CellPress

Cell Metabolism

Perspective

From microbiome to metabolism: Bridging a two-decade translational gap

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https://doi.org/10.1016/j.cmet.2025.10.011

SUMMARY

The mapping of the human genome sparked high expectations for biomedical breakthroughs, yet attention has since shifted toward the human microbiome as a key player in health and disease. Pioneering studies revealed striking inter-individual variability and numerous associations between gut microbiota and a wide range of conditions (i.e., obesity, diabetes, cardiovascular and inflammatory bowel diseases, autism, allergies, neurodegenerative diseases, and cancers). However, the field has faced a deluge of correlative "dysbiosis" studies with limited causal evidence. Although animal models have provided crucial mechanistic insights, translating these findings to humans has proven challenging. Interventions such as fecal microbiota transplantation, prebiotics, probiotics, and postbiotics often yield inconsistent or modest effects in clinical trials. This gap highlights the need for precision, functional profiling, and integration of multi-omics , for instance, through artificial intelligence. In this perspective, we discuss what microbiome research offers as a transformative shift and how we conceptualize disease, favoring systems biology and personalized interventions over reductionist approaches.

INTRODUCTION: FROM THE GENOME TO THE MICROBIOME REVOLUTION

The dawn of the 21st century coincided with a wave of optimism and elevated expectations in biomedical research, when it was announced that the human genetic code had been fully sequenced. Many hoped this landmark achievement would unveil the root causes of complex diseases and resolve many long-standing mysteries in medicine. Building on the momentum of the Human Genome Project and taking advantage of newly accessible sequencing technologies, scientists quickly turned their attention to the vast and largely unexplored genetic material residing within and on our bodies, that is, the human microbiome. The recognition that humans coexist with trillions of microbial cells and collectively harbor over 100 times more genes than the human genome prompted the conceptualization of the microbiome as our "second genome."[1]

This paradigm shift was catalyzed by two landmark international initiatives: the Human Microbiome Project (HMP) in the United States (US)[2] and the Metagenomics of the Human Intestinal Tract (MetaHIT) project in Europe.[3] Launched in 2007, the HMP aimed at characterizing the microbial communities from multiple human body sites and at exploring their role in health and disease.[2] In parallel, the MetaHIT consortium focused on the gut microbiome, producing the first comprehensive gene catalog of the human intestinal microbiota, and revealing over 3.3 million microbial genes identified from fecal samples of healthy individuals.[3] These efforts provided foundational reference datasets and demonstrated remarkable inter-individual variability in microbial composition and function, even among healthy people.

What emerged was a vision of the microbiome as a dynamic, symbiotic ecosystem that is intimately involved in processes ranging from digestion and immune maturation to host metabolism and even neurobiology.[4,5] Importantly, this shift from a human-centric to a holobiont perspective laid the groundwork for exploring how alterations in microbial communities might contribute to complex diseases, including metabolic disorders such as obesity,[6–8] diabetes,[9] cardiovascular disease,[10] and metabolic dysfunction-associated steatotic liver disease (MAFLD),[6,7,11] as well as neurological,[12,13] inflammatory,[7] and even oncological conditions.[14]

While advances in sequencing technologies, metagenomics, and metabolomics have deepened our insights into host-microbe interactions, the translation of these findings into clinically actionable interventions remains limited, a gap this perspective critically examines in relation to metabolic disorders.

THE "DYSBIOSIS DELUGE": ASSOCIATION WITH(OUT) CAUSATION

Besides the well-established causal relationship between *Clostridioides difficile* infection and colitis[15] or *Helicobacter pylori* and gastric ulcers,[16] for which there are no doubt about

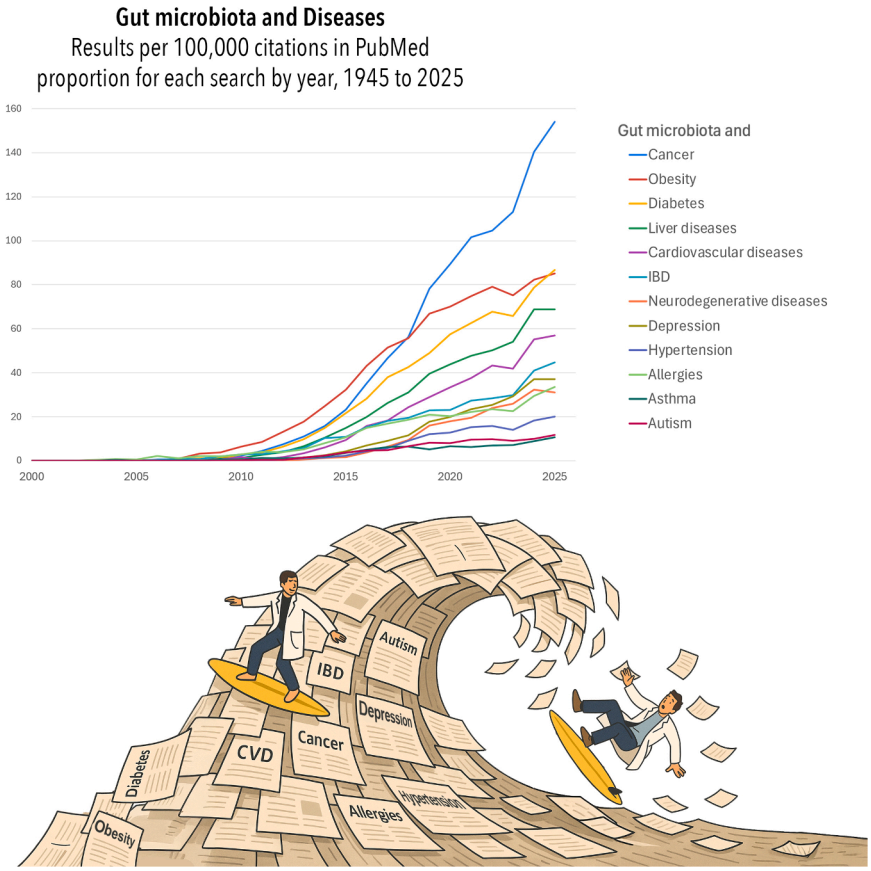

Figure 1. Surfing on the hype: The microbiome-disease association tsunami

Early microbiome research rode a wave of enthusiasm, linking dysbiosis to numerous diseases. But as the field matured, inconsistent results and limited clinical translation risked stalling momentum. The panel with the number of publications per 100,000 citations in PubMed was made with PubMed by Year (http://esperr.github.io/pubmed-by-year) using keywords "gut microbiota and (the disease listed)" on June 17, 2025.

causality, most conditions studied to date present a far more complex picture, where the microbiota may act as a driver, a modifier, or merely a bystander. In the years following the initial mapping of the human gut microbiome, there has been a surge of studies seeking to define how microbial communities differ in health and disease. This led to what might be described as a "dysbiosis deluge" with a dramatic increase in reports linking compositional shifts in the gut microbiota to nearly every chronic condition. These included classical metabolic disorders such as obesity,[17–19] type 2 diabetes,[20] liver diseases,[21,22] and cardiovascular disease,[23–25] as well as a broad range of human diseases, including allergies[26,27] and asthma[28] and inflammatory conditions like inflammatory bowel disease (IBD),[29–32] irritable bowel syndrome (IBS),[33] and rheumatoid arthritis.[34] Over time, this wave of association studies expanded to include neurological and neurodevelopmental disorders, such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, autism spectrum disorder (ASD), and major depressive disorder (MDD). As the list grew, our group, along with others in the field, began to critically reflect on the expanding scope of these associations, asking whether "the gut microbiota is at the intersection of everything"[35] (Figure 1).

As studies increasingly linked gut microbiota alterations to diverse disease states, the term "dysbiosis" started to emerge as a broad, often loosely defined label to describe alterations in the gut microbiota associated with disease (Figure 1). While lacking a universally accepted definition, it is generally used to denote a deviation from a presumed healthy microbial state, often characterized by reduced diversity, loss of putatively interesting taxa, and overrepresentation of microbes associated with inflammation or instability. Commonly cited features include a decline in butyrate-producing Firmicutes and an enrichment of Proteobacteria, a phylum frequently linked to stress or immune activation.[36] Early studies highlighted specific compositional patterns, such as an increased Firmicutes-to-Bacteroidetes ratio in obesity[37] or the depletion of *Akkermansia muciniphila*, a mucin-degrading species associated with metabolic regulation.[38,39] However, as more data accumulated, inconsistent replication across studies, populations, and sequencing methods began to reveal the limitations of compositional biomarkers as robust, generalizable biomarkers of disease.

