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Taking it Personally: Personalized Utilization of the Human Microbiome in Health and Disease

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The genomic revolution enabled the clinical inclusion of an immense body of person-specific information to an extent that is revolutionizing medicine and science. The gut microbiome, our "second genome," dynamically integrates signals from the host and its environment, impacting health and risk of disease. Herein, we summarize how individualized characterization of the microbiome composition and function may assist in personalized diagnostic assessment, risk stratification, disease prevention, treatment decision-making, and patients' follow up. We further discuss the limitations, pitfalls, and challenges that the microbiome field faces in integrating patient-specific microbial data into the clinical realm. Finally, we highlight how recent insights into personalized modulation of the microbiome, by nutritional and pre-, pro-, and post-biotic intervention, may lead to development of individualized approaches that may enable us to harness the microbiome as a central precision medicine target.

Introduction

For many decades, modern medicine has focused on the identification of disease-specific diagnostic, preventive, and therapeutic modalities. Most such methods were found to be efficient in some, but not all, patients, although the person-specific factors driving individualized disease manifestations and response to treatment remained elusive. With the advent of genomic understanding of human physiology in the past two decades, the focus has been shifting from disease-specific toward patient-specific diagnostics and therapeutics, a new field termed personalized or precision medicine (Jameson and Longo, 2015). Most early advances in precision medicine were made in human oncology (Jameson and Longo, 2015; Hamburg and Collins, 2010), in which person-specific genomic screening for germ-line encoded mutations enables implementation of patient-tailored preventative or early treatment measures. Examples include preventive mastectomy for *BRCA1/2* mutation carriers (Rebbeck et al., 2004), periodic colonoscopies for patients with familial adenomatous polyposis (FAP) syndrome (Winawer et al., 2003), and prophylactic thyroidectomy in multiple endocrine neoplasia (Skinner et al., 2005). In addition, genomic characterization of somatic mutations in sporadic cancers (Druker et al., 2006) increasingly enables an accurate diagnosis of cancer subtypes, leading to custom-made tailoring of molecular therapy, as in the case of *EML4-ALK* non-small cell lung cancer and Crizotinib treatment (Kwak et al., 2010).

Non-cancer precision medicine is also being gradually integrated into clinical practice, enabling better diagnosis of diseases and their variants in multiple conditions ranging from celiac disease (Sollid, 2000) to cardiomyopathies (Biswas et al., 2014). In addition, stratification of patients by treatment responsiveness and susceptibility to adverse effects is attainable through characterization of allelic gene variations, such as the risk of cardiovascular events in patients receiving clopidogrel therapy correlating to *CYP2C19* gene variants (Simon et al., 2009). In some cases, medication doses may vary in accordance with patients' genetic profiles, such as in the case of oral anticoagulant warfarin, whose effective dosage was suggested to depend on allelic variations in the *VKORC1* and *CYP2C9* genes (Wizemann et al., 2010).

Parallel to the "genomic revolution," the recent decade has witnessed the advent of microbiome research, a complex ecosystem of microorganisms living on and inside our bodies whose genome outnumbers that of the host and influences multiple physiological functions. The association of the microbiome with host health and disease risk has materialized in the pioneering works of Jeffrey Gordon and colleagues, who were among the first to link the microbiome with obesity. Since these works, numerous other studies showed associations between alterations in the composition and function of the microbiota, termed dysbiosis, with "multi-factorial" disorders such as glucose intolerance (Zhang et al., 2013; Suez et al., 2014), obesity (Le Chatelier et al., 2013), type 2 diabetes mellitus (T2DM) and insulin resistance (Qin et al., 2012; Vijay-Kumar et al., 2010; Le Chatelier et al., 2013), aging-related disease (Claesson et al., 2012) and non-alcoholic fatty liver disease (Yan et al., 2011). At present, microbiome research is moving beyond description of community structure and disease associations, toward a deeper molecular understanding of its contributions to the pathogenesis of complex disorders. As such, recent next-generation DNA sequencing-based studies are suggesting that the utilization of person-specific microbiome

