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FOOD & MEDICINE HOMOLOGY RESOURCES
ISSN 3136-103X (online)
https://doi.org/10.26599/FMHR.2026.9740006

Review Article

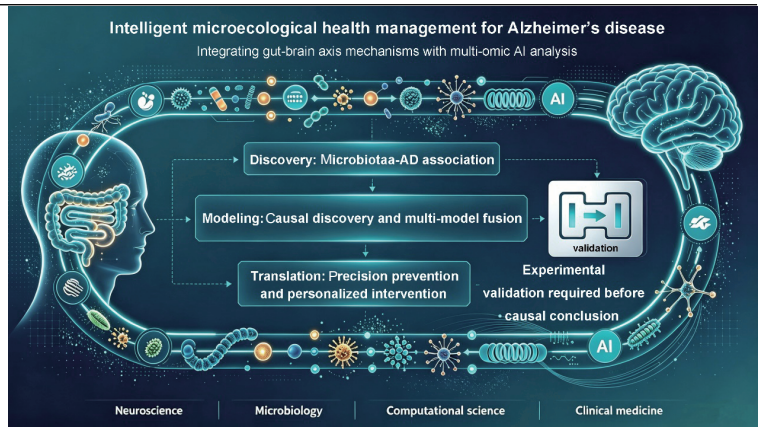
Artificial intelligence in gut-brain axis research for Alzheimer's disease: From associative insights toward causal inference and personalized interventions

Yan Gao[1, 2, †] (✉), Can Zhang[1, 2, †], Zhi-Wen Zhou[3, †], Hong-Shuo Sun[4], Zhong-Ping Feng[4], Shan-Shan Jie[5], Qian-Hang Shao[6], Song-Wei Yang[7, 8], Qi-Di Ai[7, 8], Jia-Qing Yan[9] (✉), Nai-Hong Chen[10] (✉), Shi-Feng Chu[10] (✉), Zhao Zhang[10] (✉), Wen-Bin He[11] (✉)

[1] *Biomedical Innovation Center, Beijing Shijitan Hospital, Capital Medical University, Beijing* 100038, *China*
[2] *Beijing Key Laboratory for Therapeutic Cancer Vaccines, Beijing* 100038, *China*
[3] *Department of Systems Neuropharmacology, Research Institute of Environmental Medicine, Nagoya University, Nagoya* 464-8601, *Japan*
[4] *Department of Physiology, Faculty of Medicine, University of Toronto, Toronto M5S 1A8, Canada*
[5] *The Institute of Basic Theory of Chinese Medicine, China Academy of Chinese Medical Sciences, Beijing* 100700, *China*
[6] *Department of Pharmacy, Peking University People's Hospital, Beijing* 100044, *China*
[7] *College of Pharmacy, Hunan University of Chinese Medicine, Changsha* 410208, *China*
[8] *Hunan Engineering Technology Center of Standardization and Function of Chinese Herbal Decoction Pieces, Changsha* 410208, *China*
[9] *Department of Pharmacy, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing* 100021, *China*
[10] *State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing* 100050, *China*
[11] *National International Joint Research Center for Molecular Chinese Medicine, Shanxi University of Chinese Medicine, Taiyuan* 030024, *China*
† *Yan Gao, Can Zhang, and Zhi-Wen Zhou contributed equally to this work.*

Received: 12 August 2026 / **Revised:** 19 August 2026 / **Accepted:** 19 August 2026

Abstract: Alzheimer's disease (AD) research is shifting from a "brain-centered" model toward a "gut-brain axis system" perspective. However, the causal roles of gut microbiota in AD pathology remain elusive, as traditional association studies struggle to distinguish potential drivers from passengers. This review examines the potential applications and inherent limitations of artificial intelligence (AI) in dissecting this complex relationship. We discuss three emerging and conceptually promising directions: First, AI-frameworks for hypothesis-generating causal discovery that can prioritize microbiota-AD relationships for subsequent experimental validation, while recognizing that current approaches predominantly identify statistical associations rather than empirically supported causal conclusions; second, multi-modal fusion networks, when applied with appropriate regularization and validation, can assist in constructing cross-scale molecular maps across microbiota, metabolites, and the brain, though such pathway inferences require careful interpretation as they represent statistical associations rather than biochemically validated mechanisms; and third, the conceptual application of digital twin-based simulation as a long-term vision for personalized microbiota modulation, which currently lacks the ecological resolution and validation required for predictive modeling. By integrating multi-pathway mechanisms of the gut-brain axis and characteristic profiles of gut microbiota, we critically evaluate the technical advantages of machine learning in microbiome data analysis alongside major challenges including overfitting, batch effects, confounding factors, and reproducibility. Leveraging AI-driven microbiota analysis, we propose a phased translational roadmap, from near-term standardization and cross-cohort validation, through mid-term endophenotyping and trial enrichment strategies, to the long-term exploration of personalized interventions, toward the



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Address correspondence to Yan Gao, gaoyantalentback@126.com; Jia-Qing Yan, yanjiaqing@cicams.ac.cn; Nai-Hong Chen, chennh@imm.ac.cn; Shi-Feng Chu, chushifeng@imm.ac.cn; Zhao Zhang, zhangzhao@imm.ac.cn; Wen-Bin He, hewb@sxtcm.edu.cn

eventual goal of precision prevention. This article outlines a full-cycle framework from discovery to clinical translation and envisions a conceptual ecosystem for future intelligent microecological health management. Achieving the promise of AI-empowered gut-brain axis research will require deep integration across neuroscience, microbiology, computational science, and clinical medicine, alongside rigorous experimental validation, standardized protocols, and realistic appraisal of current technological boundaries.

Keywords: Alzheimer's disease; gut-brain axis; gut microbiota; artificial intelligence; precision medicine

Citation: Gao Y., Zhang C., Zhou Z. W., et al. Artificial intelligence in gut-brain axis research for Alzheimer's disease: From associative insights toward causal inference and personalized interventions. *Food & Medicine Homology Resources*, 2026, 1: 9740006. https://doi.org/10.26599/FMHR.2026.9740006

1 Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, is characterized by complex etiology and an insidious onset[1]. For decades, research has been dominated by the "brain-centered model" with a primary focus on central neuropathological processes such as neuronal damage, amyloid-β (Aβ) deposition, and tau protein hyperphosphorylation[2]. However, in recent years, increasing recognition of the host-microbiome symbiotic system has provided a novel perspective on AD pathogenesis through the gut-brain axis framework[3]. This theory conceptualizes the gut microbiota as a critical bridge connecting the peripheral environment and the central nervous system, with accumulating evidence implicating microbial metabolites, immune signals, and neuroendocrine pathways in the regulation of brain function and behavior[4]. Clinical studies have consistently reported significant gut microbiota dysbiosis in AD patients, with compositional changes correlated with cognitive decline and levels of pathological biomarkers such as $A\beta_{42}$ and phosphorylated tau, suggesting that the gut microbiome may serve as a potential target for early warning and intervention in AD[5-7].

Despite these correlative insights, the fundamental question of whether gut microbiota alterations act as causal drivers of AD pathology or primarily represent secondary consequences of disease processes remains unresolved. This "driver versus passenger" dilemma is compounded by several inherent challenges in microbiome research: The high dimensionality and sparsity of microbial sequencing data[8,9], the non-linear and bidirectional nature of host-microbe interactions[10,11], the difficulty of integrating heterogeneous multi-omics datasets[12,13], and the persistent problem of confounding factors such as diet, medication use, and comorbidities[14]. Traditional statistical approaches, while valuable for hypothesis generation, are often insufficient to address these complexities. In this context, artificial intelligence (AI) has emerged as a potentially powerful set of computational approaches capable of extracting patterns from high-dimensional data, integrating disparate data types, and generating testable hypotheses about causal relationships[15]. From deep feature extraction to multi-modal integration and from time-series forecasting to simulation modeling, AI techniques are increasingly being explored in biomedical research, including early applications in microbiome and neurodegenerative disease (NDD) studies[16].

