Semin Immunopathol (2015) 37:5–16
DOI 10.1007/s00281-014-0456-2

REVIEW

Toward the comprehensive understanding of the gut ecosystem via metabolomics-based integrated omics approach

Wanping Aw · Shinji Fukuda

Received: 15 September 2014 / Accepted: 9 October 2014 / Published online: 22 October 2014

Abstract Recent advances in DNA sequencing and mass spectrometry technologies have allowed us to collect more data on microbiome and metabolome to assess the influence of the gut microbiota on human health at a whole-systems level. Major advances in metagenomics and metabolomics technologies have shown that the gut microbiota contributes to host overall health status to a large extent. As such, the gut microbiota is often likened to a measurable and functional organ consisting of prokaryotic cells, which creates the unique gut ecosystem together with the host eukaryotic cells. In this review, we discuss in detail the relationship between gut microbiota and its metabolites like choline, bile acids, phenols, and short-chain fatty acids in the host health and etiopathogenesis of various pathological states such as multiple sclerosis, autism, obesity, diabetes, and chronic kidney disease. By integrating metagenomic and metabolomic information on a systems biology-wide approach, we would be better able to understand this interplay between gut microbiome and host metabolism. Integration of the microbiome, metatranscriptome, and metabolome information will pave the way toward an improved holistic understanding of the complex mammalian superorganism. Through the modeling of metabolic interactions between lifestyle, diet, and microbiota, integrated omics-based understanding of the gut ecosystem is the new avenue, providing exciting novel therapeutic approaches for optimal host health.

Keywords Gut ecosystem · Metabolomics · Choline · Bile acid · Phenol · Short-chain fatty acid

This article is a contribution to the Special Issue on Microbiome, Immunity and Inflammation - Guest Editor: Hiroshi Ohno

Wanping Aw and Shinji Fukuda contributed equally to this work.

W. Aw · S. Fukuda (✉)
Institute for Advanced Biosciences, Keio University, 246-2 Mizukami, Kakuganji, Tsuruoka, Yamagata 997-0052, Japan
e-mail: sfukuda@sfc.keio.ac.jp

S. Fukuda
RIKEN Center for Integrative Medical Sciences, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

Metabolomics-based omics approach and gut microbiota

With recent advances in DNA sequencing and mass spectrometry technologies, we have been able to collect more data on microbiome and metabolome to evaluate the impact of the gut microbiota on human health on a whole-systems level [1]. Understanding the organ and systemic metabolism is vital on health and nutrition status [2]. Major advances in metagenomics and metabolomics technologies have revealed that the gut microbiota plays a vital role in contributing to host overall health status. Apart from the obvious role in digestion, gut microbiota has been implicated in not only the host health but also etiopathogenesis of various pathological states such as obesity [3], diabetes [4, 5], allergy [6], multiple sclerosis [7], autism [8], chronic kidney disease (CKD) [9], inflammatory bowel diseases (IBD) [10], and colon cancer [11]. In addition, gut microbiota is also with diverse body functions like drug metabolism and toxicity [12], dietary calorific bioavailability [13], immune response [14], and post-surgical recovery [15] (Fig. 1).

The gut microbiota consists of all the microorganisms inhabiting the gastrointestinal tract. In most mammals, the gut microbiota is dominated by four bacterial phyla that perform roles that define the role of the host: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* [5]. The gut is mainly populated by bacteria amounting to about 100 trillion cells, which is approximately threefold larger than the number of human body cells [16] and has extensive metabolic capabilities [5]. Thus, the gut microbiota is often likened to a

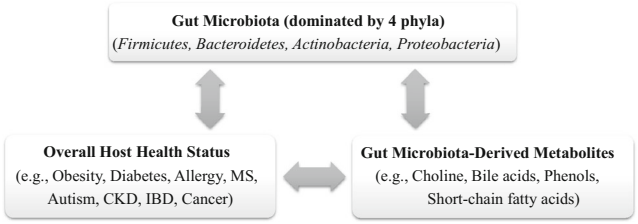

Fig. 1 Overview of the review. The gut microbiota and its metabolites like choline, bile acids, phenols, and short-chain fatty acids are highly implicated in the etiopathogenesis of various pathological states such as obesity, diabetes, allergy, multiple sclerosis(MS), autism, chronic kidney disease (CKD), inflammatory bowel diseases (IBD), and cancer, thereby playing a vital role in host health

measurable and functional organ consisting of prokaryotic cells, which creates the unique gut ecosystem together with the host eukaryotic cells [17]. According to the lifestyle and nutritional status of the host, the bacterial communities vary in composition along the digestive tract and adapt through life [18]. In recent years, we are beginning to understand the systemic influence of the gut microbial community on the whole host metabolic repertoire.

