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Modulating the human gut microbiome as an emerging therapeutic paradigm

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ABSTRACT

The human body is actually a vast and changing ecosystem comprised of billions of microbial organisms, known collectively as the microbiome. Within the last few years, the study of the microbiome and its impact on human health has been a rapidly growing area of biomedical science. The gut intestinal tract microbiome has been a particular focus of research given its potential role in many inflammatory and metabolic diseases as well as drug metabolism. Although a nascent field, the potential for modulating the gut microbiome or human host interactions associated with these microbes offers new therapeutic strategies for many chronic diseases, in particular obesity, diabetes and inflammatory bowel diseases. Here we provide an overview of present knowledge about the gut microbiome, its putative role in metabolic diseases and the potential for microbiome focused treatments from the drug development perspective.

Keywords: *microbiome, metabolic disease, obesity, diabetes, drug discovery, human genome*

1. Introduction

Studies of the human genome have led to the advancement of biomedical science and drug discovery. However, it is increasingly apparent that determinants of our health are not solely controlled by our own genomes. Rather, many disease pathologies involve the interplay between the human body, the external environment and the complex communities of microorganisms residing on respiratory[1], vaginal/urogenital[2] and gastrointestinal (GIT) tract[3] and skin[4] surfaces. The complement of microbial cells co-inhabiting an individual, the microbiota, exceeds at least 10-fold the number of cells in the human body[5,6]. Furthermore, the gene collection of this residing microbial community, the microbiome, exceeds by at least 100 times the complement of genes present in the human nuclear genome[7]. While our knowledge of the detrimental disease impact of many bacterial, viral and eukaryotic pathogens is well-established, the roles of complex non-pathogenic microbiota communities in sustaining health or promoting disease are only recently studied.

Over the last five years, the microbiome has been one of the fast growing areas of biomedical research. The advent of sensitive, high volume DNA sequencing and metabolomics technologies has led to a rapid expansion of datasets from microorganism populations associated with various chronic disease phenotypes. Major public funding

initiatives such as the US National Institutes of Health (NIH) Human Microbiome Project, initiated in 2007[8], and the EU MetaHIT Consortium, started in 2008, are driving the characterization of microbiomes from hundreds, soon thousands, of individuals of different ages, geographical, dietary and disease backgrounds. New tools and methodologies are also being developed for modulating the microbiota in model organisms as well as sampling not only genomes but also metabolites. Concurrently, more powerful computational biology approaches are necessary to make sense of the vast volumes of genomic, metabolic and phenotypic data being generated from both pre-clinical and clinical microbiome related studies.

2. Defining the normal gut microbiome

With over 200 m^2 of mucosal surface area and a nutrient rich environment, the GIT hosts the majority of the human microbiota[5,8]. Furthermore, absorption in the distal gut results in approximately 10% of the metabolites in the host systemic blood flow being of bacterial origin[9]. Changes in gut microbiota have been linked with numerous GIT and other systemic diseases[3]. The broad implications of GIT microbiota in human physiology combined with sample accessibility – about 60% of fecal material is microbial biomass[3,10] – has facilitated the exploration of the human intestinal microbial ecosystem in health and disease (Figure 1).

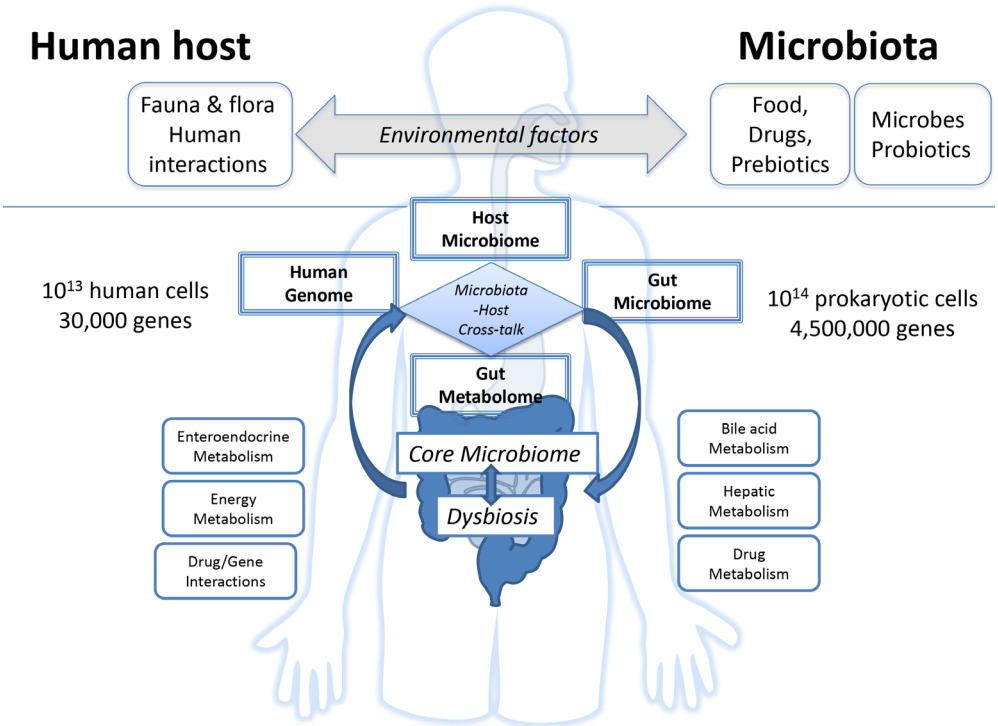

Figure 1 *Schematic diagram showing potentially disease relevant interactions between the human host and our microbiome.*

In healthy individuals, changes in the GIT microbiota have been associated with host genetics[11], aging[12] and dietary patterns[13]. A recent population study involving 39 individuals from different cultures, geographical locations, and races, as well as gut-associated and dietary disease patients, showed that subjects' GIT microbial communities could be segregated into three statistically robust clusters[14]. These clusters, known as enterotypes, were also found to be consistent across major populations, including 85 European[15] and 154 American individuals[16]. Enterotypes were differentiated by the relative abundances of three bacterial genera, Bacteroides, Prevotella (both of the phylum Bacteroidetes) and Ruminococcus (of the phylum Firmicute)[14]. However, the enterotype model has been critiqued for being based on relatively too few individuals and awaits further validation on larger cohorts[17].

