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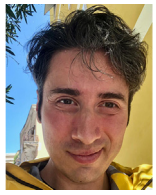
Machine Learning and Artificial Intelligence in the Multi-Omics Approach to Gut Microbiota



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The gut microbiome is involved in human health and disease, and its comprehensive understanding is necessary to exploit it as a diagnostic or therapeutic tool. Multi-omics approaches, including metagenomics, metatranscriptomics, metabolomics, and metaproteomics, enable depiction of the gut microbial ecosystem's complexity. However, these tools generate a large data stream in which integration is needed to produce clinically useful readouts, but, in turn, might be difficult to carry out with conventional statistical methods. Artificial intelligence and machine learning have been increasingly applied to multi-omics datasets in several conditions associated with microbiome disruption, from chronic disorders to cancer. Such tools have potential for clinical implementation, including discovery of microbial biomarkers for disease classification or prediction, prediction of response to specific treatments, and fine-tuning of microbiome-modulating therapies. The state of the art, potential, and limits, of artificial intelligence and machine learning in the multi-omics approach to gut microbiome are discussed.

Keywords: Machine Learning; Artificial Intelligence; Gut Microbiome; Precision Medicine.

The human gut microbiome refers to the ecosystem formed by trillions of micro-organisms, spanning bacteria, viruses, fungi, and archaea, that inhabit the gastrointestinal tract. These microbes are critical to human health because they influence immune maturation, regulation of metabolic pathways, processing of nutrients, drug metabolism, and several other functions.[1,2] The microbiome is a dynamic entity and it changes depending on several factors, such as the type of delivery, diet, drugs, and other environmental factors.[2] Moreover, the gut itself consists of several distinct local ecosystems that make microbial communities change significantly across different regions of the gastrointestinal tract.[3,4] The imbalance of the microbiome homeostasis has been linked to a number of chronic diseases, including metabolic disorders,[5] systemic autoimmune conditions,[6] and cancer.[7] Increasing evidence highlights that the gut microbiome plays a pivotal role in modulating immune responses, influencing carcinogenesis, and shaping therapeutic responses.[6,7] A comprehensive understanding of gut microbiome composition and functions and disease-related shifts is necessary to exploit the microbiome as a diagnostic and/or a therapeutic tool in clinical medicine. To unravel the dynamics that regulate the gut microbiome in health and disease, integrative approaches able to retrieve not only data on microbial composition and taxonomy but also on their functions are required.

In recent years, a comprehensive set of cutting-edge biotechnologies, collectively referred to as "multi-omics," have appeared in the landscape of biomedical research. Multi-omics integrate multiple layers of biological information to comprehensively and transversally analyze complex ecosystems, such as gut microbiome. When applied to the microbiome field, they mainly differentiate in

Abbreviations used in this paper: **AI, artificial intelligence; AUC, area under the curve; CRC, colorectal cancer; EHR, electronic health record; IBD, inflammatory bowel disease; ML, machine learning; RF, random forest; SL, supervised learning.**

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metagenomics, metatranscriptomics, metabolomics, and metaproteomics.[8] Metagenomics explores the DNA pool of microbial communities, offering insights into taxonomic and functional potential structure of the microbiome.[9,10] Metatranscriptomics focuses on the analysis of RNA sequences, highlighting active gene expression, RNA-based regulations, and microbial interactions.[10] Metabolomics is the analysis of small, biologically active molecules, products of metabolism and interactions between the microbes and their host.[10] Finally, metaproteomics investigates the protein content, identifying functional mediators and microbial contributions to host biochemistry and physiology.[11] Taken together, these methods provide a powerful framework to dissect the complex interplay between microbial communities and host biology.[8] For example, metagenomic and proteomic analyses offer detailed maps of microbial composition and functional properties,[8] and transcriptomic and metabolomic approaches add dynamic layers by revealing microbial activity and the biochemical interplay within the host environment.[12]

These technologies generate large amounts of data that may be used to identify microbial disease–related biomarkers, as well as infer putative mechanistic pathways underlying dysbiosis-related conditions, including metabolic; inflammatory; or neoplastic disorders.[13] These data-driven inferences are only achieved by means of integration of the partially independent data streams, which are proving to be difficult to carry out with conventional statistical methods. Therefore, the development and application of advanced analysis tools to multi-omics data are the current critical challenges needed to advance our knowledge on the gut microbiome and its application in medicine.

In recent years, artificial intelligence (AI) and machine learning (ML) have revolutionized the biomedical research field with the development, implementation, validation, and application of a wide range of tools to analyze and interpret complex biological data.[14,15] AI involves a variety of approaches that are designed to simulate human cognitive functions. ML, a subfield of AI, concerns algorithms that learn patterns directly from data and tend to improve with the amount of available training data.[16] These technologies are particularly useful for those data-intensive fields in which factors such as volume, variety, and velocity, challenge conventional approaches of analysis.

AI and ML have shown very promising capabilities in life sciences, including applications such as drug discovery,[17] improvement of diagnostic accuracy,[18] and personalized medicine.[19]

Due to its intrinsically complex nature, microbiome-applied multi-omics is a key area that can benefit tremendously from advancements in the AI/ML fields.[20] Due to the capacity of integrating datasets from multiple sources, these methods can detect relationships between large-scale heterogeneous multi-omics datasets[21,22]; predict biological functions of gut microbiome; and identify microbial biomarkers,[21] improving the understanding of host–microbiota interactions and inferring mechanisms that rule these relationships.[22] These technologies have been used mainly for strain-level resolved analysis and prediction of functional pathways and, being able to identify disease biomarkers and personalize therapeutic interventions has potential for exploitation of the microbiome in clinical medicine.[23] Considering the continuously growing complexity of microbiome data, AI and ML represent indispensable approaches that can enable discoveries that could not be achieved using more conventional methods.[24] In this review article, we provide an updated landscape of AI and ML in the multi-omics approach to gut microbiome.

