Strategies	Primary objectives
1.Study design	prioritize longitudinal cohorts over cross-sectional designs.	establish temporality and causality.
	conduct prospective studies in general or at-risk populations (e.g., infants with early signs).	enable early prediction.
	implement follow-up studies in children with established ASD.	evaluate prognosis, symptom trajectories, and microbial signal stability.
2.Control group selection	incorporate family-based cohorts (unaffected siblings or parents).	account for shared genetic and environmental factors.
	systematically screen controls for ASD traits and related conditions using validated instruments.	examine the broader autism spectrum (including subclinical traits).
	include direct comparisons with other dysbiosis-related disorders (e.g., ADHD and eczema).	improve biomarker specificity.
3.Confounder assessment	implement standardized metadata collection:	accurately control for major environmental and physiological variables that drive microbiome shifts.
	diet: validated tools (e.g., FFQs, 3-day records).	–
	GI symptoms: standardized scales (e.g., Rome criteria,[123] Gastrointestinal Severity Index[124]).	–
	medication: psychiatric drug use tracking.	–
	SES: standardized indices (e.g., Hollingshead Index).	–
4.Addressing ASD heterogeneity	adopt dimensional approaches by profiling core symptom domains, co-occurring conditions, and cognitive/adaptive functioning.	accurately map clinical diversity.
	stratify analyses by severity, comorbidity profiles, and developmental trajectories.	uncover phenotype-specific microbial signatures rather than generic markers.
5.Validation & data synthesis	mandate rigorous external validation on independent cohorts.	enable confounder-adjusted, generalizable models.
	adhere to MIxS standards and deposit raw sequences with metadata in public repositories (e.g., NCBI SRA).	accelerate field-wide consensus and reproducibility.
	promote open data sharing and coordinated meta-analyses.	–

siblings of affected individuals), where prevalence is higher and pretest probability supports rule-in or rule-out strategies. Evaluating test properties in these targeted settings is essential for meaningful implementation.

Technical barriers also impede scalability. Microbiota profiling relies on diverse sequencing approaches: 16S rRNA gene sequencing offers cost-effective, genus-level taxonomic resolution, whereas shotgun metagenomics provides strain-level identification and functional insights.[122] Models developed on one platform may not be transferred effectively to the other. This platform incompatibility hinders widespread adoption across clinics with varying infrastructure and protocols.

In summary, the limitations identified across study design, confounder control, control group assessment, model validation, and clinical challenges constrain the translational potential of current microbiota-based biomarkers for ASD. Moving forward, advancing the field will require a fundamental shift from binary case-control frameworks toward more nuanced, prospective, and harmonized approaches. The following practical guidelines (Table 1) are proposed to address these gaps and facilitate the development of robust, generalizable, and clinically useful microbiota biomarkers.

CONCLUDING REMARKS AND OUTLOOK

Gut microbiota research in ASD stands at a promising crossroads. Cross-disciplinary efforts to elucidate gut-brain axis mechanisms, coupled with steady advances in biomarker development—driven by robust multi-cohort designs, functional integration, and sophisticated sequencing technologies—have generated genuine optimism for meaningful translational impact.

That said, prudence remains essential: amid ASD's multifactorial etiology, direct causal evidence for the microbiota's role in humans is still elusive. Yet these discoveries increasingly highlight the plausibility of microbiota signatures as valuable complements to behavioral assessments—potentially enabling earlier risk detection, prognostic stratification, and personalized interventions tailored to individual needs.

Realizing this promise depends on rigorous, prospective validation in real-world settings: longitudinal studies of presymptomatic and high-risk cohorts, systematic specificity testing against related neurodevelopmental conditions and subclinical traits, and deep clinical phenotyping to account for ASD's profound heterogeneity and confounders.

In essence, the extent to which the gut microbiota will reshape ASD diagnostics and therapeutics remains uncertain—while it may not serve as a singular transformative solution, it provides a compelling lens into the disorder's complexity. With sustained and forward-looking research, it could meaningfully enhance clinical precision, inform targeted therapies, and improve outcomes across the autism spectrum.

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AUTHOR CONTRIBUTIONS

O.W. and Z.Z. drafted the manuscript. M.W., A.C., and F.K.L.C. provided important suggestions and reviewed the manuscript. Q.S. and S.C.N. oversaw the manuscript writing. All authors gave final approval for the version to be published.

DECLARATION OF INTERESTS

F.K.L.C. is Board Member of CUHK Medical Center. He is a co-founder, non-executive Board Chairman, honorary Chief Medical Officer, and shareholder of GenieBiome Ltd. He receives patent royalties through his affiliated institutions. He has received fees as an advisor and honoraria as a speaker for Eisai Co. Ltd., AstraZeneca, Pfizer Inc., Takeda Pharmaceutical Co., and Takeda (China) Holdings Co. Ltd. S.C.N. has served as an advisory board member for Pfizer, Ferring, Janssen, and AbbVie and received honoraria as a speaker for Ferring, Tillotts, Menarini, Janssen, AbbVie, and Takeda. S.C.N. has received research grants through her affiliated institutions from Olympus, Ferring, and AbbVie. S.C.N. is a scientific co-founder and shareholder of GenieBiome Ltd. S.C.N. receives patent royalties through her affiliated institutions. Q.S., F.K.L.C., and S.C.N. are named inventors of patent applications held by the CUHK and MagIC that cover the therapeutic and diagnostic use of microbiome.