Metabolomics and metabolite profiling are widely used in the identification of disease biomarkers. Capturing the metabolome can be achieved by a wide range of analytical methods, with nuclear magnetic resonance (NMR) and mass spectrometry providing robust and sensitive identification of metabolites produced by microbiota and host cells in fecal, blood, urine, and tissue samples [19]. Ranging from targeted to untargeted methods, methodologies have been reported for screening biochemical pathways, for example, central carbon metabolism, glycolysis, tricarboxylic acid cycle, amino acid pathways, lipid pathways, and selected secondary metabolism pathways [20–22]. These tools allow researchers to determine the effects of treatments have on the host's metabolic profile by analyzing the presence and quantity of thousands of metabolites simultaneously.

Metabolic analyses allow the relationship between metabolism of the gut microbiota to be directly compared with metabolic outcomes in host. In a report by Wikoff et al., the effect of gut microbiota on the host was tested by plasma metabolomic profile comparison from germ-free (GF) and conventionally raised mice. It was reported that in mice with and without gut microbiota, concentrations of more than 10 % of all metabolites differed by more than 50 %. In addition, many metabolites were detected only in conventionally raised mice, and not in GF mice [23]. Another study evaluated the systemic influence of probiotics, prebiotics, and their combination in initially GF mice colonized with a combination of microbes representing those found in a human infant [24]. Dietary supplementation significantly altered the relative proportions of the microbiota community leading to systemic changes in the metabolic profiles of different tissues. Prebiotics increased proportions of *Bifidobacterium breve*, *Bifidobacterium longum*, and *Bacteroidesdistasonis*; decreased proportions of *Escherichia coli* and *Clostridium perfringens*; and modulated lipid metabolism by reducing plasma levels of glucose and hepatic triglycerides [24].

Human global metabolism at the systemic level is the integration between the activities of our gene and the gut microbiome. As the human gastrointestinal tract provides nutrients to cells and tissues via the circulatory system, similarly, so are the metabolites originating from gut microbiota. This intricate interaction among gut microbiota-derived metabolites, the gut microbiota itself, and the host immune system is transmitted via a large array of signalling pathways that extend beyond the immune system. Conjointly, the direct chemical interactions between gut microbiota and the host, and the immune-mediated signalling mechanisms, influence various organs such as the gut, the skeletal muscle, the liver, and the brain. These complex inter-relationships come together to form a series of host-microbe metabolic axes. Within these axes, bacterial genomes can regulate metabolic reactions, resulting in metabolism of substrates by both the gut microbiome and host genome. This is exemplified by the production of choline, bile acids, phenols, and short-chain fatty acids (SCFAs) that are fundamental to host health [25] (Fig. 2).

Choline

Choline, a vital component of cell membranes which is mostly obtained from foods such as red meat and eggs, is primarily metabolized in the liver [26]. In addition, choline is essential in lipid metabolism and hepatic synthesis of very-low-density lipoproteins. This microbial conversion of dietary choline is an imminent characteristic that is associated with altered gut microbial ecology resulting in obesity and liver steatosis in mice [27] and humans [28], and cardiovascular disease (CVD) [29]. Markedly, low quantities of γ-proteobacteria and high levels of *Erysipelotrichia* in human fecal microbiota are correlated with hepatic steatosis [28]. Gut microbial enzymes catalyze the conversion of dietary choline to trimethylamine (TMA) [26] which is then further metabolized by the flavin monooxygenase (FMO) system in the liver to produce trimethylamine-N-oxide (TMAO) [30, 31]. These transformations may result in lower levels of bioavailable choline and are suggested to trigger non-alcoholic fatty liver disease (NAFLD) in mice [30]. Moreover, plasma levels of TMAO and its metabolites are correlated with CVD [29]. This effect of microbial choline metabolism in CVD was observed when atherosclerosis was reduced in atherosclerosis-prone *Apoe*$^{-/-}$ mice after broad-spectrum antibiotics treatment [29]. In a study to investigate the relationship between oral intake of phosphatidylcholine and the involvement of the gut microbiota in proatherogenic TMAO production in humans, it was