Other studies suggest that long-term diet is important in shaping GIT microbial communities. Comparisons between children from Europe on a typical Western diet, high in animal protein and fat, with children from Burkina Faso in Africa, on a low animal protein and high carbohydrate diet, found the Bacteroides enterotype higher in Europeans while the Prevotella enterotype predominated in African children[18]. Another recent study also found that animal fat and high protein versus carbohydrate rich diets correlated with the Bacteroides versus Prevotella enterotypes, respectively[12]. In the same study, controlled feeding of 10 subjects with high fat, low fiber versus low fat, high fiber diets produced detectable changes in their microbiome within 24 hours. However, overall individuals' enterotypes remained stable for the duration of the 10-day study, suggesting that long-term rather than transient dietary trends determine the ecological structure of the gut microbial communities.

Other factors such as environmental exposure also influence biodiversity and the gene content of the GIT microbiota. Babies delivered naturally have microbial communities most similar to that of the mother's vagina while the microbiota from neonates born by Caesarean section closer resembles the mother's skin bacteria[19]. A fascinating case of lateral gene transfer (LGT) from marine bacteria genomes to the human microbiome involved genes encoding carbohydrate-hydrolyzing enzymes acting on marine algal polysaccharides being frequently detected in the microbiome of Japanese individuals who regularly ingest seaweed as part of their diet[20]. Further understanding of the microbiota variation across human populations, as well as the environmental and genetic factors shaping it, will be important in designing future therapeutic strategies.

3. Gut microbiome and metabolic diseases

Obesity is possibly one of the most important diseases of the 21st century[21]. According to the latest estimates from the World Health Organization (WHO) in 2008, more than 1.4 billion adults aged 20 and older worldwide were overweight (body mass index or BMI > 25 kg m^{-2}), with over 200 and 300 million men and women, respectively, being obese (BMI > 30 kg m^{-2}). Alarmingly, these estimates also point out that more than 40 million children (less than 5 years) were overweight in 2010[22]. Obesity substantially increases the risk of individuals to conditions such as metabolic syndrome, dyslipidemia, hypertension, cardiovascular disease, type 2 diabetes (T2D), liver nonalcoholic steatohepatitis (NASH), obstructive sleep apnea, and polycystic ovarian syndrome. There is also an elevated risk for obese individuals to various types of cancers and several degenerative conditions, and medical costs of obesity alone have risen to an estimated $147 billion US in 2008[21,23].

Considering the crucial role microbiota plays on host metabolism, understanding the influence of microbiome on various metabolic diseases is an intense area of research[24]. Studies of gut microbiota in lean and obese mice suggest that it has the ability to impact energy homeostasis by not only influencing the efficiency of calories harvested from the diet but also on how this harvested energy is utilized and stored[24–26]. Early reports of decreased proportion of Bacteroidetes in obese individuals relative to lean individuals as well as correlations between increases in Bacteriodetes proportions with weight loss on two different types of low-calories diet suggested that obesity might have a profound microbial component[27]. Further animal studies support linkages between microbiota dysbiosis and low grade inflammation, obesity and T2D[28]. In human populations, a recent study found decreased proportions of Bacteroidetes species in the feces of obese Kazakh girls, with no significant changes in the abundance of another common gut bacterial phylum, the Firmicutes[29]. However, bacterial abundance estimates are not consistent between different obesity studies. One report found a reduction in Bacteroidetes species in obese patients, and a higher abundance of the Firmicutes genus Lactobacillus in obese subjects, when compared to lean controls[30]. Examining the composition of gut microbiota during pregnancy in overweight and normal-weight women, another study found that Bacteroides and Staphylococcus had higher abundances in overweight compared to normal weight-women, and that high Bacteroides abundance was associated with excessive weight gain over pregnancy[31]. However, agreement is not universal and there are reports of different gut microbiota compositions in obese versus lean individuals, which conflict with early observations of decreased proportions of Bacteroidetes[14,31–33]. It has also been suggested that any deviations from the 'core microbiome' might be associated with different physiological states such as obesity, for example, when compared with lean subjects, as it represents a functional shift of microbiome[16].

There is a critical need for new therapeutics to treat obesity. The US Food and Drug Administration (FDA) has approved Orlistat, a lipase inhibitor, which reduces the availability of absorbable fatty acids and monacylglycerols, by preventing the breakdown of triglycerides by inhibiting pancreatic and intestinal lipases[34]. Until recently, this was the only drug available specifically for obesity patients. Qsymia, a combination of the drugs phentermine, topiramate and Belviq (Lorcaserin) has been recently approved for obese (BMI > 30) and overweight subjects (BMI > 27) that have various associated co-morbidities such as T2D, increased levels of cholesterol, and hypertension[35]. Lorcaserin, a selective agonist of the serotonin 2C receptor, has been reported to reduce weight and improve glucose homeostasis in subjects with T2D[36]. Previously, for short-term treatment of obesity, the US FDA approved the use of phentermine, which is an adrenergic reuptake inhibitor that increases adrenergic signaling. With the existing pharmacotherapy options, the weight reductions achieved by various obese subjects are modest in many cases, especially when compared to gastrointestinal weight-loss surgery[37]. Drug discovery programs have a critical need to find less invasive and highly effective therapeutic interventions for obesity, and explore novel ways of addressing this obesity epidemic. Needless to say that understanding the gut microbiome's influence on obesity appears to be an avenue to explore to develop innovative therapeutic intervention strategies[38].

