Microbiome at the Frontier of Personalized Medicine

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Abstract

The genomic revolution promises to transform our approach to treat patients by individualizing treatments, reducing adverse events, and decreasing health care costs. The early advances using this have been realized primarily by optimizing preventive and therapeutic approaches in cancer using human genome sequencing. The ability to characterize the microbiome, which includes all the microbes that reside within and upon us and all their genetic elements, using next-generation sequencing allows us to now incorporate this important contributor to human disease into developing new preventive and therapeutic strategies. In this review we highlight the importance of the microbiome in all aspects of human disease, including pathogenesis, phenotype, prognosis, and response to treatment, as well as their role as diagnostic and therapeutic biomarkers. We provide a role for next-generation sequencing in both precise microbial identification of infectious diseases and characterization of microbial communities and their function. Taken together, the microbiome is emerging as an integral part of precision medicine approach as it not only contributes to interindividual variability in all aspects of a disease but also represents a potentially modifiable factor that is amenable to targeting by therapeutics.

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The focus of biomedical research for most of its existence has been the ability to identify and target specific disease-associated pathways, leading to therapeutic strategies targeting a pathway. This approach remains mostly naive to interindividual variability in development of disease and response to therapy especially relevant in multifactorial diseases. However, the genomic revolution has provided a window into individual-specific information and its effect on human physiology, paving the way for personalized or precision medicine.[1] Over the past decade, efforts in oncology have allowed human genomic screening to identify a spectrum of germline-encoded sequence variations, enabling individual-specific application of preventive and therapeutic strategies. In addition to personalization of treatment based on genetic contribution to disease pathogenesis, precision medicine efforts have allowed stratification of patients based on response to treatment and development of adverse events.

The advent of microbiome research has identified the microbiome as an important contributor to human health, and in this review we highlight why the microbiome is an integral component of the precision medicine initiative (Figure). The microbiome represents the complex collection of microorganisms both within and upon us, their genomes, and collective functions.[2] The field has benefited vastly from the genomic revolution, allowing DNA-based identification of nonculturable bacteria inhabiting various body sites. Alteration in microbial communities (often referred to as *dysbiosis*) has been shown to be associated with diseases ranging from infectious (*Clostridium difficile* infection) to inflammatory (inflammatory bowel disease [IBD] and rheumatoid arthritis) and metabolic (diabetes and obesity) diseases, suggesting an important role for them in the pathogenesis of multifactorial conditions.[3] An important aspect about the microbiome is its resilience as well as its plasticity, making it more mutable than human cells. Although on first impression these appear opposing concepts, the resilience of the microbiome is evident in health, in which, in spite of temporary insults (travel, diet, antibiotics, etc), the microbiome maintains a relatively stable steady state. In contrast, it represents a malleable organ and can be modified by dietary and other directed therapies (Figure). Furthermore, the interindividual variability in composition and metabolic capacity of the microbiome play an important role in interactions with the environment, resulting in the development of disease as well as response to treatment and development of adverse events. The microbiome has been shown to be determined in part by the host genome, but this contribution seems small when compared with the vast environmental microbiome modulation. Hence, the important role of the microbiome in human health, the interindividual variability and contribution to host function in health, and its plasticity making it a targetable factor all point toward the importance of incorporating the microbiome into precision medicine (Figure).

The current methods use a spectrum of strategies to characterize the microbiome, the simplest being the marker gene approach using variable regions within the highly conserved 16S ribosomal RNA gene. This approach, although valuable in assessing alterations in microbial community structure, fails to provide resolution at species or strain level and does not provide sufficient functional insight into the community. Complimentary approaches including metagenomics (study of all genomes in an ecosystem), metatranscriptomics (characterization of gene expression from all microbes in an ecosystem), metabolomics (characterization of all small molecule metabolites in an ecosystem), and metaproteomics (characterization of all proteins in an ecosystem) provide greater insight into functional potential as well as the expression of microbiome-derived bioactive molecules necessary to understand the therapeutic implications for the microbiome. Although the microbiome represents an attractive target for the development of personalized treatment approaches, standardization of methods to develop reliable and reproducible microbiome-based diagnostic and therapeutic strategies remains a challenge. The strong effort by the scientific community, as well as collaboration with rapidly emerging biotech companies, provides an optimistic outlook for developing microbiome-dependent and microbiome-targeted diagnostics and therapeutics.