Crucially, most of these studies were largely cross-sectional and correlational. As a result, the directionality of the observed associations remained ambiguous. Were microbial shifts a cause, a consequence, or merely a byproduct of disease-related factors such as lifestyle, diet, inflammation, or medication use? This challenge is exemplified by metagenome-wide association studies (MGWASs) in type 2 diabetes, which consistently reported depletion of "butyrate producers,"[40] but later analyses showed that these patterns could be largely attributed to metformin use, rather than the disease itself.[41] Similarly, while certain taxa like Proteobacteria are often elevated in IBD, this may reflect an adaptive response to environmental stressors, rather than a pathogenic role. This perspective is particularly relevant in the context of post-bariatric surgery microbiota profiles, where compositional shifts are pronounced but not necessarily detrimental.[42–44] Indeed, Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy trigger profound ecological shifts within hours to days, driven by altered gastric pH, accelerated nutrient flow to the jejunum, and a surge in luminal bile acids.[44,45] These changes select for taxa such as facultative Proteobacteria (e.g., *Escherichia*, *Klebsiella*) and oral-derived genera (e.g., *Streptococcus*, *Veillonella*), and they coincide with marked improvements in weight, insulin sensitivity, and metabolic health. Multi-omics analyses revealed that post-RYGB microbiota are

functionally enriched for propionate production, 7α-dehydroxylation, bile-acid deconjugation, and branched-chain amino acid catabolism, together with functions that plausibly enhance GLP-1 secretion, bile acid-activated farnesoid X receptor (FXR)/TGR5 signaling, and host energy expenditure.[44,46–48] Hence, these compositional shifts represent adaptive responses to a restructured gut environment, rather than pathological signatures. This illustrates our broader point: microbiota should not be classified as "healthy" or "dysbiotic" solely on the basis of taxonomy; rather, functional readouts, host physiology, clinical context, and temporal dynamics must frame the interpretation.

This need for multidimensional assessment extends beyond post-surgical contexts. Even foundational biomarkers like the Firmicutes/Bacteroidetes ratio have proven unreliable across cohorts. A 2016 reanalysis showed no consistent direction of change across obesity studies, and population-level research suggests that intra-individual variability over time may exceed differences between disease states.[49] This observational bias has raised concerns in microbiome research, where correlative patterns are often overinterpreted as indicative of mechanistic or pathogenic relationships. However, correlation is neither necessary nor sufficient to establish causation, emphasizing the need for more rigorous causal inference methods in microbiome studies[50] or clear causality studies.[35,50] As noted in a 2020 *Cell* editorial, the field risks losing credibility when every disease is seen as transmissible through gut microbiota or correctable by probiotics, despite insufficient causal evidence.[51]

Still, studies revealing states of dysbiosis are not without merit. These association studies have established the microbiota as a point of interest across a wide range of human diseases and generated a wealth of hypotheses to test. The next step is to move toward causality through mechanistic studies, to consider how microbes interact as communities and how their collective functions influence host pathways, and to refine the concept of dysbiosis. Only by doing so can the microbiota be confidently integrated within mainstream biomedical discourse on health and disease.

Of note, beyond bacteria, emerging work shows that gut Archaea (e.g., *Methanobrevibacter smithii*), fungi (the mycobiome), and the gut virome can modulate health and metabolism. This perspective will not cover these aspects, although they are extremely important, and strain-resolved multi-kingdom profiling remains challenging. Therefore, integrating these data streams is essential for a complete view in the future.

FROM CORRELATION TO CAUSATION: INSIGHT FROM ANIMAL MODELS

To move beyond association and investigate causal links between the gut microbiota and host metabolism, researchers turned to controlled animal models (e.g., germ-free, gnotobiotic, and antibiotic-treated mice). These systems allow for targeted manipulation of microbial communities while controlling for confounding factors such as diet, environment, and host genetics, revealing that gut microbes are not passive bystanders but active modulators of host physiology.[25]

One of the seminal discoveries in this field came from experiments showing that colonization of germ-free mice with microbiota from conventionally raised counterparts led to increased fat deposition and insulin resistance, even in the absence of increased caloric intake. This microbial-induced adiposity was attributed to enhanced energy extraction from the diet and suppression of host pathways that inhibit fat storage (e.g., fasting-induced adipocyte factor [FIAF]). These findings, by Bäckhed and colleagues, offered some of the first causal evidence that gut microbes influence host metabolism.[6]

Building on these insights, other studies explored potential mechanisms by which the microbiota might drive metabolic disease. Cani and colleagues proposed that gut microbes might also contribute to the low-grade inflammation characteristic of metabolic diseases. They introduced the term "metabolic endotoxemia" to describe a modest but chronic elevation in circulating lipopolysaccharide (LPS), a pro-inflammatory component of Gram-negative bacteria. High-fat feeding increased plasma LPS levels, coinciding with altered gut microbiota composition and impaired barrier function.[7,9] Mechanistically, they demonstrated that chronic low-dose LPS infusion could recapitulate features of metabolic disease, including insulin resistance, adipose tissue inflammation, and hepatic steatosis, while mice lacking the LPS co-receptor CD14 were resistant to these effects.[7,9] More recent evidence suggests that metabolic endotoxemia is driven less by overall Gram-negative burden and more by impaired gut barrier integrity, which allows translocation of pro-inflammatory bacterial products into the circulation.[9] In addition, the structural class of LPS is now recognized as a critical determinant of its immunological impact: hexa-acylated LPS species are strongly pro-inflammatory, whereas penta-acylated LPS or lipooligosaccharides (LOSs) tend to be neutral or even anti-inflammatory.[52,53]

Further evidence came from microbiota transplantation experiments. Turnbaugh et al.[18] demonstrated that germ-free mice colonized with microbiota from mice with obesity gained significantly more fat than those colonized with microbiota from lean mice, despite similar diets.[18] This study introduced the concept of an "obese microbiome" capable of transferring metabolic phenotypes. This finding was later extended to human microbiota in the now-famous "twin transfer" experiment, in which microbiota from human donors with obesity induced greater adiposity in mice than microbiota from their lean co-twin.[54]

Beyond fat accumulation, gut microbiota also modulate glucose homeostasis, lipid metabolism, bile-acid transformation, and immune signaling. Vijay-Kumar et al.[55] showed that mice lacking Toll-like receptor 5 (TLR5), a component of the innate immune system, developed metabolic syndrome-like features (obesity, insulin resistance, and hyperlipidemia), which were transmissible to wild-type mice via co-housing, implicating altered microbiota as a driver of disease in this model.[55]

Similarly, inducible deletion of MyD88 (myeloid differentiation primary response gene 88) specifically in intestinal epithelial cells confers partial protection against high-fat diet-induced obesity, insulin resistance, hepatic steatosis, and inflammation. This protective effect was associated with enhanced energy expenditure, improved glucose homeostasis, reduced adiposity, and a more anti-inflammatory immune profile. Notably, these benefits were transferrable to germ-free mice via microbiota transplantation, underscoring the key role of host-microbe interactions in mediating metabolic outcomes.[56] Mechanistically, MyD88 deletion restored antimicrobial peptide expression, increased

intestinal regulatory T cells, and elevated anti-inflammatory endocannabinoid levels. Remarkably, even when targeted after obesity had developed, MyD88 deletion led to reduced adipose inflammation and fat accumulation, identifying intestinal epithelial MyD88 as a nutrient-responsive metabolic sensor and a promising therapeutic target for metabolic disease intervention.[56] Although MyD88 is best known as the adaptor protein mediating inflammatory signaling downstream of TLRs, accumulating evidence indicates it also functions as a nutrient-responsive metabolic node. Saturated fatty acids, other TLR4 agonists, and diet-enhanced LPS act as ligand pools that engage epithelial and adipose TLRs in a MyD88-dependent manner. Conditional deletion of Myd88 in intestinal epithelial cells attenuates high-fat diet weight gain, elevates energy expenditure, and alters endocannabinoid signaling, while adipose-tissue MyD88 is required for high-fat diet-induced macrophage recruitment and insulin resistance.[56] Thus, the same adaptor that relays microbial danger also integrates diet-derived molecular patterns, bridging gut barrier status, immune tone, and systemic energy metabolism.

Another striking example concerns MAFLD. Henao-Mejia et al.[57] found that mice deficient in inflammasome components (e.g., NLRP6) developed exacerbated steatosis and metabolic inflammation, linked to a microbiota that promoted gut permeability and hepatic TLR4/9 activation.[57] This gut-liver axis, modulated by microbial communities, has since become a central focus in metabolic disease research.

In addition to endogenous microbiota, the role of microbial metabolites in mediating host effects has become increasingly clear (see the translational wall: why most interventions fail in humans). Preclinical studies have shown that short-chain fatty acids (SCFAs), bile-acid derivatives, and novel microbial metabolites (e.g., imidazole propionate) influence insulin sensitivity, inflammation, and energy expenditure via host receptors and signaling pathways.[58,59] These studies not only underscore the mechanistic potential of microbial function but also highlight the complexity and context dependence of these effects. While these insights have pushed the field forward, it is important to recognize the limitations of mouse models.[60] Differences in microbiota composition, immune development, and metabolism between humans and mice can constrain translatability. Moreover, housing conditions, diet, and even cage effects can confound results. Most of the time none of the studies are verifying whether the original microbiota is transferred in recipient mice and thereby conclude a direct causal relationship, when in fact some taxa are not transferred.[61] Nonetheless, preclinical models remain indispensable for uncovering mechanistic links and testing microbiota-targeted interventions *in vivo* and especially when combined with human data in integrative frameworks.[62]

Establishing a causal link between the gut microbiota and a host phenotype (such as obesity or metabolic changes) requires more than observing correlations or transferring whole communities. Rigorous causal inference can be guided by four complementary criteria.