As reviewed recently, the gut microbiota mechanisms that contribute to obesity and metabolic diseases potentially involve regulation of energy production by the processing of dietary polysaccharides that are otherwise indigestible by mammals, which leads to a modulation of absorption of short-chain fatty acids and promoting fat deposition in adipose

tissue by gene regulation mechanisms[39]. Short-chain fatty acids are potential signaling molecules for G-protein coupled receptors (GPCRs) such as GPR41 and GPR43, and also are substrates for lipogenesis. These receptors, along with gut microbiota, potentially influence the release of gut enteroendocrine hormones such as glucagon-like peptide 1 (GLP1), glucagon-like peptide 2 (GLP2) and peptide tyrosine–tyrosine (PYY), which play critical role in satiety regulation and glucose homeostasis. Also, gut microbiota have been reported to be associated with suppression of fasting-induced adipocyte factor (FIAF) also now known as angiopoetin-like 4 (ANGPTL4) from the gut epithelium, which results in increased storage of triacylglycerol in the adipose tissue by reduction of skeletal muscle and liver fatty acid oxidation with concomitant increase in the activity of lipoprotein lipase (LPL) (reviewed in ref. 38). Among the factors leading to low grade inflammation associated with metabolic disorders such as obesity, gut microbiota derived lipopolysaccharide (LPS) has been reported to be important. Gut epithelium, which acts as a healthy barrier, is compromised under various conditions like stress, higher dietary lipid intake and alcohol usage, leading to translocation of LPS and other bacteria-derived metabolites. Such endotoxin-mediated inflammation could potentially be an important factor for insulin resistance and metabolic disturbances associated with obesity[40,41].

Since pharmacotherapy options offer limited to moderate help, gastrointestinal weight-loss surgery remains one of the most effective treatments for individuals with BMI greater than 35 and significant co-morbidities such as hypertension and/or diabetes or for individuals with BMI greater than 40 (with or without associated co-morbidity conditions) as recommended by the National Institutes of Health Consensus Panel on Gastric Surgery for Severe Obesity (1992). Recently, these guidelines have been modified by FDA advisory committee on obesity to include any patient that has BMI > 30[42]. From the early studies that reported T2D to be controlled by gastric bypass surgery for as long as 14 years, to date, there have been many advances in the application as well as the utility of gastrointestinal weight-loss surgeries for patients[43]. Many studies report that gastrointestinal weight-loss surgery leads to not only weight loss but also long-term remission in many individuals of various co-morbidities such as T2D, hypertension, hypertension, gastroesophageal reflux disease, polycystic ovary syndrome, nonalcoholic steatotic hepatitis, and adult asthma[42]. It is critical to gain better mechanistic understanding of metabolic improvements on the basis of how various critical physiological parameters are affected by different gastrointestinal weight-loss surgical methodologies[44]. The multitude of complex mechanisms involved in weight regulation and the evolutionarily conserved pathways recruited to guard against starvation make it unlikely that modulation of a single cell, pathway, or target will prove to be an effective long-term therapeutic intervention[45]. It will potentially require a multi-pronged approach that would result in the modulation of more than one mechanism and also includes lifestyle management approaches in order to achieve significant weight reductions and metabolic improvements in subjects[46].

Some recent studies have aimed at understanding the influence of gastrointestinal weight-loss surgery on host metabolic parameters, including gut-microbiome and cross talk between microbiota and host. Significant shifts in the GIT microbiome composition have also been seen as a consequence of Roux-en-Y gastric bypass (RYGB) surgery which suggests a potential role of the bacterial metabolome in post-operative improvements in obesity and hyperglycemic control in these patients[39,47]. In a non-obese rodent study, RYGB surgery resulted in a higher abundance of *Proteobacteria* after surgery (predominantly

Enterobacter hormaechei) with decreased abundances of *Firmicutes* and *Bacteroidetes*. The altered nutrient flow as a result of surgery resulted in a different metabolite profile and fermentation of nutrients, profoundly influencing gut-microbiome and host cross-talk[48]. Another study reported a decrease in *Firmicutes* following gastric-bypass surgery, which were dominant in normal-weight and obese individuals as well as an increase in the abundance of Gammaproteobacteria[49]. The same study reported that *Prevotellaceae*, which produce hydrogen gas (H_2) were highly enriched in the obese individuals, as well as the *Archaea*, which were mainly H_2 oxidizing *Methanobacteriales*. This led to the suggestion that interspecies H_2 transfer between bacterial and archaeal species constitutes a mechanism of increased energy uptake in the large intestines of obese individuals.