SEQUENCING REVOLUTION ALLOWS DEVELOPMENT OF PRECISE MICROBIAL DIAGNOSTICS

Awareness of the role of the microbiome in health has both benefited from and been spurred by sequencing technology. Once considered milestone achievements requiring

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the resources of dedicated genomic centers, the sequencing of a complete bacterial genome can now be performed on a laboratory bench for about a hundred dollars per sample. Rapidly declining costs and continuing development of software and algorithms for assembling genomes, either from existing reference databases or de novo, promise to fundamentally alter the clinical paradigm by improving our ability to track, understand, and identify disease-causing agents.[4]

Here we will describe some of the applications of bacterial genome sequencing and attempt to summarize some of the many efforts going on worldwide to bring genomic data to various problems ranging from bacterial typing[5,6] to antiterrorism.[7] Although these might seem like disparate use cases, what unites them is the data contained within the genome, which contains sequence variations that reflect evolutionary relationships[8] and genes that underlie important phenotypes such as antibiotic resistance.[9-11]

Infectious disease tracking involves the ability to detect and trace outbreaks of disease. This assists hospitals in preventing the spread of nosocomial infections, food distributors in tracing back contaminated food sources, and governments in protecting people from biological agents. The most common of these uses is in the hospital in which an indication of nosocomial disease spread can be used to improve the practice of medicine. However, these efforts have largely relied on event count and statistics; that is, they are more reactive than proactive.

The most prominent bacterial typing technique is pulse-field gel electrophoresis (PFGE),[12] which relies on restriction enzymes and gels to obtain a rough distribution of genome fragment sizes and in essence provides little detailed information and must generally be used with care and attention to detail.[13] Great effect and effort has been made through the standardization of PFGE techniques to enhance the comparability of results between different gels run at different laboratories.[14-16] However, PFGE retains its difficulties in detecting infectious outbreaks across multiple centers.[17]

Where PFGE falls short on finer resolution and reproducibility, genome sequencing excels. Genomic data provide a base-by-base genomic "fingerprint" that enhances the resolution with which monitoring becomes possible. The fact that this may one day enable us to identify potential outbreaks sooner and act to prevent them before they become larger has prompted numerous studies on the efficacy of different comparison methodologies.[18-20] These methods have been tested across various species and range from single nucleotide polymorphism—based tests to use of whole-genome comparisons.[21,22] This has also given rise to a large number of publically available phylogenetic reconstruction algorithms that analyze genomic data for signatures of relatedness to track relationships between different pathogens.[23-25] These algorithms use the evolutionary principle of descent with modification to assess which strains descended from a recent common ancestor.

In addition, sequencing provides a great deal of information about the characteristics of an