First, strain-level engraftment should be verified when the hypothesized mechanism depends on long-term colonization and community reshaping. While early landmark studies using the obese microbiome[18] or the ''twin microbiome''[54] demonstrated phenotype transfer, they rarely confirmed whether specific strains persisted in recipients. Modern strain-tracking now allows this, ensuring that the proposed causal organism or its functional genes are active in the host. However, for transient-acting microbes or postbiotics, persistence is not required, as functional activity during passage can be sufficient.

Second, the causal mechanism should involve the modulation of a defined functional pathway that changes in tandem with the phenotype. Broad taxonomic labels, such as butyrate producers, are insufficient. SCFAs, for example, act locally and systemically, yet stool levels may misrepresent their functional impact because of absorption and utilization dynamics. For example, SCFAs are produced and utilized by other bacteria (cross-feeding) and host cells such as the colonocytes (i.e., butyrate) but are also absorbed to influence host metabolism at a distance from the gut (e.g., liver, brain, adipocytes).[63–65] Similar considerations apply to bile acids, imidazole propionate, and other microbial metabolites. Stronger evidence comes from showing that an intervention alters a specific biochemical or signaling pathway, such as receptor activation or metabolite flux, in a way that plausibly explains the host effect.[66–68]

Third, reversibility should be demonstrated through gain- and loss-of-function experiments. Removing or blocking the microbial factor should diminish the effect, while reintroducing it should restore it. Examples include blocking SCFA receptors to abolish benefits or supplementing specific bile acids to reproduce a microbial effect. Such manipulations provide strong evidence that the microbial factor actively drives, rather than merely associates with, the host phenotype.

Finally, cross-cohort reproducibility is essential (see the way forward). True causal factors should produce consistent effects across independent studies, populations, and experimental systems, guarding against context-specific artifacts and ensuring generalizability. This may involve validating mouse findings in human studies, testing in different dietary or genetic backgrounds, or confirming efficacy in multiple trials.

While these criteria set a high bar for proving causality, it is important to acknowledge that the cost, technological requirements, and experimental effort needed to meet them remain a major barrier for many researchers. The reality is that implementing these rigorous gold standards often requires costly technologies, advanced infrastructures, and large-scale efforts that remain inaccessible to many researchers. Advanced strain-resolved metagenomics, targeted metabolomics, functional assays, and multi-cohort validations demand resources that are not equally accessible across research settings. Bridging this gap will require both methodological innovation and broader access to the necessary tools, enabling more investigators to rigorously test causal hypotheses and translate microbiome science into actionable interventions. Pragmatic alternatives can nevertheless help: targeted strain-tracking panels, focused metabolomics of priority pathways, and the use of functional ''reporter assays'' can provide lower-cost entry points. In parallel, data-sharing frameworks across laboratories can support reproducibility without requiring each group to independently bear the full technological burden.

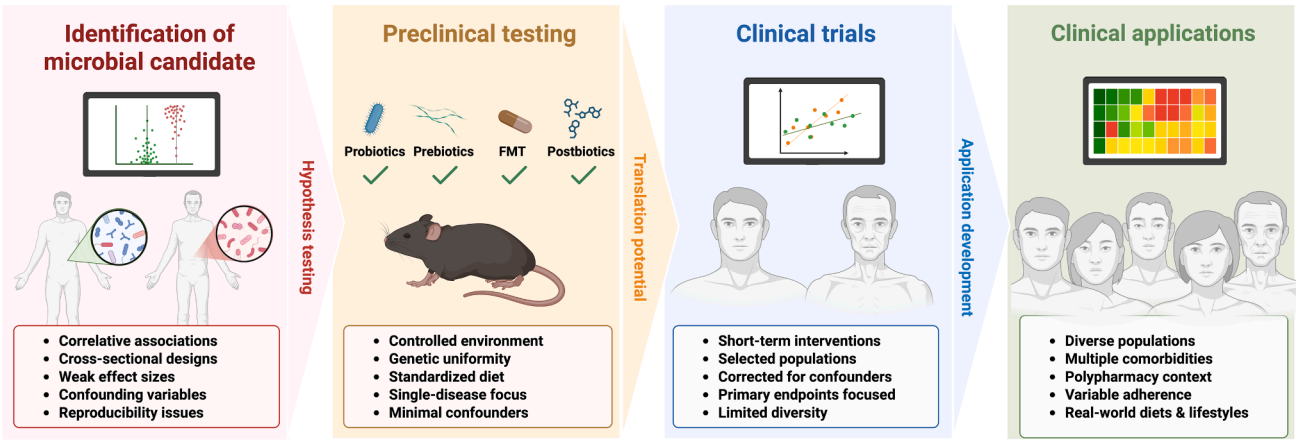

Figure 2. From bench to bedside: Lost in translation?

The schematic follows a microbiota-targeted therapy as it moves from discovery to real-world use and pinpoints the sources of attrition at each step. Identification of a microbial candidate (pink): microbiome-wide association studies (MWASs) link specific taxa or functions to host traits, but evidence is often correlative, cross-sectional, and of small effect size; confounding variables and poor reproducibility further weaken the signal.

Preclinical testing (beige): candidate probiotics, prebiotics, fecal microbiota transplantation (FMT) formulations, or postbiotic compounds are validated in genetically uniform mice kept on standardized diets under tightly controlled conditions. Such homogeneity increases internal validity but fails to capture the complexity of human disease.

Clinical trials (blue): early-phase studies in carefully selected patient subsets use short interventions, strict monitoring, and narrowly defined primary endpoints. While safety and proof-of-concept efficacy are established, these "ideal" cohorts still under-represent the diversity found in routine care.

Clinical application (green): once products enter everyday practice they face heterogeneous populations, multiple comorbidities, polypharmacy, variable adherence, and unconstrained diets/lifestyles. These real-world factors often blunt or obscure the benefits seen in controlled settings. The slanted arrows and graduated background colors illustrate the increasing translational barrier that must be overcome from left to right. MWAS, microbiome-wide association study; FMT, fecal microbiota transplantation. Figure created with BioRender.com.

THE TRANSLATIONAL WALL: WHY MOST INTERVENTIONS FAIL IN HUMANS

Despite compelling mechanistic insights from preclinical models,[59,69] the optimism generated by *in vivo* studies in rodent models has not fully translated into clinically effective microbiota-targeted therapies in human trials. Approaches such as fecal microbiota transplantation (FMT), probiotics, prebiotics, and microbial metabolite supplementation have repeatedly shown clear metabolic effects in rodents. Yet in humans, these same interventions often yield modest, inconsistent, or transient outcomes. This translational gap underscores the complex interplay between microbial ecology, host physiology, and environmental context, thereby calling for a critical reassessment of how we move from mechanistic discovery in mice to interventions in humans (Figure 2).

Fecal microbiota transplantation

FMT was among the earliest attempts to harness the gut microbiota for metabolic benefit. The rationale was intuitive: let's replace a dysbiotic microbial community with one from a healthy donor to restore metabolic balance. Some early human trials generated excitement. For example, a randomized controlled trial in which men with metabolic syndrome received FMT from healthy, lean donors showed that 6 weeks post-transplant, recipients exhibited improved insulin sensitivity.[70] These findings mirrored results from rodent studies and demonstrated the therapeutic potential of FMT in metabolic diseases. However, the intervention did not affect body weight, fat mass, or markers of inflammation, and the metabolic improvements were modest and transient. Microbiota analysis revealed an increase in butyrate-producing bacteria such as *Roseburia intestinalis*, *Faecalibacterium prausnitzii*, and *Anaerobutyricum soehngenii* (formerly *Eubacterium hallii*), pointing to a potential mechanistic link with enhanced insulin sensitivity.[70] In a follow-up study in 2017, the same research group emphasized the importance of baseline microbiota composition in determining FMT efficacy, identifying distinct microbial profiles between metabolic responders and non-responders.[71] Nevertheless, subsequent trials struggled to reproduce these effects consistently, highlighting the variability in response. For instance, the Gut Bugs trial in adolescents with obesity found no significant effects of oral encapsulated FMT on body mass index (BMI), insulin sensitivity, or metabolic markers after 6–12 weeks, despite evidence of donor strain engraftment.[72] A long-term study investigating FMT for metabolic syndrome reported sustained microbial diversity up to 12 months post-transplant. However, no significant improvements in biochemical or anthropometric parameters were observed, and this is possibly because key anaerobic species, including *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, were lost during the transfer process.[73] Meta-analyses of FMT trials for metabolic disorders conclude that while some short-term improvements in glycemia and lipids are possible, FMT does not consistently reduce weight or induce durable metabolic changes.[71,74–76]

These inconsistencies underscore the substantial challenges in translating FMT efficacy from controlled experimental conditions to heterogeneous real-world clinical settings. Multiple factors can limit success, including colonization resistance from the recipient's resident microbiota, variable strain-level engraftment of donor organisms (see from correlation to causation: insight from animal models), host dietary patterns, immune system compatibility, and the ecological stability of introduced communities over time. Emerging evidence suggests that functional compatibility between donor and recipient microbiomes—rather than mere taxonomic similarity—may be a more decisive determinant of clinical benefit. Consequently, interventions may be more effective if they prioritize the transfer of key microbial functions (e.g., high-capacity butyrate synthesis, bile-acid remodeling) and select donors on the basis of these functional traits. While FMT remains a promising therapeutic strategy for metabolic disease, its optimal application will require mechanistic insight into host-microbe and microbe-microbe interactions, validated criteria for donor-recipient matching, and prospective evaluation of functional engraftment alongside clinical endpoints (Figure 2).