Overview of Machine Learning and Artificial Intelligence Models in Multi-Omics Analysis

Despite the promising capabilities exhibited by AI and ML approaches for the integration and analysis of multi-omics data, their characteristics in terms of heterogeneity, high dimensionality, and sparsity of such types of data introduce important challenges for their understanding.[25] We provide an overview of the most common AI/ML methodologies in the multi-omics field and discuss their strengths and limitations (Table 1).

Supervised learning (SL) aims to establish a relationship between the input features (eg, omics data) and a predefined output label (eg, disease status or other phenotypic characteristics).[26] They need labeled datasets to build the models and are used for several predictive applications in multi-omics research. Random forests (RFs)[27] and gradient boosting machines[28,29] are among the most popular ensemble approaches for both classification (ie, for predicting categorical values) and regression (ie, for predicting continuous values) tasks. Such approaches gained popularity for their consistent accuracy performances due to their capabilities in preventing overfitting issues and managing high-dimensional feature spaces. Another important tree-based ensemble methodology is represented by XGBoost,[30] which usually exhibits high accuracy when integrating different data types and provides insights about feature importance and interpretability of the model. Further approaches include support vector machines,[31] an algorithm able to deal with nonlinear relationships thanks to kernel functions and that can be applied in both binary (ie, for discriminating between 2 categories/classes) and multicategory (ie, for discriminating among more than 2 categories) classification problems. More recently, deep learning models have become very popular[32] and have revolutionized the AI field, including applications in the multi-omics domain.[33] Because of their ability to learn complex nonlinear relationships, especially when dealing with large sample sizes, they represent a fundamental technique, given the continued increase in complexity and size of current and future datasets. Popular deep learning models rely on artificial neural networks[34] and convolutional neural networks.[35] Recurrent neural networks[36] and transformer architectures can be also be applied on time-series data, enabling the understanding of temporal patterns in longitudinal and time-series multi-omics data. Autoencoders,[37] a form of unsupervised deep learning, are often combined with supervised approaches to reduce

Table 1. Key Concepts of Some Artificial Intelligence and Machine Learning Methodologies in the Context of Multi-Omics Microbiome Research

Method	Key concept	Strengths	Disadvantages
Supervised learning	Make predictions from labeled data	Accurate predictions for classification and regression tasks	Can requires large labeled datasets; risk of overfitting
Random forests	Ensemble-based supervised learning	Prevents overfitting; robust to noise	Limited interpretability compared with other models
Gradient boosting machines	Sequential ensemble-based learning for improved accuracy	Accurate predictions	Computationally intensive; sensitive to hyperparameters
Neural networks	Deep networks for complex data	Capture highly nonlinear patterns; good scalability	"Black box" nature and limited interpretability; computationally intensive
Unsupervised learning	Identified patterns without labeled data	Useful for exploratory analysis (eg, clustering and dimensionality reduction)	Results can be subjective; sensitive to parameters
K-means clustering	Groups data into clusters based on similarity	Simple, fast	Requires specifying the cluster number; suboptimal for nonspherical clusters
Hierarchical clustering	Builds a tree-like structure to capture data similarity	No need to specifying the cluster number	Computationally intensive for large datasets
DBSCAN	Density-based clustering	Robust to noise	Requires fine-tuning of density parameters
Consensus clustering	Merge results from multiple clustering runs	Increase reliability of clusters	Sensitive to algorithm choice; computationally intensive
Principal component analysis	Linear dimensionality reduction; identifies variance sources	Simple interpretation; useful for dimension and noise reduction	Limited to linear relationships
t-distributed stochastic neighbor embedding	Nonlinear dimensionality reduction	Useful for visualization; preservers local structure	Sensitive to hyperparameter choice; computationally intensive
Uniform manifold approximation and projection	Nonlinear dimensionality reduction	More scalable than t-distributed stochastic neighbor embedding; preserves both local and global structure	Sensitive to hyperparameter choice; limited interpretability
Feature selection	Identifies relevant variables	Reduce noise; improves interpretability	Risks removing important variables
Semi-supervised learning	Combines unlabeled and labeled data	Useful in situations with scarce labeled information	Complex implementation
Explainable AI	Improve model interpretability	Increases usability in clinical contexts	Possibly reducing accuracy
Multi-omics integration	Combines datasets from various omics	Handles source heterogeneity; enables more comprehensive analyses	Methodological challenging
Federated learning	Integrates datasets from different institutions without data sharing	Promotes collaboration; maintains data privacy	Difficult implementation; requires dedicated infrastructure
Causal inference	Distinguishes causative from correlation relationships	Provides more mechanistic insights	Challenging implementation
Longitudinal analysis	Handles time-series data	Detect dynamic relationships	Requires advanced modeling; prone to overfitting

Table 1. Continued

Method	Key concept	Strengths	Disadvantages
Bayesian methods	Incorporates prior knowledge into modeling	Useful for small datasets	Requires good prior knowledge; can be computationally expensive
Batch effect correction	Aligns different datasets	Reduces variability across datasets; enable meta-analyses	Risk of introducing biases due to bad parameter selection
Scalability	Enhances computational efficiency for large datasets	Supports big data analysis	Requires dedicated infrastructure

dimensionality and extract latent features, enhancing prediction accuracy for tasks like disease classification or phenotype prediction.