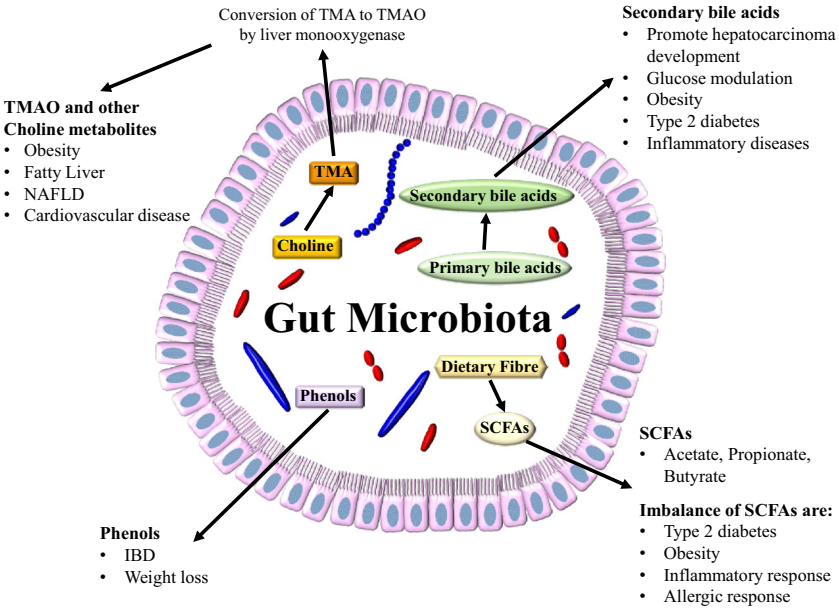

Fig. 2 The influence of gut microbiota-derived metabolites on host physiology. Choline metabolites including trimethylamine-N-oxide (TMAO) have been implicated in the pathogenesis of obesity, fatty liver, non-alcoholic fatty liver disease (NAFLD), and cardiovascular diseases. Secondary bile acids are reported to promote hepatocarcinoma development, glucose modulation, obesity, type 2 diabetes, and inflammatory diseases. Phenols are involved in inflammatory bowel disease (IBD) and weight loss. Short-chain fatty acids (SCFAs) have been reported as health-promoting metabolites, and the imbalance of these metabolites mediates type 2 diabetes, obesity, inflammatory, and allergic responses

reported that fasting plasma TMAO levels predict the risk of incident major adverse events, independently of traditional cardiovascular risk factors, even in low-risk cohorts. Modulating gut microbiota could prove interesting for therapeutic purposes in relation to these events [32]. As reported by Koeth et al., metabolism of dietary L-carnitine by gut microbiota, a TMA present in large amounts in red meat, also produces TMAO and accelerates atherosclerosis in mice. In addition, vegans produced lesser TMAO than omnivorous human subjects following ingestion of L-carnitine through a microbiota-dependent mechanism. The presence of specific bacterial taxa in human fecal samples was correlated with both plasma TMAO levels and dietary status. L-carnitine concentrations in plasma in subjects undergoing cardiac evaluation predicted increased risks for both prevalent CVD and incident major adverse cardiac events, but only amongst subjects with co-existing elevated TMAO levels. Modified cecal microbial composition in mice by chronic dietary L-carnitine supplementation significantly enhanced synthesis of TMA and TMAO and increased atherosclerosis but was not observed when gut microbiota was suppressed at the same time. In mice with an intact gut microbiota, dietary supplementation with TMAO or either carnitine or choline reduced reverse cholesterol transport in vivo. Gut microbiota may thus contribute to the well-established link between high levels of red meat consumption and CVD risk [33]. These studies provide a potential link between gut microbiota, dietary choline, and disease risk.

Bile acids

The primary bile acids (or bile salts) cholic acid and chenodeoxycholic acid are synthesized in the human liver from cholesterol and secreted in bile, mainly function to facilitate the metabolism of dietary fat and the absorption of fat-soluble vitamins and cholesterol. Primary bile acids undergo an enterohepatic cycle between the gut and the liver eight times per day, with 90–95 % of the bile acids being reabsorbed by the intestine and returned to the liver, whereupon they are conjugated to taurine in mice and to glycine in humans, to form bile salts [34, 35]. Approximately 5–10 % of the bile acids are biotransformed to a large extent through degradation by gut microbiota, while some are lost in the feces. Gut microbiota involved in the biotransformation are mainly anaerobic and belong to the genera *Bacteroides*, *Eubacterium*, and *Clostridium*. Taurine- and glycine-conjugated bile acids are deconjugated via bile acid hydrolases to their respective unconjugated free bile acids which then form secondary bile acids such as deoxycholic acid (DCA) and lithocholic acid [35, 36] which are then reabsorbed, mainly by both bile acid transporters in the ileum and passive absorption in the intestine [36]. Bile acid hydrolase enzymes have been identified in several bacterial species, mainly anaerobes of the genera *Bacteroides*, *Eubacterium*, *Clostridium*, *Lactobacillus*, and *Escherichia*. A minor portion of bile acid biotransformation has also been reported to be conducted by aerobic bacteria

