Title

Chapter 18 The gut microbiome in personalized precision medicine

ISBN

9780323988087

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Publication Date

2025

DOI

10.1016/b978-0-323-98808-7.00012-6

Peer reviewed

CHAPTER 18

The gut microbiome in personalized precision medicine

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Introduction

The human microbiome is comprised of dynamic communities of microorganisms that colonize the body and fulfill important molecular and metabolic functions that are vital for processes including pathogen control, immune regulation, digestion, neurophysiology, and metabolite production [1]. The term "microbiome" refers to the collection of bacteria, archaea, viruses, and fungi that reside in distinct anatomical sites throughout the body [2]. In the past decade, our understanding of microbial ecology has grown exponentially, creating new clarity on the microbial composition, and signaling mechanisms that underpin both human health and disease. The importance for the microbiome in promoting human health is emphasized when the vast number of clinical conditions and diseases associated with imbalances in gut microbial composition and downstream alterations are considered. These include obesity and metabolic syndrome [3,4], inflammatory bowel disease [5], neurological disorders [6], cardiovascular disease [7], and cancer [8].

Integrating clinical screening of human genomic information into patient care and diagnosis is now routine to identify host polymorphisms associated with disease susceptibility and that might affect treatment response. Fortunately, the next generation sequencing (NGS) technologies that make these clinical tests possible have also become the standard microbiome analysis tool. When NGS data are paired with data generated from mass spectrometry, clinical outcomes, and highly novel bioinformatic approaches, the data bonanza can generate new discovery and hypotheses linking structure to function [9,10].

This ever-increasing understanding of how the microbiome affects human health and disease creates the strong argument that human microbiome data should be included in clinical precision medicine strategies. Taking further this argument, microbiome "states" are highly individual, even between coraised identical twins [11]. The general composition of the gut

Implementation of Personalized Precision Medicine. https://doi.org/10.1016/B978-0-323-98808-7.00012-6

microbiome also remains relatively stable and unique for a person throughout their adult life. Interestingly, less than 2% of microbiome diversity is explained by host genetics, suggesting that microbiome data are unique from the host. The gut microbiome also creates a prime opportunity for intervention, as the proportions of the microbial members can be influenced by diet, lifestyle practices, and the environment, among other factors [12,13]. Thus, the human microbiome may serve as a valuable complementary approach to traditional genomic medicine to create novel opportunities for individualized treatments.

In the following sections we outline the current state of knowledge of the human gut microbiome and highlight how precision medicine approaches can be applied.

Human biome in health and disease

Resident commensal microorganisms, including gram-positive Firmicutes, gram-negative Bacteroidetes and Proteobacteria, and methanogenic archaea within the intestines [14], have adapted complex ecological networks with the host and other microbes to acquire nutrients and thrive within the intestinal environment. Host-microbe, microbe-microbe, and microbe environment interactions determine the proportions and distributions of individual organisms throughout the gastrointestinal tract, with nutrient dependencies predominately determining niches of individual microbes. The gut microbiota provides numerous functions critical for human health and homeostasis, including, but not limited to, biosynthesis of steroid hormones, neurotransmitters, and vitamins, xenobiotic metabolism, cell proliferation, neurologic signaling, vascularization, and regulation of host immune maturation [15]. An abundance of data now suggests that gut microbial synthesis and secretion of metabolites may be equally, if not more, important for the maintenance of health and disease prevention as the specific composition of microbes, but the composition and their metabolic activity are relatively well correlated [16,17]. Microbial metabolic products such as SCFAs, for example, promote production of intestinal mucus, local immune activation, and antimicrobial peptides, which are important for intestinal barrier integrity and robust immunity. Not surprisingly, metabolites are also variable between individuals. For example, choline and short-chain fatty acids (SCFAs) also seem to vary between individuals [18,19], which was supported by a study using nontargeted shotgun mass spectrometry metaproteomics that showed microbiota composition and function differed between individuals at the protein level [20].

That microbiomes vary between people is valuable if we are to use microbial features to predict disease or health states. However, defining a health- or disease-associated microbiome has proved difficult. This has been captured in the Anna Karenina hypothesis based on Tolstoy's books opening sentence "All happy families are alike; each unhappy family is unhappy in its own way" [21]. While a microbiome, in equilibrium with the body, is associated with health, a disease-associated microbiome can be different for each disorder. This has led to the term "dysbiosis," wherein the microbiome and the body are no longer in equilibrium [22].