However, it is important to recognize that the application of AI to gut-brain axis research remains in its early stages, and several critical limitations must be acknowledged[17]. First, AI models trained on observational microbiome data primarily identify statistical associations[18]; genuine causal inference requires either specific modeling assumptions that are difficult to verify or experimental validation that is rarely integrated with computational predictions[19]. Second, the concept of "digital twin" simulations for personalized microbiota modulation, while intellectually appealing, currently lacks the ecological resolution, functional redundancy modeling, and validation data necessary for predictive clinical application[20]. Third, multi-modal fusion networks can generate comprehensive molecular maps, but these represent correlative patterns rather than biochemically validated pathways, and are susceptible to overfitting, batch effects, and cohort-specific artifacts[21]. The translational distance between computational discoveries and clinical implementation remains substantial, and realizing the promise of AI in this field will require iterative integration with experimental models, rigorous validation standards, and realistic appraisal of current technological boundaries[22,23].

Given this context, the present review aims to provide a balanced and critical examination of how AI is being applied, and might realistically be applied, to gut-brain axis research in AD. Rather than presenting AI as a solution to all methodological challenges, we focus on three promising application scenarios where AI offers unique capabilities: Hypothesis-generating causal discovery frameworks[24], multi-modal integration for cross-scale molecular mapping[25], and computational simulation as a long-term vision for personalized intervention modeling. For each scenario, we discuss both the potential value and the current limitations, with an emphasis on what can be achieved with existing approaches and what remains as a research agenda for the coming decade[26,27].

It is worth acknowledging that non-AI approaches remain indispensable in this research domain. Traditional statistical methods such as mediation analysis, Mendelian randomization, and structural equation modeling offer more transparent assumptions and well-established inference frameworks for testing specific hypotheses about microbiota-AD relationships. Experimental approaches including gnotobiotic animal models, fecal microbiota transplantation (FMT), and pathway-specific pharmacological interventions provide the definitive causal evidence that AI models cannot deliver from observational data alone. AI is best positioned not as a replacement for these methods, but as a complementary tool for pattern discovery, hypothesis generation, and integration of heterogeneous data types at scales beyond human intuition. The most productive path forward lies in iterative cycles of AI-driven discovery and traditional experimental validation.

It is necessary at the outset to clarify what this review means by AI and to distinguish it from related but distinct methodological categories. As used here, AI refers broadly to computational approaches that learn patterns from data without being explicitly programmed for a specific rule, with an emphasis on machine learning and deep learning methods that can handle high-dimensional, non-linear, and heterogeneous data types. Conventional statistical modeling, including linear regression, generalized linear models, mediation analysis, and structural

equation modeling, is not classified as AI in this review, although these methods remain indispensable for testing specific hypotheses and for making causal inferences under strong assumptions. Machine learning, by contrast, encompasses supervised techniques such as random forest and gradient boosting as well as unsupervised methods including clustering and dimensionality reduction, which prioritize prediction and pattern discovery over parameter estimation and hypothesis testing. Deep learning represents a further subset of machine learning that uses multi-layered neural networks to learn hierarchical representations from raw or minimally preprocessed data, and is particularly relevant for image analysis, sequence modeling, and multi-omics feature extraction. Causal inference is treated here as a distinct intellectual enterprise with its own formal language of directed acyclic graphs, counterfactuals, and identification assumptions; although machine learning tools can be applied within causal frameworks for tasks such as propensity score estimation or heterogeneous treatment effect discovery, causal reasoning itself is not reducible to a machine learning algorithm. Network biology methods, including similarity network fusion and graph neural network (GNN), are discussed as integrative tools that sit at the intersection of machine learning and systems biology, but their outputs are explicitly interpreted as correlational maps requiring external validation rather than as mechanistic models. Finally, mechanistic and ecological modeling, such as genome-scale metabolic reconstruction and community dynamics simulation, relies on biophysical and biochemical principles that are largely independent of data-driven learning, and these methods are discussed in this review primarily as a long-term aspiration rather than as current AI practice. This taxonomy guides the structure of the following sections: Conventional statistical methods are referenced as benchmarks and validation tools, machine learning and deep learning are presented as the primary engines for pattern discovery and prediction, causal inference is addressed with careful attention to its assumptions and limitations, network biology is treated as an integration strategy with clear interpretive caveats, and mechanistic modeling is positioned as a future direction that requires substantial foundational advances before it can be meaningfully integrated with AI-driven microbiome research.

2 Gut-brain axis in AD: Evidence, mechanisms, and evidence gaps

Gut microbiota dysbiosis in AD has emerged as a critical pathological feature, with research progressing from descriptive observations to mechanistic elucidation across multiple levels of evidence. An integrated overview of the four major pathways, immune-inflammatory, neuroendocrine, metabolic, and direct pathological, through which the gut microbiota communicate with the central nervous system in AD is presented in Fig. 1.

Accumulating evidence from clinical observational studies has established robust associations between gut microbiota composition and AD status[28]. Metagenomic sequencing has revealed distinct alterations in the gut microbial composition of AD patients compared with cognitively normal (CN) controls[29]. Characteristic changes include reduced microbial diversity, decreased abundance of anti-inflammatory genera such as *Faecalibacterium* and *Bifidobacterium*, and enrichment of pro-inflammatory taxa including *Escherichia* and *Bacteroides*[30,31]. These compositional shifts have been correlated with cerebrospinal fluid biomarkers including Aβ_{42} and phosphorylated tau, as well as with cognitive assessment scores, suggesting clinical relevance[32,33]. Furthermore, multi-cohort studies have begun to identify both geography-specific and conserved microbial signatures associated with AD pathology[34]. The characteristic atlas of AD-associated gut microbiota dysbiosis presented in Fig. 2.

Experimental animal models have provided evidence supporting a potential causal role for gut microbiota in AD pathology[35]. Studies using transgenic AD mice and microbiota-depleted models receiving FMT from AD patients have demonstrated that recipient animals exhibit impaired cognitive

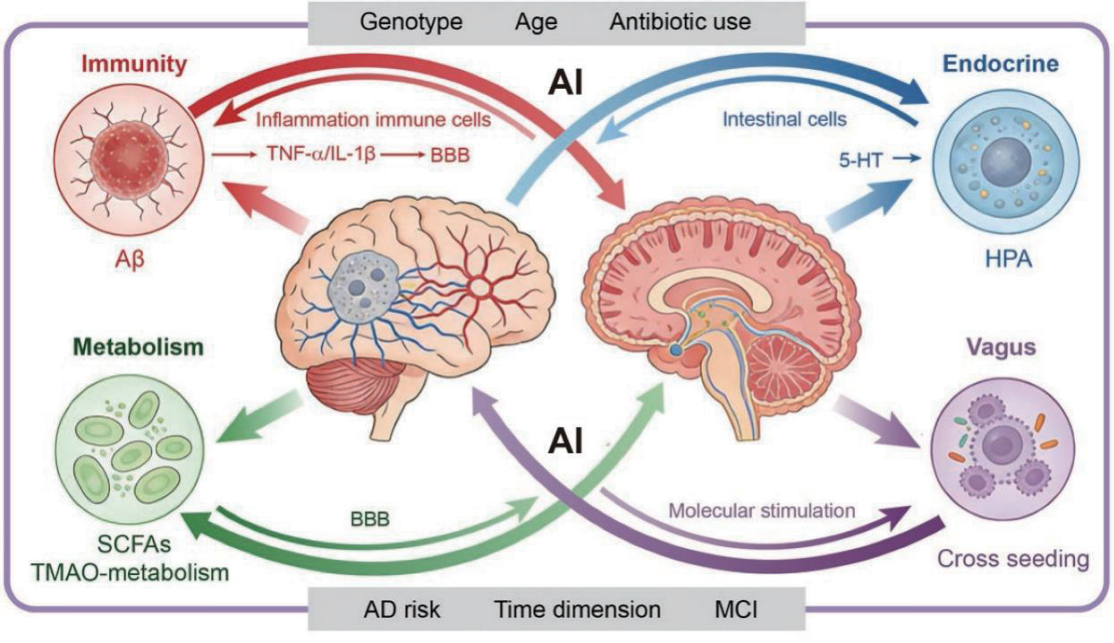

Figure 1 Integration of gut-brain axis bidirectional communication and multi-pathway mechanisms in AD pathology. This figure illustrates the four major pathways, immune-inflammatory, neuroendocrine, metabolic, and direct pathological, through which gut microbiota communicate with the central nervous system in AD. Key mediators include microbial metabolites such as short-chain fatty acids (SCFAs), trimethylamine-*N*-oxide (TMAO), and bile acids; immune signals such as LPS and cytokines; and neural pathways such as the vagus nerve and HPA axis.