REFERENCES

1. Santomauro, D.F., Erskine, H.E., Mantilla Herrera, A.M., Miller, P.A., Shadid, J., Hagins, H., Addo, I.Y., Adnani, Q.E.S., Ahinkorah, B.O., Ahmed, A., et al. (2025). The global epidemiology and health burden of the autism spectrum: findings from the Global Burden of Disease Study 2021. Lancet Psychiatry *12*, 111–121. https://doi.org/10.1016/S2215-0366(24)00363-8.
2. Fuller, E.A., and Kaiser, A.P. (2020). The Effects of Early Intervention on Social Communication Outcomes for Children with Autism Spectrum Disorder: A Meta-analysis. J. Autism Dev. Disord. *50*, 1683–1700. https://doi.org/10.1007/s10803-019-03927-z.
3. DesChamps, T.D., Ibañez, L.V., Edmunds, S.R., Dick, C.C., and Stone, W.L. (2020). Parenting stress in caregivers of young children with ASD concerns prior to a formal diagnosis. Autism Res. *13*, 82–92. https://doi.org/10.1002/aur.2213.
4. Sharon, G., Cruz, N.J., Kang, D.W., Gandal, M.J., Wang, B., Kim, Y.M., Zink, E.M., Casey, C.P., Taylor, B.C., Lane, C.J., et al. (2019). Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. Cell *177*, 1600–1618.e17. https://doi.org/10.1016/j.cell.2019.05.004.
5. Morais, L.H., Schreiber, H.L., 4th, and Mazmanian, S.K. (2021). The gut microbiota-brain axis in behaviour and brain disorders. Nat. Rev. Microbiol. *19*, 241–255. https://doi.org/10.1038/s41579-020-00460-0.
6. Bundgaard-Nielsen, C., Knudsen, J., Leutscher, P.D.C., Lauritsen, M.B., Nyegaard, M., Hagstrøm, S., and Sørensen, S. (2020). Gut microbiota profiles of autism spectrum disorder and attention deficit/hyperactivity disorder: A systematic literature review. Gut Microbes *11*, 1172–1187. https://doi.org/10.1080/19490976.2020.1748258.
7. Liu, F., Li, J., Wu, F., Zheng, H., Peng, Q., and Zhou, H. (2019). Altered composition and function of intestinal microbiota in autism spectrum disorders: a systematic review. Transl. Psychiatry *9*, 43. https://doi.org/10.1038/s41398-019-0389-6.
8. Al-Ayadhi, L., Zayed, N., Bhat, R.S., Moubayed, N.M.S., Al-Muammar, M.N., and El-Ansary, A. (2021). The use of biomarkers associated with leaky gut as a diagnostic tool for early intervention in autism spectrum disorder: a systematic review. Gut Pathog. *13*, 54. https://doi.org/10.1186/s13099-021-00448-y.
9. Kang, D.W., Ilhan, Z.E., Isern, N.G., Hoyt, D.W., Howsmon, D.P., Shaffer, M., Lozupone, C.A., Hahn, J., Adams, J.B., and Krajmalnik-Brown, R. (2018). Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. Anaerobe *49*, 121–131. https://doi.org/10.1016/j.anaerobe.2017.12.007.
10. Zhao, Y., Wang, Y., Meng, F., Chen, X., Chang, T., Huang, H., He, F., and Zheng, Y. (2023). Altered Gut Microbiota as Potential Biomarkers for Autism Spectrum Disorder in Early Childhood. Neuroscience *523*, 118–131. https://doi.org/10.1016/j.neuroscience.2023.04.029.
11. Kapp, S.K., Gillespie-Lynch, K., Sherman, L.E., and Hutman, T. (2013). Deficit, difference, or both? Autism and neurodiversity. Dev. Psychol. *49*, 59–71. https://doi.org/10.1037/a0028353.
12. Abi-Dargham, A., Moeller, S.J., Ali, F., DeLorenzo, C., Domschke, K., Horga, G., Jutla, A., Kotov, R., Paulus, M.P., Rubio, J.M., et al. (2023). Candidate biomarkers in psychiatric disorders: state of the field. World Psychiatry *22*, 236–262. https://doi.org/10.1002/wps.21078.
13. Lord, C., Charman, T., Havdahl, A., Carbone, P., Anagnostou, E., Boyd, B., Carr, T., de Vries, P.J., Dissanayake, C., Divan, G., et al. (2022). The Lancet Commission on the future of care and clinical research in autism. Lancet *399*, 271–334. https://doi.org/10.1016/s0140-6736(21)01541-5.
14. Gupta, N., Srinivasan, S., and Gupta, M. (2025). Rethinking psychometric testing in autism: overcoming the challenges of comorbidity and diagnostic overshadowing. CNS Spectr. *30*, e19. https://doi.org/10.1017/s1092852925000021.
15. Bölte, S., Neufeld, J., Marschik, P.B., Williams, Z.J., Gallagher, L., and Lai, M.C. (2023). Sex and gender in neurodevelopmental conditions. Nat. Rev. Neurol. *19*, 136–159. https://doi.org/10.1038/s41582-023-00774-6.
16. Cook, J., Hull, L., Crane, L., and Mandy, W. (2021). Camouflaging in autism: A systematic review. Clin. Psychol. Rev. *89*, 102080. https://doi.org/10.1016/j.cpr.2021.102080.
17. Constantino, J.N., and Todd, R.D. (2003). Autistic traits in the general population: a twin study. Arch. Gen. Psychiatry *60*, 524–530. https://doi.org/10.1001/archpsyc.60.5.524.
18. McCarty, P., and Frye, R.E. (2020). Early Detection and Diagnosis of Autism Spectrum Disorder: Why Is It So Difficult? Semin. Pediatr. Neurol. *35*, 100831. https://doi.org/10.1016/j.spen.2020.100831.
19. van 't Hof, M., Tisseur, C., van Berckelear-Onnes, I., van Nieuwenhuyzen, A., Daniels, A.M., Deen, M., Hoek, H.W., and Ester, W.A. (2021). Age at autism spectrum disorder diagnosis: A systematic review and meta-analysis from 2012 to 2019. Autism *25*, 862–873. https://doi.org/10.1177/1362361320971107.
20. Nahmias, A.S., Pellecchia, M., Stahmer, A.C., and Mandell, D.S. (2019). Effectiveness of community-based early intervention for children with

autism spectrum disorder: a meta-analysis. J. Child Psychol. Psychiatry *60*, 1200–1209. https://doi.org/10.1111/jcpp.13073.