Unsupervised learning[38] aims, instead, at finding hidden patterns and structure from data without the requirement to define labels *a priori*. Such models are very useful and important for different tasks, such as clustering, dimensionality reduction, and network analyses. The most popular method for clustering is K-means,[39] a partitioning technique that separates data into a certain number of clusters defined *a priori*. Its iterative process is very efficient computationally, but it assumes multidimensional spherical and similarly sized clusters, therefore, it can give poor results when dealing with noisy data or when cluster shapes are irregular. Another widely used strategy is represented by hierarchical clustering,[40] which provides a tree-like structure called dendrogram, exploitable at various levels of granularity. It relies on agglomerative (ie, it starts with each data point as its own cluster and merges clusters based on a linkage criterion in an iterative way) or divisive (ie, it starts with everything in a single cluster, then repeatedly splits it) approaches, which do not require to specific the number of clusters *a priori*, however, they are computationally costly for large datasets. Arbitrarily shaped clusters can be characterized through the density-based spatial clustering of applications with noise algorithm,[41] which identifies clusters as the high-density regions of the data points. It can filter out outliers effectively and is, therefore, quite robust in noisy datasets. More advanced clustering techniques include spectral clustering,[42] which performs clustering after reducing the dimensionality of the original feature set and can be effective when dealing with nonconvex clusters. There is also the opportunity to perform consensus clustering.[43] It relies on the summarization of several clustering runs, usually performed with different algorithms or different initializations, which can lead to higher robustness and reliability of the result. Another important task that usually relies on unsupervised learning is represented by dimensionality reduction, which is widely used to simplify high-dimensional multi-omics datasets into lower dimensions that can be explored for finding patterns, reducing noise, and identifying key biomarkers. Methodologies include both linear and nonlinear approaches, and some of them leverage deep learning for advanced feature extraction. The most popular linear dimensionality reduction method is principal component analysis,[44] which transforms the original dataset into a set of orthogonal components known as principal components. This technique is particularly useful for sample relationships, detecting batch effects, and identifying main sources of variation in data. Another linear methodology is singular value decomposition,[45] which decomposes the dataset into singular vectors and values through a matrix factorization technique, being quite efficient for denoising and dimensionality reduction in large datasets. Limitations of linear methodologies can be overcome by nonlinear ones, such as the case of multidimensional scaling.[46] Another nonlinear method is t-distributed stochastic neighbor embedding,[47] which aims at projecting the data in a low-dimensional feature space by means of preserving the local structure. This makes this approach useful for cluster visualization; however, it is computationally expensive and sensitive to hyperparameters (eg, perplexity). Compared with t-distributed stochastic neighbor embedding, the uniform manifold approximation and projection[48] algorithm is faster and tries to preserve both local and global structures of the data. Reduction of the dimensionality of the data can also be used through deep learning approaches, such as the case of autoencoders.[37] They represent a family of neural networks that comprise an encoder (which condenses original data into a latent representation) and a decoder (which instead reconstructs the original data from the latent representation). An extension of autoencoders is given by variational autoencoders,[49] which provides better generalization and interpretability by considering a probabilistic structure on the latent space. More advanced deep learning approaches, such as convolutional neural network and recurrent neural network, can be also exploited to extract features in a hierarchical way from multi-omics data, along with pretrained deep neural networks that can be transferred from natural language processing and image analysis to the multi-omics domain.[50] Unsupervised approaches can be also investigated for network-based modeling devoted to the identification of interaction networks from multi-omics data.[51,52] Examples are represented by weighted correlation network analysis[53] and Gaussian graphical models.[54]

Beyond SL and UL, semi-supervised learning[55,56] can be also adopted to incorporate both labeled and unlabeled data

to mitigate the problem in obtaining enough samples for building predictive models. Common techniques include self-training[57] (in which the model is trained on the small labeled dataset, labels for the unlabeled data are predicted, the confident predictions are added to the labeled set, and the process is iterated) and co-training[58] (in which multiple models are built on different sets of features, unlabeled data are labeled by each model, and predictions are used to train the other models) along with more advanced methodologies, such as those based on generative adversarial networks[59] and semi-supervised variational autoencoders.[49,60] The integration of different multi-omics sources can be used through quite sophisticated models, with the aim of dealing with issues related to heterogeneity, noise, and missing data. Although early integration relies on concatenating the different omics into 1 data structure, followed by single modeling, late integration instead processes the different layers separately and then integrates them at a later state.[61]

In addition to the relatively traditional approaches introduced above, other emerging lines of development are contributing to multi-omics data analysis through AI/ML solutions. Of great interest is the investigation of explainable AI,[62,63] which aims at proposing models that are not only accurate but also explainable and transparent in their output. This is very relevant to try to close the gap between the "black box" nature of many AI/ML algorithms, especially deep learning models,[64] and the interpretability of results to reinforce trustability in decision-making processes and extract meaningful biological connections for experimental validation. Advancements can be also achieved by integrating causal inference with AI/ML models to better infer mechanistic relationships in multi-omics data.[65,66] Hybrid models that connect statistical frameworks to deep learning approaches are also important to provide a probabilistic interpretation of complex relationships.[67] It is also relevant to consider scalability issues because multi-omics analysis is usually computationally intensive and the development of efficient workflows and distributed computing platforms is necessary.[68] Another relevant aspect is federated learning, which allows integration of data from multiple sources and institutions without necessarily sharing sensitive data directly.[69,70]

Artificial Intelligence and Machine Learning for Microbiome-Based Precision Medicine: State of the Art

The Potential of Machine Learning and Artificial Intelligence for Microbiome-Based Precision Medicine

Due to its inter-individual variability and plasticity, the gut microbiome represents a modulable target for precision medicine. However, the complexity of the communities characterizing the human microbiota, along with their intricate interactions with the host, have represented important limitations for their deep understanding using traditional analytical methods. Given the continuous increase in the number of samples and variety of acquired datasets, the integration of more advanced AI/ML techniques into microbiota and multi-omics research enables the identification of patterns not previously detectable.