Characterizing the elusive "health-" and "disease"-associated microbiome pattern may be much harder than previous envisioned. This is in part due to the highly stochastic nature of microbial community assembly, driven by a wide variety of external and internal influences that can shape the accumulation of functional traits by the community. For example, pathogens and commensal bacteria alike require particular ecological niches within the intestine to colonize and proliferate, and as such, competitive mechanisms have evolved. Bacteriocins and protein toxins produced by commensal bacteria, specifically, can inhibit growth or function of the same or other bacterial species [23]. Other examples include microbial generation of SCFAs to alter the local pH, which can inhibit growth and colonization of other species driving changes in the ecological structure [24–26].

A brief outline of gut microbiome-mediated disorders are summarized in Table 1.

Table 1 A summary of gut microbiome-mediated disorders highlights the involvement of the human microbiome across the chronic disease spectrum.

Disease/ disorder	Microbial changes and consequence [27]	Microbial-based therapeutics and outlook [27,28]
Inflammatory bowel disease	Gut microbiota dysbiosis [29]; Decrease Firmicutes including *Roseburia hominis* and *Faecalibacterium prausnitzii* [30,31]; Increases in pathogenic *Mycobacterium avium paratuberculosis,* adherent invasive *Escherichia coli*, *Clostridium difficile*, Campylobacter, and Salmonella [32].	Microbial therapeutics based on underlying mechanisms and identifying features for successful fecal–microbial transplant [3,33–36].
Crohn's disease	Higher proportion of fungi to bacteria; increased Basidiomycota:Ascomycota ratio; decreased proportion of *Saccharomyces cerevisiae*, increased *Candida albicans* [37,38].	Dietary management [39].
Irritable Bowel syndrome	Increase in SCFA producing Firmicutes, particularly unclassified Clostridium cluster IV and XIV [40]; higher levels of mucin-degrading *Ruminococcus torques* and *Akkermansia muciniphila* [40].	Dietary management [41].
Obesity	Gut microbiota dysbiosis associated with increased Firmicutes:Bacteroidetes [42]; increased systemic lipopolysaccharides arising from Gram-negative bacteria [43,44].	Microbiome therapeutics in trials [45–49] with validation studies needed.
Type 2 diabetes	Depleted fiber-degrading and SCFA-producing bacteria such as Roseburia, Eubacterium, and Fecalibacterium [50]	Next-generation microbial therapeutics [51]; microbiome-guided dietary and drug therapy [52,53].

Continued

Table 1 A summary of gut microbiome-mediated disorders highlights the involvement of the human microbiome across the chronic disease spectrum—cont'd

Disease/ disorder	Microbial changes and consequence	Microbial-based therapeutics and outlook [27,28]
Cardiovascular disease	Gut microbiota metabolism of choline, phosphatidylcholine, and carnitine leads to trimethylamine and oxidation to trimethylamine, associated with atherosclerotic plaque development [7,18].	Therapeutic targets against bacterial metabolism under development [54,55].
Cancer	Gut microbiota can serve as diagnostic tool for early-stage cancer [56,57]; production of metabolites and modulation of immune states [58–60].	Microbial therapeutics (pre- and probiotics, metabolites) [56]; activity-based protein profiling for predicting efficacy and adverse events [61]
Neurological disorders	Gut microbiota may interact with the nervous system through production of neuroactive molecules [62,63], implicated in Parkinson's, Alzheimer's, pain [64], depression [65], other neurodegenerative diseases [66–68].	Dietary management; GABA manipulation [69].
NAFLD	Microbiota-mediated mechanism can result in deleterious changes in the liver [70]; ethanol production by bacteria might contribute to steatosis [71–73].	Bacteria and bacteriophage-based therapies show potential in liver disease [74,75].
Clostridioides difficile infection	The gut microbiome is a determinant of *C. difficile* infection [76]; strain-specific differences might determine antibiotic resistance [77].	FMT to treat recurrent *C. difficile* infection with ongoing studies in replacement with use of defined microbial communities [78].