such as actinobacteria and proteobacteria [35]. The enterohepatic circulation of DCA induces senescence-associated secretory phenotype (SASP) in hepatic stellate cells (HSCs) [37], which then secretes various hepatic inflammatory and tumor-promoting factors, thus facilitating murine hepatocarcinoma (HCC) development after exposure to chemical carcinogen. Importantly, the muting of DCA production or gut microbe reduction efficiently prevents HCC development in obese mice. Similar results were also observed in mice lacking an SASP inducer [38] or depleted of senescent HSCs, indicating that the DCA–SASP axis in HSCs plays a vital role in obesity-associated HCC development. In addition, it was also observed that there were signs of SASP in the HSCs in the area of HCC arising in patients with non-alcoholic steatohepatitis [39], suggesting that there might be a similar pathway that contributes to certain aspects of obesity-associated HCC development in humans [40]. As gut microbiota is heavily involved in the transformation of bile acids, GF rodents have more bile acid and a less diverse profile than their conventionally raised counterparts [34, 41]. In addition, bile acids also function as signalling molecules and bind to cellular receptors [36] like nuclear receptor farnesoid X receptor (FXR) [42] and G protein-coupled receptor (GPCR) TGR5 [43]. Both FXR andTGR5 are involved in glucose metabolism modulation in rodents whereby FXR impairs, whereas TGR5 promotes glucose homeostasis [42, 43]. FXR is activated by primary bile acids while TGR5 binds secondary bile acids such as DCA and lithocholic acid. In enteroendocrine L-cells, TGR5 signaling induces secretion of glucagon-like peptide-1 (GLP-1), thereby leading to enhanced hepatic and pancreatic function and improved glucose tolerance in obese mice [43]. Furthermore, the activation of TGR5 in brown adipose tissue and muscle has been reported to increase energy expenditure and protects against diet-induced obesity [36]. As reported by David et al., in 2014, *Bilophila wadsworthia* growth is stimulated in mice by secreted bile acids while consuming saturate fats from milk and in humans who consume high-fat diets. Levels of *B. wadsworthia* which contains microbial genus *Bilophila* increased, which correlated with long-term daily saturated fat intake. The animal-based diet also led to elevated fecal bile acid levels, increases in the abundance of microbial DNA and RNA encoding sulfite reductases, leading to the conclusion that animal-based diets may induce changes to bile acid concentration, gut microbiota composition, and thereby lead to the development of IBD [44]. It has been demonstrated that all major bacterial groups are involved in bile salt hydrolase activity, and as such, the gut microbiota may therefore contribute to effective obesity and type 2 diabetes management by controlling lipid and glucose metabolism via the composition of bile-acid pools and the modulation of FXR and TGR5 signaling [45].

Phenols

Human beings excrete approximately between 50 to 100 mg of volatile phenols daily, mainly in the form of 4-cresol and phenol (predominantly as glucuronide and sulphate conjugates) with lower levels of 4-ethylphenol [46]. Production of cresols in mammalians is associated to various genera in the *Clostridium*, *Bifidobacterium*, and *Bacteroides*. Altered levels of urinary 4-cresol metabolites in humans are correlated to a diverse physiology and pathological conditions ranging from IBD to weight loss. In addition, it has also been reported that these conditions are associated with altered gut microbiota composition, particularly a lowered diversity of microbiota due to loss of *Lactobacillus* and *Bacteroides* species in IBD [47], and differences in the ratio between *Firmicutes* and *Bacteroidetes* in weight loss [3, 48].

Dietary fiber fermentation and short-chain fatty acid production

Dietary fibre (complex carbohydrates) are digested and subsequently fermented in the colon by gut microbiota into SCFAs such as acetate, propionate, and butyrate. These SCFAs are sensed by the GPCRs expressed by gut enteroendocrine cells—GPR41 and GPR43 [49]. SCFAs can suppress inflammation through GPR43 signaling in immune cells, such as neutrophils [50, 51], and insulin signaling in adipocytes [52]. In addition, SCFAs modulate secretion of the hormone GLP-1, which improves insulin secretion and exerts antidiabetic effects [53]. Furthermore, the gut microbiota induces peptide YY expression by L-cells via a GPR41-dependent mechanism as observed in the study where conventional *Gpr41*-deficient mice exhibited reduced adiposity compared with conventional wild-type mice, whereas GF wild type and *Gpr41*-deficient mice had similar adiposity [49]. Of the SCFAs produced from microbial fermentation, butyrate is particularly important as an energy substrate for cellular metabolism in the colonic epithelium [54]. It is mainly produced by microbial order *Clostridiales*, a dominant order in gut microbiota. Recent study using a metabolomics-based omics approach has also showed that the colonization of *Clostridiales* into GF mice fed with high-fiber diet promotes gut microbial fermentation, resulting in accumulation of luminal SCFAs [55]. Among SCFAs, butyrate induces the differentiation of regulatory T (Treg) cells in vitro. Administration of butyrylated starch induces colonic Treg cell differentiation and ameliorates the development of colitis in vivo. Treatment of naive T cells under the Treg-polarizing conditions with butyrate enhances histone H3 acetylation, particularly in the promoter and CNS 3 enhancer regions of the *Foxp3* gene, the master transcriptional regulator of Treg cells. Taken together, butyrate derived from dietary fiber fermentation by commensal microbial order *Clostridiales*

epigenetically induces the differentiation of colonic Treg cells which have a pivotal role in suppressing inflammatory and allergic responses [55–57].