In this scenario, integrative multi-omic technologies represent a promising approach that can be exploited to implement several aspects of precision medicine, including the discovery of disease biomarkers for the early diagnosis or prediction of disorders, and the personalization of treatments (Figure 1). Beyond human health, such advances

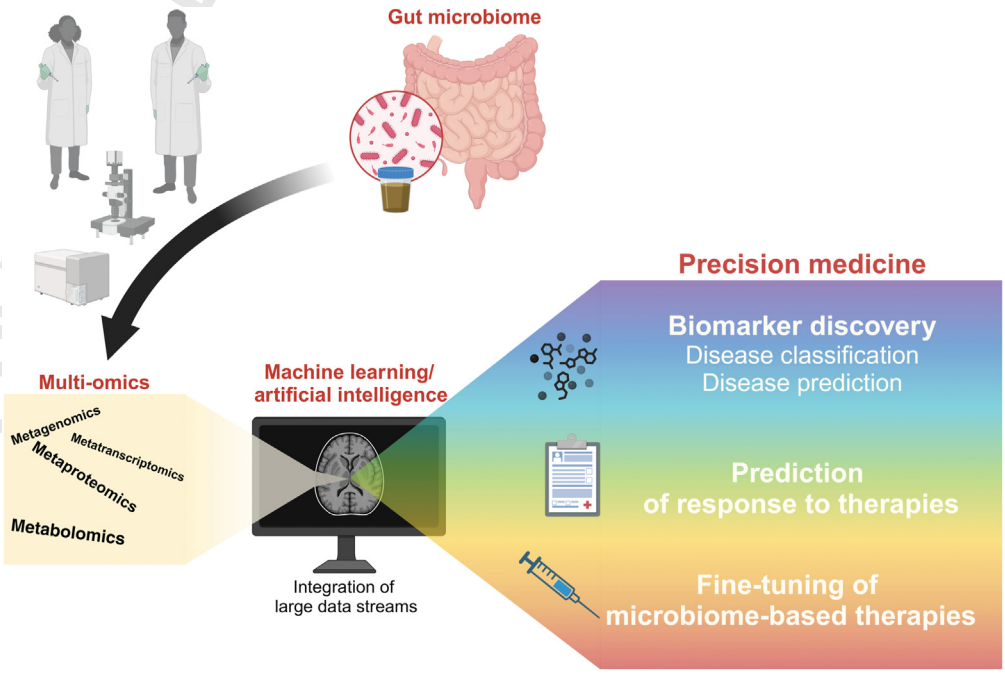

Figure 1. Machine learning and artificial intelligence-driven analysis of microbiome-derived multi-omics data: potential clinical applications.

can also contribute to other important and related areas, such as food safety[71,72] and environmental health,[73] raising the impact of microbiome science. As these AI/ML technologies continue to improve, health care management is gradually moving from a "one-size-fits-all" approach to a personalized one[74,75] with the microbiome playing a key role in many aspects.[76]

Biomarker Discovery for Disease Classification and Prediction

The proper integration of AI/ML approaches with multi-omics data can offer a better understanding of microbiome-associated diseases and corresponding dysbiosis and evolutionary trajectories.[21,77] These may also potentially contribute to the development of new diagnostic approaches that can help in early host-phenotype identification.

Advanced pipelines like DADA2 combined with Recursive Ensemble Feature Selection, for instance, integrate amplicon datasets with ML to enhance reproducibility and robustness in identifying biomarkers.[78] These tools have been investigated in several digestive and extra-digestive disorders associated with microbiome disruption, including cancer; chronic gastrointestinal disorders, such as inflammatory bowel diseases (IBDs); neuropsychiatric and neurologic disorders; metabolic disorders; and others.

Cancer. Colorectal cancer (CRC) probably represents the field in which the application of AI and ML to microbiome omics analysis has generated the most reliable results, with high potential of exploitation in clinical practice in the near future. CRC is a leading cause of cancer-related morbidity and mortality worldwide,[79] and its pathogenesis is strongly influenced by genetic and environmental factors, of which gut microbiome appears to be fundamental.[80] The development of multiclass ML classifiers (ie, models designed to classify data into multiple categories or classes) has improved diagnostic accuracy, addressing limitations of binary models. For instance, an RF-based multiclass model was able to distinguish CRC from other diseases with high accuracy (area under the receiver operating characteristic = 0.90–0.99) using species-level gut microbiome metagenomic data.[81] In another study, based on 3 different datasets collecting stool samples analyzed by 16S amplicon sequencing, a comparison of ML algorithms revealed RFs as the optimal classifier for CRC detection, with a precision of 0.729 and an area under the precision-recall curve of 0.668, further corroborating its utility in microbiome-based diagnostics.[82] Similarly, an explainable AI framework using Shapley Additive Explanations reproducibly uncovered key microbial markers of CRC, including *Fusobacterium*, *Peptostreptococcus*, and *Parvimonas*, while enhancing model interpretability by quantifying individual feature contributions.[82,83] Furthermore, integrating Shapley Additive Explanations values into principal component analysis enabled clearer separation of CRC subgroups.[83] Recently, 2 large metagenomic meta-analyses of 5 cohorts, in which the leave-one-dataset-out model and cross-cohort validation were applied, were able to identify taxonomic and functional microbial CRC signatures (relying specifically on oral bacteria as well as gluconeogenesis, putrefaction, and fermentation pathways). These microbial features were geographically reproducible and showed consistently high accuracy (mean area under the curve [AUC] = 0.84) in detecting CRC.[84,85] Another RF classifier effectively predicted KRAS mutation status in patients with CRC using amplicon-based microbiome profiles, identifying microbial markers, such as *Bifidobacterium* spp, which are associated with tumor–immune interactions and gene expression pathways.[86]