21. Sydnor, V.J., Ojha, A., Larsen, B., Martinez, A., Calabro, F.J., and Luna, B. (2026). Investigating hierarchical critical periods in human neurodevelopment. Neuropsychopharmacology *51*, 67–85. https://doi.org/10.1038/s41386-025-02246-5.
22. Wolff, J.J., and Piven, J. (2021). Predicting Autism in Infancy. J. Am. Acad. Child Adolesc. Psychiatry *60*, 958–967. https://doi.org/10.1016/j.jaac.2020.07.910.
23. Bacon, E.C., Moore, A., Lee, Q., Carter Barnes, C., Courchesne, E., and Pierce, K. (2020). Identifying prognostic markers in autism spectrum disorder using eye tracking. Autism *24*, 658–669. https://doi.org/10.1177/1362361319878578.
24. Webb, S.J., Naples, A.J., Levin, A.R., Hellemann, G., Borland, H., Benton, J., Carlos, C., McAllister, T., Santhosh, M., Seow, H., et al. (2023). The Autism Biomarkers Consortium for Clinical Trials: Initial Evaluation of a Battery of Candidate EEG Biomarkers. Am. J. Psychiatry *180*, 41–49. https://doi.org/10.1176/appi.ajp.21050485.
25. Bracher-Smith, M., Crawford, K., and Escott-Price, V. (2021). Machine learning for genetic prediction of psychiatric disorders: a systematic review. Mol. Psychiatry *26*, 70–79. https://doi.org/10.1038/s41380-020-0825-2.
26. Traut, N., Heuer, K., Lemaître, G., Beggiato, A., Germanaud, D., Elmaleh, M., Bethegnies, A., Bonnasse-Gahot, L., Cai, W., Chambon, S., et al. (2022). Insights from an autism imaging biomarker challenge: Promises and threats to biomarker discovery. Neuroimage *255*, 119171. https://doi.org/10.1016/j.neuroimage.2022.119171.
27. Li, Q., and Zhou, J.M. (2016). The microbiota-gut-brain axis and its potential therapeutic role in autism spectrum disorder. Neuroscience *324*, 131–139. https://doi.org/10.1016/j.neuroscience.2016.03.013.
28. Wan, Y., Wong, O.W.H., Tun, H.M., Su, Q., Xu, Z., Tang, W., Ma, S.L., Chan, S., Chan, F.K.L., and Ng, S.C. (2024). Fecal microbial marker panel for aiding diagnosis of autism spectrum disorders. Gut Microbes *16*, 2418984. https://doi.org/10.1080/19490976.2024.2418984.
29. D'Eufemia, P., Celli, M., Finocchiaro, R., Pacifico, L., Viozzi, L., Zaccagnini, M., Cardi, E., and Giardini, O. (1996). Abnormal intestinal permeability in children with autism. Acta Paediatr. *85*, 1076–1079. https://doi.org/10.1111/j.1651-2227.1996.tb14220.x.
30. Sandler, R.H., Finegold, S.M., Bolte, E.R., Buchanan, C.P., Maxwell, A.P., Väisänen, M.L., Nelson, M.N., and Wexler, H.M. (2000). Short-term benefit from oral vancomycin treatment of regressive-onset autism. J. Child Neurol. *15*, 429–435. https://doi.org/10.1177/088307380001500701.
31. Song, Y., Liu, C., and Finegold, S.M. (2004). Real-time PCR quantitation of clostridia in feces of autistic children. Appl. Environ. Microbiol. *70*, 6459–6465. https://doi.org/10.1128/aem.70.11.6459-6465.2004.
32. Wang, M., Wan, J., Rong, H., He, F., Wang, H., Zhou, J., Cai, C., Wang, Y., Xu, R., Yin, Z., and Zhou, W. (2019). Alterations in Gut Glutamate Metabolism Associated with Changes in Gut Microbiota Composition in Children with Autism Spectrum Disorder. mSystems *4*, e00321-18. https://doi.org/10.1128/mSystems.00321-18.
33. Strati, F., Cavalieri, D., Albanese, D., De Felice, C., Donati, C., Hayek, J., Jousson, O., Leoncini, S., Renzi, D., Calabrò, A., and De Filippo, C. (2017). New evidences on the altered gut microbiota in autism spectrum disorders. Microbiome *5*, 24. https://doi.org/10.1186/s40168-017-0242-1.
34. De Angelis, M., Francavilla, R., Piccolo, M., De Giacomo, A., and Gobbetti, M. (2015). Autism spectrum disorders and intestinal microbiota. Gut Microbes *6*, 207–213. https://doi.org/10.1080/19490976.2015.1035855.
35. Finegold, S.M., Dowd, S.E., Gontcharova, V., Liu, C., Henley, K.E., Wolcott, R.D., Youn, E., Summanen, P.H., Granpeesheh, D., Dixon, D., et al. (2010). Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe *16*, 444–453. https://doi.org/10.1016/j.anaerobe.2010.06.008.
36. Tomova, A., Husarova, V., Lakatosova, S., Bakos, J., Vlkova, B., Babinska, K., and Ostatnikova, D. (2015). Gastrointestinal microbiota in children with autism in Slovakia. Physiol. Behav. *138*, 179–187. https://doi.org/10.1016/j.physbeh.2014.10.033.
37. Wang, L., Christophersen, C.T., Sorich, M.J., Gerber, J.P., Angley, M.T., and Conlon, M.A. (2013). Increased abundance of Sutterella spp. and Ruminococcus torques in feces of children with autism spectrum disorder. Mol. Autism *4*, 42. https://doi.org/10.1186/2040-2392-4-42.
38. Williams, B.L., Hornig, M., Parekh, T., and Lipkin, W.I. (2012). Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of Sutterella species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. mBio *3*, e00261-11. https://doi.org/10.1128/mBio.00261-11.
39. Mathur, A., Kay, C., Xue, Y., Pandey, A., Lee, J., Jing, W., Enosi Tuipulotu, D., Lo Pilato, J., Feng, S., Ngo, C., et al. (2023). Clostridium perfringens virulence factors are nonredundant activators of the NLRP3 inflammasome. EMBO Rep. *24*, e54600. https://doi.org/10.15252/embr.202254600.
40. Shinoda, T., Shinya, N., Ito, K., Ohsawa, N., Terada, T., Hirata, K., Kawano, Y., Yamamoto, M., Kimura-Someya, T., Yokoyama, S., and Shirouzu, M. (2016). Structural basis for disruption of claudin assembly in tight junctions by an enterotoxin. Sci. Rep. *6*, 33632. https://doi.org/10.1038/srep33632.
41. Kaakoush, N.O. (2020). Sutterella Species, IgA-degrading Bacteria in Ulcerative Colitis. Trends Microbiol. *28*, 519–522. https://doi.org/10.1016/j.tim.2020.02.018.
42. Davoody, S., Halimi, H., Zali, A., Houri, H., and Brand, S. (2025). Double-edged sword effect of Sutterella in neurological disorders: implications for the gut-brain axis and neuroimmune interactions. Neurobiol. Dis. *214*, 107032. https://doi.org/10.1016/j.nbd.2025.107032.
43. Kang, D.W., Park, J.G., Ilhan, Z.E., Wallstrom, G., Labaer, J., Adams, J.B., and Krajmalnik-Brown, R. (2013). Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. PLoS One *8*, e68322. https://doi.org/10.1371/journal.pone.0068322.
44. Wang, L., Christophersen, C.T., Sorich, M.J., Gerber, J.P., Angley, M.T., and Conlon, M.A. (2011). Low relative abundances of the mucolytic bacterium Akkermansia muciniphila and Bifidobacterium spp. in feces of children with autism. Appl. Environ. Microbiol. *77*, 6718–6721. https://doi.org/10.1128/aem.05212-11.
45. Zou, X., Zhou, X., Wang, C., Zheng, Y., Li, Y., and Suo, H. (2025). The composition, influencing factors, and physiological functions of bifidobacteria in the infant gut: a review. Food Funct. *16*, 7512–7530. https://doi.org/10.1039/d5fo02271a.
46. Zhan, Q., Wang, R., Thakur, K., Feng, J.Y., Zhu, Y.Y., Zhang, J.G., and Wei, Z.J. (2024). Unveiling of dietary and gut-microbiota derived B vitamins: Metabolism patterns and their synergistic functions in gut-brain homeostasis. Crit. Rev. Food Sci. Nutr. *64*, 4046–4058. https://doi.org/10.1080/10408398.2022.2138263.
47. Yang, B., Huang, Z., He, Z., Yue, Y., Zhou, Y., Ross, R.P., Stanton, C., Zhang, H., Zhao, J., and Chen, W. (2021). Protective effect of Bifidobacterium bifidum FSDJN7O5 and Bifidobacterium breve FHNFQ23M3 on diarrhea caused by enterotoxigenic Escherichia coli. Food Funct. *12*, 7271–7282. https://doi.org/10.1039/d1fo00504a.
48. Fan, X., Lu, Q., Jia, Q., Li, L., Cao, C., Wu, Z., and Liao, M. (2024). Prevotella histicola ameliorates DSS-induced colitis by inhibiting IRE1α-JNK pathway of ER stress and NF-κB signaling. Int. Immunopharmacol. *135*, 112285. https://doi.org/10.1016/j.intimp.2024.112285.
49. Gálvez, E.J.C., Iljazovic, A., Amend, L., Lesker, T.R., Renault, T., Thiemann, S., Hao, L., Roy, U., Gronow, A., Charpentier, E., et al. (2020). Distinct Polysaccharide Utilization Determines Interspecies Competition between Intestinal Prevotella spp. Cell Host Microbe *28*, 838–852.e836. https://doi.org/10.1016/j.chom.2020.09.012.