In a recent study by Trompette et al., it was reported that dietary fiber content interestingly changed the composition of both gut and lung microbiota, via the alteration of the ration of *Firmicutes* to *Bacteroidetes* [58]. In mice fed high-fiber diets, there were increased levels of SCFAs and mice were protected against allergic inflammation in the lung, whereas the contrary was observed in mice fed low-fiber diets. Mice that were treated with SCFA propionate had altered bone marrow hematopoiesis where enhanced generation of macrophage and dendritic cell precursors and subsequent seeding of the lungs by dendritic cells of high phagocytic capacity. It was reported that the effects of propionate on allergic inflammation were defended on GPR41 but not GPR43 [58]. Other SCFAs such as acetate and propionate are taken up by the liver and used as substrates for lipogenesis and gluconeogenesis [59]. SCFAs have been reported to modulate the function of histone deacetylases by stimulating the sympathetic nervous system, thereby influencing social behavior in rats [60]. SCFAs, propionate and butyrate, which are generated by the fermentation of soluble fibers, activate intestinal gluconeogenesis (IGN) that improves glucose and energy homeostasis [61]. Butyrate activates IGN gene expression via a cAMP-dependent mechanism while propionate, a substrate of IGN, activates IGN gene expression through the gut–brain neural circuit involving the fatty acid receptor FFAR3 [61]. Butyrate, a commensal metabolite from fermentation of dietary fiber in the colon, activates GPR109a niacin receptor and suppresses colonic inflammation and carcinogenesis [62]. In the view of protection of enteropathogenic infection, acetate produced by probiotic bifidobacteria improves intestinal defense mediated by upregulation of epithelial cell barrier function and thereby protects the host against lethal infection using a simplified model of lethal infection with enterohaemorrhagic *Escherichia coli* O157:H7, combined with an integrated omics approach [63]. In a study by Okada et al., it has been demonstrated that microbiota-derived lactate is a major factor in the induction of enterocyte hyperproliferation in starvation-refed mice. Colonic epithelial cell turnover is interrupted during a 12- to 36-h period of starvation and increases 12–24 h after refeeding. Enhanced epithelial cell proliferation is also dependent on the increase in live *Lactobacillus murinus*, lactate production, and dietary fiber content [64]. As such, SCFAs is a vital product of microbial fermentation of dietary fibers and influence a range of host processes with significant effects.

Dysbiosis and extraintestinal disease

The gastrointestinal tract, a complex and well-balanced ecosystem, is one of the largest interfaces between the outside world and the human internal environment. Under normal conditions, commensal microbes and their hosts are in a symbiotic relationship. However, any imbalance in this equilibrium via qualitative and quantitative changes in the gut microbiota itself, changes in their metabolic activities, and changes in their local distribution leads to a condition where microbial imbalance exerts adverse effects on the host known as dysbiosis. Many factors like antibiotic consumption, dietary habits, and environmental conditions can harm the microbial stability and thus, contribute to intestinal dysbiosis [65], leading to various chronic diseases like multiple sclerosis, autism, obesity, diabetes, and CKD which we will discuss in the following sections (Fig. 3).

Multiple sclerosis

The intestinal microbiota has been reported to affect the development of autoimmune central nervous system (CNS) disorders. In mice that exhibit symptoms of experimental autoimmune encephalomyelitis (EAE), broad spectrum antibiotics are administered orally [66]. In a particular model of multiple sclerosis, mice are immunized with self-antigen myelin oligodendrocyte glycoprotein (MOG) in complete Freund's adjuvant (CFA). Disease symptoms in both spontaneous EAE and MOG-CFA induced EAE were diminished when the mice were housed under GF conditions [7, 67]. Monocolonization of GF mice with segmented filamentous bacteria (SFB) that are related to the genus *Clostridium*

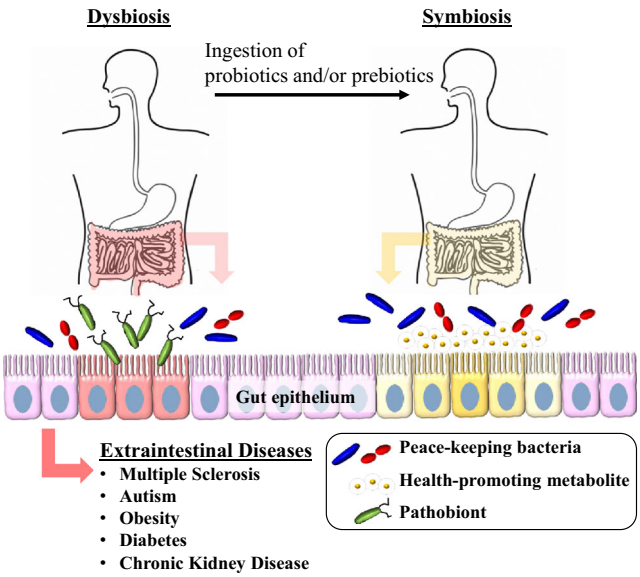

Fig. 3 Dysbiosis-related extrsaintestinal diseases and therapeutic modulation of gut microbiota. Dysbiosis refers to the imbalance between the peacekeeping bacteria and pathobionts leading to extraintestinal diseases like multiple sclerosis, autism, obesity, diabetes, and chronic kidney disease (*left*). Therapeutic modulation of gut microbiota by treatment with probiotics and/or prebiotics modifies gut luminal metabolite composition, and thereby, the host condition is improved (*right*)

resulted in an increased number of interleukin-17 producing helper T (T_H17) cells in both the intestinal lamina propria and the CNS, leading to severe EAE [67]. However, it is currently unknown whether EAE is a result of the migration of SFB-specific T_H17 cells into the CNS or by the expansion of pathogenic autoantigen specific T cells that are promoted by T_H17 cell responses. Conversely, certain populations of commensal bacteria like polysaccharide A $(PSA)^+$*Bacteroides fragilis* can attenuate CAS inflammation via $Foxp3^+$ Treg cell differentiation, thereby preventing EAE symptoms. As such, the pathogenesis of CNS disorders like multiple sclerosis may be dependent on the balance of different community members in the gut microbiota.