After proving effective in diagnosing CRC, microbiome-applied ML has started to be investigated in the detection of precancerous and early colorectal lesions. In a large (n = 971) cohort of patients undergoing colonoscopy, a cross-validated elastic-net regularized regression model was applied to the analysis of fecal metagenomes and was able to identify specific strains negatively (*Flavonifractor plautii*) or positively (*Bacteroides stercoris*) associated with colorectal adenomas and early carcinogenesis, and to infer functional pathways associated with tubular adenomas (depletion of microbial methanogenesis and mevalonate metabolism) or serrated adenomas (microbial antioxidant defense pathways[87]). Moreover, several ongoing initiatives (eg, ClinicalTrials.gov, number NCT06588166 and other projects[88]) are aimed to address, by means of exploiting ML, the reliability of gut microbiome for the institutional screening of CRC in subjects undergoing colonoscopy because of fecal immunochemical test positivity, with a potentially critical impact on CRC prevention.

Interestingly, ML was also used to identify gut microbiome biomarkers for early-stage lung cancer, using data derived from amplicon sequencing of stool samples collected from 76 patients with lung cancer. Using the minimal redundancy maximal relevance algorithm and backward selection with cross-validation, researchers refined 13 OTUs for optimal predictive accuracy. A support vector machine model achieved an AUC of 97.6% in the discovery cohort and 76.4% in the validation cohort, and the simplified Patient Discrimination Index maintained high accuracy for clinical application.[89]

Chronic gastrointestinal and extraintestinal disorders. Chronic gastrointestinal disorders are another subfield of ML application in microbiome research due to the large amount of evidence for a microbial contribution to their pathogenesis.

IBDs, which include Crohn's disease and ulcerative colitis, are characterized by dysregulated immune responses and microbial disruption within the gastrointestinal tract.[90] In IBDs, AI and ML algorithms have been found to be potentially useful in identifying microbial signatures and dysbiosis patterns associated with disease onset, progression, and treatment response.[91] Specifically, in a large multicohort dataset of nearly 6000 metagenomes, RF models identified specific bacterial clusters able to discriminate between IBDs and healthy controls, as well as Crohn's disease and ulcerative colitis, with high accuracy (AUC > 0.90).[92] Comparable findings were achieved using AI-driven tools to analyze microbial data across metagenomics, metatranscriptomics, metabolomics, proteomics, and host fecal calprotectin levels.[93]

AI and ML have also been applied to microbiome research involving metabolic disorders, providing a robust framework to uncover microbial features linked to conditions such as obesity, type 1 diabetes, type 2 diabetes, and metabolic dysfunction–associated steatotic liver disease. For instance, in 777 patients with or without insulin resistance, supervised ML algorithms, specifically RFs, analyzing 16S ribosomal RNA gene sequencing data, identified 10 microbial genera that reliably identified the presence of metabolic dysfunction–associated steatotic liver disease in insulin-resistant individuals, achieving superior predictive accuracy compared with traditional methods, but failing to get the same results in insulin-sensitive subjects.[94] ML frameworks have also depicted integrated microbial, lipidomic, and metabolomic signatures associated with type 1 diabetes, highlighting pathways, such as short-chain fatty acid biosynthesis and glycerolipid metabolism, that may mediate host–microbiota interactions.[95,96] Large-scale studies integrating microbial multi-omics data from obese patients have identified 42 species as consistent biomarkers across populations, with good performance in distinguishing obese people from healthy people (AUC = 0.85).[97] In type 2 diabetes, interpretable ML models like Light Gradient Boosting Machine[98] or based on an RF algorithm,[99] have demonstrated strong associations between microbial features (several species and functional pathways) and blood metabolites and glycemic control—assessed at different time points during follow-up visits[98]—suggesting their role in disease modulation and clinical translation.[98,99] In addition, population-based studies have linked shifts in the gut microbiota, particularly the decrease of butyrate-producing bacteria, with prediabetes and type 2 diabetes, paving the way for microbiome-targeted interventions.[99]

Recently, ML and AI have been investigated in neurologic and neuropsychiatric disorders. ML also identified a multikingdom gut microbiome panel as biomarkers for autism spectrum disorder using stool metagenomic sequencing and phenotype data from more than 1600 children, revealing 14 archaea, 51 bacteria, 7 fungi, 18 viruses, 27 microbial genes, and 12 metabolic pathways significantly altered in autism spectrum disorder.[100] An RF model achieved an AUC of 0.91, demonstrating high diagnostic accuracy, while pathways like ubiquinol-7 and thiamine diphosphate biosynthesis, identified through R package MaAsLin2, emerged as key contributors to classification, making this multikingdom approach a significant step forward in biomarker discovery for autism spectrum disorder.[100] Moreover, RF classifiers and the Boruta algorithm were able to identify key microbial taxa, such as *Dorea formicigenerans*, *Oscillibacter* sp 57_20, and *Faecalibacterium prausnitzii*, as potential biomarkers for distinguishing preclinical Alzheimer's disease from cognitively normal individuals, even in the absence of canonical Alzheimer's disease biomarkers, like amyloid-β and τ protein.[101]