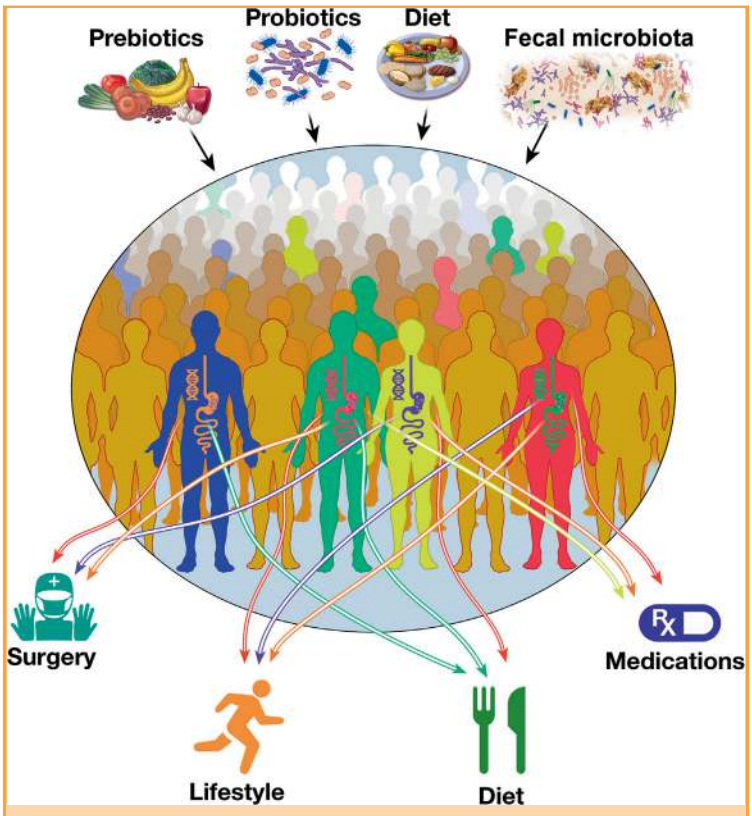

FIGURE. Gut microbiome as a determinant of human health and response to therapeutic intervention. The gut microbiome plays an important role in an individual's response to interventions ranging from dietary and lifestyle changes to medications and surgical interventions; hence, in addition to host genetics, it is important to consider the role of the gut microbiome in selecting appropriate therapy. The gut microbiome, unlike host genes, represents a modifiable factor that can be targeted by probiotics, prebiotics, diet, as well as community replacement approaches such as fecal microbiota transplant.

infection. One can query for antibiotic resistance genes, identifying susceptibilities in antibiotic-resistant pathogens. This can be done using polymerase chain reaction amplicon sequencing[26,27] or whole-genome sequencing.[9,10] Although polymerase chain reaction—based approaches currently have an advantage in turnaround time and cost, whole-genome approaches provide more information and a common platform for evaluating multiple species. Both methods have the potential to directly assess antibiotic resistance without culture, a feature that becomes especially important in the case of slow-growing bacteria, such as tuberculosis, in which culture-based tests can take weeks to complete.

The utility of sequencing bacteria goes beyond pathogen or pathogen-complex evaluation. They can be used to directly assess more complex specimens revealing microbial ecosystems with multiple species present and represent a potential tool for diagnosing infections of unknown origin.[28-30] Such broad searches require even greater bioinformatics and database support. This need has spurred a rapid growth in the number of publicly available resources for identifying potential infectious agents from complex microbiome data.[31,32]

MICROBIOME SEQUENCING

The revolutionary change in our ability to understand the role of the microbiome came with the advent of next-generation sequencing that has allowed in-depth characterization of the gut microbiota using multi-omics approaches without the need to culture individual microbes, which in some instances can be quite challenging. The most popular method to characterize microbial communities is the marker gene approach using the 16S ribosomal RNA gene, which is highly conserved in bacteria with little evidence of horizontal gene transfer. However, this approach lacks species and strain level resolution, which often requires metagenomic sequencing and de novo assembly of genomes, providing better compositional as well as functional resolution of the microbiome.[2] Metatranscriptomics compliments metagenomics by allowing identification of microbial genes that are expressed under different conditions. Metabolomics and metaproteomics help identify metabolites and proteins resulting from microbe-host cometabolism, which can serve as reliable biomarkers given that they represent end products of metabolic interactions among the microbe and host. The combination of multi-omic technologies increases confidence in identified diagnostic and therapeutic biomarkers as well as provides testable hypotheses. To test emergent hypotheses generated as a result of these technologies and delineate mechanisms by which microbes influence the host, germ-free animal models provide a highly controllable experimental system with reduced complexity to study interactions between the host and its resident microbiota.