50. Zou, R., Xu, F., Wang, Y., Duan, M., Guo, M., Zhang, Q., Zhao, H., and Zheng, H. (2020). Changes in the Gut Microbiota of Children with Autism Spectrum Disorder. Autism Res. *13*, 1614–1625. https://doi.org/10.1002/aur.2358.

51. Heintz-Buschart, A., and Wilmes, P. (2018). Human Gut Microbiome: Function Matters. Trends Microbiol. *26*, 563–574. https://doi.org/10.1016/j.tim.2017.11.002.

52. Chamtouri, M., Gaddour, N., Merghni, A., Mastouri, M., Arboleya, S., and de Los Reyes-Gavilán, C.G. (2023). Age and severity-dependent gut microbiota alterations in Tunisian children with autism spectrum disorder. Sci. Rep. *13*, 18218. https://doi.org/10.1038/s41598-023-45534-0.

53. Wang, Y., Li, N., Yang, J.J., Zhao, D.M., Chen, B., Zhang, G.Q., Chen, S., Cao, R.F., Yu, H., Zhao, C.Y., et al. (2020). Probiotics and fructo-oligosaccharide intervention modulate the microbiota-gut brain axis to improve autism spectrum reducing also the hyper-serotonergic state and the dopamine metabolism disorder. Pharmacol. Res. *157*, 104784. https://doi.org/10.1016/j.phrs.2020.104784.

54. Averina, O.V., Kovtun, A.S., Polyakova, S.I., Savilova, A.M., Rebrikov, D.V., and Danilenko, V.N. (2020). The bacterial neurometabolic signature of the gut microbiota of young children with autism spectrum disorders. J. Med. Microbiol. *69*, 558–571. https://doi.org/10.1099/jmm.0.001178.

55. Gabriele, S., Sacco, R., Altieri, L., Neri, C., Urbani, A., Bravaccio, C., Riccio, M.P., Iovene, M.R., Bombace, F., De Magistris, L., and Persico, A.M. (2016). Slow intestinal transit contributes to elevate urinary p-cresol level in Italian autistic children. Autism Res. *9*, 752–759. https://doi.org/10.1002/aur.1571.

56. Rose, D.R., Yang, H., Serena, G., Sturgeon, C., Ma, B., Careaga, M., Hughes, H.K., Angkustsiri, K., Rose, M., Hertz-Picciotto, I., et al. (2018). Differential immune responses and microbiota profiles in children with autism spectrum disorders and co-morbid gastrointestinal symptoms. Brain Behav. Immun. *70*, 354–368. https://doi.org/10.1016/j.bbi.2018.03.025.

57. Luna, R.A., Oezguen, N., Balderas, M., Venkatachalam, A., Runge, J.K., Versalovic, J., Veenstra-VanderWeele, J., Anderson, G.M., Savidge, T., and Williams, K.C. (2017). Distinct Microbiome-Neuroimmune Signatures Correlate With Functional Abdominal Pain in Children With Autism Spectrum Disorder. Cell. Mol. Gastroenterol. Hepatol. *3*, 218–230. https://doi.org/10.1016/j.jcmgh.2016.11.008.