Multidrug-resistant bacteria. AI and ML have also been applied to fight the growing burden of antibiotic resistance, which claims more than 1 million lives annually.[102] By integrating genomic, metagenomic, and proteomic data, these tools have identified key resistance genes, such as β-lactamases and efflux pumps, and clarified how gut bacteria adapt under antibiotic pressure, particularly in vulnerable populations like formula-fed infants.[103,104]

ML models have also been used to map how antibiotic treatments reshape microbial communities, uncovering patterns of resistance gene enrichment and persistence.[104] Moreover, AI has enabled the discovery of new antimicrobial peptides from vast microbiome datasets, as exemplified by the AMPSphere catalog, which predicted nearly 1 million antimicrobial peptides with potential to combat drug-resistant pathogens.[105] These discoveries support global efforts against this increasingly critical issue.

Prediction of Treatment Response and Fine-Tuning of Microbiome Therapeutics

One of the most promising applications is represented by the stratification of patients according to their microbial signatures and multi-omics profiles.[106] This can enable the identification of subgroups of patients that can respond, or fail to respond, to specific interventions. Advanced AI/ML methodologies may further provide predictions on the individual responses to therapies, reducing trial-and-error prescription and improving clinical outcomes. The use of predictive models may also have a significant role in evaluating the response to therapy in a longitudinal way, with the possibility to change the therapeutic strategies.[107] Moreover, AI modeling may contribute to the identification of novel targets and microbiome-targeting therapeutics that could consider metabolites of microbial origin and host–microbe interactions. These approaches have generated evidence in different fields of medicine.

Prediction of response to treatments. AI and ML have been applied to gut microbiome data with the aim of predicting response to specific treatments. This approach has been increasingly investigated in cancer immunotherapy. ML models have linked baseline gut microbiota composition to response to immune checkpoint inhibitors, with specific taxa like *Akkermansia muciniphila* and *F prausnitzii* emerging as biomarkers predictive of efficacy across diverse cancer types.[108,109] Strain-resolved metagenomics has further refined predictive accuracy, revealing functional variation within microbial species associated with therapeutic outcomes.[109] In a cohort of 245 patients with non–small cell lung cancer, a metagenome-based, species-level co-abundance network was capable of identifying 2 main microbial clusters, negatively and positively associated, respectively, with response to immune checkpoint inhibitors, and was also successfully validated in other cancer types.[110]

In addition, in a cross-cohort analysis of 172 patients undergoing CD19 CAR-T cell therapy for lymphoma, ML models based on shotgun metagenomic sequencing data, identified key microbial taxa, such as *Akkermansia*, *Bacteroides*, and *Eubacterium*, that were strongly associated with immune activation and long-term responses. The study also found microbiome functional pathways, such as peptidoglycan biosynthesis, that predicted CAR-T therapy success and may potentially guide patient selection and management.[111]

In addition, AI tools using RF classifiers have identified, using metagenomic and transcriptomic data, microbial signatures that distinguish immune-related adverse events with high accuracy (AUC = 0.88), implicating microbial metabolites, like menaquinone, as mediators of toxicity.[112]

Artificial modeling approaches have also been preliminarily investigated in nonpharmacologic treatments. A 2-year longitudinal study of a nonsurgical weight-reduction program identified bacterial genera, including *Akkermansia*, as key predictors of sustained weight loss. Based on metagenomic analysis of gut microbiome, ML-driven statistical modeling accurately classified 14 of 15 participants into responder or nonresponder categories.[113] Further evidence of the microbiome predictive potential comes from studies on diabetes prevention. A randomized controlled trial involving prediabetic individuals used ML algorithms to assess baseline microbiome features and predict response to exercise. Responders exhibited microbiota-driven metabolic benefits, such as increased short-chain fatty acid production and improved insulin sensitivity.[114] These findings suggest that microbiome-targeted interventions could maximize the metabolic benefits of lifestyle modifications.

Fine-tuning of microbiome therapeutics. AI and ML may be potentially exploited in a pioneering field that is the metagenome-informed targeting of microbiome-modulating therapies. For example, in a study investigating prebiotics for calcium absorption in adolescents, support vector machine based on amplicon sequencing identified 19 microbial features able to predict responders to soluble corn fiber administration with 96% accuracy, paving the way for a targeted microbiome-driven improvement of bone health during critical growth phases.[115] In a multicenter randomized controlled trial of patients with irritable bowel syndrome, AI-assisted diets based on the individual patient microbiome profile were compared with standard low FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) regimens. Although both treatments improved symptoms and the Irritable Bowel Syndrome Symptom Severity Scale, the personalized diet demonstrated superior efficacy over low-FODMAP diet in improving quality of life and microbiome α-diversity.[116]

Finally, in a metagenomic meta-analysis of 24 cohorts, a leave-one-dataset-out model was able to predict the most effective donor to provide a successful microbiome engraftment in patients undergoing fecal microbiota transplantation for different disorders, paving the way for a more refined and efficacious selection of stool donors.[117]

Roadmap to Clinical Implementation

These applications exemplify how AI bridges the gap between microbiome complexity and actionable insights, potentially enabling clinicians to tailor interventions with unprecedented precision. This integration of computational and biological sciences is happening now more than ever, driving research advancements and making a tangible impact on clinical practice, marking the dawn of a new era of precision medicine shaped by the gut microbiome's intricate contributions to health and disease. However, this advancement is encumbered by a number of pitfalls and challenges in microbiota research and in AI/ML approaches.