Prebiotics

Prebiotics are considered a promising approach for promoting health by "feeding" specific gut microbes. Initially introduced by Roberfroid and Gibson[77] and more recently defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as "substrates that are selectively utilized by host microorganisms conferring a health benefit,"[78] prebiotics offer a simple, diet-based means of modulating microbial composition and function. Because they are derived from common dietary components, such as non-digestible carbohydrates, certain polyphenols, and omega-3 fatty acids,[79] their appeal lies in their safety, accessibility, and capacity to influence the microbiota without introducing foreign species.[69,78,80–82]

In animal models, fermentable fibers such as inulin, oligofructose, and galacto-oligosaccharides consistently enhance gut barrier function and improve host metabolic outcomes, including insulin sensitivity and lipid homeostasis. These effects are often mechanistically linked to the growth of SCFA-producing taxa and downstream signaling through G protein-coupled receptors and histone deacetylase inhibition.[83–85]

In humans, the story is more complex. While several trials have reported modest benefits on glycemia, satiety, or inflammation, others have failed to detect consistent effects.[86,87] Importantly, both the source and the structural characteristics of prebiotics appear to be critical. For example, variations in chain length of inulin-type fructans can lead to markedly different metabolic outcomes.[88,89] Another major challenge is inter-individual variability: the metabolic and microbial response to prebiotics depends heavily on the baseline microbiota composition, with "responders" typically harboring a higher abundance of taxa capable of utilizing the substrate (e.g., *Bifidobacterium*, *Faecalibacterium*).[81,90] Habitual diet, host genotype, gut transit time, and immune status further influence the outcome.[91]

Another limitation is the non-specificity of many prebiotics. Despite their "selectivity" by definition, fibers like inulin can also promote growth of non-target taxa, with context-dependent outcomes. Additionally, gastrointestinal side effects such as bloating and discomfort at effective doses (>5 g/serving) may limit long-term adherence.

In addition, fundamental questions continue to shadow the field: to what extent do prebiotics represent true biomedical innovation? And to what extent are they a rebranding of long-standing dietary recommendations? After all, the core message of public health nutrition, that is, "eat more fiber, eat more plants,"[92–96] has been promoted since the 1970s, and it long predates the rise of microbiome science. Recent research has added mechanistic depth, revealing that certain prebiotic substrates and other microbiota-accessible carbohydrates (MACs) can selectively fuel defined microbial guilds. We now also know that these effects depend on substrate structure, dose, and the host's baseline microbiome, ultimately shaping metabolite production and influencing host physiology via immune and endocrine pathways. However, because these dietary recommendations are already widely known to the general public, prebiotics are rarely perceived as a novel innovation emerging from microbiome science. The nuance of these findings is often lost in public and even some clinical discourse. A simple but important example is that not all dietary fibers are prebiotics. While most prebiotics are fermentable carbohydrates, many fibers, such as cellulose or wheat bran, provide bulk and support bowel function but do not selectively stimulate beneficial microbes; produce specific bioactive metabolites; and confer consistent, demonstrable health benefits, and therefore, they do not meet the formal definition of prebiotics. When this distinction is blurred, the unique contributions of microbiome science risk being overshadowed by the perception that it simply reiterates decades-old nutrition advice.

As a result, prebiotics struggle to gain recognition as a real translational product of gut microbiota research. This disconnect contributes to skepticism about the clinical relevance of microbiome science, especially when outcomes appear to reinforce already entrenched public health messages.

Despite these caveats, we still believe that prebiotics remain a promising and well-tolerated class of microbiota-modulating agents. Future progress may depend on precision matching of substrates to microbial configurations, integration with other strategies (e.g., synbiotics or postbiotics), and reframing prebiotics not merely as "fiber supplements" but as tools for modulating host-microbe interactions in targeted ways (Figure 2).

Probiotics

In his 1907 book *The Prolongation of Life: Optimistic Studies*, Élie Metchnikoff proposed that consuming lactic acid bacteria from fermented milk could promote health and longevity, a hypothesis inspired by the exceptional lifespans of Bulgarian peasants who regularly consumed yogurt. The implication was clear—if certain microbes could promote health, then isolating and supplementing these strains could offer a new path to disease prevention.

More than a century later, and over two decades since the FAO/WHO formally defined probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host,"[97] we are left wondering—where are the probiotics we were promised?

Despite decades of research on traditional "probiotic" strains, such as *Lacticaseibacillus rhamnosus* GG and *Bifidobacterium animalis* subsp. *lactis* BB-12, clinical translation has proven

difficult. These strains have been extensively studied and shown to modulate inflammation, gut barrier integrity, and metabolic parameters in animal models. However, systematic reviews and meta-analyses of human trials consistently show that while these probiotics may improve outcomes such as glycemic control, insulin sensitivity, or blood lipid profiles, the effects are often modest in the general population and may be limited to specific subgroups.[98–102]

Next-generation candidates, like *Akkermansia muciniphila*, have generated considerable interest owing to their mechanistic links to metabolic health in preclinical models.[39,103,104] In rodents, *Akkermansia muciniphila* has been associated with improved glucose metabolism, reduced adiposity, and enhanced gut barrier function.[38,105–108] Yet human data remain limited to early-phase studies (i.e., phases 1 and 2),[104,109] and while initial results are promising, larger (i.e., phase 3), controlled trials are needed to determine efficacy, safety, and long-term impact in diverse populations (Figure 2).

One major issue is that even under controlled conditions, probiotic colonization is not guaranteed. A pivotal study by Zmora et al. showed that individuals vary greatly in their ability to support probiotic engraftment, depending on baseline microbiota composition and host mucosal features.[110] However, colonization is context dependent rather than uniformly unstable. While Zmora and colleagues showed highly personalized, often transient colonization in healthy adults, multiple studies demonstrate that persistence varies by strain, substrate/niche availability, host factors, and study context (e.g., antibiotic exposure). For example, niche-matching can enable durable engraftment of specific strains (e.g., *Bifidobacterium longum* AH1206) in individuals with compatible baseline ecologies.[111] Conversely, poorly matched strains rarely persist. Wastyk et al. also demonstrated that a fermented-food diet increases diversity and modulates immune markers, but they highlighted that benefits may occur without durable engraftment of the ingested microbes.[112] Altogether, these data suggest that additional determinants are strongly contributing and include doses, formulations, gastric survival, delivery route and timing (especially post-antibiotics), dietary context (availability of complementary carbohydrates), mucosal vs. luminal niches, and host genetics (e.g., secretor status). Thus, rather than a binary ''colonization/no-colonization,'' outcomes may reflect strain/host/diet interactions that should be considered best practice. Still, in many cases, administered probiotics are transient visitors and are excreted without lasting ecological or functional impact.

This biological variability and regulatory inertia have led to growing disillusionment. One might question whether the term probiotic has devolved into a marketing label rather than a meaningful therapeutic concept. This critique is understandable, given the disconnect between scientific evidence and commercial practice, as well as the inconsistent outcomes observed in human trials. It reflects not only mounting frustration within the research community but also growing skepticism from clinicians and the broader public.

Yet abandoning probiotics altogether would be premature. The problem lies not with the concept itself but with the overly broad, ''catch-all'' use of the term probiotic as a definition that stands in stark contrast to the rigorous classification systems applied in pharmacology. In medicine, we categorize drugs according to their class, active compound(s), mechanism of action (when known), therapeutic target, dosage, and, crucially, their approved indication. By comparison, probiotics are still evaluated and marketed under a vague umbrella that lumps together a wide array of microbial strains, formulations, and intended outcomes.

This lack of specificity poses a fundamental problem. After all, no medical professional would accept grouping all pharmaceuticals, ranging from antibiotics to antihypertensives, under a single label like ''chemical therapy'' and expect consistent results. Just as it would be unthinkable to assess drug efficacy without specifying the compound or the disease being treated, it is equally misguided to evaluate probiotics without accounting for strain specificity, host context, and targeted clinical outcomes.

The current generic use of the term probiotic obscures the critical nuances that determine efficacy: strain identity, mechanisms of action, and the pathology being addressed. As a result, drawing reliable comparisons across studies or translating findings into clinical practice becomes nearly impossible. To move forward, the field must adopt a more pharmacologically informed mindset and view probiotic strains not as universal ''elixirs'' but as biologically active agents with context-dependent effects. The same applies to prebiotics—precision in definition must precede precision in therapy. Only by shedding the illusion of a one-size-fits-all solution can we begin to unlock the full therapeutic potential of microbiota-targeted interventions, with the same rigor and specificity that define modern medicine (Figure 2).

Postbiotics

Having examined strategies aimed at modulating the gut microbiota directly through FMT, prebiotics, and probiotics, we must also turn to the latest development in the field, that is, the ''postbiotics.'' This approach bypasses the complexity of reshaping microbial communities by instead targeting the potential bioactive compounds that microbes produce or display on their surfaces. In theory, this marks a major step forward, sidestepping ecological variability, colonization barriers, and safety concerns associated with live microbes. In practice, however, the gap between mechanistic insights and clinically effective applications remains significant.