MICROBIOME AS A TOOL FOR PRECISION DIAGNOSIS AND PERSONALIZED TREATMENT STRATEGIES

There is an emerging role of the gut microbiome as a biomarker for disease phenotype, prognosis, and response to treatment in addition to the well-described associations of alterations in microbial community structure in different disease states. Inflammatory bowel disease is one of the best-studied conditions associated with dysbiosis, with the microbiome serving as an important marker of disease phenotype and response to treatment. Inflammatory bowel disease is heterogeneous with 3 major subtypes: ulcerative colitis, Crohn disease (CD), and indeterminate colitis, which not only differ in their presentation and location but also have different therapeutic strategies, making it important to obtain a precise diagnosis. The microbial populations are quite distinct even within CD with a decrease in *Faecalibacterium prausnitzii* and increase in *Escherichia coli* as well as antibodies against *E coli* outer membrane protein C seen in ileal CD compared with colonic CD[33,34] as well as extraintestinal manifestations such as peripheral spondyloarthritis.[35] Gut microbiome signatures have also been associated with surgical outcomes in CD with an increase in *F prausnitzii* in the ileal mucosa associated with decreased disease recurrence at 6 months. In spite of several studies highlighting changes in the microbiome in IBD, there is lack of agreement among studies, making it imperative to have large cohorts from different geographic locations to overcome the effect of disease subtype, antibiotic use, diet, and other factors affecting the gut microbiome. This was highlighted in a study of treatment-naive patients with CD, in which a large patient cohort was needed to identify discriminatory taxa.[36] The study further found the need to study mucosa-associated bacteria, which may be

more relevant in inflammatory diseases such as IBD. In addition to IBD, microbiome signatures have been described in several other gastrointestinal diseases. *Fusobacterium nucleatum* has been implicated in colorectal cancer through its FadA adhesion serving as both a diagnostic and a therapeutic marker.[37] *Clostridium difficile* infection has been associated with decreased microbial diversity and a decrease in secondary bile acid production.[38] In addition, recently 2 studies have identified microbiome signatures in *Clostridium difficile* infection that allow prediction of disease outcome enabling therapeutic stratification.[39,40] An expansion of Proteobacteria in the setting of dysbiotic microbiota was described in patients with celiac disease with gastrointestinal symptoms compared with those with extraintestinal manifestations of celiac disease.[41] In addition to diseases within the gastrointestinal tract, it is interesting to note that several studies have described gut microbiome signatures in systemic disorders such as rheumatoid arthritis. An expansion of *Prevotella copri* has been described in new-onset rheumatoid arthritis.[42] Another recent study identified enrichment of *Collinsella*, *Eggerthella*, and *Faecalibacterium* in patients with rheumatoid arthritis and a strong association of *Collinsella* with high levels of α-aminoadipic acid and asparagine as well as production of the α-aminoadipic cytokine interleukin 17A and experimental arthritis.[43] These few examples are just a window into accumulating experimental evidence for the role of the microbiome in human disease and the future of microbiome-based diagnostic and therapeutic biomarkers. Although these studies are helpful in identifying biomarkers, much work still needs to be done in validating these signatures in large multicenter cohorts as well identifying potential causative role using a combination of in vitro and in vivo models.

MICROBIOME AS A DETERMINANT OF HUMAN THERAPEUTICS

The ecology of a microbial population, as in any ecosystem, involves a lot of cross talk between different species. Microbial survival and growth is governed strongly by their chemical environment, and unsurprisingly, they have evolved gene cassettes for chemical warfare.[44,45] Indeed, the discovery of antibiotics first occurred in microbial culture as a unique characteristic of colonies[46] and since then broader surveys of the soil microbiota have revealed an even greater array of antibiotic compounds.[47,48] Recently, this has been extended to the human microbiome as well across multiple sites along the human body,[49] which means the source of compounds we need to harness control over our microbiome might already be within us.

In addition to antibiotics and signaling agents, the discovery of the so-called bacterial immune system, that is, the CRISPR-Cas system, allows bacteria to resist and exclude bacteriophages from the population by targeting specific sequences for cleavage.[50] Although providing adaptive immunity to viruses, the industrial uses of this biological system have been widely recognized, leading to the implementation of synthetic CRISPR-Cas systems[51] that have led to the implementation of species-specific antimicrobial agents[52] that may be able to preserve the bulk of the microbiome while still making key alterations.