Gaps and Challenges in Microbiota Research

Several factors related to the methodology of microbiome studies might influence the correct application of AI/ML tools to microbiome data, and then their further clinical implementation.

A first set of issues concerns the design of microbiome trials. Generally, several microbiome studies have been criticized, as they often fail in determining causality of association between microbial signatures and clinical outcomes,[118] which is crucial for the clinical application of their findings. Moreover, the correct selection of the study population is critical to answer specific questions. For example, current associative data of microbiome signatures and CRC are mostly based on patients with a diagnosis of advanced cancer,[84] and although highly interesting, are not applicable to screening populations, who deserve specific investigations.[88] Also the discrepancy in patient eligibility criteria and disease definition might bias study outcomes and, consequently, the data to be elaborated by AI/ML tools. Finally, a comprehensive evaluation of host variables and assessment of their potential confounding effect on final results is another aspect to be included in the design of microbiome studies.[119]

Another block of gaps relates to the technicalities of microbiome research, including sample collection and storage, DNA extraction and sequencing, processing, and profiling. Differences in these technical steps of the analysis workflow may confound results as well.[120–122]

In recent years, a number of efforts have been directed toward standardizing different aspects of the microbiome field. For example, the STORMS (Strengthening The Organization and Reporting of Microbiome Studies) checklist has been built to provide guidance for reliable and comprehensive reporting of microbiome studies, with the aim of facilitating their understanding and the comparative analysis of their findings.[123] Other standardization approaches include the definition of criteria to release microbiome testing[124]; role of microbiome biomarkers[125]; guidelines on different methodological steps of microbiome analysis, from sample collection, to genome extraction; library preparation; and others.[126,127]

Altogether, these initiatives are expected to fill the current gaps of microbiome research, facilitating the correct application of AI/ML to this field.

Pitfalls and Potential Concerns About Artificial Intelligence and Machine Learning Approaches

Despite the very promising opportunities exhibited by AL/MI approaches in the microbiota field, some bottlenecks still need to be overcome before they can be successfully translated into clinical practice.[128] Table 2 summarizes challenges and limitations that arise when AI and ML are applied to multi-omics approaches to gut microbiota. To overcome these challenges, complex, multidimensional, and

Table 2. Challenges and Limitations of Artificial Intelligence and Machine Learning in Multi-Omics Research

Challenge/limitation	Explanation
Data heterogeneity and complexity	Multi-omics datasets often comprise different data formats and noise levels, which complicates their integration and analysis.
Data incompleteness	Multi-omics data are often incomplete, which requires the use of imputation methods that may introduce biases in the analysis.
Data scarcity	High-quality datasets are often limited, especially when dealing with labeled information, which makes the development of robust and generalizable models challenging.
Lack of standardization	Acquisition of data along with their processing are usually performed using different experimental platforms and protocols in different studies, which introduce batch effects that are challenging to correct and can bring inconsistent results.
Computational complexity	The collection, processing, and integration of multiple data sources require substantial computational resources and time, aspects that are further highlighted when dealing with deep learning.
High dimensionality	The large number of features, especially when dealing with several sources, compared with the relatively small sample size reduces model performance due to possible overfitting issues.
Scalability issues	Large-scale studies generate large datasets, which poses challenges for model scalability and real-time analysis.
Accessibility of tools	Many researchers lack the required expertise to design and interpret AI/ML methodologies, which can come with the lack of access to user-friendly tools and interfaces.
Limited biological context	Many AI/ML models do not incorporate prior biological knowledge, which limits interpretability and relevance.
Biological and clinical interpretability	Many AI/ML models, especially based on deep learning technologies, are still considered "black boxes," which makes it difficult to derive biologically meaningful insights.
Identification of causative relationships	Although AI/ML models tend to identify correlations, it is still challenging to distinguish causative relationships from associations.
Model validation	Proper validation of predictive models is challenging due to the scarcity of independent datasets and benchmarks.
Cross-study generalization	Many AI/ML models trained on specific studies fail to generalize across cohorts and populations.
Dynamics of the gut microbiome	The gut microbiota is highly dynamic and influenced by multiple factors, while most available datasets usually provide single snapshots of the microbial community that do not capture temporal changes.
Ethical and privacy issues	Using sensitive data raises concerns about patient privacy, data sharing, and compliance with data protection regulations.

multidisciplinary work that involves validation of the models, standardization of regulatory aspects, and integration into electronic health records (EHRs) is needed.