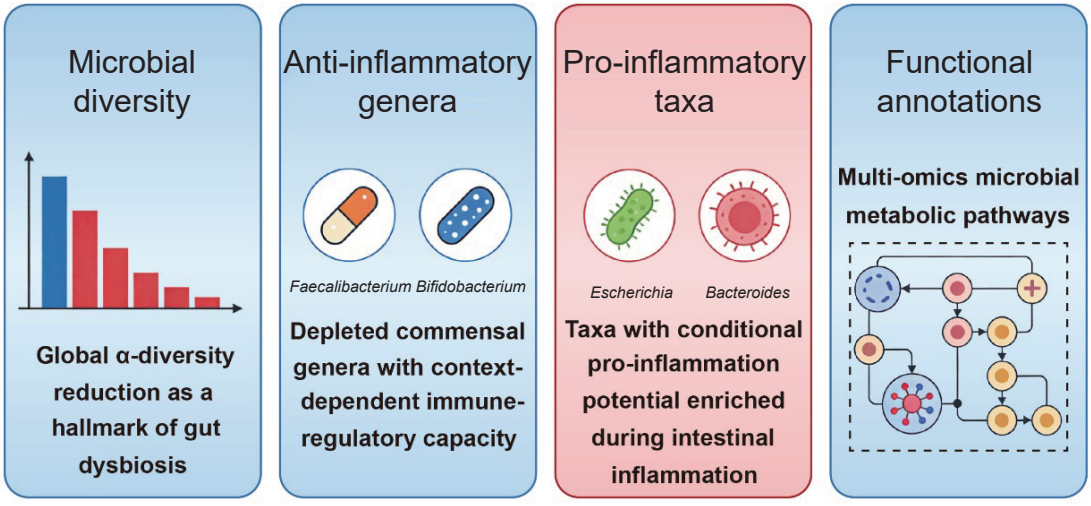

Figure 2 Characteristic profiles of AD-associated gut microbiota dysbiosis, including reduced global α-diversity, depletion of anti-inflammatory commensal genera, enrichment of conditionally pro-inflammatory taxa, and functional annotations of microbial metabolic pathways.

function, increased cerebral Aβ deposition, and enhanced tau hyperphosphorylation[36, 37]. Conversely, intervention with healthy donor microbiota or specific probiotic strains has been shown to ameliorate cognitive deficits and neuropathological changes in AD models[38, 39]. These findings suggest that AD-associated microbiota can transmit pathological features and that microbiota modulation holds therapeutic potential.

Multiple signaling pathways have been proposed to mediate gut-brain communication in AD, though the causal strength of evidence varies across pathways. The immune-inflammatory pathway involves dysbiosis-induced intestinal barrier disruption, leading to increased translocation of lipopolysaccharide (LPS) and pro-inflammatory cytokines, which may activate microglial neuroinflammation *via* TLR4 signaling[40, 41]. The neuroendocrine pathway encompasses alterations in hypothalamic-pituitary-adrenal (HPA) axis function and neurotransmitter modulation, particularly the influence of microbiota on serotonin, gamma-aminobutyric acid (GABA), and catecholamine metabolism[42]. The metabolic pathway features microbiota-derived metabolites including SCFAs, which exert neuroprotective effects through epigenetic regulation and G-protein-coupled receptor signaling, alongside metabolites such as TMAO and secondary bile acids that may promote neuroinflammation and protein aggregation[43, 44]. Direct pathological pathways involve microbiota-derived amyloid proteins that may cross-seed cerebral Aβ aggregation, as well as microbial metabolites that influence blood-brain barrier permeability[45].

Table 1 (see below) summarizes the current evidence across these four pathways with explicit grading of causal strength. Human association studies provide the largest body of evidence but are inherently limited by confounding factors and reverse causation. Animal FMT studies offer stronger support for causal relationships by demonstrating that microbiota from AD donors can transmit disease-relevant phenotypes. Probiotic and metabolite supplementation trials provide proof-of-concept for therapeutic modulation but often lack mechanistic specificity. Mechanistic blocking experiments offer the most direct causal evidence but are largely restricted to animal models and may not fully translate to human disease. It is essential to recognize that across all pathways, the evidence remains predominantly correlational, and definitive demonstration of causality in human AD requires further investigation.

In summary, although substantial evidence supports that gut microbiota plays an important role in AD pathology, the strength

Table 1 Evidence grading for gut-brain axis mechanisms in AD

Pathway	Key mediators	Types of evidence	Key findings	Causal strength	Grading criteria
Immune-inflammatory	LPS, cytokines, TLR4	Human correlation; Animal FMT; mechanistic (TLR4 blockade)	Dysbiosis increases intestinal permeability; microbial products activate microglial inflammation	Moderate	Multi-level evidence exists, and mechanistic blockade experiments support causal direction, but direct human evidence remains correlational
Neuroendocrine	HPA axis, serotonin, GABA, catecholamines	Human correlation; animal models; probiotic trials	Microbiota influences neurotransmitter precursor availability and HPA axis reactivity	Weak to moderate	Human correlational and animal model evidence exists, but direct mechanistic blockade experiments are lacking, and probiotic trial results are inconsistent
Metabolic	SCFAs, TMAO, bile acids	Human correlation; Animal FMT; metabolite supplementation	SCFAs neuroprotective; TMAO and secondary bile acids pro-inflammatory	Moderate	Metabolite supplementation provides relatively strong causal support, but human evidence remains predominantly correlational, and the strength of evidence varies across different metabolites
Direct pathological	Microbial amyloids, BBB-modulating metabolites	Human correlation; animal models; *in vitro*	Bacterial amyloids may cross-seed Aβ aggregation; metabolites affect barrier integrity	Weak	Evidence is predominantly from *in vitro* and animal models; human evidence is extremely limited, and specific blockade experiments are lacking

of evidence varies considerably across the four pathways, and the vast majority of studies remain at the correlational level. Returning to the central question raised in the Introduction regarding whether microbial alterations act as drivers or passengers: Current evidence demonstrates that gut microbiota dysbiosis can transmit disease-relevant phenotypes at least in animal models, which supports a certain degree of causal involvement. However, in human AD, whether microbial changes represent early drivers of disease pathogenesis or are merely secondary consequences of AD pathological processes such as neuroinflammation, dietary changes, and reduced physical activity remains unresolved with existing data. Although AI-driven causal discovery frameworks can generate testable hypotheses about causal directions from observational data, these hypotheses still urgently require rigorous experimental validation.

Despite substantial progress in characterizing AD-associated gut microbiota dysbiosis and elucidating candidate mechanistic pathways, several fundamental limitations must be acknowledged. Traditional approaches struggle to determine whether microbial alterations represent drivers of AD pathology, modifiers of disease progression, or merely secondary consequences of the disease process or its treatment. Furthermore, the integration of multi-omics data across different scales, from microbial genes to host metabolism to brain imaging, remains technically challenging, limiting the ability to construct comprehensive causal models of the gut-brain axis. These unresolved questions set the stage for the application of AI, which offers unique capabilities to address the high-dimensional, non-linear, and causal inference challenges inherent in gut-brain axis research, as discussed in the following sections.

3 Foundational machine learning approaches for microbiome data analysis

The integration of AI with microbiome research has catalyzed the development of sophisticated methodological frameworks for processing high-dimensional microbial data, but the value of these approaches in AD research must be assessed by their ability to address specific clinical questions. The most immediate challenge is whether gut microbiome features can improve discrimination along the AD continuum, distinguishing CN individuals from those with mild cognitive impairment (MCI), prodromal AD, and established dementia. Supervised learning methods, particularly random forest and gradient boosting, have been applied to metagenomic and 16S rRNA data to build classifiers distinguishing AD patients from controls, though these estimates are uniformly optimistic without external validation[46]. However, classifiers trained on AD versus control cohorts perform poorly when applied to MCI populations, indicating that microbial signatures of early disease are either too weak or too heterogeneous for reliable discrimination. Gradient boosting frameworks show, including Extreme Gradient Boosting (XGBoost) and Light Gradient Boosting Machine (LightGBM), superior performance in integrating heterogeneous data types and handling missing values, making them suitable for combining microbiome data with clinical assessments, but the incremental predictive gain beyond established markers. As shown in Fig. 3, a comprehensive overview of the technical workflow from raw data generation through preprocessing, feature extraction, machine learning modeling, to biological interpretation and clinical application is presented.