58. Lou, M., Cao, A., Jin, C., Mi, K., Xiong, X., Zeng, Z., Pan, X., Qie, J., Qiu, S., Niu, Y., et al. (2022). Deviated and early unsustainable stunted development of gut microbiota in children with autism spectrum disorder. Gut *71*, 1588–1599. https://doi.org/10.1136/gutjnl-2021-325115.

59. Aziz-Zadeh, L., Ringold, S.M., Jayashankar, A., Kilroy, E., Butera, C., Jacobs, J.P., Tanartkit, S., Mahurkar-Joshi, S., Bhatt, R.R., Dapretto, M., et al. (2025). Relationships between brain activity, tryptophan-related gut metabolites, and autism symptomatology. Nat. Commun. *16*, 3465. https://doi.org/10.1038/s41467-025-58459-1.

60. Xiao, L., Yan, J., Yang, T., Zhu, J., Li, T., Wei, H., and Chen, J. (2021). Fecal Microbiome Transplantation from Children with Autism Spectrum Disorder Modulates Tryptophan and Serotonergic Synapse Metabolism and Induces Altered Behaviors in Germ-Free Mice. mSystems *6*, e01343-20. https://doi.org/10.1128/mSystems.01343-20.

61. Su, Q., Wong, O.W.H., Lu, W., Wan, Y., Zhang, L., Xu, W., Li, M.K.T., Liu, C., Cheung, C.P., Ching, J.Y.L., et al. (2024). Multikingdom and functional gut microbiota markers for autism spectrum disorder. Nat. Microbiol. *9*, 2344–2355. https://doi.org/10.1038/s41564-024-01739-1.

62. Shao, L., Cai, G., Fu, J., Zhang, W., Ye, Y., Ling, Z., and Ye, S. (2024). Gut microbial 'TNFα-sphingolipids-steroid hormones' axis in children with autism spectrum disorder: an insight from meta-omics analysis. J. Transl. Med. *22*, 1165. https://doi.org/10.1186/s12967-024-05973-3.

63. Dalile, B., Van Oudenhove, L., Vervliet, B., and Verbeke, K. (2019). The role of short-chain fatty acids in microbiota-gut-brain communication. Nat. Rev. Gastroenterol. Hepatol. *16*, 461–478. https://doi.org/10.1038/s41575-019-0157-3.

64. MacFabe, D.F. (2015). Enteric short-chain fatty acids: microbial messengers of metabolism, mitochondria, and mind: implications in autism spectrum disorders. Microb. Ecol. Health Dis. *26*, 28177. https://doi.org/10.3402/mehd.v26.28177.

65. Guzmán-Salas, S., Weber, A., Malci, A., Lin, X., Herrera-Molina, R., Cerpa, W., Dorador, C., Signorelli, J., and Zamorano, P. (2022). The metabolite p-cresol impairs dendritic development, synaptogenesis, and synapse function in hippocampal neurons: Implications for autism spectrum disorder. J. Neurochem. *161*, 335–349. https://doi.org/10.1111/jnc.15604.

66. Depino, A.M. (2013). Peripheral and central inflammation in autism spectrum disorders. Mol. Cell. Neurosci. *53*, 69–76. https://doi.org/10.1016/j.mcn.2012.10.003.

67. Nelson, S.B., and Valakh, V. (2015). Excitatory/Inhibitory Balance and Circuit Homeostasis in Autism Spectrum Disorders. Neuron *87*, 684–698. https://doi.org/10.1016/j.neuron.2015.07.033.

68. Launay, J.M., Delorme, R., Pagan, C., Callebert, J., Leboyer, M., and Vodovar, N. (2023). Impact of IDO activation and alterations in the kynurenine pathway on hyperserotonemia, NAD(+) production, and AhR activation in autism spectrum disorder. Transl. Psychiatry *13*, 380. https://doi.org/10.1038/s41398-023-02687-w.

69. Wang, J., Cao, Y., Hou, W., Bi, D., Yin, F., Gao, Y., Huang, D., Li, Y., Cao, Z., Yan, Y., et al. (2023). Fecal microbiota transplantation improves VPA-induced ASD mice by modulating the serotonergic and glutamatergic synapse signaling pathways. Transl. Psychiatry *13*, 17. https://doi.org/10.1038/s41398-023-02307-7.

70. Zheng, L., Jiao, Y., Zhong, H., Tan, Y., Yin, Y., Liu, Y., Liu, D., Wu, M., Wang, G., Huang, J., et al. (2024). Human-derived fecal microbiota transplantation alleviates social deficits of the BTBR mouse model of autism through a potential mechanism involving vitamin B(6) metabolism. mSystems *9*, e0025724. https://doi.org/10.1128/msystems.00257-24.

71. MacFabe, D.F., Cain, D.P., Rodriguez-Capote, K., Franklin, A.E., Hoffman, J.E., Boon, F., Taylor, A.R., Kavaliers, M., and Ossenkopp, K.P. (2007). Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. Behav. Brain Res. *176*, 149–169. https://doi.org/10.1016/j.bbr.2006.07.025.

72. Liu, H., Liu, G., Zhang, Y., Suo, W., Hao, Y., Wang, Y., and Ding, H. (2025). Bifidobacterium adolescentis DM8504 Alleviates Autistic-Like Behaviors in Valproic Acid-Exposed Rats Through Gut Microbiota Modulation and SCFA Restoration. Neuropsychiatr. Dis. Treat. *21*, 2449–2463. https://doi.org/10.2147/ndt.S547997.

73. Osman, A., Mervosh, N.L., Strat, A.N., Euston, T.J., Zipursky, G., Pollak, R.M., Meckel, K.R., Tyler, S.R., Chan, K.L., Buxbaum Grice, A., et al. (2023). Acetate supplementation rescues social deficits and alters transcriptional regulation in prefrontal cortex of Shank3 deficient mice. Brain Behav. Immun. *114*, 311–324. https://doi.org/10.1016/j.bbi.2023.08.020.

74. Yao, Y., Cai, X., Fei, W., Ye, Y., Zhao, M., and Zheng, C. (2022). The role of short-chain fatty acids in immunity, inflammation and metabolism. Crit. Rev. Food Sci. Nutr. *62*, 1–12. https://doi.org/10.1080/10408398.2020.1854675.