To accomplish clinical applicability,[129] the deployed models need to be rigorously validated. The models should be robust, reproducible, and generalizable across diverse datasets.[130] This includes multisite validation using independent cohorts.[85] Variations in microbiome composition due to geographical,[131] dietary,[132] and host genetic[133] factors should also be considered in such a validation study. Such studies should also consider data incompleteness, as multi-omics datasets often have missing values that require proper imputation techniques,[134] which, however, may introduce biases. High dimensionality, coming from the large number of features relative to sample size, poses overfitting risks, which further emphasize the need for robust validation.[135] Prospective studies are also very important to ensure good reliability of the models over time, given the dynamic nature of the gut microbiota. These temporal variations, in addition to detecting causative relationships rather than just correlations, demand proper modeling techniques able to capture the dynamics of microbial communities and causal inference.[136]

This should also come with understandable and explainable models that can bring trust from clinicians and successive adoption in the practice.[137] Many AI/ML models are often perceived as "black boxes."[138] Utility in a clinical setting is limited by this lack of interpretability, and clinicians require clear and transparent decision-making processes to ensure the safety of the patients. Interpretability can also be enhanced by considering models that incorporate prior biological knowledge. This should be also complemented by

open-access databases and standardized pipelines to ensure reproducibility in analyzing multi-omics data.[139] The lack of standardization in data acquisition and processing methods often introduces batch effects that must be properly addressed to have cross-study generalization.[140]

Moreover, assuming that the application of AI/ML to gut microbiome may be useful in clinical practice, these tools should then be provided with a timely and high-throughput fashion and on a per-patient basis, as other diagnostic tools, to allow a prompt optimization of the patient management. This may represent a challenge, as proper frameworks for microbiome analysis are not yet widespread. However, the diffusion of sequencing machines, and of AI/ML approaches, is increasing progressively, along with a decrease in related costs.[141] In addition, recent efforts to standardize the field of microbiome testing for clinical practice are paving the way to overcome this barrier.[124]

Another important translational clinical challenge is represented by regulatory approval.[142,143] Strong evidence needs to be provided in terms of safety, along with clinical utility, including improvement in diagnosis, decisions on treatments, or patient outcomes over current care standards.[144,145] It is also important to provide high standards regarding data protection and privacy issues.[146,147] These issues are further impacted by the lack of proper guidelines for evaluating performance and safety of AI/ML models in health care.[148] Processes for regulatory approvals should comprise collaboration among multiple parties, such as clinicians, developers, and experts in regulations including those involving AI aspects.

Successful implementation of AI/ML models should involve their proper integration in EHR systems[149] for effective real-time clinical decisions.[150] Such integration should also involve interoperability between current EHR devices and future AI/ML systems using standardized protocols to ensure that data are ported easily. Multi-omics data coming from microbiome sequencing platforms and other laboratory equipment should be automatically imported into EHRs. At the same time, output of the AI models, such as predicted risk scores or recommended interventions, should be presented in a proper intuitive format to the clinician.[151] Such outputs should be provided with additional information, such as patient history, laboratory results, and other relevant data, to provide personalized treatment strategies. Proper training and education on AI systems should also be performed to give clinicians the skills required for the use of such tools.[152,153]

In this scenario, user-friendly platforms integrating AI/ML for biomarker discovery and disease prediction have also been developed. Among others, IntelliGenes[154] integrates statistical and ML methods, providing a customizable interface that is easy to understand, even for nontechnical users. This platform supports comprehensive analysis workflows, from data preparation to model visualization, and emphasizes interpretability and reproducibility, making AI a potentially useful item for every research team. However, the scalability of such platforms remains challenging, especially for large-scale studies that can generate big datasets requiring non-negligible computational resources for processing, integration, and real-time analysis.[155]

Finally, despite the promising results achieved so far, further evidence is still needed to understand the real value of AI/ML in the microbiome field. AI/ML is currently a catchy and attractive field, therefore, it is tempting to consider it superior to current standard predictors. However, the mere availability of such tools is not sufficient for their established implementation in clinical practice, which should be supported by reliable proof of superiority over standard approaches in pertinent comparative studies.

Practical Insights for the Application of Artificial Intelligence and Machine Learning Methodologies

The adoption of AI and ML methodologies in gastroenterology requires a multidisciplinary approach involving clinical, technical, and organizational expertise.[156] We provided some insights that could lead to their rapid and successful implementation. First, the training of clinicians is important for acquisition of basic methodological concepts and prospects on their applications (eg, through interdisciplinary seminars and practical workshops that show how to interpret AI/ML results). The phase of model development should also be conducted in a multidisciplinary environment in which gastroenterologists should collaborate with data scientists and engineers[157] to develop techniques in agreement with gastroenterology workflows (the output must be clinically meaningful and actionable). This should also encompass the proper integration of models within EHRs and existing infrastructures. Although meta-omics analyses are often conducted in single cohorts, validation across different cohorts and populations is important to obtain models that are robust to geographic and demographic patterns. This should also be performed by limiting batch effects through standardization of data collection and processing protocols,[139] in addition to guaranteeing reproducibility of the results.[158] Methodological advancements should also be prioritized toward explainable AI solutions,[83] so outputs from these models would be trusted by clinicians. AI implementation must be fully compliant with data privacy regulations, sensitive data should be processed on secured platforms, and interaction with regulatory agencies is important to determine the best procedures for implementation and evaluation of AI tools.[159] Federated learning approaches can be useful to enforce collaboration among different institutions without actual sharing of data. Clinicians should also report feedback about model performance and usability to provide active mechanisms of improvement of the framework. Patients should also be educated to improve their trust in AI tools and limit their concerns about data security and transparency of decisions.[160] It is also important to invest in the necessary computational infrastructures (including graphics processing unit–based[161] and cloud-based[162] platforms) to have real-time processing and integration of large data volumes. By following these recommendations, gastroenterology teams can advance the field to establish the

frameworks necessary to integrate AI/ML solutions into standard practice with the final aim of enhancing patient care and clinical outcomes.

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CrediT Authorship Contributions Q7
■■■

Conflicts of interest Q8
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