The shifts in bacterial populations following gastric-bypass surgery may reflect changes of surgical affect as well as altered food ingestion and digestion. In another human study, the *Bacteroides/Prevotella* group was seen to increase three months after RYGB[50]. *Escherichia coli* as well as *Lactobacillus/Leuconostoc/Pediococcus*, which are associated with lactic acid production and *Bifidobacterium* decreased three months after surgery. Interestingly, *Faecalibacterium prausnitzii* species, a butyrate producer, was found to be inversely correlated with inflammatory markers before surgery and throughout the follow-up in diabetic individuals. Another recent study on obese individuals with T2D reported shifts in the gut microbiota composition which consisted of higher abundances of *Proteobacteria,* such as *Enterobacter cancerogenus,* and lower representation of Firmicutes and Bacteroidetes[51]. Changes in dissolved oxygen status and pH levels as a result of altered gastric acid secretion might also be influencing the gut microbiota composition. Analyses suggested that higher and lower abundances numbers of the Proteobacterium *E. cancerogenus* and the Firmicutes *Faecalibacterium prausnitzii* and *Coprococcus come*, respectively, correlate with BMI and C-reactive protein. *F. prausnitzii* levels appear to correlate well to fasting blood glucose. Microbiota composition and function changes as a consequence of gastrointestinal weight-loss surgery could be driven by a multitude of factors including the altered anatomical structure of the GIT, changes in hormone levels and life-style shifts in the type and quantity of nutrition[39]. Gastrointestinal weight-loss surgery might impact the gut microbiota capacity to produce butyrate, process bile salts and utilize nutrients. The associated changes in urinary and fecal metabolite profiles, along with adaptations to changes in gut hormone profiles following surgery, are expected to influence interactions between the human host and their microbiota[52]. Carefully designed studies will be needed to understand the impact of preoperative metabolic state and post gastrointestinal weight loss surgery on gut microbiota composition, function and host metabolism.

Altered gut permeability leading to endotoxin-mediated inflammation, insulin resistance as well as hyperinsulinemia could also play a role in metabolic disturbances leading to T2D and metabolic syndrome. There is also emerging literature suggesting links between the composition and function of gut microbiota, gut hormone changes, leptin modulation and endocannabinoid system with altered metabolic phenotype[41,53]. There have been reports of reduced abundances of Firmicutes and *Clostridia sp.* in diabetic subjects when compared to non-diabetic control subjects. For example, in diabetic subjects Betaproteobacteria and plasma glucose were positively correlated[54]. A metagenome-wide association study reported gut microbial dysbiosis in T2D subjects which suggests a decrease in butyrate-producing bacteria and altered microbiota functions related to sulfate metabolism and oxidative stress[7,54]. A better understanding of

the metabolites associated with altered microbiome functions in patients with metabolic diseases could potentially result in novel ways of intervening therapeutically.

A manifestation of metabolic syndrome is non-alcoholic fatty liver disease (NAFLD) that occurs from 20 to 30% in the general population and as high as 75 – 100% in obese individuals. A recent study found that inflammasome-deficient mice developed NASH and that these disease phenotypes could be transmitted to co-housed wild-type mice[55]. This suggests that gut microbiota changes mediated by defective NLRP3 and NLRP6 inflammasome sensing could exacerbate liver steatosis and obesity. In humans patients with infective endocarditis, treatment with vancomycin has shown an increased weight gain which is thought to be caused by dysbiosis due to colonization of *Lactobacillus* sp., intrinsically resistant to this antibiotic[56].

Gut microbiota generated metabolites can result in the modulation of host metabolic pathways, and negative cross-talk between the gut microbiota and host could play a critical role in dysbiosis. Microbiota and host interactions possibly affect various biologically active axes linking gut, muscle, liver and brain as well as other physiological systems (Table 1)[57].

4. The gut microbiome and other human diseases

The status of gut microbiome is increasingly being studied in a wide variety of other diseases. One central aspect of the human-microbiota symbiosis is the dialogue between the microbiota and the immune system[58,59]. The microbiota contributes to the development of both the mucosal and systemic immune systems. It is now appreciated that loss of homeostasis or dysbiosis in the GIT immune system plays a central role in numerous disease conditions. Clinically, antibiotic usage has also been associated with increased incidence of Crohn's disease and ulcerative colitis in both adults and children[60,61]. Homeostasis of the mucosal immune system requires the development of both tolerance to the residential microbiota and, at the same time, regulation to avoid overgrowth and invasion of internal tissues. Both the microbiota and the innate and adaptive immune systems contribute to the establishment of an optimal equilibrium whereas disturbances can lead to dysbiosis[62] and disease states through the development of intestinal inflammation[63,64]. This is reflected on the host side by genetic predispositions to inflammatory bowel diseases (IBD), which point to the importance of the immune system and microbial sensing. For example, mutations in human genes NOD2, ATG16L1 and those encoding defensins are known predispositions to IBD. Recent meta-analysis of 15 GWAS studies of Crohn's disease and/or ulcerative colitis with a combined total of more than 75,000 cases and controls revealed a significant overlap between susceptibility loci for IBD and mycobacterial infection which reinforces the role of host-pathogen responses in IBD pathology[65].

Specific microbiome changes have also been observed in patients with colon cancer[66,67]. Moreover, mechanisms for the microbiome to promote colon cancer progression have been suggested. These include microbial stimulation of the c-Jun/JNK and STAT3 signaling pathways[68] and the production of genotoxin colibactin, a polyketide synthase encoded by the gene *pks* found in enteric *E. coli* strains[69]. Other areas of interest are the potential roles of the GIT microbiota in diseases of distal organs such as asthma[70].

Early stage but intriguing research suggests that some behavioral disorders might be manifestations of a CNS–gut microbiota axis mediated by immune, neural and

endocrine pathways[71]. Many bioactive neural activating metabolites, including GABA, norepinephrine, serotonin, dopamine and acetylcholine, are produced by microbial species[72]. Chronic administration of *Lactobacillus rhamnosus* to mice was shown to modulate GABA receptor expression via the vagus nerve and reduce anxiety and depression behaviors[73]. Children with autism spectrum disorders have been found to have abnormal amino acid metabolism, increased oxidative stress, and altered gut microbiomes[74].