In addition to being a source of therapeutics with implications for human disease, the microbiome serves as both a modulator of traditional therapies and a target for therapies. The interindividual variability in response to therapy and development of adverse events has been attributed to individual specific disease phenotype and host genetics, but gut microbiota is often overlooked. However, the gut microbiota plays an important role in drug transformation affecting their efficacy. Acetaminophen, a commonly used analgesic drug, may compete with bacteria-generated *p*-cresol for O-sulfonation, resulting in acetaminophen glucuronidation, which can explain in part interindividual variability in analgesic response[53] as well as differences in adverse events due to accumulation of its toxic metabolite *N*-acetyl-*p*-benzoquinone imine. Microbiome markers of drug efficacy ranging from chemotherapeutic agents to statins have been widely described. *Bifidobacterium* has been found to augment tumor control in mouse models of melanoma treated with anti—programmed death-ligand 1.[54] Similarly in humans, *Bacteroides* have been suggested to be responsible for antitumor effects of cytotoxic T-lymphocyte associated protein 4 blockade, commonly used for cancer immunotherapy.[55] Irinotecan (7-ethyl-1O-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin), a chemotherapeutic agent commonly used for colorectal cancer, can undergo β-glucuronidation by gut bacteria, resulting in an active metabolite that causes severe diarrhea.[56] It is important to

note that host genetic variation also plays an important role in shaping the gut microbiome.[57] Bacteria-derived coprostanol levels have been associated with clinical response to statins, which are commonly used as low-density lipoprotein cholesterol–lowering agents. Digoxin, a cardiac glycoside with a narrow therapeutic window, can be inactivated by *Eggerthella lenta* in the gut. Finally, a recent study highlights the role of the gut microbiota in mediating the antidiabetic effects of metformin.[58] These examples clearly highlight the importance of considering the gut microbiota when determining drug responses akin to pharmacogenomics (Figure). The combination of the 2 approaches will allow us to impart more precise and effective therapeutics while decreasing overall adverse events.

TARGETING THE MICROBIOME TO IMPROVE HEALTH

In addition to serving as diagnostic and therapeutic biomarkers and modulating therapeutic responses to drugs, the appealing aspect of the microbiome is its plasticity and our ability to modify components of the microbiome. The traditional approach to target microbial populations has been with the use of antibiotics, which are both essential and effective for treating systemic infections typically resulting from pathogen invasion. However, the unintended off-target effects on microbial community structure as well as adverse effects in humans makes it less appealing as precise therapies to target the microbiome.[59-61] There is a continued role for developing pathogen-targeted antibiotics by identifying specific targets, which narrow the spectrum of the antibiotic. A novel approach includes mining the microbiota for therapeutic targets by identifying specific functions that affect the host, allowing us to modify microbial community functionality without harming the community itself.[62] An example is the role of trimethylamine oxidase in atherosclerosis and the inhibition of bacterial trimethylamine lyases by 3,3-dimethyl-1-butanol, decreasing bacterial trimethylamine production in a high choline diet–fed murine model.[63] Even with precise targeting of a single pathway, there were still alterations in the microbiome by 3,3-dimethyl-1-butanol, highlighting the complexity of microbial interactions within these ecosystems. There are several other approaches to target the microbiome, including use of probiotics, prebiotics, as well as dietary interventions. The early probiotics (live microorganisms that, when given in sufficient amounts, confer a health benefit on the host) were dominated by members of the genera *Lactobacillus* and *Bifidobacterium*, but lacked precision in terms of targeting a biological function. A recent systematic review of medium- to high-quality randomized controlled trials using probiotics found that there was no significant effect on the gut microbiota compared to placebo. The clinical efficacy of currently available probiotics is difficult to assess given the small sample sizes limiting the power, heterogeneity in strains of bacteria used, end points, duration of treatment and molecular methods of studying the gut microbiota, recording of baseline measurements such as diet, and often a lack of good preclinical mechanistic data.[64] However, recent work highlights the promise of next-generation probiotics that will be developed using targeted approaches to alter microbial metabolism in a disease-specific manner. A precision approach using *Clostridium scindens* to augment resistance to *C difficile* infection by targeting secondary bile acid pathway[38] is one such example. Similarly, a multicomponent probiotic was shown to modulate the gut microbiome with resultant suppression of hepatocellular carcinoma in a mouse model.[65] The advent of genetic engineering and synthetic biology approaches also hold promise for the development of precision probiotics.[66] An example is the engineering of a common gut commensal to secrete the molecular signal cholera autoinducer-1, inhibiting *Vibrio cholerae* virulence in a mouse model.[67] Furthermore, tunable expression tools in robust colonizers of the human gut provides us the ability to further calibrate delivery of bioactive compounds by these precision probiotics.[68] Prebiotic (ingredients that are selectively fermented by gut microbes and confer a health benefit) approaches aim to modulate the microbial community in a way that is beneficial to human health. Although the early prebiotics have focused on promoting the growth of a single or group of beneficial bacteria, they fail to account for the downstream effects on other microbial members. Similar to probiotics, prebiotics that are mainly composed of fermentable oligosaccharides such as inulin and fructooligosaccharides have focused on increasing growth of potentially beneficial bacteria such as *Bifidobacterium*. The lack of an ecosystem approach is reflected in the modest clinical efficacy of available prebiotics. The development of next-generation prebiotics will require careful modeling of the metabolic interactions among the members of the ecosystem to better understand the overall effects