Autism

Autism spectrum disorder (ASD) is a serious neurodevelopmental condition that is diagnosed based on presence of stereotypic behavior and deficits in language and social interaction. Among several comorbidities in ASD, gastrointestinal distress is of particular interest due to its reported prevalence [68, 69] and correlation with symptom severity [70]. There have been studies that reported altered composition of intestinal microbiota in ASD [70–75]. Children with autism had lower levels of species of *Bifidobacterium*, higher levels of species of *Lactobacillus*, and lower levels of total SCFAs including lower levels of acetate, propionate, and valerate [70]. *Bacteroidetes* was found at high levels in the severely autistic group, while *Firmicutes* were more predominant in the control group. There were also small and significant differences in the *Actinobacteria* and *Proteobacteria* phyla. *Desulfovibrio* species and *Bacteroides vulgatus* are present in significantly higher numbers in stools of severely autistic children than in controls [70]. Another study showed significantly lower abundances of the genera *Prevotella*, *Coprococcus*, and unclassified *Veillonellaceae* in samples from autism children. In addition, the members of the family *Alcaligenaceae* are present in children with autism and gastrointestinal dysfunction [72]. Hsiao et al., has also recently revealed that oral administration of PSA^+*Bacteroides fragilis* in the offspring of maternal immune activated (MIA) mice corrected gut permeability, altered gut microbial composition, and attenuated defects in behavioral and physiological abnormalities associated with ASD [76]. PSA^+*B. fragilis* treatment also corrected the levels of MIA-induced serum metabolites by restoring the levels of metabolite 4-ethylphenylsulfate which in naive mice, results in certain behavior abnormalities [76]. Taken together, these findings indicate that relationship between the gut microbiota, metabolome, and the brain identifies novel therapeutic opportunities in human neurodevelopmental disorders.

Obesity

In 2005, the first study that suggested that obesity could be due to gut microbial composition revealed that phylum *Bacteroidetes* was lower and phylum *Firmicutes* was higher in abundance in obese ob/ob mice than their lean littermates when fed the same diet [48]. Subsequently, a study with obese human twins showed a correlation between the decrease in *Bacteroidetes* proportion with an increase in *Firmicutes* proportion to the enrichment of microbial genes encoding key enzymes involved in carbohydrate metabolism which is implicated in the host's ability to digest food and supply energy such as SCFAs [3, 77]. Colonization of gut microbiota from both obese mice [78] and obese humans [3, 79] into GF recipient mice also reproduced the obese phenotype. In addition, it has also been reported that in obese children, bifidobacterial numbers were lower while levels of *Staphylococcus aureus* were higher [80]; Gram-negative family *Enterobacteriaceae* was significantly higher, and levels of *Desulfovibrio* and *Akkermansia muciniphila*-like bacteria were significantly lower in obese children than those in children with normal weight [81]. It has also been reported that the number of *Akkermansia muciniphila*, a novel mucin-degrading bacterium that colonizes in the mucus layer constituting of 3–5 % of the bacterial community [82], is dramatically decreased in both genetically and high-fat diet-induced obese mice [83]. Further studies have also shown that this bacterium is negatively correlated with body weight [81, 84–86] as well as type 1 [87] and type 2 diabetes [88]. Improvement in several metabolic disorders including fat-mass gain, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance were observed upon oral administration of *Akkermansa mucinphila* or oligofructose. Recently, it has been reported that in GF mice fed a high-fat diet, mono-association with an endotoxin-producing *Enterobactercloacae* B29 strain isolated from an obese human subject induced obesity and glucose homeostasis disorders. This implies that lowering plasma endotoxin levels could be a viable strategy in controlling metabolic diseases [89]. In addition, in human patients presenting with metabolic syndrome, fecal transplantation of gut microbiota from lean healthy donors resulted in improved insulin sensitivity. This improvement was correlated with an increase in the number of butyrate-producing bacteria, indicating that microbial butyrate may play a role in promoting this improvement [90].