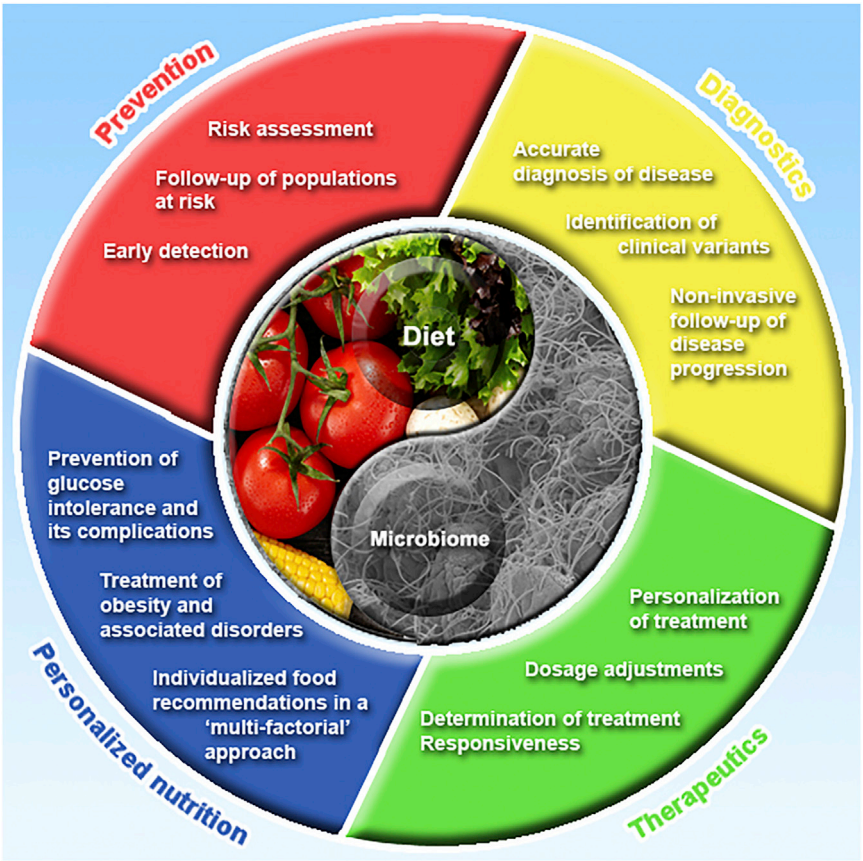

Figure 1. Microbiome and Precision Medicine
The microbiome, and its rapid modulation by factors such as diet, may impact multiple aspects of personalized medicine.

data may contribute to the development of precision medicine, personalized diagnostic and treatment modalities. Here, we will review these recent advances and their relevance to potential future application of microbiome-based knowledge in personalized patient care (Figure 1).

Microbiome in Personalized Disease Prevention and Risk Stratification

Assessing disease risk in susceptible subpopulations is one of the hallmarks of precision medicine, allowing for stratifications of these subpopulations in a manner that improves the accuracy and cost-effectiveness of follow up and treatment. In addition, such personalized diagnostics may increasingly allow, in some cases, initiation of prophylactic treatment that would otherwise be considered too aggressive for an entire population at risk. As the function, composition, and growth dynamics of the gut microbiome are associated with many host physiological and pathological states, non-invasive sampling methods and decreasing profiling costs make it a feasible avenue for early diagnosis and disease risk assessment.

Obesity research highlights the potential of microbiome-based disease risk stratification. As the obesity pandemic is becoming a substantial global health and economic burden, personalized diagnosis of individuals at risk of developing obesity and its metabolic complications becomes a critical unmet need (Ng et al., 2014). The gut microbiome is believed to be a marker and contributor to the development of obesity. Individual microbiome configurations feature differential capabilities of harvesting energy from food (Turnbaugh et al., 2006), leading to individualized effects on host energy storage (Bäckhed et al., 2004). Even in childhood, altered microbiome compositions have been suggested to be predictive of a propensity for becoming overweight later in adulthood (Koleva et al., 2015). Likewise, lower bacterial diversity and altered functional microbial pathway abundance have been strongly associated with obesity (Turnbaugh et al., 2006, 2009; Le Chatelier et al., 2013). These microbiome configurations directly contribute to obesity development, as colonization of GF mice with microbes from obese murine or human donors induce a significant weight gain in recipient mice (Turnbaugh et al., 2006; Ridaura et al., 2013). Correspondingly, these obesogenic effects were ameliorated by cohousing recipient mice with GF littermates receiving the microbiome of a lean donor (Ridaura et al., 2013). This strong association between dysbiosis and the propensity for obesity, starting early in human life (Koleva et al., 2015), may thus allow for identification, stratification, and preventive intervention of susceptible individuals at risk to develop obesity and its complications.