Postbiotics are defined by the ISAPP as ''preparations of inanimate microorganisms and/or their components that confer a health benefit on the host.''[113] These may include heat-killed bacteria, purified microbial metabolites, extracellular vesicles, or even cell wall fragments. By focusing on function rather than viability, postbiotics promise greater stability, better safety profiles, and more standardized manufacturing, compared with their live counterparts.[114,115]

Despite these advances, the transition from concept to clinic is not straightforward (Figure 2). As discussed in Box 1, postbiotics, like other microbiota-based interventions, face challenges of delivery, context dependence, biological redundancy, and regulatory ambiguity. Mechanisms are often incompletely understood, and human data are still scarce. Moreover, the diversity of postbiotic compositions complicates standardization and dose optimization, which are key difficulties for clinical approval. Still, this functional turn marks a conceptual evolution in microbiome science. Where probiotics aim to ''add'' microbes and prebiotics

Box 1. Why microbiota-based interventions struggle to translate

Despite strong mechanistic data from animal models, microbiota-targeted interventions (FMT, prebiotics, probiotics, postbiotics) have shown inconsistent efficacy in humans. This translational gap is driven by biological complexity, methodological variability, and regulatory ambiguity.

(1) Context dependence and inter-individual variability: microbiota-host interactions are shaped by genetics, diet, medications, comorbidities, and baseline microbiota composition. Unlike drugs with defined molecular targets, these interventions engage with a dynamic and personalized ecosystem. As a result, responses are often unpredictable and vary substantially between individuals.

(2) Mismatch between model systems and human disease: many mechanistic insights stem from animal models—typically germ-free or inbred mice—whose microbiota, physiology, and disease trajectories differ markedly from those of humans, limiting direct clinical translation.

(3) Ecological and functional complexity: gut microbial communities are functionally redundant and ecologically resilient, with extensive cross-feeding networks. Introduced microbes may fail to engraft, may be outcompeted by resident strains, or may exert minimal impact. Similarly, isolated microbial metabolites may not reproduce the coordinated, system-level effects of a balanced microbiome. Some compounds even have context-dependent or opposing effects, depending on host physiology and disease state.

(4) Trial design limitations: clinical trials differ widely in duration, endpoints, formulations, and inclusion criteria. Many are short term despite targeting chronic conditions and often lack controls for key confounders like diet and medication. This heterogeneity limits cross-study comparability and may obscure subtle but biologically relevant effects.

(5) Lack of standardization and biomarkers: there is no consensus on optimal strains, doses, delivery routes, or formulation stability. Moreover, validated biomarkers to assess microbial function or predict response remain scarce. As a result, many trials rely on broad clinical outcomes that may miss mechanistic signals or individual variation in response.

(6) Regulatory and practical barriers: microbiota-based products often fall between categories, such as food, drug, or biologic, which is creating uncertainty around approval, safety, and labeling. Challenges like probiotic viability, metabolite bioavailability, and quality control remain poorly addressed. Furthermore, subjective outcomes (e.g., gastrointestinal symptoms, mood) and strong placebo effects further complicate efficacy evaluation in the absence of rigorous trial design.

aim to "feed" them, postbiotics aim to replicate the effects of a healthy microbiome without relying on ecological integration. If these interventions can be refined and validated in humans, they may offer a safer, more targeted, and scalable path toward microbiome-inspired therapies.

Given the heterogeneous clinical responses to FMT, prebiotic, probiotic, and postbiotic interventions, it is critical to investigate whether baseline bacterial niche availability and functional compatibility between donor and recipient microbiomes can improve therapeutic outcomes. Prioritizing functional traits (e.g., high-capacity butyrate production or bile-acid remodeling) over purely taxonomy matching may enhance both the generalizability of findings and the feasibility of prospective patient stratification.

Although methodologically demanding and potentially costly, well-designed trials should incorporate verification of strain-level engraftment of function-carrying taxa, direct quantification of pathway activity (using either metatranscriptomics or metabolomics), and pre-specified responder definitions based on baseline functional capacity.[116,117] Notably, a recent study shows that donor colonization and recipient strain persistence were largely uncoupled from clinical outcomes[118] yet can be accurately predicted using machine learning (ML) models. These findings suggest that targeted depletion of specific recipient microbes, combined with the introduction of functionally diverse consortia of the same species (rather than a single strain), could improve colonization and turnover, although whether these ecological improvements translate into consistent clinical benefit remains to be demonstrated.

Finally, specific complementary human-relevant systems might be used to increase translational confidence and prediction. Germ-free mice colonized with human microbiota are already used and can capture certain host-microbe interactions on an organismal scale, although one must remain aware of differences in immunity and diet between mice and humans. In addition, advanced *in vitro* models such as intestinal organoids and gut-on-chip[119,120] platforms might be useful. These systems provide a more controlled environment and can incorporate features like co-culture of human cells with microbiota under aero-anaerobic conditions, the presence of mucus and fluid shear forces, and the ability to measure barrier integrity and immune responses using patient-derived tissues. Where feasible, *in situ* human sampling (mucosal biopsies or intestinal lavage samples) and spatial multi-omics should complement stool analyses, since microbiota communities and functions associated with the mucosal lining can differ substantially from those observed in feces. Adding these points clarifies how emerging human-centered models and sampling strategies can improve translational confidence in microbiome research.

WHY THIS IS NOT FAILURE BUT JUST BIOLOGY

Given the modest and inconsistent clinical outcomes of microbiota-based interventions in metabolic diseases, some have questioned whether the field has overpromised and underdelivered. But interpreting this translational gap as failure would be a fundamental misunderstanding of biology. Rather than disproving the relevance of the microbiome, the current state of the field reflects its inherent complexity and signals the need for more

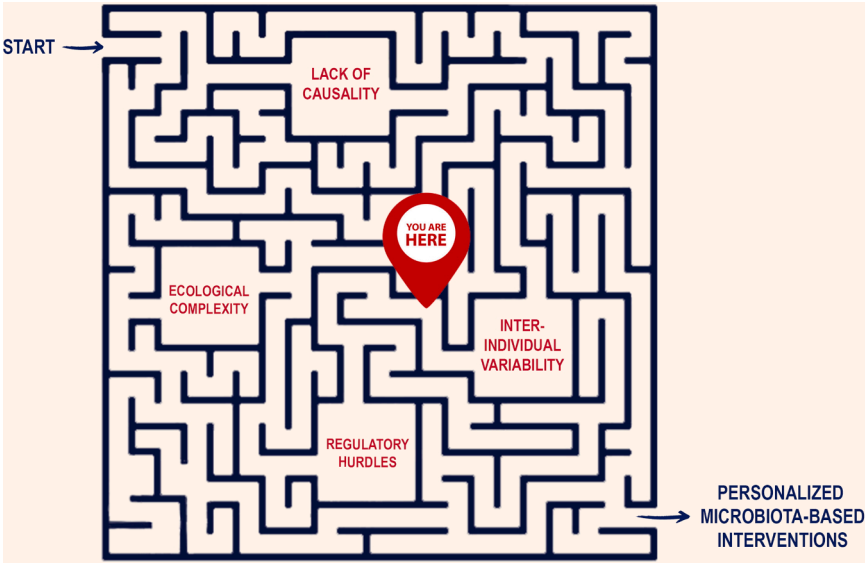

Figure 3. Navigating the microbiota maze

The labyrinth symbolizes the tortuous journey from first microbial discoveries (entry, left) to effective, patient-tailored therapies (exit, right). Red callouts mark the main barriers: lack of causality meaning associative evidence often suffices to launch a product but rarely guarantees efficacy once confounders are removed. Ecological complexity and microbe-microbe and host-microbe interactions can invert or erase an expected effect. Regulatory hurdles meaning heterogeneous classification of probiotics, LBPs, and FMT across jurisdictions slow trial design and market entry. Inter-individual variability such as genetics, diet, drugs, and lifestyle modulate both baseline microbiota and response to intervention. The red "You are here" pin situates current research: midway through the maze, aware of the obstacles but still searching for the optimal path. The figure is purely illustrative; the text in the perspective follows details to concrete criteria required to streamline translation.

sophisticated models, deeper mechanistic understanding, and realistic expectations.

Parallels can be drawn with the Human Genome Project. When the human genome was completed in 2003, it sparked high expectations for a rapid revolution in precision medicine, including the hope of identifying genetic cures for common diseases. Yet decades later, few complex disorders have been solved by single genetic variants. Instead, the project catalyzed a deeper understanding of polygenic risk, gene-environment interactions, and biological networks. It laid the foundation for personalized medicine—not by offering silver bullets, but by redefining how we think about disease mechanisms.

We believe that the same applies to the microbiome. While early enthusiasm may have focused on quick-fix interventions such as probiotics, prebiotics, FMT, and postbiotics, the true strength of microbiome science lies in its ability to reframe human health as the result of dynamic, bidirectional interactions between host and microbes. Over the past two decades, research in this field has transformed our understanding of core physiological processes, reshaping how we view digestion, immunity, neurobiology, and metabolic regulation. It has uncovered microbial metabolites that function as endocrine and immunomodulatory signals and that brought to light the importance of gut barrier integrity, bile-acid signaling, and intestinal inflammation in the development of metabolic diseases.[59,69,85] It provided early mechanistic insights into how mismatches between host and microbial functions may underlie individual differences in dietary responses, drug efficacy, and disease susceptibility.