A critical consideration is how AI models should incorporate established AD biomarkers, including Aβ deposition, phosphorylated tau, and neurodegeneration markers such as hippocampal volume. Microbiome features should be viewed not as independent predictors against these biomarkers but as potential upstream modulators that may provide information orthogonal to or antecedent to biomarker changes[47]. Machine learning models integrating microbiome data with amyloid and tau biomarkers have demonstrated only marginal improvement over biomarker-only models, suggesting that the microbiome's predictive signal overlaps substantially with information already

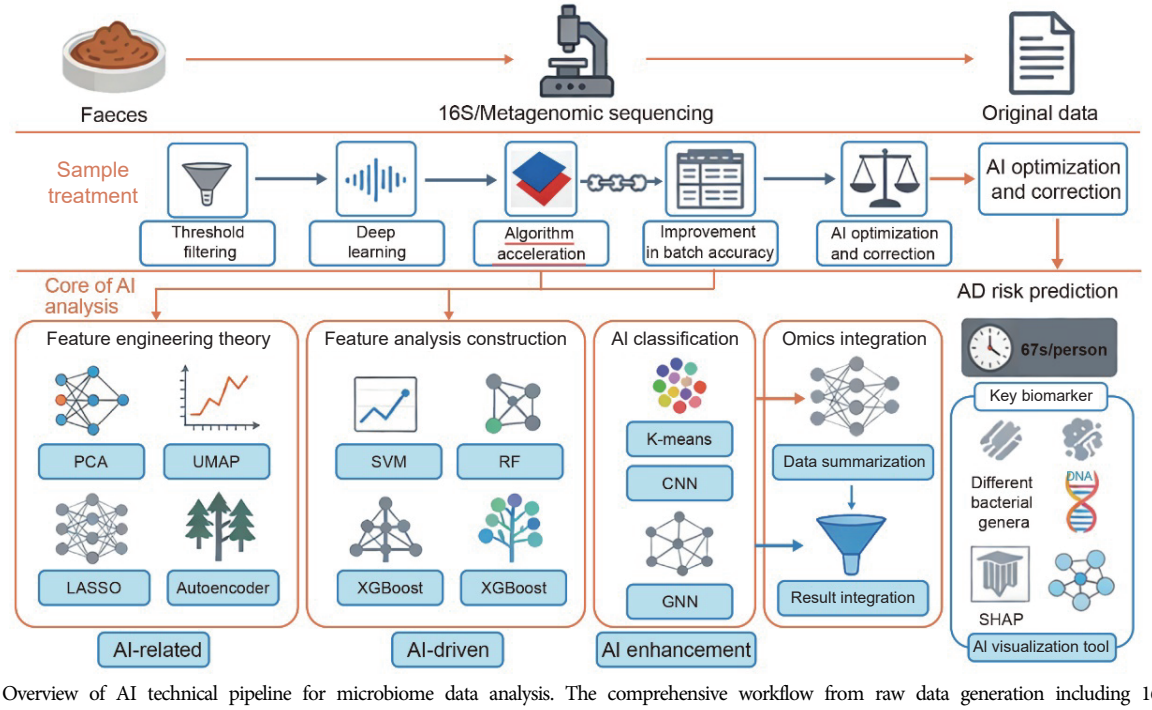

Figure 3 Overview of AI technical pipeline for microbiome data analysis. The comprehensive workflow from raw data generation including 16S rRNA, metagenomics, and metabolomics through preprocessing, feature extraction, machine learning modeling as supervised, unsupervised, time-series, to biological interpretation and clinical application were presented.

captured by core AD pathology[48]. The more valuable application lies in risk prediction for individuals without overt biomarker elevation who might be targeted for primary prevention[49]. Dimensionality reduction techniques such as principal coordinate analysis and uniform manifold approximation and projection visualize microbial community structure along the cognitive continuum, but these unsupervised methods do not address whether observed separation reflects disease-specific signals rather than other correlates of decline.

Perhaps the most challenging issue is whether AI models can distinguish AD-specific microbiome changes from confounders that accompany aging and cognitive impairment, including frailty, polypharmacy, dietary changes, reduced physical activity, and multimorbidity. Many of these factors exert effects on gut microbiota composition as large as or larger than those attributed to AD itself. Random forest and gradient boosting models that include comprehensive covariate panels can estimate the marginal contribution of AD status after adjustment, and studies performing such adjustments generally find that the unique microbial signal attributable to AD is substantially attenuated compared to unadjusted analyses[50,51]. Feature selection methods such as recursive feature elimination or regularization techniques can identify features that survive covariate adjustment, but these assume all relevant confounders have been measured without error[52]. Interpretability tools including SHapley Additive exPlanations (SHAP) and Local Interpretable Model-agnostic Explanations (LIME) reveal which microbial features drive individual classifications, enabling clinicians to assess whether predictions rely on features plausibly linked to AD pathology rather than on confounders, though these methods do not eliminate confounding but merely make model reliance transparent[53,54].

The temporal dimension adds further complexity, as disease stage and cognitive decline trajectories fundamentally alter the interpretation of microbial features. Time-series analysis methods, including dynamic Bayesian networks and recurrent neural networks, examine whether rates of microbial change correlate with rates of cognitive decline, offering a dynamic perspective beyond cross-sectional comparisons. Recurrent neural networks modeling sequential microbiome measurements can identify individuals whose microbial trajectories diverge from normative aging patterns, and such deviations may serve as early warning signals of impending deterioration. However, these methods require dense longitudinal sampling rarely available in existing cohorts, and dynamic approaches face the feedback loop where cognitive decline alters behavior that in turn affects the microbiome, complicating interpretations of temporal precedence. The incorporation of time-varying covariates is essential for distinguishing microbiome changes that drive decline from those reflecting behavioral consequences, but current longitudinal datasets are generally underpowered for such complex modeling. In sum, the foundational approaches described above, supervised classification, unsupervised clustering, feature selection, and time-series modeling, constitute the essential algorithmic infrastructure for addressing the clinical questions defining AD gut-brain axis research, and their relevance is determined not by algorithmic sophistication but by their ability to generate testable hypotheses about disease mechanisms, prognostic stratification, and potential intervention targets.

The foundational methods described above, supervised and unsupervised learning for pattern recognition, feature selection, and time-series modeling, constitute the essential algorithmic infrastructure for the more advanced application scenarios that follow. As will become evident in Section 4, causal discovery frameworks extend probabilistic graphical models with temporal constraints; multi-omics integration techniques build upon unsupervised clustering and dimensionality reduction to fuse heterogeneous data modalities; and digital twin simulation leverages time-series forecasting and reinforcement learning paradigms to model dynamic intervention responses. Recognizing this hierarchical relationship clarifies that the advanced scenarios are not entirely new methodologies but rather sophisticated integrations and extensions of the foundational approaches, inheriting both their capabilities and their inherent limitations, particularly the inability to establish causality from observational data alone.

4 AI application scenarios: From hypothesis generation to long-term vision

Building upon the foundational methodologies described above, this section examines three advanced AI application scenarios that address core challenges in gut-brain axis research. For each scenario, we discuss both the potential value and the current limitations, emphasizing that these approaches are best understood as tools for hypothesis generation and exploratory analysis rather than as definitive solutions. The relationships among these scenarios within a unified AI analytical framework are illustrated in Fig. 4.

4.1 Causal discovery frameworks for hypothesis generation

The central causal question in gut-brain axis research for AD is whether specific compositional or functional features of the gut microbiota exert a direct influence on AD pathology and clinical progression, or whether observed microbial alterations are predominantly secondary consequences of the disease process, its associated lifestyle changes, or its pharmacological treatments[24,55]. Answering this question requires a clear definition of the causal estimand: Ideally, we would seek to estimate the average effect of a sustained modification of a specific microbial taxon or metabolic pathway on a defined AD-related outcome, such as change in cerebrospinal fluid $A\beta_{42}$ levels, tau phosphorylation, or cognitive decline rate over a specified time horizon. However, because randomized manipulation of the human gut microbiome is rarely feasible in large-scale observational cohorts, AI-based causal discovery methods do not directly estimate this interventional effect. Instead, they typically estimate a statistical association that is interpretable under certain assumptions as a directional dependence, for example, whether longitudinal changes in microbial abundance precede changes in biomarkers, or whether a microbial feature is conditionally independent of the outcome given other measured variables in a probabilistic graph. These outputs are best understood not as effect size estimates but as hypothesis-generating signals that prioritize microbiota-AD relationships for subsequent experimental testing[33,56].