Similarly, the gut microbiome has been recently suggested to affect susceptibility to multiple other disorders, even those systemically occurring at remote extra-intestinal organs. Children with a high risk for developing type 1 diabetes mellitus (T1DM) exhibit dysbiosis and reduced abundance of lactate- and butyrate-producing species even before the overt manifestations of the disease (de Goffau et al., 2013; Kemppainen et al., 2015; Brown et al., 2011). Similarly, some features of dysbiosis have been correlated with asthma and atopy in children. These disorders subsided in a murine model upon reversion of bacterial composition to normality (Arrieta et al., 2015; Bisgaard et al., 2011). Increased predisposition to rheumatoid arthritis (RA) has been linked to gastrointestinal microbiota alterations, and it has been proposed that *Porphyromonas gingivalis*, which normally resides in the oral cavity, might be involved in its pathogenesis (Taneja, 2014). In all of these examples, microbiome characterization in individuals at risk, or of family members of diagnosed patients, may potentially aid in diagnosis and patient stratification leading to improved follow up and patient care. Likewise, microbiome assessment may contribute to early detection and patient stratification in a number of neoplastic disorders. Colorectal cancer, for example, was associated with

dysbiosis, characterized in some, but not all, patients by overabundance of *Fusobacterium*, among several other commensal gut microbes (Marchesi et al., 2011; Kostic et al., 2012; Castellarin et al., 2012; Sobhani et al., 2011; Ahn et al., 2013). Analysis of the salivary microbiome composition is suggested to aid in early detection of pancreatic cancer (Farrell et al., 2012; Torres et al., 2015). As such, integration of features related to the composition of the gut microbiome with other known clinical risk factors may potentially enhance early cancer detection (Zackular et al., 2014).

While personalized microbiome profiling holds promise of impacting disease risk stratification, much research is still required in order to more accurately investigate the associations between personalized microbiome signatures and the susceptibility to develop human diseases. For such predictive modeling systems to be integrated into clinical practice, personalized microbiome readouts far richer than relative composition, such as metagenomic, meta-transcriptomic, metabolomics, and metaproteomic analyses, should be included. Such extensive microbial characterization, coupled with inclusion of the gut virome and fungome into predictive modeling, would greatly add to the accuracy and reproducibility of disease risk assessment. On a positive note, in contrast to host genomics, the microbiome is readily modifiable, potentially allowing for not only the detection and risk stratification of individuals at risk for disease, but also their comprehensive follow up and reevaluation. With the automation of microbiome analysis, such longitudinal microbiome-based follow up may become accessible and cost-effective even at local community settings.

Microbiome and Precision Disease Diagnosis

Beyond the above potential utility of the microbiome in risk assessment, primary prevention, and follow up of patients at risk, microbiome analysis may aid in the actual diagnosis of diseases, as well as in the treatment decision-making process and prognosis estimation. Changes in the gut bacterial composition not only provide unique fingerprints of various conditions, but may also predict patient-specific disease activity, manifestations, severity, and responsiveness to treatment.