Dietary choices and the microbiome

The gut microbiome is influenced by several external environmental factors, as described in the earlier section. Of these, the long-term diet seems to be the primary environmental factor influencing the gut microbiota proportions, function, and host–microbiome interactions. The possibility of preventing and treating disease through modulation of the gut microbiota or their secreted metabolites by dietary interventional strategies is an active and developing area of research. Diets rich in fiber, from foods such as grains, legumes, and leafy greens, promote a diverse gut microbiome and subsequent release of diverse beneficial metabolites, including SCFAs. Conversely, diets poor in fiber display reduced microbial diversity and altered function, and are often associated with impaired host physiology and increased susceptibility to chronic inflammation and infection. The proportion of the commensal microbe, *Prevotella copri*, for example, correlates with improved glucose and insulin tolerance in subjects consuming a high fiber diet [79,80]. Subsequent NGS metagenomic analyses defined four distinct carbohydrate metabolizing clades that utilized distinct plant-derived polysaccharides [79,80].

Studies such as the one described in the earlier section have utilized African and South American populations, whose diets are high in fiber, which contrast with the low fiber Westernized diet [81–84]. With increased rates of obesity, diabetes, cancer, etc. in developed nations, the question arises regarding the extent to which coevolution between humans and their gut microbiota, and the resulting impact of a low fiber high saturated fat diet on our microbiota and health has naturally been raised. Diverse global populations have thus become important if we are to understand diet-influenced differences in the gut microbiota and human health. Not surprising, differences in the gut microbiota between populations have been consistently observed. Specifically, worldwide populations with a diet rich in fruit, vegetables, and fibrous tubers are enriched for fiber-fermenting bacteria, including *Xylanibacter, Treponema, Lachnospira,* and *Prevotella* [81,82,84]. When these are reduced, reduced beneficial metabolites, including SCFAs, and a decreased capability to degrade complex polysaccharides are also observed. [85] However, in Western populations consuming a diet rich in fiber, a greater proportion of *Prevotella* and *Lachnospira* are again observed, along with greater concentrations of SCFAs in stool and blood [86]. Together, data suggest a diet high in fiber plays an important role to promote human health through enhancing microbial diversity and production of beneficial metabolites, which raises promise that restoration of the microbiota to a healthy state could be achieved through either dietary intervention or through treatment with microbial carbohydrate-degrading enzymes to replace metabolic activity lost through diet-induced microbiome changes [87].

Current clinical knowledge and interventions

Given observed connections between the microbiome and health, numerous clinical studies have been performed to utilize the microbiome as an interventional health care strategy. Microbiome interventions fall into two primary categories, untargeted and targeted. Untargeted methods, including antibiotics, probiotics, dietary changes, and fecal microbiota transplantation (FMT) seek to impact the microbiome by restoring a healthy species and functional diversity to the entire host-associated community. Targeted methods, such as engineering microbes and specific enzymes or metabolites, seek to restore homeostasis with a specific microbe or drug.

Of the untargeted strategies, FMT and use of probiotic products are perhaps the most well-known and most explored. FMT, in which the stool of a healthy donor is transplanted into the gastrointestinal tract of a patient, is becoming a common and successful treatment for *Clostridioides difficile* (C. diff) infection. *C. difficile* infection can occur for a variety of reasons including chronic illnesses or gastrointestinal conditions [88]. With this success, many studies are exploring use of FMT to also treat metabolic diseases, including insulin sensitivity and diabetes, with alteration of the microbial population and improved disease state noted in multiple studies [88–90]. As further evidence of the potential for FMT, patients enrolled in a

recent pilot study of FMT in ulcerative colitis achieved a response rate near 90%, accompanied by changes in IL-6, CXCL5, and other immunomodulatory cytokines [91]. Despite the potential for use of FMT to treat metabolic disease, the variation in intestinal microbiome composition between individuals makes standardized treatment for clinical symptoms a challenge. Probiotics, unlike FMT, have a defined composition. Commonly used probiotics include *Lactobacillus, Bifidobacterium,* and *E. coli* Nissile 1917. Similar to FMT, probiotics have also been shown to significantly improve disease markers such as fasting glucose, insulin sensitivity, and cholesterol [92–96]. Further, studies using probiotic or prebiotic-probiotic combinations have reported improvement in hepatic fat [97] and liver enzymes [98] in NAFLD patients. However, results in probiotic studies are not always consistent, and it is unclear to date if failed outcomes are the result of other study limitations, variability in strains, or differences in subjects, for example in their endogenous microbiome.