The application of AI-based causal discovery to microbiome data rests on several strong assumptions that are rarely fully satisfied. These include the assumption of no unmeasured confounding, requiring that all common causes of the microbial feature and the AD outcome have been measured and correctly included in the model; the assumption of correct temporal ordering, which demands dense longitudinal sampling with

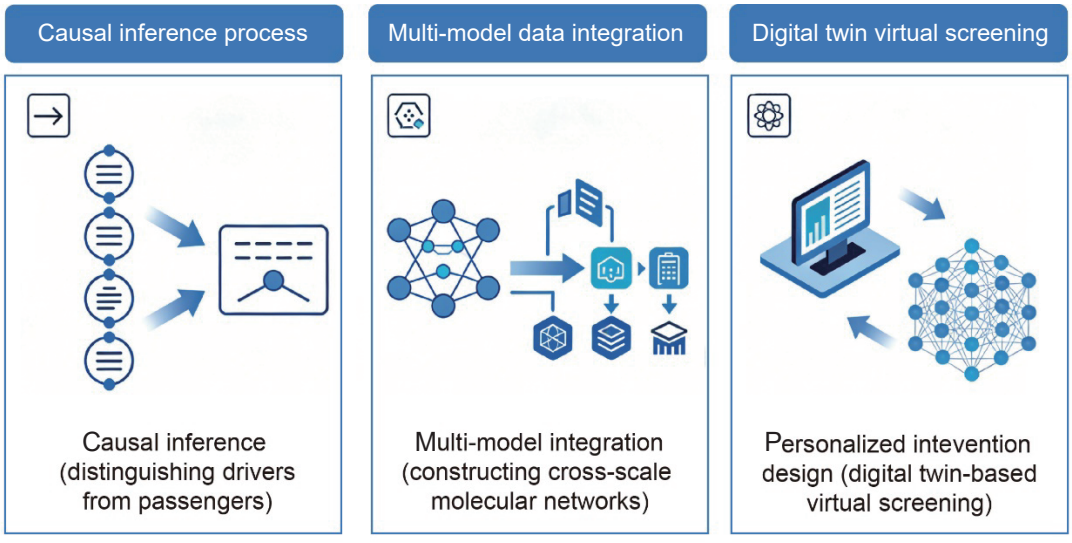

Figure 4 AI-driven application scenarios in gut-brain axis research. It illustrates the three core AI application scenarios discussed in this review: Causal inference as distinguishing potential drivers from passengers, multi-modal integration as constructing cross-scale molecular networks, and personalized intervention design as digital twin-based virtual screening.

sufficient temporal resolution to establish that microbial changes precede biomarker changes; the assumption of no measurement error, which is unrealistic given the substantial technical variability in sequencing-based microbiome profiling; and the assumption of model specification, meaning that the functional form of relationships is correctly captured, which is particularly challenging given the non-linear and interactive nature of host-microbe dynamics[57]. In practice, some confounders can be controlled, including age, sex, APOE genotype, common medications such as statins or proton pump inhibitors, and basic dietary patterns captured by food frequency questionnaires. However, critical confounders remain elusive, including detailed long-term dietary history, physical activity levels, frailty status, socioeconomic factors influencing diet and healthcare access, and the cumulative effect of past antibiotic and medication exposures. These unmeasured confounders pose a fundamental threat to causal interpretation, and their influence cannot be fully remedied by any computational approach.

Given these limitations, what AI-based causal discovery frameworks can realistically provide is a ranking of candidate microbiota-AD relationships by their statistical support under a set of transparently stated assumptions. Methods such as Bayesian network structure learning applied to cross-sectional data identify conditional independence structures that suggest possible directed pathways, but these are inherently unidentifiable from observational data without additional constraints. Longitudinal Granger-type analyses implemented in recurrent neural network architectures can assess whether time-lagged microbial abundances predict subsequent biomarker changes beyond autoregressive effects, providing evidence consistent with temporal precedence. These approaches do not, however, estimate the magnitude of an intervention effect; they merely identify patterns of statistical dependence that are compatible with a causal interpretation under the specified modeling choices. As such, their outputs are markedly different from what would be obtained from a randomized trial or a well-designed instrumental variable analysis such as Mendelian randomization, which can provide estimates of causal effects under weaker assumptions when valid genetic instruments are available. Therefore, AI-based causal discovery should be viewed not as a substitute for traditional causal inference methods but as a complementary tool for screening large feature spaces and generating hypotheses that are then prioritized for rigorous testing using established experimental and quasi-experimental designs.

The experimental validation required to move from AI-generated hypotheses to causal conclusions is substantial and multifaceted. At the most basic level, any candidate microbial driver identified by computational models should be tested in gnotobiotic animal models, where defined microbial communities can be introduced into AD-prone mice and assessed for effects on neuropathology and behavior. FMT from human donors with distinct microbial profiles into AD models provides a more holistic test of whether the overall microbial community composition, rather than individual taxa, transmits disease-relevant phenotypes[58]. For specific metabolic pathways, such as short-chain fatty acid biosynthesis or tryptophan metabolism, pathway-specific pharmacological inhibition or supplementation in animal models can establish whether the proposed metabolite mediates the observed effect[59]. Ultimately, for human translation, the strongest causal evidence would come from randomized controlled trial (RCT) of microbiome-targeted interventions, such as probiotics, prebiotics, or dietary modifications, that are stratified using the computational signatures to test whether predicted responders show better outcomes than predicted non-responders[60]. In this iterative framework, AI models do not deliver final answers but rather guide the efficient allocation of validation resources toward the most promising hypotheses, and the results of experimental validation are in turn used to refine the computational models, gradually narrowing the gap between statistical association and causal understanding[61].

Representative examples of AI-based multi-omics integration studies in AD and related neurodegenerative disorders (Table 2). This table summarizes published studies that have applied AI methods to microbiome or multi-omics data in AD or related NDDs. For each study, we list the types of data integrated, the AI method used, key findings, and validation strategy. It should be noted that most studies in this field remain exploratory and hypothesis-generating; only a minority have included independent experimental validation.

Table 2 Representative examples of AI-based multi-omics integration studies in NDDs

Study	Data types	Input modalities	AI method	Prediction/Inference target	Major limitations
Lu *et al.*[62]	LPS-induced acute lung injury in mice	Metagenomics + metabolomics	Similarity network fusion	Identification of gut microbiota-metabolite modules mediating lung injury	Non-AD model; sample size not specified; lacks human validation
Parker *et al.*[63]	Aged mice (young↔old FMT)	16S rRNA + metabolomics	Similarity network fusion	Identification of microbial-metabolite modules associated with aging phenotypes	Non-AD model; focuses on aging rather than AD; sample size not specified
Li *et al.*[64]	Bipolar depression patients	Metagenomics + neuroimaging	GNN	Prediction of regional brain glucose metabolism	Non-AD disease; no experimental validation; sample size not specified
Zheng *et al.*[65]	Traumatic brain injury in mice	Metagenomics + network pharmacology	GNN	Identification of microbiota-metabolic pathways mediating rhubarb therapeutic effects	Non-AD model; no independent validation; sample size not specified
Lin *et al.*[66]	AD continuum (CN/MCI/AD)	Multi-omics + clinical	Random forest	Identification of multi-omics features associated with AD staging	Sample size not specified; details of validation cohort not fully described
Guo *et al.*[67]	MCI to AD progression	Multi-omics + clinical	Random forest	Prediction of MCI-to-AD conversion trajectories	Sample size not specified; details of validation cohort not fully described
Huang *et al.*[68]	AD continuum	Multi-omics + clinical + imaging	Machine learning (algorithm not specified)	Modeling of disease progression trajectories	Sample size not specified; specific algorithm not clearly stated
Meng *et al.*[69]	AD patients vs. controls	Metagenomics + metabolomics + proteomics	XGBoost	AD vs. control classification	No independent external validation; sample size not specified
Dwivedi *et al.*[27]	Not specified (review-oriented work)	16S rRNA + metabolomics	Deep neural network	Identification of microbial-metabolite modules associated with tau pathology	Disease context not clearly defined; primarily a methodological review rather than original research

4.2 Multi-omics integration for cross-scale molecular mapping

The complexity of AD pathogenesis involves interplay among gut microbial composition, microbial metabolites, host immune responses, neuroendocrine signaling, and brain structure and function[70]. AI-driven multi-omics integration methods, including similarity network fusion, multi-view learning, and GNNs, provide flexible frameworks for synthesizing disparate data types into unified representations. The optimal approach depends on the characteristics of each omics layer, including dimensionality, missingness patterns, and expected interaction structures, and requires rigorous benchmarking against simpler integration strategies[16,71]. These approaches can integrate metagenomic sequencing, plasma metabolomics, and brain imaging data to identify microbial metabolic pathways that correlate with regional brain glucose metabolism or Aβ deposition patterns[66].