Bacterial (Frank et al., 2007; Manichanh et al., 2006) and viral (Norman et al., 2015) alterations in the gut microbiome have been widely described to associate with inflammatory bowel disease (IBD). These microbial alterations may contribute to inter-individual phenotypic variation in disease manifestations. For example, the microbiome may differentiate between ileal and colonic Crohn's disease (CD). This distinction is of major clinical importance, as these two IBD variants differ in their response to antibiotic regimens (Steinhart et al., 2002; Greenbloom et al., 1998) and enteral nutrition (Afzal et al., 2005). Patients with ileal CD harbor strikingly different bacterial populations as compared to patients with colonic CD or their healthy counterparts (Willing et al., 2009, 2010). Notably, the ileal CD phenotype was associated with reduced abundance of *Faecalibacterium prausnitzii* and enrichment with *Escherichia coli* as compared to the colonic CD phenotype. The increase in *E. coli* abundance coincides with previous studies, which showed elevated levels of antibodies directed against the *E. coli* outer membrane protein C (OmpC) and flagellin in ileal CD but not in colonic CD (Arnott et al., 2004; Targan et al., 2005), an effect potentially attributed to decreased production of alpha-defensins and diminished antimicrobial activity (Wehkamp et al., 2005). Furthermore, *E. coli* strains isolated from ileal CD patients were found to be more virulent and to correlate with the severity of disease (Baumgart et al., 2007). These data suggest that characterization of the microbiome holds potential as a non-invasive biomarker for disease phenotype, potentially even to a greater extent than the host genotype. Moreover, recent studies suggested that the prognosis of CD patients undergoing surgical resection of the terminal ileum may be predicted by the degree of dysbiosis, as patients who maintained post-surgical remission exhibited a higher microbial diversity as compared to individuals who experienced recurrence (Dey et al., 2013). Specifically, increased abundance of *F. prausnitzii* on resected ileal mucosa obtained during the surgery was found to be associated with decreased recurrence of the disease 6 months later (Sokol et al., 2008). Likewise, in pediatric CD, dysbiosis and diminished species richness was found to be correlated with disease severity and could predict outcome as quantified by a 6-month Pediatric CD Activity Index (PCDAI; Gevers et al., 2014). In UC patients who underwent colonic surgical resection and subsequent ileal pouch-anal anastomosis (IPAA), pouch microbiota composition and diversity was correlated with the presence and extent of pouch inflammation and CD-like complications (Tyler et al., 2013).

The emerging role of the microbiome in predicting disease manifestations, prognosis, and response to treatment can be illustrated in various other medical conditions. For example, in celiac disease a lower duodenal microbiome diversity and enhanced dysbiosis, dominated by Proteobacteria, was associated with gastrointestinal symptoms, as compared to patients suffering from extra-intestinal celiac-associated skin manifestations (Wacklin et al., 2013). Patients with new-onset rheumatoid arthritis (NORA) were recently shown to feature a striking gut microbiome expansion of *Prevotella copri*, as compared to patients with chronically treated RA, psoriatic arthritis, and healthy controls (Scher et al., 2013).

Taken together, these early studies suggest that integrating microbiome profiling into patient care may allow for a faster, more accurate, and less invasive clinical decision-making processes. Moreover, patient-specific microbiome features may be prospectively followed in a non-invasive ambulatory manner that would potentially allow for the assessment of disease activity or responsiveness to treatment, as is detailed below.

Microbiome and Personalized Treatment

Inter-personal differences in response to therapeutic regimens are often noted in medical treatment. However, despite the fact that gut commensals are notable for their capabilities to modulate drugs by a variety of bio-transformation processes, such as by hydrolysis and reduction, their potential effects on pharmacokinetics of orally and systemically administered medications have largely remained elusive. An early example of a major idiosyncratic adverse effect driven by gut microbiome activity involved the drug sorivudine, which was removed from the market in 1993 due to a lethal interaction with the chemotherapeutic agent 5-FU, secondary to intestinal bacteria-induced inactivation of the liver enzyme dihydropyrimidine dehydrogenase (DPD; Okuda et al., 1998). Similarly, the chemotherapeutic agents topotecan and irinotecan (CPT-11) are glucuronidated

into an inactive form by hepatic metabolism, but when they reach the gut they can undergo beta-glucuronidation by bacterial enzymes into the active form, thereby causing severe diarrhea (Wallace et al., 2010).