In targeted microbiome intervention strategies, engineered microbes designed with specific genetic modifications can be used to deliver microbes that carry out specific disease-relevant function. For example, Duan et al. engineered *Lactobacillus gasseri* ATCC 33323 to secrete human GLP-1 (1–37), which is a hormone that regulates glucose metabolism by stimulating intestinal epithelial cells to secrete insulin. Administration of GLP-1-expressing *Lactobacillus* increased the insulin level in diabetic rats, leading to a reduction in blood glucose level [99]. Instead of enriching or depleting such bacterial producers through engineering, another strategy has been the use of supplements of bacteria-derived metabolites to restore a depleted metabolite pool or to inhibit the action of a specific metabolite. Experimental evidence highlights the potential of "postbiotic" therapeutic application. For example, administration of SCFAs improved inflammatory conditions in colitis-mouse models [100], supplementation of flavonoids alleviated weight regain following successful dietary-weight loss in animal models with recurrent obesity [4], and inhibitors of microbial enzymes producing the metabolite, trimethylamine N-oxide from L-carnitine, can reduce stroke and myocardial infarction [101]. Although currently, most studies of targeted microbiome interventions have been undertaken in animal models; to date, results show promise as a future avenue for clinical study and disease treatment.

Microbiome analysis tools for precision medicine

Based on the direct relationship between diet, microbial composition and function, and host-related disorders, measurements of both composition and function are required for microbiome precision medicine to be implemented. Currently, to understand microbial composition within the microbiota, two approaches, 16S rRNA amplicon sequencing and shotgun metagenomics, are widely in use to extract and analyze microbial genomic DNA. The 16S rRNA gene is comprised of both highly conserved and hypervariable regions, which allows for broad use of primers, as well as identification of base pair differences that allow species

level identification [102,103]. Typical 16S amplicon studies compare differences between observed communities of bacteria between differing samples to calculate statistical correlations between microbial composition and sample descriptions. The Earth Microbiome Project's standardized amplicon protocols have become de facto industry standards and are widely available with rigorous benchmarking (https://earthmicrobiome.org/). Shotgun metagenomics is becoming increasingly popular due to the power of the approach to provide a higher resolution of the entire microbial community, the metabolic and signaling capacity of each taxon, at costs that are significantly less expensive with each passing year [104,105]. In this way, taxonomy can be determined from signature genes, and phylogeny can be assigned by comparing DNA sequences against a library of genomes from databased relatives. Genomes can also be assembled from organism in the microbiome that are resistant to culture, enabling exploration of taxa associated with each sample or person, and allows determination of metabolic and signaling capacity of each taxon to understand how it might interact with the rest of the body or its environment [104–106]. The utility of amplicon and metagenomic sequencing is best described by the Microbiome Wide Association Study [107] paradigm, whereby microbial traits (genes, species, pathways) in the microbiome are statistically associated with health or disease traits in the host population.

Both 16S rRNA amplicon sequencing and shotgun metagenomics have limitations. Primarily, the microorganism evolution, microbial horizontal gene transfer, and subtleties in characterization of different types of microbiomes (including body sampling site), can make the single snapshot of the microbiome problematic. Additionally, both approaches can have contamination from undesired DNA and biases toward culturable organisms [108], and both require specific training in microbiome analysis to identify correlations, with metagenomics being more computationally complex.