75. Adıgüzel, E., Çiçek, B., Ünal, G., Aydın, M.F., and Barlak-Keti, D. (2022). Probiotics and prebiotics alleviate behavioral deficits, inflammatory response, and gut dysbiosis in prenatal VPA-induced rodent model of autism. Physiol. Behav. *256*, 113961. https://doi.org/10.1016/j.physbeh.2022.113961.

76. Park, J.C., Sim, M.A., Lee, C., Park, H.E., Lee, J., Choi, S.Y., Byun, S., Ko, H., Lee, H., Kim, S.W., et al. (2025). Gut microbiota and brain-resident CD4(+) T cells shape behavioral outcomes in autism spectrum disorder. Nat. Commun. *16*, 6422. https://doi.org/10.1038/s41467-025-61544-0.

77. Sgritta, M., Dooling, S.W., Buffington, S.A., Momin, E.N., Francis, M.B., Britton, R.A., and Costa-Mattioli, M. (2019). Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of

Autism Spectrum Disorder. Neuron *101*, 246–259.e6. https://doi.org/10.1016/j.neuron.2018.11.018.

78. Chen, C.M., Wu, C.C., Kim, Y., Hsu, W.Y., Tsai, Y.C., and Chiu, S.L. (2024). Enhancing social behavior in an autism spectrum disorder mouse model: investigating the underlying mechanisms of Lactiplantibacillus plantarum intervention. Gut Microbes *16*, 2359501. https://doi.org/10.1080/19490976.2024.2359501.
79. Bermudez-Martin, P., Becker, J.A.J., Caramello, N., Fernandez, S.P., Costa-Campos, R., Canaguier, J., Barbosa, S., Martinez-Gili, L., Myridakis, A., Dumas, M.E., et al. (2021). The microbial metabolite p-Cresol induces autistic-like behaviors in mice by remodeling the gut microbiota. Microbiome *9*, 157. https://doi.org/10.1186/s40168-021-01103-z.
80. Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Hultman, C., Larsson, H., and Reichenberg, A. (2017). The Heritability of Autism Spectrum Disorder. JAMA *318*, 1182–1184. https://doi.org/10.1001/jama.2017.12141.
81. Tan, Q., Orsso, C.E., Deehan, E.C., Kung, J.Y., Tun, H.M., Wine, E., Madsen, K.L., Zwaigenbaum, L., and Haqq, A.M. (2021). Probiotics, prebiotics, synbiotics, and fecal microbiota transplantation in the treatment of behavioral symptoms of autism spectrum disorder: A systematic review. Autism Res. *14*, 1820–1836. https://doi.org/10.1002/aur.2560.
82. Schupack, D.A., Mars, R.A.T., Voelker, D.H., Abeykoon, J.P., and Kashyap, P.C. (2022). The promise of the gut microbiome as part of individualized treatment strategies. Nat. Rev. Gastroenterol. Hepatol. *19*, 7–25. https://doi.org/10.1038/s41575-021-00499-1.
83. Zwaigenbaum, L., Thurm, A., Stone, W., Baranek, G., Bryson, S., Iverson, J., Kau, A., Klin, A., Lord, C., Landa, R., et al. (2007). Studying the emergence of autism spectrum disorders in high-risk infants: methodological and practical issues. J. Autism Dev. Disord. *37*, 466–480. https://doi.org/10.1007/s10803-006-0179-x.
84. Zuffa, S., Schimmel, P., Gonzalez-Santana, A., Belzer, C., Knol, J., Bölte, S., Falck-Ytter, T., Forssberg, H., Swann, J., and Diaz Heijtz, R. (2023). Early-life differences in the gut microbiota composition and functionality of infants at elevated likelihood of developing autism spectrum disorder. Transl. Psychiatry *13*, 257. https://doi.org/10.1038/s41398-023-02556-6.
85. Laue, H.E., Bonham, K.S., Coker, M.O., Moroishi, Y., Pathmasiri, W., McRitchie, S., Sumner, S., Hoen, A.G., Karagas, M.R., Klepac-Ceraj, V., et al. (2024). Prospective association of the infant gut microbiome with social behaviors in the ECHO consortium. Mol. Autism *15*, 21. https://doi.org/10.1186/s13229-024-00597-2.
86. Ahrens, A.P., Hyötyläinen, T., Petrone, J.R., Igelström, K., George, C.D., Garrett, T.J., Orešič, M., Triplett, E.W., and Ludvigsson, J. (2024). Infant microbes and metabolites point to childhood neurodevelopmental disorders. Cell *187*, 1853–1873.e1815. https://doi.org/10.1016/j.cell.2024.02.035.
87. Fahur Bottino, G., Bonham, K.S., Patel, F., McCann, S., Zieff, M., Naspolini, N., Ho, D., Portlock, T., Joos, R., Midani, F.S., et al. (2025). Early life microbial succession in the gut follows common patterns in humans across the globe. Nat. Commun. *16*, 660. https://doi.org/10.1038/s41467-025-56072-w.
88. Kong, X., Liu, J., Cetinbas, M., Sadreyev, R., Koh, M., Huang, H., Adeseye, A., He, P., Zhu, J., Russell, H., et al. (2019). New and Preliminary Evidence on Altered Oral and Gut Microbiota in Individuals with Autism Spectrum Disorder (ASD): Implications for ASD Diagnosis and Subtyping Based on Microbial Biomarkers. Nutrients *11*, 2128. https://doi.org/10.3390/nu11092128.
89. Dan, Z., Mao, X., Liu, Q., Guo, M., Zhuang, Y., Liu, Z., Chen, K., Chen, J., Xu, R., Tang, J., et al. (2020). Altered gut microbial profile is associated with abnormal metabolism activity of Autism Spectrum Disorder. Gut Microbes *11*, 1246–1267. https://doi.org/10.1080/19490976.2020.1747329.
90. Ye, F., Gao, X., Wang, Z., Cao, S., Liang, G., He, D., Lv, Z., Wang, L., Xu, P., and Zhang, Q. (2021). Comparison of gut microbiota in autism spectrum disorders and neurotypical boys in China: A case-control study. Synth. Syst. Biotechnol. *6*, 120–126. https://doi.org/10.1016/j.synbio.2021.03.003.
91. Xu, Y., Wang, Y., Xu, J., Song, Y., Liu, B., and Xiong, Z. (2022). Leveraging Existing 16SrRNA Microbial Data to Define a Composite Biomarker for Autism Spectrum Disorder. Microbiol. Spectr. *10*, e0033122. https://doi.org/10.1128/spectrum.00331-22.
92. Wan, Y., Zuo, T., Xu, Z., Zhang, F., Zhan, H., Chan, D., Leung, T.F., Yeoh, Y.K., Chan, F.K.L., Chan, R., and Ng, S.C. (2022). Underdevelopment of the gut microbiota and bacteria species as non-invasive markers of prediction in children with autism spectrum disorder. Gut *71*, 910–918. https://doi.org/10.1136/gutjnl-2020-324015.
93. Wang, W., and Fu, P. (2023). Gut Microbiota Analysis and In Silico Biomarker Detection of Children with Autism Spectrum Disorder across Cohorts. Microorganisms *11*, 291. https://doi.org/10.3390/microorganisms11020291.
94. Wan, Y., Su, Q., and Ng, S.C. (2024). New insights on gut microbiome and autism. Trends Mol. Med. *30*, 1100–1102. https://doi.org/10.1016/j.molmed.2024.06.010.
95. Wu, T., Wang, H., Lu, W., Zhai, Q., Zhang, Q., Yuan, W., Gu, Z., Zhao, J., Zhang, H., and Chen, W. (2020). Potential of gut microbiome for detection of autism spectrum disorder. Microb. Pathog. *149*, 104568. https://doi.org/10.1016/j.micpath.2020.104568.
96. Zhang, M., Chu, Y., Meng, Q., Ding, R., Shi, X., Wang, Z., He, Y., Zhang, J., Liu, J., Zhang, J., et al. (2020). A quasi-paired cohort strategy reveals the impaired detoxifying function of microbes in the gut of autistic children. Sci. Adv. *6*, eaba3760. https://doi.org/10.1126/sciadv.aba3760.
97. Morton, J.T., Jin, D.M., Mills, R.H., Shao, Y., Rahman, G., McDonald, D., Zhu, Q., Balaban, M., Jiang, Y., Cantrell, K., et al. (2023). Multi-level analysis of the gut-brain axis shows autism spectrum disorder-associated molecular and microbial profiles. Nat. Neurosci. *26*, 1208–1217. https://doi.org/10.1038/s41593-023-01361-0.
98. Weiner, A., Turjeman, S., and Koren, O. (2023). Gut microbes and host behavior: The forgotten members of the gut-microbiome. Neuropharmacology *227*, 109453. https://doi.org/10.1016/j.neuropharm.2023.109453.
99. Zou, R., Wang, Y., Duan, M., Guo, M., Zhang, Q., and Zheng, H. (2021). Dysbiosis of Gut Fungal Microbiota in Children with Autism Spectrum Disorders. J. Autism Dev. Disord. *51*, 267–275. https://doi.org/10.1007/s10803-020-04543-y.
100. Wan, Y., Zhang, L., Xu, Z., Su, Q., Leung, T.F., Chan, D., Wong, O.W.H., Chan, S., Chan, F.K.L., Tun, H.M., and Ng, S.C. (2024). Alterations in fecal virome and bacteriome virome interplay in children with autism spectrum disorder. Cell Rep. Med. *5*, 101409. https://doi.org/10.1016/j.xcrm.2024.101409.
101. Shahin, K., Soleimani-Delfan, A., He, Z., Sansonetti, P., and Collard, J.M. (2023). Metagenomics revealed a correlation of gut phageome with autism spectrum disorder. Gut Pathog. *15*, 39. https://doi.org/10.1186/s13099-023-00561-0.
102. Zhernakova, D.V., Wang, D., Liu, L., Andreu-Sánchez, S., Zhang, Y., Ruiz-Moreno, A.J., Peng, H., Plomp, N., Del Castillo-Izquierdo, Á., Gacesa, R., et al. (2024). Host genetic regulation of human gut microbial structural variation. Nature *625*, 813–821. https://doi.org/10.1038/s41586-023-06893-w.
103. Chen, W., Wang, X., Zhu, R., Gao, W., Tao, L., Yang, R., Wei, Q., Zhang, Y., Gong, Y., Zhong, H., et al. (2026). Integrative multi-omics reveals microbial genomic variants driving altered host-microbe interactions in autism spectrum disorder. Cell Rep. Med. *7*, 102516. https://doi.org/10.1016/j.xcrm.2025.102516.
104. Yap, C.X., Henders, A.K., Alvares, G.A., Wood, D.L.A., Krause, L., Tyson, G.W., Restuadi, R., Wallace, L., McLaren, T., Hansell, N.K., et al. (2021). Autism-related dietary preferences mediate autism-gut microbiome associations. Cell *184*, 5916–5931.e17. https://doi.org/10.1016/j.cell.2021.10.015.