on the community and host physiology. Fecal microbiota transplant (FMT) that entails transfer of the healthy gut microbiota from a donor either orally via capsules or endoscopically has been highly successful as an ecosystem approach in treating recurrent *C difficile* infection.[69] A similar approach with FMT has been tested in multiple diseases associated with microbiome alterations but has failed to show clinical efficacy. However, the use of FMT for diseases such as IBD has provided insight into donor specificity[70] in terms of response, suggesting a role of individualizing FMT approaches in multifactorial diseases such as IBD, in contrast to the approach in *C difficile* infection.

Finally, diet has major implications for the microbiome as it is the primary nutrient source of microbes. Dietary manipulations fall with 3 distinct approaches. The use of microbiome markers in optimizing dietary interventions, modulating the diet based on the microbiome and using diet to alter the microbiome. Dietary interventions limiting fermentable oligosaccharides, disaccharides, monosaccharides, and polyols have shown to be beneficial in ameliorating symptoms in patients with irritable bowel syndrome.[71] However, long-term use of such an intervention can decrease microbial short chain fatty acid production, which, in turn, may have negative implications for human health. A recent study identified microbiome markers that predict a positive response to fermentable oligosaccharides, disaccharides, monosaccharides, and polyols[72] with the potential to allow optimization of therapy and minimizing undesirable adverse effects in individuals less likely to respond. An important aspect of the gut microbiome is its role in determining host responses to dietary components given that the microbiome plays an important role in metabolism of dietary nutrients. Zeevi et al[73] found large interpersonal differences in postprandial glycemic responses to dietary components in an elegant study of 800 participants. The prediction engine used to make the predictions incorporated multiple host and microbial parameters, and they found that the incorporation of microbiome-derived features improved the accuracy of prediction of glycemic responses.[73] In a follow-up study, the authors found significant interpersonal variability in the glycemic response to different bread types, and the glycemic response to different types of bread could be predicted solely from microbiome data before the intervention.[74] These studies highlight the ability to personalize nutritional intervention to improve host physiology based on an individual's microbiome. It is important to note that both short-term and long-term dietary patterns have a significant effect on shaping the microbiome. A diet high in protein and fat in the long-term has been associated with enrichment of *Bacteroides*, whereas a carbohydrate-rich diet has been associated with *Prevotella*.[75] Sonnenburg et al[76] reported that a Western diet low in microbiota accessible carbohydrates leads to decreased diversity in the microbiota of humanized mice, which are largely reversible within a single generation, but over several generations, this leads to a progressive loss of diversity that cannot be recovered by diet alone and needs replacement of the microbiota. This has significant implications for populations consuming a Western diet, which has been associated with decreased diversity and an increase in autoimmune diseases. The study suggests that even long-term dietary effects may be reversible within a certain time frame. Interestingly, short-term dietary effects on the microbiome seem to be easily reversible even when using extreme dietary interventions.[77] Moreover, short-term dietary interventions have shown to have beneficial effects on the host and gut microbiome. In the study by Zeevi et al[73] mentioned previously, introduction of meals associated with low postprandial glucose response led to an increase in bacteria thought to be protective against type 2 diabetes mellitus such as *Roseburia inulinivorans*, *Eubacterium eligens*, and *Bacteroides vulgatus*. Similarly, a 3-day dietary intervention with barley-based bread was associated with higher *Prevotella/Bacteroides* ratio and improved glucose metabolism.[78] It is interesting to note that changes in gut microbiota to a similar dietary intervention can vary depending on an individual's microbiome.[79] Taken together, it is apparent that although the relationship of diet and gut microbiome is complex, it is highly relevant in determining host responses to diet as well as predicting changes in the microbiome in response to the diet.