Diabetes

There have been several studies implicating that the correlation between the composition of gut microbiota with type 1 and type 2 diabetes development. Type 1 diabetes (T1D) is an

autoimmune disorder that involves the destruction of insulin-producing cells in the pancreases. Studies performed in a T1D mice model, non-obese diabetic (NOD) mice, revealed that in *Myd88*$^{-/-}$ mice, protection against diabetes diminished upon GF conditions and during antibiotic treatment [4]. Bacterial 16S ribosomal RNA gene sequencing also showed changes in the microbiota composition in *Myd88*$^{-/-}$ NOD mice as compared to their normal littermates. The *Firmicutes*/*Bacteroidetes* ratio was lowered, and this indicates that certain bacterial populations were essential in the protection against T1D. However so, it has been recently reported that MYD88 deficiency alone does not influence gut microbiota composition; therefore, it is plausible that the genetic background of NOD mice influenced the effect of MYD88 [91]. SFB has also been reported to protect against T1D [92]. However, this protection is gender-specific as male NOD mice with SFB have lowered incidence of T1D than females. Furthermore, gut microbiota in male NOD mice is different from that in females, contributing to increased testosterone levels which are associated with T1D protection [92, 93]. When gut microbiota was transferred from male NOD mice to their female counterparts, testosterone concentration increased and reduced susceptibility to T1D was observed [93]. In type 2 diabetes (T2D), Larsen et al., reported that gut microbiota of male T2D subjects had significantly lower levels of *Firmicutes*, including class *Clostridia*, as compared to non-diabetic control subjects [94]. The same study also reported that both the ratios of *Bacteroidetes* to *Firmicutes* and of the *Bacteroides–Prevotella* group to the *C. coccoides–Eubacterium rectale* were correlated to plasma glucose concentrations. As *Bacteroidetes* and *Proteobacteria* are Gram-negative bacteria and have high levels of endotoxin lipopolysaccharide (LPS) as a main component of their outer membranes, these findings suggest that T2D may be promoted via an endotoxin-induced inflammatory response [94]. In ob/ob diabetic mice model, it was also observed that antibiotic treatment for 2 weeks significantly reduced the numbers of both aerobic and anaerobic gut microbes, leading to lower hepatic triglycerides, lower plasma concentrations, elevated hepatic glycogen, and increased plasma adiponectin concentrations than non-treated counterparts [95]. In a metagenome-wide association study using data from a total of 345 T2D patients and non-T2D control subjects, it was reported that genes enriched in the control group were from various butyrate-producing bacteria like *Clostridiales* sp. SS3/4, *Eubacterium rectale*, *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, and *Roseburia inulinivorans* [88]. On the other hand, most of the genes enriched in the T2D group were mainly from opportunistic pathogens including *Bacteroides caccae*, *Clostridium hathewayi*, *Clostridium ramosum*, *Clostridium symbiosum*, *Eggerthella lenta*, and *Escherichia coli* which have been reported to cause or underlie human infections [88]. Mucin-degrading microbial species *A. mucinphilia* and sulfate-reducing species *Desulfovibrio* sp. were also enriched in the T2D group. In another metagenomic study conducted in 145 64-year-old-European women with normal, impaired, or diabetic glucose control, a mathematical model to identify T2D based on gut microbiota metagenomic profile was developed. This model was also applied to the abovementioned study using Chinese subjects, and the metagenomic markers to distinguish European T2D and Chinese T2D cohorts were different. This suggests that age and geography may play a role in gut microbial metagenome profiles towards T2D development [96].

Chronic kidney disease

Various extraintestinal non-communicable diseases are associated with gut dysbiosis as intestinal immunity is affected to the extent that physiological control of microbiota can no longer be maintained [97]. In a study by Vaziri et al., where he characterized the gut microbiota of uremic versus non-uremic rats and patients, it was found that uremia was associated with an increase in intestinal pathobionts, indicating that metabolic and hemodynamic alterations in CKD modify the composition and function of gut microbiota [98]. Renal dysfunction in CKD patients often present with not only metabolic derangments but also systemic inflammation where the intestinal microbiota has been increasingly identified as a the trigger for immune dysregulation [99, 100]. Wang et al., recently reported that rats with experimental uremia had increased bacterial translocation from the gut into mesenteric lymph nodes, liver, and spleen which was associated with increased levels of serum interleukin-6 and C-reactive protein [9]. Circulating bacterial endotoxin/LPS levels also increase along CKD progression and are the highest in patients on dialysis. LPS levels that were highest in patients under dialysis were comparable to that in patients with liver disease, gut irradiation, and decompensated heart failure. As LPS originates from the cell wall component of Gram-negative bacteria, microbiota enriched in γ-proteobacteria may be a valid source of circulating LPS [101]. In addition, it has also been reported that colonic bacteria generate uremic toxins like α-phenylacetyl-l-glutamine, 5-hydroxyindole, indoxyl glucuronide, *p*-cresol sulfate, and indoxyl sulfate; all of which contribute to end-stage renal disease [102, 103]. Gut microbiota-directed interventions improved uremic state when oral intake of non-pathogenic *Sprosarcina pasteurii* improved renal function and prolonged lifespan of uremic rats [103]. In addition, neutralization of bacteria-derived uremic toxin indoxyl sulfate in the gut delayed the progression of CKD and CVD in uremic rats [104]. Lastly, an improved 5-year survival was observed in pre-dialysis patients with the same indoxyl-sulfate-binding agent as described above [104]. Although the understanding of the relationship between gut microbiota and CKD is still in

its infancy, there is evidence that points toward the CKD-related immune derangements and complications are related to microbiota variation. It is necessary that the gut no longer be neglected as a potential trigger for CKD, and research activities are required to unravel the pathogenetic role of gut microbiota in kidney disease and to discover appropriate therapeutic interventions to manipulate the gut microbiota to correct CKD-related immune dysregulation and to prevent further complications.