These insights, even though not yet converted into widespread therapies, are essential building blocks for the future of integrative medicine. They highlight the limitations of reductionism in complex systems and point to a systems biology approach as the most promising path forward. The reality is that the gut microbiota represents a context-sensitive, self-adapting ecosystem, interacting continuously with the host and the environment. Attempting to manipulate it with "oversimplified" strategies such as supplementation or transplantation is bound to be more difficult than initially imagined. But that difficulty reflects biological reality, not scientific failure. Rather than deserting the field, we must recalibrate our goals; acknowledge the ecological nature of the problem; and focus on mechanisms, stratification, and integration (Figure 3).

THE WAY FORWARD

If the first two decades of microbiome-metabolism research were defined by enthusiasm and discovery, the next must be characterized by strategic refinement (Figure 3; Box 2). The disappointing translational yield of generalized interventions like probiotics or high-dose prebiotics underscores a key lesson—to be effective, microbiota-based strategies must embrace the biological variability and ecological complexity of the gut ecosystem.

Several guiding principles could lead the way to more realistic and actionable microbiome research as discussed below.

Shift focus from taxonomy to function

Many early studies emphasized differences in microbial composition, and mostly in terms of relative abundance.[121] However, these taxonomic shifts have proven to be unstable, inconsistent, and often non-informative across individuals or populations. A more promising approach lies in functional profiling, which focuses on microbial activities rather than solely on identity. Advances in metagenomics, metatranscriptomics, and metabolomics now allow researchers to identify key microbial pathways, such as butyrate synthesis, bile-acid dihydroxylation, and other metabolite-producing processes that may be mechanistically linked to host physiology.

Functional profiles, representing the metabolic pathways and gene families present in the microbiome, tend to be more similar across individuals than taxonomic profiles. They remain more stable over time within the same individual and show

Box 2. The path forward

FMT

Future FMT strategies could move beyond generic donor selection by incorporating functional donor-recipient matching algorithms. These would combine microbial composition with metabolic activity profiling to predict compatibility. In addition, transient depletion of colonization-resistant resident strains may create ecological space that improves engraftment of beneficial donor taxa.

Prebiotics

To improve efficacy, prebiotic interventions should be matched to the host's baseline microbiome and targeted to specific functional deficits. Combining substrates with complementary probiotics may also enhance colonization and functional output. Ultimately, rational design of prebiotic formulations requires moving from "one-size-fits-all fibers" to precision substrates selected for structure-function compatibility.

Probiotics

Probiotics should be developed and evaluated more like pharmacological agents, with clear definition of strain identity, mechanism of action, and intended clinical context. Strategies such as niche-matching (selecting strains adapted to the host's metabolic or immunological environment) may improve persistence when engraftment is necessary, while functional activity during transient passage can suffice in other cases.

Postbiotics

The postbiotic field would benefit from standardizing composition, identifying optimal delivery formats, and linking dosing to measurable host pathway modulation. By treating postbiotics as defined bioactive compounds rather than loosely formulated extracts, the field can accelerate their clinical translation.

less variation between individuals in large cohorts (i.e., lower beta-diversity). This pattern reflects the fact that different microbial species can often perform the same functions, a phenomenon known as functional redundancy.[2,122,123] In longitudinal disease cohorts, taxa often fluctuate widely, whereas functional shifts are smaller, more targeted, and correlate more closely with host metabolites and inflammatory markers. That said, this pattern is scale and context dependent (e.g., influenced by diet, antibiotic exposure, or bile-acid metabolism) and should not be overstated.

This functional perspective has clear translational implications. Patient stratification based on microbial metabolic capacities may better guide targeted interventions. One example is therapies designed to enhance butyrate production; butyrogenic live strains such as *Anaerobutyricum soehngenii* and *Clostridium butyricum* have shown improved glycemic control in randomized trials in the context of metabolic syndrome.[124–126] Such interventions illustrate how moving beyond taxonomy toward function can bridge mechanistic understanding with therapeutic application (Figure 4).

Importantly, functional profiling does not always require full multi-omics pipelines. Targeted qPCR for key microbial genes, focused metabolite panels, or pathway-specific bioassays can serve as pragmatic alternatives, lowering the barrier for groups without access to high-throughput platforms.

Stratify patients based on microbiome features

Emerging data suggest that baseline features including microbiota composition and function, microbially derived metabolites, host genetic variants (e.g., secretor status FUT2[127–129]), and immune signatures influence response to dietary fiber, probiotics, and FMT (see previous sections of this perspective).[71,81,104,130] Some individuals are responders, while others show minimal change or even adverse effects. Rather than pursuing universal recommendations, microbiome interventions should be biomarker-guided, using baseline profiles to identity likely beneficiaries and to tailor dose, strain, and dietary context accordingly.

This aligns with precision medicine and is already feasible in several domains. For instance, microbiome features have been used to predict postprandial glycemic responses to meals, likelihood of weight loss following dietary changes, and responsiveness to cancer immunotherapy. Such stratification could be critical in metabolic diseases, where heterogeneity in phenotype, microbiota, and lifestyle factors makes "one-size-fits-all" approaches ineffective (Figure 4).

Candidate predictive markers include microbial functional capacity (e.g., butyrate synthesis and bile-acid transformation pathways), specific metabolites (e.g., fecal SCFAs and bile acids), microbial ecological metrics (e.g., community stability and immunoglobulin A [IgA] coating indices[131]), host genetics (e.g., FUT2 genotype[127–129] and HLA haplotypes),[132] and immune signatures (e.g., Th17 and/or Treg balance). Integrating these into multi-omic predictive models, followed by rigorous prospective validation, could enable targeted and effective interventions. However, such development depends on standardized sampling protocols, robust wet-lab quality controls, and harmonized bioinformatics pipelines to ensure reproducibility and cross-study comparability.

A critical enabler of stratification will be the creation of open-access reference datasets linking microbiome features to intervention outcomes across diverse populations. Such shared resources would allow predictive models to be trained and validated beyond single cohorts, increasing their clinical utility.

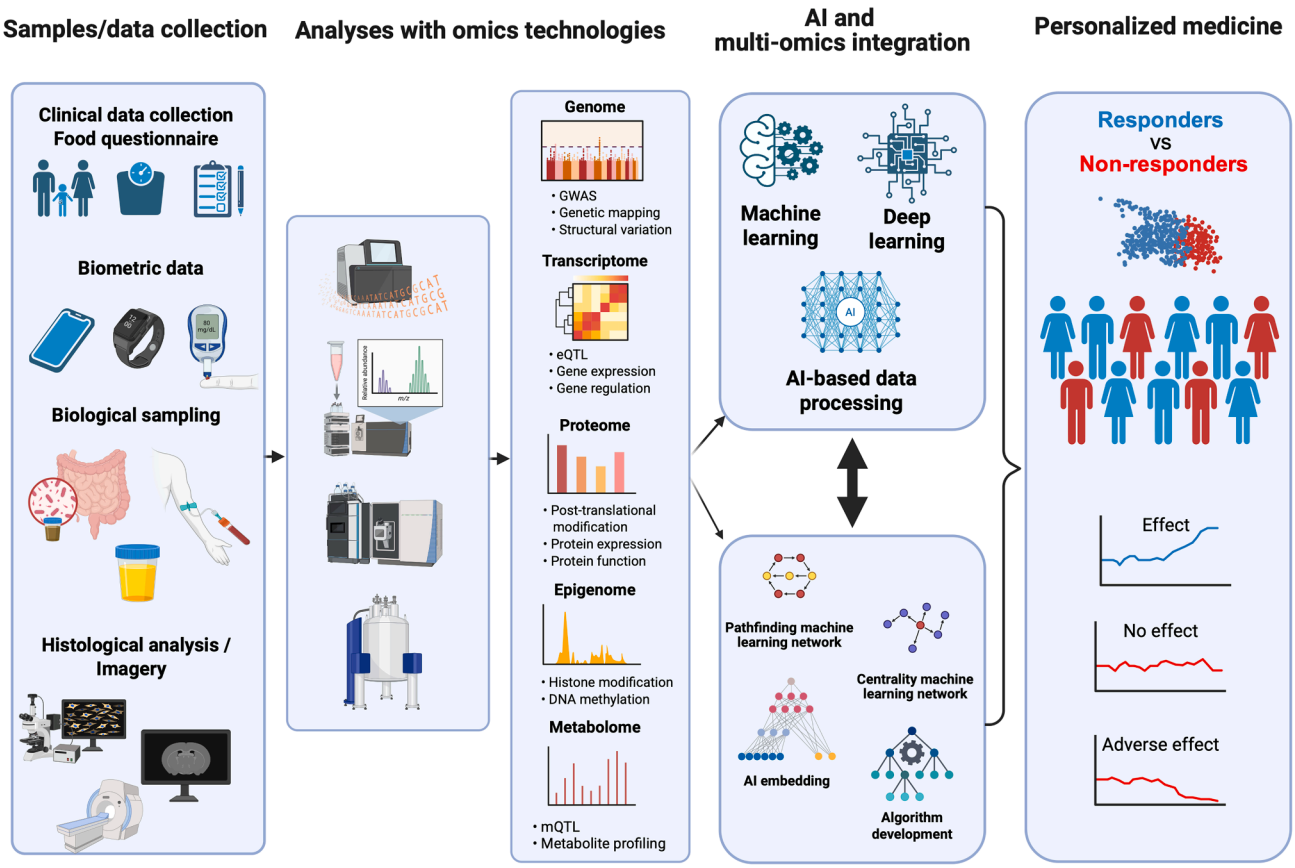

Figure 4. Overview of the translational pipeline from clinical data collection to individualized therapeutic strategies

The diagram walks through the successive steps that convert heterogeneous clinical information into personalized therapeutic recommendations.