However, cautious interpretation is required. The molecular pathway maps generated by deep learning models represent statistical associations rather than biochemically validated mechanisms[66,72]. Without independent experimental validation, such as targeted metabolomics, pathway-specific inhibitors, or animal models, these models risk fitting noise rather than true biological signals[25,73,74]. Additional challenges include data harmonization across different omics platforms, batch effect correction, missing data imputation, model evaluation in the absence of ground truth, and independent validation in external cohorts[75,76]. Currently, the most productive use of multi-omics integration is to generate novel hypotheses about molecular pathways linking the gut microbiome to AD pathology, followed by targeted experimental testing[77,78]. These integrative approaches can also enable the identification of patient subgroups characterized by distinct multi-omic profiles, a concept referred to as endophenotyping or subtyping, providing a biological basis for stratifying patients beyond clinical symptomatology (Fig. 5).

These integrative approaches not only highlight novel molecular pathways linking the gut microbiome to AD pathology but also enable the identification of patient subgroups characterized by distinct multi-omic profiles, a concept referred to as endophenotyping or subtyping, providing a biological basis for stratifying patients beyond clinical symptomatology.

It is critical to clarify, however, that the subgroups generated by multi-omics clustering do not automatically constitute validated endophenotypes. To confirm that a clustering-derived subgroup represents a biologically meaningful endophenotype in the context of the AD gut-brain axis, several lines of evidence are required: The subgroup must be reproducibly identified across independent cohorts with consistent multi-omics profiling; the subgroup-defining features must show association with established AD biomarkers; the subgroup should demonstrate differential trajectories of cognitive decline or clinical progression in longitudinal follow-up; and ideally, the subgroup should exhibit differential responses to specific interventions in proof-of-concept trials. Currently, most published studies in this field have achieved only the first level of evidence, with limited validation against clinical endpoints. The relationship between multi-omics subgroups and clinical outcomes, including conversion risk from MCI to AD, rate of cognitive decline, and treatment responsiveness, remains a critical knowledge gap that requires prospective cohort studies with standardized, longitudinal biospecimen collection.

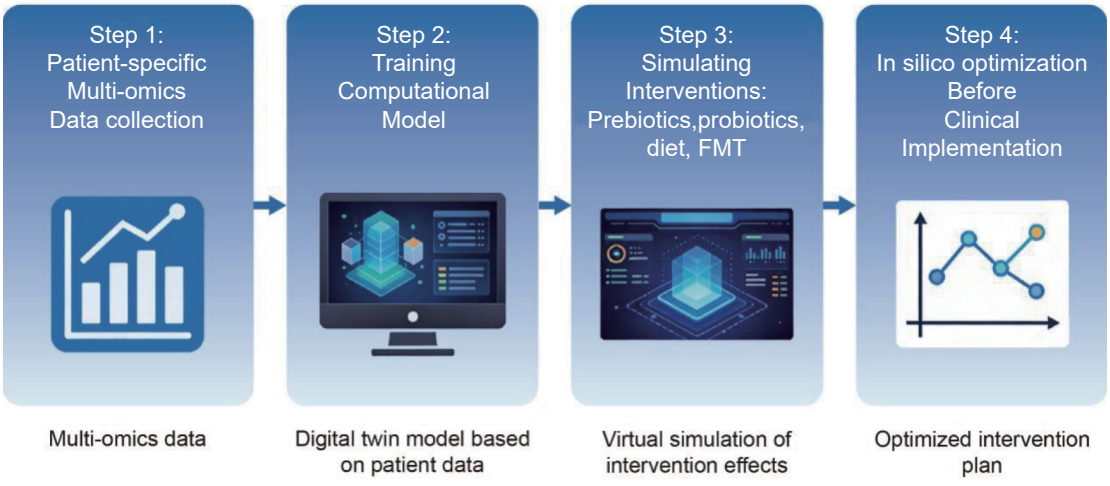

Figure 5 Schematic workflow of the AI-powered personalized microbiota intervention digital twin platform. Schematic four-step workflow of an AI-driven digital twin platform for personalized gut microbiota intervention, covering patient multi-omics profiling, individualized computational model construction, virtual simulation of diverse microbiota modulatory strategies, and in silico optimization of therapeutic regimens prior to clinical translation.

4.3 Computational simulation as a long-term vision for personalized intervention

The concept of a "digital twin", a computational representation of an individual's gut microbiome that simulates dynamic responses to potential interventions, represents an ambitious long-term vision for personalized medicine[20, 79, 80]. Using machine learning models trained on large cohorts, such a system might eventually estimate how a specific patient's microbiota would respond to various prebiotics, probiotics, dietary modifications, or FMT protocols[79, 81]. Reinforcement learning algorithms offer one promising framework for iteratively exploring intervention spaces, though their effectiveness depends critically on the fidelity of the underlying simulation environment and the availability of dense longitudinal training data. Other optimization approaches, including Bayesian optimization and evolutionary strategies, may be more appropriate in data-sparse settings[82].

The defining limitation of the digital twin vision is not generic data quality issues but the fundamental gap between current community-scale ecological models and the predictive accuracy required for clinical decision-making. Addressing this gap requires advances in modeling functional redundancy and colonization resistance, along with extensive datasets linking baseline profiles to intervention outcomes. Therefore, while digital twin-based simulation is intellectually compelling as a research agenda for the coming decade, it should be clearly distinguished from near-term feasible applications. Claims about "personalized microbiota modulation platforms" require substantial tempering, and the concept is best presented as an aspirational long-term goal that will require advances in ecological modeling, extensive validation datasets, and integration with experimental models.

Synthesizing the three application scenarios described above, it is clear that each addresses the core question of drivers versus passengers with distinct emphases. Causal discovery frameworks directly target this question by learning directed probabilistic structures from observational data to generate hypotheses about the causal direction between microbial changes and AD pathology. Multi-omics integration, through the construction of cross-scale molecular networks, can identify microbial pathways that reside upstream in the disease network, thereby indirectly providing clues about potential driver nodes. Digital twin simulation, in turn, presupposes the existence of actionable causal targets, and its feasibility itself depends on accurate determination of the driver-passenger relationship. Nevertheless, it must be acknowledged that none of these three scenarios can independently derive definitive causal conclusions from observational data alone. Their role lies in prioritizing which microbiota-AD relationships are most worthy of investment in experimental validation resources.

5 Methodological challenges and critical considerations for AI in microbiome research

Before AI-driven approaches can be reliably translated into clinical applications, several fundamental methodological challenges must be addressed. These are not peripheral issues; they determine whether the proposed AI tools are scientifically credible.

Compositionality and sparsity. Microbiome data are inherently compositional, measurements reflect relative abundances rather than absolute quantities, which violates standard statistical assumptions and can induce spurious correlations[83]. In the context of AD gut-brain axis research, this challenge is amplified by the fact that many AD-relevant microbial taxa are present at low relative abundances and are particularly susceptible to compositional distortion. A change in relative abundance of these taxa could arise either from a true biological shift or from changes in other highly abundant taxa, a distinction that has direct implications for interpreting their role in AD pathology. Similarly, the high sparsity of microbiome data poses challenges for model training and interpretation; many AD-associated microbial signatures involve low-abundance organisms that may be absent in a large proportion of samples, leading to unstable model estimates[84, 85]. Transformations such as centered log-ratio or robust methods for zero-inflation are necessary but not always sufficient.

Overfitting and validation. High-dimensional microbiome data combined with moderate sample sizes creates substantial risk of overfitting, where models capture noise rather than signal[21]. In AD microbiome research, this risk is particularly acute because AD cohorts are often modest in size, while the feature space commonly exceeds 1,000 dimensions. Moreover, the subtle effect sizes typical of AD-associated microbial shifts mean that overfitted models may yield apparently high classification accuracy that fails

completely in external validation. Cross-validation leakage, improperly using information from validation samples in model training, can produce overly optimistic performance estimates[86]. Rigorous validation requires external cohorts that are independent of the discovery dataset, with consistent sample collection, processing, and sequencing protocols[17].