Additional tools are constantly being developed to identify metabolites alongside the microbial communities in order to understand the microbial functional traits encoded in the DNA. Metabolomics refers to analysis of all metabolites in a given sample. Metabolomics seeks to analyze the metabolites in a sample so that they may be quantified and associated with human or microbial traits, in much the same MWAS analysis for DNA sequencing approaches. The current paradigm for metabolite discovery involves molecule identification by mass spectrometry coupled to liquid or gas chromatography (GC-MS). Although this method offers chromatographic resolution and reproducibility, it is limited by poor dynamic range, accurate mass, and a scan rate sufficient for more complex samples (i.e., mammalian tissue), which ultimately results in significantly less than 30% of compounds identified; and often only ~5% of spectrum peaks identified as originating from a given molecule [109]. Additionally, the polar nature of many metabolites requires specialized approaches to allow the volatilization required for chromatography. Finally, the high-sample throughput required for -omics level analysis is limited. To overcome these challenges, a next generation of mass spectrometers has been developed, including the Orbitrap series by Thermo Scientific. The Orbitrap mass spectrometer

coupled with liquid chromatography (LC) has contributed to the development of metabolite annotation in a variety of public databases by providing high mass resolving power combined with tandem MS capabilities [110–113].

Community interconnections in the microbiome

Microorganisms thrive in communities with large numbers and close interactions that benefit the population [114]. Relationships are far from simplistic, spanning the landscape of ecological relationships that include mutualisms, commensalism, synergism, competition, parasitism, and predation. In this way, microbe interactions and relationships add to the genetic diversity in microbial population. Functional genes can be dropped in a microbial genome from random mutations and selective pressures, leading to low or medium gene frequencies [115], and interactions can be reshaped by gaining genes that adapt and extend the niche [116]. The breadth of these relationships has been emphasized through data collected through the Earth Microbiome Project (EMP). The EMP is a public database and framework for sample collection with standardized sequencing and metadata curation [117]. The data has emphasized the wired pattern among microbial communities in different environments and emphasized that community characteristics can be used in conjunction with microbial taxon composition profiles. Although EMP datasets focus on environmental datasets focusing on bacterial and archaeal communities, it has broad relevance to other lifeforms on earth, especially humans, where the interactome is relevant to health outcomes. The EMP experimental and analysis framework has now been referenced over 1100 times as researchers seek to understand microbial cooccurrence patterns.

Current advances and future challenges for microbiome-mediated precision medicine

With decreasing sampling and processing costs and the development of novel sequencing technologies, it is becoming possible to sample more densely in time, in a longitudinal fashion, to capture the dynamics of microbial interactions [118]. Emerging sampling techniques, such as laser capture microdissection of intestinal crypts, are also advancing understanding of the spatial inhomogeneous nature of the microbiome and its influence on function [119], and development of automatic sampling devices is making longitudinal collection more accessible and feasible (e.g., BiomeSense Gutlab, https://www.biomesense.com). However, as identifying bacteria and their metabolisms that may be causative in health -and disease-states for unique individuals is an important facet of precision medicine, combining these approaches with novel analysis to understand how the overall ecology of the microbiome pertains to an individual's health is possibly even more vital. To do this, quantitative measures of microbiome composition and metabolites must be paired with patient health and lifestyle measures and clinical outcomes. Such rich datasets require bioinformatic approaches for modeling to predict insights

into the relationships between the host, the microbiome, health, and disease. Although combining datatypes is challenging, advances in artificial intelligence, and more specifically machine learning (ML) approaches, are making it possible to explore relationships between genetic, physical, and clinical information [120]. For example, Hollister and colleagues used ML to analyze multiomic features to understand the connections between childhood irritable bowel syndrome and nutritional interventions. They were able to identify associations between abdominal pain, microorganisms, and metabolites, with the potential do precipitate novel microbiome-mediated stratification and therapeutic strategies [121]. Novel technologies, analysis approaches, and comprehensive data collections are also being combined with data sharing approaches that publicly open up datasets for analysis. Such examples include the IBD project, which includes microbiome, host genotype, phenotype data, and transcriptomes from biopsies of greater than 1200 patients with IBD [122] and the IBD Multiomics Database, which provides comprehensive descriptions of microbial and host activities in IBD [123].

The future presents both opportunities and challenges for precision medicine. The range of bacteria present, gene expression variations, and single nucleotide polymorphisms both between regions of the gut and between individuals are vast, and often outnumber patient samples as well as time points collected. Achieving appropriate statistical power to facilitate robust AI analyses [124], and subsequent patient sampling is more important than ever for drawing meaningful conclusions. Nevertheless, the combined advances in knowledge and technology have resulted in current treatment advances and are prime for continued advances in precision medicine.

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