Could changes in the microbiome be a common underlying cause behind the rise of immune-related diseases? Some researchers have proposed the hygiene hypothesis which suggests that the rapidly changing human lifestyle throughout the western world, such as the widespread use of antibiotics and changes in diet, has resulted in predisposition to multiple currently prevalent diseases due to imbalances in immune-microbiota coupling[75]. For, example the homeostasis of the GIT is known to be affected by the presence of

Table 1 *Examples of gut microbial metabolites and their biological functions. Table adapted from Nicholson* et al.[57]

Metabolites	Related bacteria	Biological functions
SCFA	*Clostridial clusters* IV and XIVa, *Eubacterium, Roseburia, Faecalibacterium, Coprococcus*	Cholesterol synthesis, implicated in T2D, obesity, insulin resistance, colorectal cancer
Bile acids	*Lactobacillus, Bifidobacteria, Enterobacter, Bacteroides, Clostridium*	Absorb dietary fats, intestinal barrier function, signal systemic endocrine functions, energy homeostasis
Choline metabolites	*Faecalibacterium prausnitzii, Bifidobacterium*	Lipid metabolism and glucose homeostasis, involved in NAFLD, obesity, diabetes & CV disease
Phenolic, benzoyl and phenyl derivatives	*Clostridium difficile, F. prausnitzii, Bifidobacterium, Subdoligranulum*	Detox of xenobiotics, urinary metabolites
Indole derivatives	*Clostridium sporogenes, Escherichia coli*	Modulate pro-inflammatory genes, strengthen epithelial cell barrier, implicated in brain-GI axis
Vitamins	*Bifidobacterium*	Endogenous sources of vitamins, potential epigenetics
Polyamines	*Campylobacter jejuni, Clostridium saccharolyticum*	Exert genotoxic effects, potential anti-inflammatory & anti-tumor effects
Lipids	*Bifidobacterium, Roseburia, Lactobacillus, Clostridium, Proteobacteria*	LPS induction, intestinal permeability, brain-GI-liver axis & glucose homeostasis
Others: lactate, endocannabinoids, *etc*.	*Bacteroides, Pseudobutyrivibrio, Ruminococcus, Faecalibacterium, Lactobacillus, etc*.	Various pathways including endocannabinoid system

eukaryotic parasites and viruses. Interactions between helminths worms and the human GIT can positively influence immune homeostasis (reflecting host–parasite adaptations) by modulating the immune system towards an optimal anti-inflammatory status[76]. Whether or not exposure to such pathogens has a role in microbiome-driven disease etiology is still an open question. However, a better understanding of the contribution of the different partners in dysbiosis, and the cross-talk taking place between them, represents important opportunities to develop new strategies to treat chronic diseases.

5. Microbiome-related therapeutic strategies

Targeted therapeutics for manipulating the microbiome are still very rudimentary when compared to other pharmaceutical products. Prebiotics and probiotics are the most commonly marketed generic supplements for gastrointestinal disorders[77,78]. Probiotics are usually supplements of bacterial strains which integrate into the broader microbiota with limited global GIT effect[79,80], unless the microbiota is temporarily significantly depleted by antibiotic treatments[81]. While our understanding of probiotics mechanistic effects is limited, a recent study by Atarashi *et al*.[82] using a defined mix of *Clostridium* strains demonstrated induction of Treg cells in mice leading to enhanced resistance to colitis and systemic immunoglobin E responses.

Prebiotics, nutrients aimed at stimulating the growth of specific microbial species, have shown greater potential for manipulating the environmental pressures which shape the microbial ecology, especially in the developmental stages of life[83], although the effects of these supplements are short term and can be overshadowed by the overall diet[78]. It is widely accepted that a full complement of biodiversity is important for a healthy microbiota and single species supplements or substitutions have little effect on the long-term host microbiota-phenotype[3]. Lactic acid bacteria species that thrive on specific co-factors are the closest the probiotic field has come to targeted microbial therapies [84]. Most lactic acid species do not require iron and they can be out-competed by normal pathogens in high iron environments such as that accompanied by trauma and internal bleeding prior to surgery. By introducing *Streptococcus thermophilus* strains that have a positive response to increased iron concentrations, the negative effects of pathogenic bacteria could be potentially counteracted. Other more extreme therapies are intentional infections by parasitic helminths worm[75] and fecal transplants[85] which have been tested as alternatives to invasive surgery in IBD patients. In preliminary clinical trials, these approaches seem to somewhat regulate the host gut inflammatory response, although more thorough controls are desirable and the duration of relief is unclear[85,86].

Traditionally, antibiotics have been considered the most common course of treatment against infectious disease, microbial disorders and inflammation. However, growing evidence for microbial contribution to health and advanced understanding of complex microbial diseases has resulted in a re-evaluation of some antibiotic[87,88] and immunosuppressant treatments[89]. Antibiotics have been considered as a poor choice for GIT microbiota modification because of tolerance issues associated with long term dosing and the lack of bacterial species specificity. However, antibiotics can positively modulate chronic disease conditions, such as diabetes and obesity, at least in rodent models[90]. Desirable pharmacological properties for a potential GIT microbiota modulator would be selective bacterial species activity and high bioavailability in the gut. Intriguingly, these are precisely the type of molecules which are considered to be failed candidates in

antibiotic drug development[91]. The fact that over 80% of the gut microbial species cannot be cultured using conventional laboratory methods restricts the use of high throughput compound screening campaigns to discover anti-microbiota compounds[92]. However, the development of *in vitro* human gut models[93,94] as well as using bacteria phylum specific antibiotics, for example against Gram-positive Firmicutes, might accelerate the development of narrow spectrum drugs for microbiota modulation. As the human gut microbiome encodes hundreds of unique protein families, it is a rich source of potential high specificity drug targets[95].