Sample and data collection (far left): standardized clinical records and food-frequency questionnaires are complemented by *in vivo* readouts from wearables (biometric data). Biospecimens (blood, feces, urine, intestinal tissue) and histology/imaging datasets complete the multidimensional input.

High-throughput measurements (second panel): dedicated platforms generate multi-omics readouts from each sample type.

Omics layers and typical outputs (third panel): genome (genome-wide association studies [GWASs], genetic mapping, structural variant calling), transcriptome (expression quantitative-trait loci [eQTL], gene expression levels, regulatory signatures), proteome (posttranslational modifications, protein abundance profiles, functional annotation), epigenome (histone mark landscapes, DNA methylation patterns), metabolome (metabolite quantitative-trait loci [mQTL], untargeted metabolite profiling).

AI-driven multi-omics integration (fourth panel): ML and deep-learning frameworks fuse the layered datasets, generate low-dimensional embeddings, construct network features (path-finding and centrality), and iterate algorithm development.

Personalized medicine output (far right): predictive models stratify patients into responders (blue) vs. non-responders (red) and forecast the magnitude or absence of therapeutic benefit, as well as potential adverse effects, thereby informing individualized treatment choices.

Bidirectional arrows emphasize that insights fed back to earlier stages iteratively refine both data acquisition and model performance. GWAS, genome-wide association study; eQTL, expression quantitative-trait locus; mQTL, metabolite quantitative-trait locus. Figure created with BioRender.com.

Standardize and harmonize methodologies

One persistent barrier to reproducibility in microbiome studies is the lack of methodological standardization. Variability in sample collection procedures, sequencing platforms, bioinformatics pipelines, and metadata annotation often leads to conflicting results, even across studies investigating the same condition.

While this challenge is well recognized in academic research, it becomes even more striking in the context of growing public interest in microbiome-based diagnostics. Direct-to-consumer tests, in particular, often operate without regulatory oversight and offer questionable clinical value. To address these issues, an international panel of experts recently developed standardized recommendations for microbiome testing in clinical settings, outlining key principles, protocols, and reporting guidelines.[133]

Their goal is to lay the foundation for a regulatory framework that curbs inappropriate testing and facilitates the evidence-based integration of microbiome diagnostics into clinical medicine (Figure 4).

To overcome these challenges, the field must adopt community-wide standards encompassing sample collection and storage, consistent reporting of dietary and clinical metadata, and the use of reference-based bioinformatics workflows. Equally important is the implementation of rigorous quality control measures for microbiota-targeted interventions, including verification of strain identity, viability, and metabolite stability. Initiatives such as the Human Microbiome Standards project and the Microbiome Quality Control (MBQC) project are already laying the groundwork for such harmonization, offering valuable frameworks for improving reproducibility and comparability across

studies.[133–135] These concrete success stories illustrate both feasibility and impact of harmonization efforts. However, cross-study comparability remains limited, and broad adoption of these standards is urgently needed to ensure that biomarker-based patient stratification and microbiota-targeted interventions can be reliably translated into clinical practice.

At the same time, some researchers also caution against "over-standardization," warning that rigid protocols may obscure biologically meaningful variation and limit innovation in a rapidly evolving field (Figure 4).

Standardization efforts should balance rigor with feasibility. Adopting a minimal essential framework (core metadata fields, agreed quality control thresholds, and transparent reporting) can ensure comparability across studies while allowing smaller laboratories to participate without prohibitive costs.

Contextualize findings in real-world settings

The gut microbiome is shaped by diet, medications, geography, age, comorbidities, stress, and more. To be truly impactful, clinical studies must evolve from idealized, tightly controlled interventions to designs that better reflect real-world complexity, particularly for chronic diseases like obesity and diabetes, which are profoundly affected by lifestyle and environmental factors. Hence, multicenter trials, long-term follow-ups, and integration with electronic health records and wearable data are essential to bridge the gap between discovery and practical application (Figures 2 and 4).

Real-world also means longitudinal and circadian-aware designed studies. Indeed, microbiota and metabolic profiles fluctuate with diet,[136,137] circadian rhythms,[138] and medications. Consequently, related investigations should incorporate longitudinal sampling for both discovery and clinical monitoring, coupled with dynamic modeling of microbiome/metabolites-host interactions using time-series sampling and related multi-omics. Practically, this entails more than 2 baseline samples spaced by at least 3 days pre-intervention (i.e., to maximize potential dietary confounders), then precise dietary patterns and records of meals, sleeping behaviors, and drug intake. Such modeling should leverage mixed-effects or state-space approaches with medication and circadian covariates and should include external validation to ensure generalizability.

However, while calls for multicenter, longitudinal, and real-world microbiome studies are increasingly common and are becoming recurring closing remarks in many research papers, their implementation remains still very limited. High costs, logistical challenges, and insufficient infrastructure—particularly in under-resourced settings or investigator-led research—continue to pose major barriers to the large-scale integration of clinical and lifestyle data.

Predictive biomarkers for patient selection and trial design

Building on the preceding discussion, we contend that translating microbiome science into dependable therapeutics will require predictive biomarkers to progress from exploratory associations to prospectively validated tools that help guide who receives what, when, and how.

Developing such biomarkers demands a practical, stepwise pipeline. Discovery should begin with multi-omic profiling (metagenomics, metatranscriptomics, metabolomics), complemented by host genetics and immune phenotyping, while rigorously controlling for confounders. Model development should prioritize parsimonious, interpretable predictors or risk scores that integrate microbial functions with host and clinical covariates, and it should transparently report not only discrimination metrics but also on calibration and clinical decision-curve analyses.

Validation must be pre-specified and performed in both temporal and external cohorts, explicitly assessing transportability across geographies, populations, dietary contexts, and sampling and sequencing methods.

Finally, demonstrating real-world utility is essential, for example, by embedding biomarkers into trial designs such as the enrichment of likely responders, stratified randomization, adaptive dosing, or the N-of-1 crossover approaches for dietary or "-biotics" interventions.

Ultimately, biomarkers must actively inform clinical decisions rather than remain observational correlates. Achieving this requires adherence to standards and equity principles to ensure reproducibility and broad applicability. This includes sharing code and reference materials, adopting reporting checklists, implementing rigorous assay quality control, and evaluating performance and bias across diverse demographic and dietary groups. Without these safeguards, biomarker-guided recommendations risk being irreproducible, inequitable, or both.

Safety and regulatory considerations

Although a comprehensive review of safety and regulatory issues lies beyond the primary scope of this perspective, these factors represent critical hurdles for translating microbiome-targeted interventions from bench to bedside and warrant explicit consideration. Regulatory agencies worldwide, including the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), and the US Food and Drug Administration (FDA), increasingly emphasize the need for rigorous, long-term safety evaluation.

Safety

Microbiome-targeted therapies require systematic surveillance that extends well beyond the short observation windows of most clinical trials. For FMT, rare but serious cases of pathogen transmission underline the need for stringent donor screening, traceability, and batch-level release testing. For live biotherapeutic products (LBPs), safety frameworks must cover strain identity, genetic stability, antimicrobial-resistance profiling, and documentation of dose and exposure at the indented site of action. Postbiotics (e.g., purified metabolites, inactivated cell preparations) generally avoid the risks associated with live organisms, but they shift the safety focus toward formulation quality, local pharmacokinetics, off-target effects, and the need for rigorous toxicology and consistency assessments. Recent consensus statements, such as that from the Asia-Pacific Microbiota consortium, highlight the urgency of embedding such safeguards into development pipelines.[139]

Regulatory pathways

Approval routes for microbiome interventions differ substantially by modality and jurisdiction, and they may be further tailored to the targeted population (e.g., healthy individuals vs. patients with specific diseases). LBPs typically follow regulatory pathways similar to biologics, requiring detailed Chemistry, Manufacturing, and Controls (CMC) documentation for living organisms and

demonstration of potency or viability, as well as compliance with Good Manufacturing Practices (GMPs) for both fermentation and final product formulation. By contrast, postbiotics and small molecules align more closely with traditional drug frameworks or, in some cases, with food regulation (e.g., food-for-special-medical-purposes, FSMP) depending on claims and jurisdiction (e.g., EU vs. US). FMT remains regulated under specific national or regional guidelines, often with additional requirements for donor selection and material handling. In all cases, clear early definition of the intended regulatory classification is essential, as it shapes preclinical requirements, trial design, and the likelihood of eventual market authorization. Without such clarity, promising microbiome-based interventions risk delays, redesigns, or failure at late stages of development.