Confounding factors. Gut microbiota composition is influenced by numerous factors that also correlate with AD status, including diet, medication use, physical activity, comorbidity, and genetic background, particularly APOE genotype[87]. In AD gut-brain axis research, these confounders pose a specific and serious threat: AD patients are frequently elderly, have distinct dietary patterns, are more likely to be medicated, and have higher rates of comorbidities compared to controls. Distinguishing disease-associated microbial signals from these confounders requires careful study design, comprehensive covariate collection, and analytical methods such as propensity score matching or negative control analyses[88, 89]. AI models that ignore confounding risk learning patterns that reflect medication use or dietary differences rather than AD pathology, a critical concern given that many published AI-based microbiome classifiers for AD have not adequately adjusted for these factors.

Batch effects and technical variability. Differences in sample collection, DNA extraction, sequencing protocols, and bioinformatics pipelines across studies introduce technical variability that can overwhelm true biological signals[90]. AI models trained on data generated with one set of protocols often perform poorly when applied to datasets generated with different methodologies[91]. Data harmonization, batch effect correction, and standardized protocols are essential for model generalizability[92].

Reproducibility and generalizability. Many AI-microbiome findings have failed to replicate across cohorts. Factors contributing to poor reproducibility include small sample sizes, cohort-specific artifacts, overfitting, lack of standardized reporting, and publication bias favoring positive results[93]. Multi-center collaborative efforts and federated learning approaches, which allow models to be trained across distributed data sources without requiring centralized data sharing, offer promising solutions, but require coordinated infrastructure and governance[94, 95].

Minimum reporting standards. To enhance reproducibility, AI-microbiome studies in AD should adhere to minimum reporting standards, including: Clear description of sample collection, processing, and sequencing protocols[94, 96]; specification of train-test splitting and cross-validation procedures; reporting of model performance on internal and external validation sets[97]; disclosure of all covariates and confounding adjustment methods; provision of code and sufficient metadata for replication; and explicit discussion of limitations and potential sources of bias[98]. Transparent reporting enables critical evaluation and appropriate clinical deployment.

6 Translational roadmap: Bridging the gap from discovery to clinic

Translating AI-driven insights from gut-brain axis research into clinical practice requires a phased approach that acknowledges current limitations while establishing realistic milestones. The timeline divisions are based on three considerations: (1) data infrastructure readiness, large-scale longitudinal cohort studies with standardized protocols typically require 2–3 years for planning and recruitment, with initial validation possible at year 5; (2) regulatory and validation requirements, prospective clinical validation and regulatory clearance for AI-based diagnostic tools typically require 3–5 years from technology maturity; and (3) technological maturity, foundational methods are mature and ready for standardization now, whereas digital twin simulation requires fundamental advances in ecological modeling that are estimated to take more than 10 years, based on current research progress.

The following roadmap outlines near-term, mid-term, and long-term priorities, with specific milestone events anchoring each time period:

Near-term (2–5 years): Standardization, validation, and interpretability. The immediate priority is establishing robust infrastructure for reproducible research. Milestone events: (A) publication of consensus standardized protocols for AD microbiome sample collection and sequencing (target: Year 2–3); (B) completion of at least 3 independent external validation studies of existing AI models (target: Year 3–4)[92]; (C) adoption of minimum reporting standards for AI-microbiome studies by major journals in the field (target: Year 4–5).

Mid-term (5–10 years): Subtyping, trial enrichment, and prospective validation. With robust infrastructure in place, the field can advance to patient stratification and clinical trial integration[99, 100]. Milestone events: (A) identification and cross-cohort validation of at least 2 AD microbiome endophenotypes with demonstrated prognostic value (target: Year 5–7); (B) initiation of the first prospective clinical trial using AI-based microbiome enrichment strategy for patient selection (target: Year 6–8)[101]; (C) completion of a multi-center longitudinal study establishing temporal precedence of microbial changes relative to AD biomarker emergence (target: Year 8–10)[102].

Long-term (more than 10 years): Personalized intervention modeling. Milestone events: (A) successful computational prediction of individual intervention response validated in a prospective trial with $n \geq 200$ (target: Year 10–13); (B) regulatory clearance of a microbiome-based AI tool as a software-based medical device in at least one major jurisdiction (target: Year 12–15); (C) demonstration of clinical utility in a RCT showing AI-guided intervention improves cognitive outcomes compared to standard care (target: Year 15 and beyond). The aspirational goal of computational simulation for personalized microbiota modulation remains a research agenda requiring substantial advances. Rather than treating this as a single monolithic goal, we propose breaking it into progressive, multi-step milestones:

Step 1 (years 10–12): Development and validation of ecological models that capture functional redundancy, colonization resistance, and emergent properties of gut microbial ecosystems[103]. This requires longitudinal intervention studies with dense sampling to parameterize community dynamics models.

Step 2 (years 12–15): Generation of large-scale public datasets linking baseline microbiome profiles to intervention responses across diverse populations and intervention types[104, 105]. These datasets must include sufficient sample sizes and covariate information to enable machine learning model training.

Step 3 (years 15–18): Prospective validation of computational predictions against real-world outcomes in RCTs, where AI-generated intervention recommendations are compared against standard-of-care or random allocation. Success at this stage would require the AI model to demonstrate statistically significant and clinically meaningful improvement in predicted versus actual outcomes.

Step 4 (years 18 and beyond): Deployment-ready systems with

regulatory approval, including infrastructure for continuous learning that updates models as new data become available[106], integration with electronic health records, and clinical decision support interfaces for healthcare providers.

It must be emphasized that throughout this phase, digital twin concepts should be clearly distinguished from near-term feasible applications, and each milestone should be accompanied by specific validation criteria rather than aspirational language (Fig. 6).

Cross-cutting challenges. Across all phases, several challenges require sustained attention. Ethical considerations include ensuring algorithmic fairness across diverse populations, avoiding perpetuation of health disparities through biased training data, maintaining patient privacy and data security, and obtaining informed consent for AI-guided interventions[107]. Regulatory considerations include classification of AI tools as software as a medical device, requirements for clinical validation and performance monitoring, and adaptation of regulatory frameworks to accommodate iterative model refinement. Implementation barriers include infrastructure needs, trained personnel, and reimbursement pathways for microbiome testing and AI-guided interventions. Addressing these challenges requires coordinated efforts among researchers, clinicians, industry partners, regulatory agencies, and patient advocacy groups.

7 Conclusion

The convergence of AI with gut-brain axis research represents an evolving frontier in our understanding of AD pathogenesis and the development of novel intervention strategies. As this review has illustrated, the shift from a brain-centered model to a systems-level perspective that encompasses the gut microbiome as an active participant in neurological health and disease signals a fundamental reorientation in how we approach the complexity of AD. However, it is essential to recognize that AI applications in this field remain at an early stage, with substantial methodological challenges and translational distance yet to be addressed.

Three application scenarios offer particular promise while requiring realistic appraisal of current limitations. First, causal discovery frameworks enable the generation of testable hypotheses about driver-passenger relationships in microbiota-AD connections, but their outputs require experimental validation and should not be mistaken for definitive causal conclusions. Second, multi-omics fusion techniques can construct comprehensive maps of the gut-brain axis, revealing candidate molecular pathways for targeted investigation, but these represent statistical associations rather than biochemically validated mechanisms. Third, digital twin-based simulation represents an ambitious long-term research agenda rather than a near-term clinical tool, requiring substantial advances in ecological modeling and validation before achieving predictive capability.

The successful translation of AI-driven advances into clinical practice depends on several prerequisites: Establishing large-scale, diverse, longitudinal cohort studies with standardized protocols[93, 108]; developing rigorous validation standards and minimum reporting requirements[109]; advancing explainable AI methods that foster clinical trust; creating regulatory and reimbursement frameworks that support deployment of AI-guided interventions[110]; and cultivating interdisciplinary expertise that bridges microbiology, neuroscience, and data science[111].