Another avenue for potential therapies is targeting host genes involved with microbiota cross-talk. Our knowledge of human receptors engaged in the maintenance of the GIT microbial community balance is growing but far from complete[96]. Toll-like receptors (TLRs) are responsible for cellular responses against bacterial infections, initiation of inflammation, production of antimicrobial peptides, maturation of antigen-presenting cells and activation of cellular repair and survival pathways[97]. While TLR2 and TLR4 are primary sensors of pathogenic bacteria, they are also important in maintaining bacterial gut flora homeostasis. Disruptions of TLR and NOD (nucleotide-binding oligomerization domain) pathways have been associated with colorectal cancer[97], IBD[98] and other intestinal diseases[62]. Microorganisms synthesize a wide range of bio-activate signaling low molecular weight molecules and metabolites, many of which are similar to human or eukaryotic produced metabolites[99]. For example, *Lactobacillus plantarum* secretes a peptide that when cleaved by intestinal proteases, results in a fragment called STp which stimulates the production of IL-10 in human intestinal dendritic cells[100].

6. The gut microbiome and drug metabolism

Besides being a potential therapeutic target for chronic disease, the gut microbiota also play a key role in the metabolism or biotransformation of xenobiotics, including many marketed drugs. Over 30 drugs are known substrates of bacterial enzymes in the GIT[101] which can have considerable impact on drug development. A tragic example was the reported death of several patients co-prescribed a new antiviral drug, sorivudine, along with an oral 5-fluorouracil which was attributed to secondary drug metabolites generated by gut flora[102]. Selective inhibition of gut microbiome enzymes can be potentially used to improve drug efficacy and safety. The colon cancer chemotherapeutic CPT-11 has a dose-limiting side-effect of severe diarrhea caused by gut bacteria producing the enzyme β-glucuronidase that modify the native drug into a toxic pro-drug molecule. In rodent models, Wallace *et al*. introduced an inhibitor of bacterial β-glucuronidase which allowed for higher CPT-11 dosing[103]. Interestingly, β-glucuronidase is not essential for bacterial viability, so inhibition of this enzyme blocked the drug metabolism function while minimizing disturbance of the gut microbial community. Clinically, oral administration of antibiotics neomycin plus bacitracin has been shown to reduce 5-fluorouracil/leucovorin (CPT-11) induced diarrhea in colorectal cancer patients[104]. The development of selective modulators of bacterial enzymes or species responsible for drug biotransformation could be an intriguing strategy for improving the clinical efficacy and safety profiles of particular drugs. Furthermore, the efficacies of many widely used drugs, including statins, are likely determined by both microbiota and host genetic factors[105], which further prompts integration of microbiome, human genetics and metabolomics into personalized medicine initiatives[106].

7. Concluding remarks

As discussed above, considerable evidence exists for the association of gut microbiota changes with human diseases. However, how certain are the cause–effect relationships between microbiota-human host dysbiosis and any particular disease? This is an essential question to answer before launching major therapeutic or drug development initiatives that focus on the microbiome as a drug target. Many human gene families are widely pursued as targets for pharmaceuticals because of the deep knowledge about their roles in diseases such as specific GPCRs for neurological disorders or inhibitors of certain kinases in the treatment of cancer. Common to all human drug targets is the strength of evidence linking modulation of the target, by small molecules, vaccines, antibodies or other peptides, to a pathway that results in a measurable change in the disease phenotype.

No doubt, as research expands the application of present DNA sequencing and metabolomics technologies to the level of individual diagnostics, data sources will become more mature and the complex nature of the human-microbiota interactions will become better defined. However, while considerable effort has been focused on the associations between bacterial communities and different disease populations, specific causal studies are still lagging. In part, a major limitation is the complexity of the microbiome and its cross-talk with the human host itself. Knocking out or adding some particular bacterial species to the microbiome and observing the effects on disease is a major challenge but not an insurmountable one. Present tools for modulating the microbiome, such as antibiotics, probiotics, prebiotics and fecal transplantation, are still crude and non-specific. Having a greater range of microbiome "knock-out" or "knock-in" technologies, such as very selective anti-bacterial compounds and vaccines, and applying them to well-established disease models will be key to elucidating disease-microbiome cause and effect relationships. Another approach is the use of metabolites, peptides and small molecules which are implicated in bacteria-to-bacteria sensing as well as human host–bacteria cross-talk. Such molecules could also be used in experiments to define disease effects and might even be the genesis of future microbiome-targeted therapeutics.

Over the coming years, the human microbiota will emerge as a major consideration in sustaining health and fighting disease. The potential therapeutic implications of modulating the microbiota are enormous, but this remains a young field of science and drug discovery. Hopefully, our understanding of the host-microbiome super-organ will develop to the point where microbial targeted therapies are a major consideration in pharmaceuticals and personalized medicine.