Only recently has the paradigm been shifting toward a more comprehensive investigation of the gut microbiome contribution to drug metabolism (Nicholson et al., 2005). The critical roles the microbiome plays in drug metabolism are exemplified in the case of digoxin, a cardiac glycoside used to treat congestive heart failure, which features a narrow therapeutic window leading to risk of toxicity. Long before the advent of microbiota research, Lindenbaum et al. (1981) noticed that some patients tend to chemically reduce the drug, thus rendering it inactive. Even stool cultures from these subjects converted the drug to its reduced form, while the administration of antibiotics eliminated the secretion of the inactive form and resulted in a 2-fold increase in plasma digoxin concentration, leading to a conclusion that enteric bacteria may modulate digoxin metabolism. A more recent study (Haiser et al., 2013) demonstrated that digoxin indeed undergoes inactivation by the species *Eggerthella lenta* and that the administration of antibiotics can offset this effect.

Acetaminophen, a compound found in many commonly prescribed analgesic drugs, exhibits a profound inter-individual variation in its clinical effects. A potential explanation of this personalized response has been recently linked to variation in microbiome function, with some individuals harboring p-cresol-generating bacteria, which favor acetaminophen glucuronidation over O-sulfonation due to competitive O-sulfonation of p-cresol. The same mechanism may apply to metabolism of other drugs that rely on sulfonation for their metabolism and excretion (Clayton et al., 2009). Statins, widely used for reduction of plasma low-density lipoprotein (LDL) cholesterol levels, are another example of microbiome-driven personalized drug responsiveness. Favorable responders to statin therapy, exhibiting a marked improvement in their plasma LDL levels, were found to feature certain secondary bile acids that are modulated by intestinal bacteria. Furthermore, a positive correlation was found between clinical response rate to statins and pre-treatment bacterial-derived coprostanol (COPR) levels, suggesting that the abundance of coprostanol-producing bacteria may predict the efficacy of statin therapy (Kaddurah-Daouk et al., 2011).

Likewise, the efficacy of chemotherapeutic agents is considerably influenced by commensal bacteria (Iida et al., 2013). Two recent papers shed light on the essential role of distinct gut microbiota in cancer immunotherapy. Sivan et al. (2015) studied the effect of commensal bacteria on anti-PD-L1 treatment in mouse models of melanoma and found that a defined microbiota composition was associated with an augmented T cell-mediated antitumor immunity, which was transferable to other mice by gastric gavage and abrogated by cohousing. The *Bifidobacterium* genus was identified as the causative agent for this favorable effect, with oral administration of this bacterium improving tumor control. Vétizou et al. (2015) investigated the interplay between gut bacteria and anti-CTLA-4 antibodies in murine models of cancer and in melanoma patients. They demonstrated that the microbiota induced an inflammatory response and conferred a beneficial effect on tumor growth in mice, which was abrogated in germ-free mice or after the administration of antibiotics. In humans, CTLA-4 blockade resulted in dysbiosis with propensity toward an increase of several *Bacteroides* species, which were suggested to be responsible for the observed antitumor properties.

Together, the notion that the microbiome plays a predominant role in drug modification is now gaining wider acceptance. Future pharmaceutical developments should take into account the unique effects that differential microbiota compositions and functions may have on drug metabolism, absorption, efficacy, and toxicity. These differences may also shed light on the varying efficacies of generic drugs with similar active compounds. Compiling these data may aid in prescribing the appropriate medical treatment, in a custom-made personalized fashion, to achieve safer and more effective treatment outcomes while minimizing adverse effects.

Microbiome and Personalized Nutrition

The past century has seen a pandemic of metabolic diseases including obesity, T2DM, non-alcoholic fatty liver disease, and associated cardiovascular diseases that impact large populations, thereby posing a substantial medical and economic burden on modern society. As such, there is a growing awareness of the need for primary preventive measures to modify the risk for the development of these disorders. Although dietary intake has long been known to play a role in the pathogenesis of obesity, T2DM and their complications, various generalized nutritional recommendations, available and updating for over four decades, do not seem to abate their rising incidence. In a recent paper (Zeevi et al., 2015), we presented evidence for marked inter-individual variability in postprandial (post-meal) glycemic responses to identical meals and that this variability associates with microbiome composition and function.