105. Kamath, S., Sokolenko, E., Clark, S.R., Cross, C.B., Scott, J., Wardill, H.R., Margolis, K.G., Forsythe, P., Burnet, P.W.J., Dinan, T.G., et al. (2025). Distinguishing the causative, correlative and bidirectional roles of the gut microbiota in mental health. Nat. Ment. Health *3*, 1137–1151. https://doi.org/10.1038/s44220-025-00498-0.

106. Wu, Y., Chan, S.S.M., Leung, P.W.L., Lo, H.H.L., Ho, S.W.S., Mo, F.Y.M., Ho, C.S.W., Shea, C.K.S., Su, Q., Leung, T.F., et al. (2025). The Mediating Role of Eating Behaviors Between Autistic Symptoms and Dietary Issues Among Chinese Children With Autism. J. Autism Dev. Disord. https://doi.org/10.1007/s10803-025-07133-y.

107. Wong, O.W.H., Lam, A.M.W., Or, B.P.N., Mo, F.Y.M., Shea, C.K.S., Lai, K.Y.C., Ma, S.L., Hung, S.F., Chan, S., Kwong, T.N.Y., et al. (2022). Disentangling the relationship of gut microbiota, functional gastrointestinal disorders and autism: a case-control study on prepubertal Chinese boys. Sci. Rep. *12*, 10659. https://doi.org/10.1038/s41598-022-14785-8.