References

1. Kiley, J.P. (2011) *Chest,* **140**, 497–501.
2. Danielsson, D. *et al.* (2011) *Ann. N. Y. Acad. Sci.,* **1230**, 48–58.
3. Sekirov, I. *et al.* (2010) *Physiol. Rev.,* **90**, 859–904.
4. Grice, E.A. and Segre, J.A. (2011) *Nat. Rev. Microbiol.,* **9**, 244–253.
5. Ley, R.E. *et al.* (2006) *Cell,* **124**, 837–848.
6. Savage, D.C. (1977) *Annu. Rev. Microbiol.,* **31**, 107–133.
7. Qin, J. *et al.* (2012) *Nature,* **490**, 55–60.
8. Whitman, W.B. *et al.* (1998) *Proc. Natl. Acad. Sci. USA,* **95**, 6578–6583.
9. Wikoff, W.R. *et al.* (2009) *Proc. Natl. Acad. Sci. USA,* 106, 3698–3703.
10. O'Hara, A.M. and Shanahan, F. (2007) Gut microbiota: mining for therapeutic potential. *Clin. Gastroenterol. Hepatol.,* 5, 274–284.

11. Sparo, M. *et al.* (2012) *Lett. Appl. Microbiol.,* **54**, 119–125.
12. Wu, G.D. *et al.* (2011) *Science,* **334**, 105–108.
13. Turnbaugh, P.J. and Gordon, J.I. (2009) *J. Physiol.,* **587**, 4153–4158.
14. Arumugam, M. *et al.* (2011) *Nature,* **473**, 174–180.
15. Qin, J. *et al.* (2010) *Nature,* **464**, 59–65.
16. Turnbaugh, P.J. *et al.* (2009) *Nature,* **457**, 480–484.
17. Jeffery, I.B. *et al.* (2012) *Nat. Rev. Microbiol.,* **10**, 591–592.
18. De, F.C. *et al.* (2010) *Proc. Natl. Acad. Sci. USA,* **107**, 14691–14696.
19. Dominguez-Bello, M.G. *et al.* (2010) *Proc. Natl. Acad. Sci. USA,* **107**, 11971–11975.
20. Hehemann, J.H. *et al.* (2010) *Nature,* **464**, 908–912.
21. O'Brien, P.E. (2010) *J. Gastroenterol. Hepatol.,* **25**, 1358–1365.
22. World Health Organization: Obesity and overweight – Fact sheet N°311, May 2012 (http://www.who.int/mediacentre/factsheets/fs311/en/index.html).
23. Finkelstein, E.A. *et al.* (2009) *Health Aff. (Millwood.),* **28**, w822–w831.
24. Backhed, F. *et al.* (2004) *Proc. Natl. Acad. Sci. USA,* **101**, 15718–15723.
25. Turnbaugh, P.J. *et al.* (2006) *Nature,* **444**, 1027–1031.
26. Turnbaugh, P.J. *et al.* (2008) *Cell Host. Microbe,* **3**, 213–223.
27. Ley, R.E. *et al.* (2006) *Nature,* **444**, 1022–1023.
28. Cani, P.D. and Delzenne, N.M. (2011) *Pharmacol. Ther.,* **130**, 202–212.
29. Xu, P. *et al.* (2012) *BMC. Microbiol.,* **12**, 283.
30. Armougom, F. *et al.* (2009) *PLoS. One,* **4**, e7125.
31. Collado, M.C. *et al.* (2008) *Am. J. Clin. Nutr.,* **88**, 894–899.
32. Duncan, S.H. *et al.* (2008) *Int. J. Obes. (Lond),* **32**, 1720–1724.
33. Mai, V. *et al.* (2009) *Nutr. J.,* **8**, 49.
34. Derosa, G. and Maffioli, P. (2012) *Expert. Opin. Drug Saf.,* **11**, 459–471.
35. Wong, D. *et al.* (2012) *Nat. Rev. Drug Discov.,* **11**, 669–670.
36. O'Neil, P.M. *et al.* (2012) *Obesity. (Silver. Spring),* **20**, 1426–1436.
37. Kaplan, L.M. (2010) *Gastroenterol. Clin. North Am.,* **39**, 69–79.
38. Hullar, M.A. and Lampe, J.W. (2012) *Nestle. Nutr. Inst. Workshop Ser.,* **73**, 67–79.
39. Aron-Wisnewsky, J. *et al.* (2012) *Nat. Rev. Gastroenterol. Hepatol.,* **9**, 590–598.
40. Cani, P.D. (2012) *Clin. Microbiol. Infect. Suppl. 4,* **18,** 50–53.
41. Cani, P.D. *et al.* (2012) *Gut Microbes,* **3**, 279–288.
42. Williams, S. *et al.* (2012) *Med. Princ. Pract.,* **21**, 301–309.
43. Pories, W.J. *et al.* (1995) *Ann. Surg.,* **222**, 339–350.
44. Stefater, M.A. *et al.* (2012) *Endocr. Rev.,* **33**, 595–622.
45. Greenway, F.L. and Bray, G.A. (2010) *Curr. Diab. Rep.,* **10**, 108–115.
46. Tam, C.S. *et al.* (2011) *Obes. Rev.,* **12**, 984–994.
47. Jeppsson, B. *et al.* (2011) *Nutrients,* **3**, 604–612.
48. Li, J.V. *et al.* (2011) *Gut,* **60**, 1214–1223.
49. Zhang, H. *et al.* (2009) *Proc. Natl. Acad. Sci. USA,* **106**, 2365–2370.
50. Furet, J.P. *et al.* (2010) *Diabetes,* **59**, 3049–3057.
51. Graessler, J. *et al.* (2012) *Pharmacogenomics. J.,* doi: 10.1038/tpj.2012.43 [Epub ahead of print].
52. Murphy, E.F. and Quigley, E.M. (2012) *Gastroenterology,* **142**, 399–401.
53. Cani, P.D. and Delzenne, N.M. (2010) *Acta Gastroenterol. Belg.,* **73**, 267–269.
54. Larsen, N. *et al.* (2010) *PLoS. One*., **5**, e9085.
55. Henao-Mejia, J. *et al.* (2012) *Nature,* 482, 179–185.
56. Thuny, F. *et al.* (2010) *PLoS. One,* **5**, e9074.
57. Nicholson, J.K. *et al.* (2012) *Science,* **336**, 1262–1267.
58. Hooper, L.V. and Macpherson, A.J. (2010) *Nat. Rev. Immunol.,* **10**, 159–169.
59. Wells, J.M. *et al.* (2011) *E. Proc. Natl. Acad. Sci. USA, Suppl. 1*, **108**, 4607–4614.
60. Hviid, A. *et al.* (2011) *Gut,* **60**, 49–54.
61. Shaw, S.Y. *et al.* (2011) *Am. J. Gastroenterol.,* **106**, 2133–2142.
62. Round, J.L. and Mazmanian, S.K. (2009) *Nat. Rev. Immunol.,* **9**, 313–323.
63. Abraham, C. and Medzhitov, R. (2011) *Gastroenterology,* **140**, 1729–1737.
64. Willing, B.P. *et al.* (2010) *Gastroenterology,* **139**, 1844–1854.
65. Jostins, L. *et al.* (2012) *Nature,* **491**, 119–124.