Previous works have presented similar evidence from different perspectives. Works by Stanley L. Hazen and colleagues (Koeth et al., 2013, Tang et al., 2013) demonstrated that the gut microbiome mediates the well-known link between red meat consumption and atherosclerosis. The metabolism by gut microbiota of L-carnitine, a nutrient abundant in red meat, produced trimethylamine-N-oxide (TMAO), a proatherogenic species. It was further shown that omnivorous human subjects produced more TMAO than vegan or vegetarian participants via this microbiome-dependent pathway. This suggests that global recommendation to reduce consumption of red meat (Hu et al., 2000; Sinha et al., 2009; Rohrmann et al., 2013) as a means of reducing cardiovascular diseases may be more relevant for people with specific microbiome configurations, calling for personalized adjustment of universal recommendations.

Similarly, we have previously presented (Suez et al., 2014) a microbiome-dependent induction of glucose intolerance caused by consumption of non-caloric artificial sweeteners (NAS). In a pilot prospective study, we proposed that even short-term consumption of NAS may cause glucose intolerance in a subpopulation of human individuals and that NAS-sensitive individuals harbored distinct microbial composition prior to NAS consumption. Our study suggests that general recommendations for the reduction of sugar consumption via the widespread use of NAS may be harmful to some population subsets and that we might need to personalize this recommendation according to the individual's microbiome.

These highly personalized associations between the microbiome, nutrition, and metabolic consequences have led us to believe that the microbiome can be helpful in characterizing the metabolic state of healthy and prediabetic individuals. To this end, we conducted a personalized nutrition study, in which we continuously monitored blood glucose levels in an 800-person cohort and measured the postprandial glycemic response (PPGR) to more than 45,000 real-life meals and more than 5,000 standardized meals (Zeevi et al., 2015). We found large inter-personal differences in the response to both types of meals. Correlated with this highly variable range of responses were many metabolic markers, such as glycated hemoglobin (HbA1c%), BMI and wakeup glucose, and many microbiome markers. We then devised a machine-learning algorithm that integrates blood parameters, dietary habits, anthropometrics, physical activity, and gut microbiome composition and function measured in this cohort and showed that it accurately predicts personalized PPGR to real-life meals (Zeevi et al., 2015).

To gain insight into the contribution of different features to algorithm predictions, we utilized partial dependence (PD) analysis (Elith et al., 2008), observing the marginal effect of a given feature on PPGR prediction outcome after accounting for the average effect of all other features. As such, we termed the features for which predicted PPGR increased with feature value as non-beneficial; and features for which predicted PPGR decreased with feature value as beneficial, and were able to discern 21 and 28 beneficial and non-beneficial microbiome-derived features, respectively. For example, growth of *Eubacterium rectale* was mostly beneficial, as 430 participants with high inferred growth for *E. rectale* were associated with a lower PPGR (Zeevi et al., 2015). Interestingly, *E. rectale* was also found to be negatively associated with T2DM in a Chinese cohort (Qin et al., 2012). As another example, relative abundances of *Parabacteroides distasonis* were found non-beneficial by our predictor, and this species was also suggested to have a positive association with obesity (Ridaura et al., 2013).

Incorporating microbiome-derived features significantly improved PPGR prediction accuracy. We note, however, that predictive power does not imply causality, and establishing such links necessitates future studies. Nevertheless, since we found that microbiome-derived features can replace most other personal features (e.g., blood tests, medical questionnaires) with little loss of accuracy, an intriguing possibility is that further research may be able to utilize microbiome features as a simple and cost-effective means of profiling for individuals and providing personalized dietary insights.