From a policy perspective, we offer the following recommendations. Funding agencies should prioritize multi-center, interdisciplinary initiatives that coordinate data standards, facilitate resource sharing, and ensure that the benefits of AI-driven precision medicine are equitably distributed across populations[112, 113]. Regulatory bodies should develop adaptive frameworks that accommodate the iterative nature of AI model refinement while ensuring patient safety through rigorous validation requirements[114]. Educational institutions should establish cross-disciplinary training programs to cultivate a workforce capable of navigating the intersection of AI, microbiology, and clinical neuroscience[115, 116]. Research communities should embrace open science practices, including

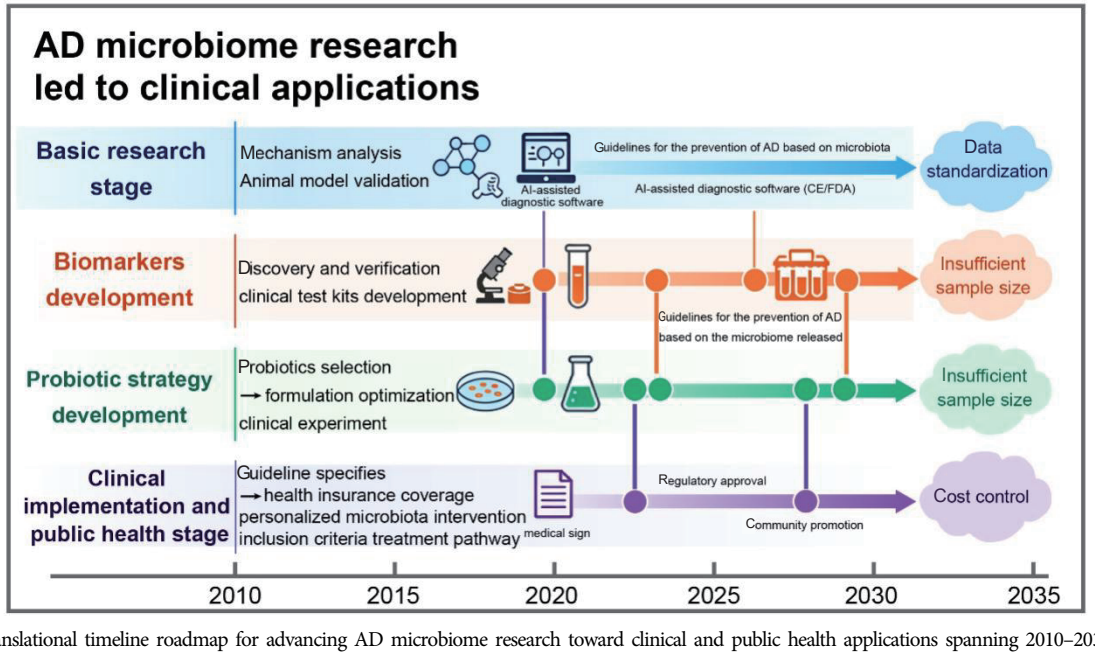

Figure 6 Translational timeline roadmap for advancing AD microbiome research toward clinical and public health applications spanning 2010–2035. Schematic timeline illustrating the translational pipeline from basic AD microbiome research to clinical and public health deployment, outlining four sequential developmental stages alongside key technical milestones and prevailing translational bottlenecks.

data sharing, code transparency, and reproducible workflows, to accelerate progress and mitigate the risk of irreproducible findings[117]. Patient communities and advocacy groups should be engaged in the design and evaluation of AI tools to ensure they align with patient values and needs[118].

Ultimately, the AI-empowered gut-brain axis research paradigm exemplifies the broader transformation occurring across biomedical sciences: The movement toward systems-level thinking, data-driven discovery, and precision approaches that recognize and leverage the complexity of human biology. For AD, a condition that has long challenged conventional therapeutic approaches, this new paradigm offers a research agenda grounded in testable hypotheses, rigorous validation, and realistic appraisal of current capabilities. By embracing the complexity of the gut-brain axis and harnessing the power of AI to generate novel insights, the field is laying the foundation for a future in which the prevention and treatment of AD may eventually be guided by the unique microbial and molecular signatures of each individual, moving incrementally toward the goal of truly personalized medicine (Fig. 7).

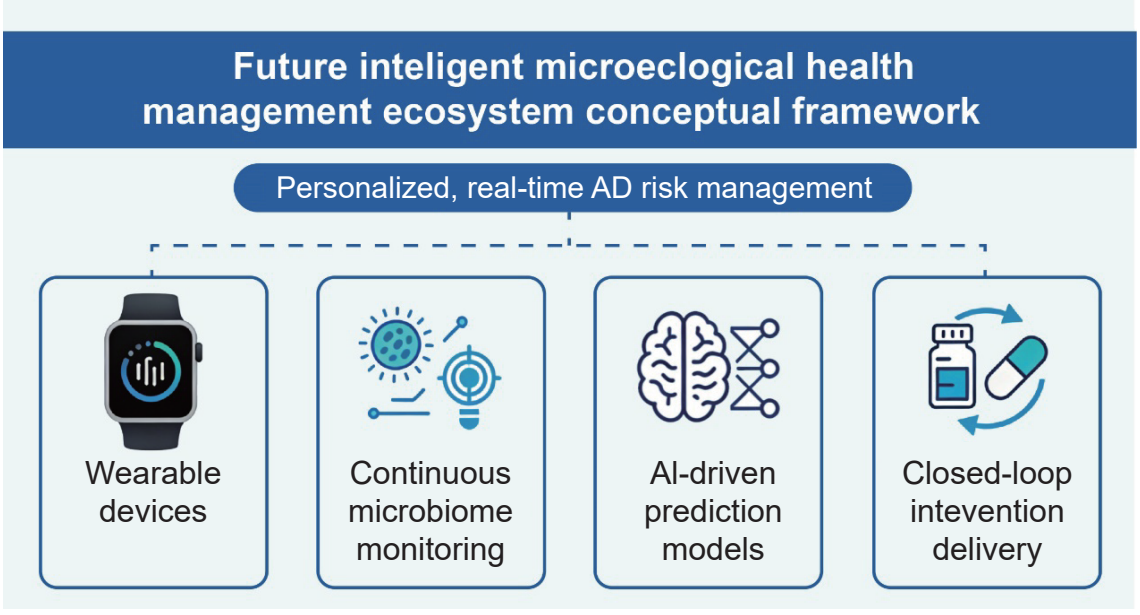

Figure 7 Schematic conceptual framework of the next-generation intelligent microecological health management ecosystem oriented toward personalized, real-time AD risk management. Conceptual framework of a future intelligent microecological health management ecosystem for personalized, real-time AD risk surveillance, integrating wearable sensing, continuous gut microbiome profiling, AI predictive modeling, and closed-loop microbiota-targeted therapeutic delivery.

Returning to the core question posed at the beginning of this review regarding drivers versus passengers, our critical examination of existing AI application scenarios leads to the following conclusion. What AI can effectively achieve at present is to prioritize the relationships most likely to have causal significance from a large number of candidate associations, thereby guiding the focus of subsequent experimental validation. However, AI itself cannot independently prove causation from observational data alone. Clarifying this boundary is essential for forming reasonable expectations about the role of AI in gut-brain axis research. In the future, an iterative research paradigm that combines AI-generated hypotheses with causal experimental validation such as FMT and pathway-specific interventions will represent a viable path toward ultimately answering this question.

Data availability

Not Applicable.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

Acknowledgements

None.

Funding

This work was supported by the National Natural Sciences Foundation of China (No. 82204645).

Author contribution statement

Yan Gao: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, validation, visualization, writing-original draft, writing-review & editing. Can Zhang: Conceptualization, data curation, investigation, methodology, project administration, validation, writing-original draft, writing-review & editing. Zhi-Wen Zhou: Conceptualization, data curation, investigation, methodology, project administration, validation, writing-original draft, writing-review & editing. Hong-Shuo Sun: Investigation, methodology, writing-original draft. Zhong-Ping Feng: Investigation, methodology, writing-original draft. Shan-Shan Jie: Investigation, writing-review & editing. Qian-Hang Shao: Investigation, writing-review & editing. Song-Wei Yang: Investigation, writing-review & editing. Qi-Di Ai: Investigation, writing-review & editing. Jia-Qing Yan: Conceptualization, funding acquisition, project administration, supervision, writing-review & editing. Nai-Hong Chen: Conceptualization, funding acquisition, project administration, supervision, writing-review & editing. Shi-Feng Chu: Conceptualization, funding acquisition, project administration, supervision, writing-review & editing. Zhao

Zhang: Conceptualization, funding acquisition, project administration, supervision, writing-review & editing. Wen-Bin He: Conceptualization, funding acquisition, project administration, supervision, writing-review & editing. All authors have read and approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

Not applicable.

Use of AI statement

During the preparation of this work the authors used deepseek in order to revised the grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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