66. Marchesi, J.R. *et al.* (2011) *PLoS. One,* **6**, e20447.
67. Sobhani, I. *et al.* (2011) *PLoS. One,* **6**, e16393.
68. Li, Y. *et al.* (2012) *Carcinogenesis,* **33**, 1231–1238.
69. Arthur, J.C. *et al.* (2012) *Science,* **338**, 120–123.
70. McLoughlin, R.M. and Mills, K.H. (2011) *J. Allergy Clin. Immunol.,* **127**, 1097–1107.
71. Cryan, J.F. and Dinan, T.G. (2012) *Nat. Rev. Neurosci.,* **13**, 701–712.
72. Lyte, M. (2011) *Bioessays,* **33**, 574–581.
73. Bravo, J.A. *et al.* (2011) *Proc. Natl. Acad. Sci. USA,* **108**, 16050–16055.
74. Ming, X. *et al.* (2012) *J. Proteome. Res.,* **11**, 5856–5862.
75. Weinstock, J.V. and Elliott, D.E. (2009) *Inflamm. Bowel. Dis.,* **15**, 128–133.
76. Elliott, D.E. and Weinstock, J.V. (2009) *Adv. Exp. Med. Biol.,* **666**, 157–166.
77. Preidis, G.A. *et al.* (2012) *FASEB. J.,* **26**, 1960–1969.
78. Preidis, G.A. and Versalovic, J. (2009) *Gastroenterology,* **136**, 2015–2031.
79. Shanahan, F. *et al.* (2012) *Clin. Gastroenterol. Hepatol.,* **10**, 1220–1224.
80. Quigley, E.M. (2011) *Curr. Opin. Pharmacol.,* **11**, 593–603.
81. Hickson, M. *et al.* (2007) *BMJ,* **335**, 80.
82. Atarashi, K. *et al.* (2011) *Science,* **331**, 337–341.
83. Arslanoglu, S. *et al.* (2007) *J. Nutr.,* **137**, 2420–2424.
84. Bailey, J.R. *et al.* (2011) *PLoS. One.,* **6**, e26507.
85. Landy, J. *et al.* (2011) *Aliment. Pharmacol. Ther.,* **34**, 409–415.
86. Borody, T.J. and Campbell, J. (2012) *Gastroenterol. Clin. North Am.,* **41**, 781–803.
87. Dethlefsen, L. *et al.* (2008) *PLoS. Biol.,* **6**, e280.
88. Blaser, M. (2011) *Nature,* **476**, 393–394.
89. Proal, A.D. *et al.* (2011) *Cell Mol. Immunol.,* **8**, 213–225.
90. Kootte, R.S. *et al.* (2012) *Diabetes Obes. Metab.,* **14**, 112–120.
91. Payne, D.J. *et al.* (2007) *Nat. Rev. Drug Discov.,* **6**, 29–40.
92. Eckburg, P.B. *et al.* (2005) *Science,* **308**, 1635–1638.
93. Feria-Gervasio, D. *et al.* (2011) *Appl. Microbiol. Biotechnol.,* **91**, 1425–1433.
94. Kim, H.J. *et al.* (2012) *Lab Chip.,* **12**, 2165–2174.
95. Ellrott, K. *et al.* (2010) *PLoS. Comput. Biol.,* **6**, e1000798.
96. Zaneveld, J. *et al.* (2008) *Curr. Opin. Chem. Biol.,* **12**, 109–114.
97. Saleh, M. and Trinchieri, G. (2011) *Nat. Rev. Immunol.,* **11**, 9–20.
98. Knight, P. *et al.* (2008) *Br. Med. Bull.,* **88**, 95–113.
99. Shenderov, B.A. (2011) *Anaerobe,* **17**, 490–495.
100. Bernardo, D. *et al.* (2012) *PLoS. One,* **7**, e36262.
101. Sousa, T. *et al.* (2008) *Int. J. Pharm.,* **363**, 1–25.
102. Okuda, H. *et al.* (1998) *J. Pharmacol. Exp. Ther.,* **287**, 791–799.
103. Wallace, B.D. *et al.* (2010) *Science,* **330**, 831–835.
104. Alimonti, A. *et al.* (2003) *Ann. Oncol.,* **14**, 805–806.
105. Kaddurah-Daouk, R. *et al.* (2011) *PLoS. One,* **6**, e25482.
106. Nicholson, J.K. *et al.* (2011) *Pharmacogenomics,* **12**, 103–111.