A central determinant of microbiome composition and function is the host diet. The prototypical dietary patterns across mammalian phylogeny were shown to drive convergence in the microbiomes of these mammals (Muegge et al., 2011). For example, carnivorous and herbivorous microbiomes promote opposing directionality for amino acid metabolism, regardless of their phylogeny. Carnivore microbiomes are enriched with amino acid degradation pathways, while the microbiomes of herbivores are enriched with amino acid biosynthesis pathways (Muegge et al., 2011). Even within a single species, the microbiome composition and function was found to be dominated by diet, rather than by host phylogeny (Carmody et al., 2015). Diet was shown to alter the gut microbiome on two separate timescales. Long-term dietary patterns, obtained from food-frequency questionnaires, were shown to strongly associate with core microbial properties. High long-term protein and fat consumption was associated with a microbiome dominated by bacteria of the *Bacteroides* genus, whereas high long-term carbohydrate consumption was associated with genus *Prevotella* (Wu et al., 2011). Notwithstanding, extreme dietary changes can quickly alter the microbiome in a reproducible manner. Community differences in the microbiome were shown to occur after only a single day on a strictly animal-based diet, and the microbiome of these individuals reverted to its normal state 2 days after the animal-based diet ended. Plant-based diet was also shown to have a significant effect on the microbiome (David et al., 2014). Notably, the effect of these short-term diets mirrored the aforementioned differences between carnivores and herbivores (Muegge et al., 2011). In a short-term experiment on healthy subjects consuming only white rice, we have shown that such an extreme change in diet is also immediately reflected in the growth dynamics of species within the microbiome (Korem et al., 2015). Likewise, a recent study showed that a 3-day consumption of barley kernel-based bread resulted in improved glucose metabolism, which was attributed to a higher *Prevotella*/*Bacteroides* ratio (Kovatcheva-Datchary et al., 2015).

Similarly, during the personalized nutrition study, we conducted a blinded randomized controlled study, which assigned personalized nutritional intervention based on either the PPGR-prediction algorithm or on expert examination of continuous glucose measurements. For each participant, we constructed two 1-week diets: a diet composed of the meals expected to have low PPGRs (the "good" diet) and a diet composed of the meals expected to have high PPGRs (the "bad" diet). We detected changes in the microbiome following both the "good" and the "bad" dietary interventions, and while many of these significant changes were person specific, several taxa changed consistently in most participants (Zeevi et al., 2015). For example, T2DM has been associated with low levels of *Roseburia inulinivorans* (Qin et al., 2012), *Eubacterium eligens* (Karlsson et al., 2013), and *Bacteroides vulgatus* (Ridaura et al., 2013), and all these bacteria increase following the "good" diet and decrease following the "bad" diet.

Personally tailored dietary interventions aimed at altering the microbiome to a more beneficial configuration may thus hold promise, but also face important challenges. The microbiome is modified by both host nutrition and its own metabolic state and in turn regulates the host metabolic homeostasis. As such, personalized nutritional interventions must take into account these intricate and bilateral relationships between the microbiome and host, which may be "reset" to a new steady state by longstanding personalized nutritional modifications. Likewise, such changes may necessitate further nutritional modifications to be implicated once this new host-microbiome equilibrium has been reached.

Future studies focusing on personalized nutrition-based microbiome and host metabolic modification will further characterize the nutrition-microbiome-host metabolism axis at a larger scale. Moreover, they may allow integration of a personalized diet and its effects on the host as part of a multi-disciplinary therapeutic approach toward multi-factorial diseases, thereby

harnessing nutritional considerations into the clinical decision-making process.

Limitations and Challenges of Microbiome Integration into Personalized Medicine

While microbiome impact on diagnosis, follow up, and treatment of disease holds promise in potentially transforming personalized medicine, many challenges, pitfalls, and limitations still need to be addressed in order for microbiome profiling to be fully integrated into common medical practice.

The microbiome shows a remarkable degree of inter-personal variability among people (Eckburg et al., 2005), both in steady-state conditions and in response to a variety of lifestyle changes including dietary alterations, use of medication (Carmody et al., 2015; Mikkelsen et al., 2015), and aging (Claesson et al., 2012). The microbiome composition and function even tends to oscillate diurnally in an hour-scale resolution (Thaiss et al., 2014). All of these microbial fluctuations may potentially introduce clinically irrelevant "noise" to microbiome-related data, thereby introducing potential biases to the interpretation of microbiome-based results. Moreover, different collection and analysis techniques, reagents, and parameters may introduce variations into microbiome results, further confounding the biologically relevant personalized variability (Flores et al., 2015; Hamady and Knight, 2009). Thus, better standardization in collection methods, reagent use, storage, and processing is greatly needed in the microbiome research community to assure that microbiome-based human data feature the degree of reproducibility that is adequate for its inclusion into routine clinical practice.