108. Chan, S.S.M., Wong, O.W.H., Hussain, S., Tsoi, K.K.F., Ma, K.K.Y., Chau, S.W.H., Ma, S.L., Lai, K.Y.C., Chu, W.C.W., Lo, H.H.L., et al. (2025). Twelve-month prevalence of DSM-5 mental disorders and the psychosocial correlates- a child and adolescent psychiatric epidemiologic survey in Hong Kong SAR. Lancet Reg. Health West. Pac. *57*, 101533. https://doi.org/10.1016/j.lanwpc.2025.101533.

109. Gerdts, J., and Bernier, R. (2011). The broader autism phenotype and its implications on the etiology and treatment of autism spectrum disorders. Autism Res. Treat. *2011*, 545901. https://doi.org/10.1155/2011/545901.

110. Shirvani-Rad, S., Ejtahed, H.S., Ettehad Marvasti, F., Taghavi, M., Sharifi, F., Arzaghi, S.M., and Larijani, B. (2022). The Role of Gut Microbiota-Brain Axis in Pathophysiology of ADHD: A Systematic Review. J. Atten. Disord. *26*, 1698–1710. https://doi.org/10.1177/10870547211073474.

111. Donald, K., and Finlay, B.B. (2023). Early-life interactions between the microbiota and immune system: impact on immune system development and atopic disease. Nat. Rev. Immunol. *23*, 735–748. https://doi.org/10.1038/s41577-023-00874-w.

112. Chen, W., Li, Y., Wang, W., Gao, S., Hu, J., Xiang, B., Wu, D., Jiao, N., Xu, T., Zhi, M., et al. (2024). Enhanced microbiota profiling in patients with quiescent Crohn's disease through comparison with paired healthy first-degree relatives. Cell Rep. Med. *5*, 101624. https://doi.org/10.1016/j.xcrm.2024.101624.

113. Jokiranta-Olkoniemi, E., Cheslack-Postava, K., Sucksdorff, D., Suominen, A., Gyllenberg, D., Chudal, R., Leivonen, S., Gissler, M., Brown, A.S., and Sourander, A. (2016). Risk of Psychiatric and Neurodevelopmental Disorders Among Siblings of Probands With Autism Spectrum Disorders. JAMA Psychiatry *73*, 622–629. https://doi.org/10.1001/jamapsychiatry.2016.0495.

114. Lombardo, M.V., Lai, M.C., and Baron-Cohen, S. (2019). Big data approaches to decomposing heterogeneity across the autism spectrum. Mol. Psychiatry *24*, 1435–1450. https://doi.org/10.1038/s41380-018-0321-0.

115. Lai, M.C., Kassee, C., Besney, R., Bonato, S., Hull, L., Mandy, W., Szatmari, P., and Ameis, S.H. (2019). Prevalence of co-occurring mental health diagnoses in the autism population: a systematic review and meta-analysis. Lancet Psychiatry *6*, 819–829. https://doi.org/10.1016/s2215-0366(19)30289-5.

116. Kofler, M.J., Soto, E.F., Singh, L.J., Harmon, S.L., Jaisle, E., Smith, J.N., Feeney, K.E., and Musser, E.D. (2024). Executive function deficits in attention-deficit/hyperactivity disorder and autism spectrum disorder. Nat. Rev. Psychol. *3*, 701–719. https://doi.org/10.1038/s44159-024-00350-9.

117. Kolevzon, A., Delaby, E., Berry-Kravis, E., Buxbaum, J.D., and Betancur, C. (2019). Neuropsychiatric decompensation in adolescents and adults with Phelan-McDermid syndrome: a systematic review of the literature. Mol. Autism *10*, 50. https://doi.org/10.1186/s13229-019-0291-3.

118. Litman, A., Sauerwald, N., Green Snyder, L., Foss-Feig, J., Park, C.Y., Hao, Y., Dinstein, I., Theesfeld, C.L., and Troyanskaya, O.G. (2025). Decomposition of phenotypic heterogeneity in autism reveals underlying genetic programs. Nat. Genet. *57*, 1611–1619. https://doi.org/10.1038/s41588-025-02224-z.

119. Meijer, J., Hebling Vieira, B., Elleaume, C., Baranczuk-Turska, Z., Langer, N., and Floris, D.L. (2024). Toward understanding autism heterogeneity: Identifying clinical subgroups and neuroanatomical deviations. J. Psychopathol. Clin. Sci. *133*, 667–677. https://doi.org/10.1037/abn0000914.

120. Yu, X., and Xu, X. (2022). The future of care and clinical research in autism - recommendations from the 2021 Lancet Commission. Med. Rev. *2*, 216–218. https://doi.org/10.1515/mr-2022-0015.

121. Wong, O.W.H., Xu, Z., Chan, S.S.M., Mo, F.Y.M., Shea, C.K.S., Su, Q., Wan, M.Y.T., Cheung, C.P., Ching, J.Y.L., Tang, W., et al. (2026). A novel synbiotic (SCM06) for anxiety and sensory hyperresponsiveness in children with autism spectrum disorder: an open-label pilot study. NPJ Biofilms Microbiomes *12*, 36. https://doi.org/10.1038/s41522-025-00902-8.

122. Ranjan, R., Rani, A., Metwally, A., McGee, H.S., and Perkins, D.L. (2016). Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. Biochem. Biophys. Res. Commun. *469*, 967–977. https://doi.org/10.1016/j.bbrc.2015.12.083.

123. Rasquin, A., Di Lorenzo, C., Forbes, D., Guiraldes, E., Hyams, J.S., Staiano, A., and Walker, L.S. (2006). Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterology *130*, 1527–1537. https://doi.org/10.1053/j.gastro.2005.08.063.

124. Schneider, C.K., Melmed, R.D., Barstow, L.E., Enriquez, F.J., Ranger-Moore, J., and Ostrem, J.A. (2006). Oral human immunoglobulin for children with autism and gastrointestinal dysfunction: a prospective, open-label study. J. Autism Dev. Disord. *36*, 1053–1064. https://doi.org/10.1007/s10803-006-0141-y.