CONCLUSION

In this review we highlight the importance of incorporating the microbiome as a component of personalized or precision medicine to improve diagnosis, reduce disease risk, and

optimize early detection and treatment. Microbial fingerprints could serve as precise, noninvasive, accessible, and economic tools that could be used for personalized disease diagnosis including phenotypes, severity, and prognosis. The role of the microbiome in the metabolism of many chemical compounds makes it a key player in determining drug availability, efficacy, and toxicity, making it indispensable for developing personalized drug therapies. Finally, the ability to manipulate the microbiome makes it appealing in developing personalized treatment approaches by using precision microbiome targeting approaches. The use of approaches targeting specific microbial pathways tailored to an individual's microbiota may enable the development of treatment of multifactorial disorders such as IBD, obesity, and diabetes mellitus. The development of precision probiotics using genetic engineering approaches, next-generation prebiotics resulting from a better understanding of metabolic interactions among members of the microbial ecosystem, and personalized dietary therapies tailored to an individual's microbiota will form the new frontier in the field of personalized medicine.

Overall, the outlook is optimistic, but there are also substantial challenges in the field. To implement microbiome-based diagnostics and therapeutics, we need to develop uniform collection, sequencing, and analysis standards that would enhance reproducibility of results across centers and reduce biases in their interpretation. Most current studies are based on disease association, but we need to better define the mechanisms by which microbiota influence aspects of human disease to develop more reliable biomarkers. Furthermore, we are only beginning to appreciate the contribution of other microorganisms such as fungi, bacteriophages, and parasites as well as the interkingdom signaling among the microorganisms and the host. As we unravel aspects of these complex interactions, we will begin to develop more robust strategies to address the effect of the microbiome on the host.

The plasticity of the microbiome, while being advantageous in terms of making it amenable to intervention, also poses a challenge in terms of stability of changes. This was highlighted above, wherein dietary interventions can be developed on the basis of an individual's microbiome; however, it has the potential to change the microbiome itself. Hence, a systems approach to better understand the diet-microbiome interaction will allow the identification of dependencies between dietary compounds and bacterial taxa as well as prediction of trends in their variation resulting from dietary intervention.

These challenges apart, the integration of microbiome-based diagnostics and therapeutics into other components of personalized medicine such as pharmacogenomics and epigenomics will be an integral part of the new era in patient care. This integration will further enhance our ability to find the right treatment for the right patient while, at the same time, reducing adverse events and health care cost.

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Abbreviations and Acronyms: **CD** = Crohn disease; **FMT** = fecal microbiota transplant; **IBD** = inflammatory bowel disease; **PFGE** = pulse-field gel electrophoresis

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