Data processing and interpretation pose another layer of potentially confounding variability. This represents an immense challenge, as many researchers employ different microbiome analysis tools that do not always produce similar results, even when generated from identical datasets. Moreover, microbiome-based biomarkers for personalized diagnostics and prognostics may not be uniformly applicable among populations and may diverge based on lifestyle, nutrition, genetics, and biogeography. Furthermore, interpretation of microbiome associations with clinical features of "multi-factorial" diseases may be complicated by difficult-to-recognize clinical confounders. This caveat has been exemplified in two studies, which identified compositional and functional alterations in the gut microbiome to be associated with impaired glucose metabolism in European and Chinese cohorts (Karlsson et al., 2013; Qin et al., 2012). Recently, a follow-up study suggested that this association was at least partially driven by metformin usage by some diabetic individuals in these cohorts (Forslund et al., 2015).

While the plasticity of the microbiome holds promise as a modifiable disease intervention target, it also poses a challenge related to the stability of the imposed changes. For example, devising personalized dietary interventions based on microbiome characteristics may be tricky, as diet itself is a main driver of microbiome composition, and thereby dietary alteration may trigger changes in the microbiome (Zeevi et al., 2015). This modifiability should merit periodic reassessment and occasional readjustment of the nutritional planning per individual, or the utilization of more elaborate algorithms, which can identify dependencies between dietary compounds and specific bacterial taxa and predict trends of their variation over time. Equally important, with the present "microbiome hype," it should be emphasized that the microbiome composition and function is only one component of the multiple factors affecting human physiology and propensity for disease. Thus, only an integrative multivariable approach, which would integrate human and microbiome genetics, as well as other environmental variables, may ensure that precision medicine is implemented to its fullest potential.

Discussion

Personalized medicine is emerging as potential means of reducing disease risk, improving diagnosis, enhancing treatment, and whenever possible, preventing disease. Future microbiome-based methods for risk assessment could provide early identification of personal disease risk at all stages of life. Screening of neonates' or infants' microbiomes may provide means for early detection of allergic disorders, childhood obesity, T1DM, and asthma and serve as an attractive target for preventive intervention in these conditions. In early adulthood, microbiome assessment may be useful in diagnosis and risk assessment of metabolic diseases such as obesity and T2DM. Later in life, microbiome assessment may aid in early detection of cancer, autoimmunity, and neurodegenerative disease and may be incorporated as part of the therapeutic arsenal in these disorders. As such, deciphering the characteristic microbiome configurations, or "microbial fingerprints," of different disorders could facilitate its future application in personalized disease diagnosis, as a precise, non-invasive, and economically viable tool that may boost massive population screening for early detection of multiple disorders.

The microbiome is also emerging as a central "player" in many aspects of personalized drug therapy. Gut commensal bacteria actively participate in the metabolism of many chemical compounds, thereby potentially impacting drug availability, levels, and toxicity.

Finally, patient-tailored manipulation of the human microbiome may enable the development of precision microbiome-targeting treatment for a variety of multi-factorial disorders. To date, such interventions were mainly limited to fecal microbiota transplantation (FMT) for the treatment of refractory *Clostridium difficile*-induced colitis. However, extensive research is underway in assessing FMT in other diseases, such as inflammatory bowel disease (Colman and Rubin, 2014). Other novel and attractive microbiome-modifying approaches may include personalized probiotics and prebiotics, personalized diet devised to alter microbiota composition and function, "postbiotic" treatment composed of microbiome-modulated metabolites designed to orchestrate host-microbiome interactions, and microbiome manipulations using phage therapy.

For these potential microbiome-based usages to be clinically implementable, the field needs to address several substantial challenges, mainly related to the development of robust and uniform collection, sequencing, and analysis standards that would improve reproducibility of results and reduce biases in their interpretation. With those challenges met, incorporating microbiome-related diagnostics and therapies into common medical practice may emerge as an integral part of modern patient care, thereby introducing thrilling new dimensions into the prospect of precision medicine.

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