THE ROLE OF AI AND MULTI-OMICS

As the microbiome field moves from descriptive catalogs to mechanistic inquiry, it faces a defining challenge—data complexity. Modern microbiome studies generate vast, multidimensional datasets that encompass not just taxonomic profiles but also metagenomics, transcriptomics, metabolomics, proteomics, host genetics, clinical metadata, dietary records, and environmental exposure. Unlocking actionable insight from this high-dimensional, high-noise space requires tools that can integrate, model, and predict across scales. This is where artificial intelligence (AI) and multi-omics integration becomes indispensable.

ML has rapidly emerged as a powerful tool for uncovering complex, non-linear patterns in microbiome data to reveal associations that very often remain undetected by traditional statistical approaches. By leveraging high-dimensional datasets, ML algorithms have been applied to classify disease states such as obesity, type 2 diabetes, and MAFLD based on microbial profiles. They have also been used to predict individual postprandial glycemic responses from microbiota composition and dietary inputs,[137] to stratify individuals as likely responders or non-responders to interventions, such as specific diets,[136] probiotics,[104] or FMT,[71] and to identify microbial signatures that may predict treatment outcomes in contexts like cancer immunotherapy.[140,141] Unlike traditional biomarkers, which often rely on a handful of variables, these models can incorporate hundreds or thousands of features, including rare taxa, microbial genes, or metabolite levels. However, ML approaches require large, well-annotated datasets to perform reliably, and their findings must be validated in independent cohorts to ensure robustness and avoid overfitting. Without rigorous validation, there is a risk that models may capture dataset-specific noise rather than true biological signals. Moreover, the interpretability of complex ML models remains a challenge, often limiting their clinical applicability. As a result, translating ML-derived insights into actionable, generalizable biomarkers or therapeutic targets in microbiome research remains difficult and requires careful methodological and biological validation.

Beyond prediction, integrating multiple-omics layers is essential to unravel the mechanistic complexity of host-microbe interactions.[142] Metagenomics reveals the repertoire of microbial genes present in the gut, while metatranscriptomics identifies which of these genes are actively expressed under specific environmental or host conditions. Metabolomics adds a functional dimension by quantifying the biochemical end products of microbial activity. Complementing these microbial insights, host genomics and transcriptomics provide a parallel view of the systemic responses elicited by microbial stimuli. Together, these layers enable researchers to build ''causal'' models, tracing how microbial pathways translate into metabolic outputs and host effects (Figure 4).

For instance, tools like MOFA+ (multi-omics factor analysis),[143] DIABLO (mixOmics),[144] and Bayesian hierarchical models are increasingly used to dissect the interplay between host and microbial systems, enabling identification of cross-domain biomarkers and therapeutic targets.[142,145]

Another frontier is the integration of real-time digital health data (from wearables, apps, or continuous monitoring devices) with microbiome and omics data. These so-called digital phenotypes[146] means high-frequency, real-world behavioral and physiological data streams captured passively or semi-passively by any sensors, wearables, smartphones, or connected devices. Examples of these are continuous glucose monitoring, step count, sleep architecture, heart-rate variability, or meal-time logging systems.

Early platforms that combine microbiome profiling with digital coaching and AI-based diet recommendations, such as ''DayTwo'' and ''Zoe,'' have provided promising proof-of-concept results, suggesting that personalized nutrition may improve metabolic outcomes like postprandial glycemic control.[136,137] However, robust, large-scale clinical trials are still needed to assess the long-term efficacy and sustainability of these interventions. Moreover, the underlying algorithms often lack full transparency, making it difficult to evaluate the biological plausibility of their recommendations. Many of these platforms also rely on proprietary datasets and models, limiting reproducibility and independent validation. As with other AI-driven health tools, concerns remain about overfitting, generalizability across diverse populations, and the risk of oversimplifying complex host-microbe-diet interactions (Figure 4).

Despite its promise, the integration of AI and multi-omics in microbiome research faces several critical challenges. Data standardization and interoperability remain major obstacles, hindering the comparison and replication of findings across platforms and studies. Many predictive models struggle to generalize across diverse populations, geographic regions, and dietary patterns. Moreover, the complexity of ML models raises concerns about interpretability and clinical usability. Ethical considerations such as data privacy, the commercial exploitation of personal health information, and the potential for algorithmic bias are further complicating their implementation.

These hurdles are substantial, but not insurmountable. When addressed thoughtfully, the fusion of AI with multi-omics technologies offers a powerful avenue for advancing microbiome science toward precision, personalized, and mechanistically informed metabolic interventions (Figure 4). In practice, precision functional profiling can be implemented as a staged process. An initial, cost-efficient tier could combine strain-resolved metagenomics with targeted pathway transcriptomics and focused metabolomics panels to capture core taxonomic and functional markers. As analytical capabilities and budgets expand, this framework can be iteratively enhanced with long-read assemblies to resolve structural variation, untargeted

metabolomics to discover novel metabolites, and single-cell host transcriptomic or proteomic readouts to link microbial activity directly to host cell responses. Such a phased approach allows incremental gains in resolution and functional depth, while remaining adaptable to technological progress and resource constraints.

Ethical and governance considerations are central to the responsible deployment of AI-driven microbiome-based precision nutrition and therapeutics. As these approaches mature, three categories of risk should be addressed from the outset. First, data privacy must be safeguarded, since raw metagenomes can reveal host genetic variants and potential disease predispositions. Second, algorithmic bias and fairness require attention since training datasets often under-represent non-Western diets, ethnicities, and low-resource settings, leading to models that may perform poorly or inequitably across populations. Third, commercialization and conflicts of interest can arise when proprietary scoring algorithms and closed datasets limit reproducibility and restrict equitable access to validated tools. Emerging frameworks provide practical safeguards: the STORMS reporting checklist[147] and FAIR principles[148] support transparent and interoperable data sharing; the Global Alliance for Genomics and Health (GA4GH)[149] offers guidance for secure and standardized genomic and microbiome data governance; and the EU "Trustworthy AI" framework sets requirements for fairness, transparency, and accountability in AI systems. Embedding these standards alongside with the GDPR-compliant consent that covers secondary AI use should be considered best practice for microbiome trials.

CONCLUSION

Nearly 2,500 years ago, Hippocrates is believed to have declared that "all disease begins in the gut." In recent years, this ancient intuition has found new life in modern science, as an explosion of research on the intestinal microbiome has positioned it as a central player in the pathogenesis of a wide variety of disorders ranging from metabolic and inflammatory diseases to neuropsychiatric conditions and even cancers. This resurgence has not only revived the idea that all disease might begin in the gut but also sparked the hope that all disease could be stopped there. It is perhaps fitting then that the Hippocratic Oath, the ethical cornerstone of Western medicine, calls upon healing deities including Panakeia, the goddess of universal remedy. Like Panakeia, the microbiota has come to symbolize the alluring promise of a common root solution for diverse ailments.

But just as physicians have yet to fulfill the vow of delivering a cure for all diseases, microbiome researchers too have not delivered on the therapeutic promise once so fervently imagined. Despite immense scientific progress, the field has struggled to translate its discoveries into interventions that are robust, replicable, and clinically impactful. The early hope that microbial modulation would offer a near-universal solution to complex diseases has given way to a humbler, more granular understanding, with one that embraces complexity rather than bypassing it.

And therein lies the true strength of microbiome-based interventions—their potential for precision and personalization. Not *despite* the fact that but *because* the gut microbiota sits at the crossroads of so many influencing factors including diet, genetics, medication, immunity, and lifestyle, it remains one of the most promising and dynamic therapeutic targets in modern biomedicine. While it may not yield to simple solutions, it opens the door to interventions that can be tailored to specific contexts, conditions, and individual profiles. We are still learning the rules of this game, but as our understanding deepens, so too does our capacity to intervene with precision. And when we finally know how to play, we just might be able to play to win.

As researchers working within this field ourselves, we offer these reflections not as an outside critique, but as part of an ongoing conversation among colleagues. The challenges we have outlined are familiar to many of us. These are not reasons to step back but reasons to push forward with greater clarity and purpose. If anything, they reinforce our belief in the potential of the microbiota as a therapeutic target. There's still much to learn and translating that knowledge into meaningful interventions will not be easy. By combining tangible measures like scaled-down functional assays, donor-recipient matching, precision prebiotic and probiotic design, and early-stage patient stratification, the field can begin to close the translational gap even before full causality frameworks are universally achievable. With thoughtful study design, methodological transparency, and a willingness to embrace complexity, the field is well positioned to make real progress in the years ahead.

ACKNOWLEDGMENTS

P.D.C. is an honorary research director at the Fonds de la Recherche Scientifique (FNRS) and a recipient of grants from the FNRS (Projet de Recherche PDR-convention, FNRS T.0032.25; CDR-convention, J.0027.22; FRFS-WELBIO, WELBIO-CR-2022A-02P; and EOS program no. 40007505).

DECLARATION OF INTERESTS

P.D.C. is an inventor on patent applications dealing with the use of bacteria on metabolic disorders. P.D.C. is a co-founder of Enterosys.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, we used ChatGPT to prepare some of the figures and in polishing English spelling. After using this tool, we reviewed and edited the content as needed, and we take full responsibility for the content of the publication.

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