Therapeutic implications of gut microbiota modulation

Therapeutic interventions such as bacteriotherapy [105] and bioecological control [106, 107] have been broadly used in the modulation of gut microbiota for human health. Modulation of intestinal microbiota populations either by the pro-morbid gut microbiota of the host by prebiotics, probiotics, or synbiotics may be beneficial to human health [106, 107]. Fecal microbiota transplantations potentially modulate gut microbiota for different diseases, including chronic gastrointestinal infections, IBD, insulin resistance, multiple sclerosis, and idiopathic thrombocytopenic purpura [108]. In antibiotic-associated diarrhea or *Clostridium difficile* infection, fecal transplantation has reported to be beneficial [109, 110]. In GF mice inoculated with human baby microbiota, populations of *Bifidobacterium longum* and *Bifidobacterium breve* increased, whereas *Clostridium perfringens* decreased when probiotics *Lactobacillus paracasei* or *Lactobacillus rhamnosus*, and two galactosyl-oligosaccharide prebiotics were administered. The changes in gut microbiota composition resulted in a range of host metabolic pathways which were reflected in lipid profiles, gluconeogenesis, and amino acid metabolism [111]. Conjugated linoleic acid is a naturally occurring conjugated isomer of linoleic acid found in ruminant-derived meat and dairy products [112]. It has been shown to protect against colon carcinogenesis, arteriosclerosis, and obesity in mice [113, 114]. Mice that were supplemented with *L. rhamnosus* GG and *Lactobacillus sakei* NR28 had lowered relative abundances of both *Firmicutes* and *Clostridium* cluster XIVa in the small intestine, resulting in the reduction of body weight gain, fat mass, and expression of lipogenesis-related genes [115]. However, supplementation with *L. acidophilus* NCDC13 in diet-induced obese mice increased the total number of *Bifidobacterium*in cecal and fecal contents without reducing adiposity [116]. In healthy overweight humans, reduction of abdominal visceral and subcutaneous fat was reduced after oral administration of *Lactobacillus gasseri* SBT2055 [116]. Infants who were supplemented with *L. rhamnosus* GG in infant formula for 6 months presented with better growth and larger weight gain [117]. In a follow-up study, pre- and post-natal administration of *L. rhamnosus* GG protected against excessive weight gain in children [118]. Prebiotic fibers have also been reported to reduce the ratio of *Firmicutes* to *Bacteriodetes* in obese rats [119] and ameliorate NAFLD by lowering hepatic de novo lipogenesis [119]. Supplementation of chitin glucan increases the number of bacteria closely related to the *Clostridium* cluster XIVa including *Roseburia* spp., which is accompanied by attenuated fat fain and fat mass development [120]. In mice fed high-fat diet supplemented with wheat-derived arabinoxylans, it has been reported that the number of *Bacteroides*/*Prevotella* spp. and *Roseburia* spp. restored, and the number of *Bifidobacterium* spp., in particular *Bifidobacterium animalis* lactis, was increased [121]. Inulin supplementation in obese women increased *Bifidobacterium* spp. and *F. prausnitzii*, and decreases *Bacteroides intestinalis*, *Bacteroides vulgates*, and *Priopionibacterium* [122]. Lastly, in healthy human subjects, consumption of galacto-oligosaccharides for 12 weeks increased several types of *Bifidobacterium* spp. and decreased in the number of *Bacteroides* [123]. Collectively, improvement of gut microbiota composition by treatment with probiotics and/or prebiotics may be beneficial in the prevention and early medical treatment of several dysbiosis-associated disorders.

Conclusion

By integrating metagenomic and metabolomics information on a systems biology-wide approach, we would be better able to understand this interplay between gut microbiome and host metabolism. Integration of the microbiome, metatranscriptome, and metabolome information will pave the way toward an improved holistic understanding of the complex mammalian superorganism. Through the modeling of metabolic interactions between lifestyle, diet, and microbiota, integrated omics-based understanding of the gut ecosystem is the new avenue, providing exciting novel therapeutic approaches for optimal host health.

References

1. Tringe S, Hugenholtz P (2008) A renaissance for the pionnering 16S rRNA gene. Curr Opin Microbiol 11:442–446
2. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S (2012) Host-gut microbiota metabolic interactions. Science 336:1262–1267
3. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027–1031
4. Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, Gordon JI, Chervonsky AV (2008) Innate immunity and intestinal microbiota in the development of type 1 diabetes. Nature 455:1109–1113

5. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Meta HITC, Bork P, Ehrlich SD, Wang J (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59–65
6. Kirjavainen P, Arvola T, Salminen S, Isolauri E (2002) Aberrant composition of gut microbiota of allergic infants: a target of bifidobacterial therapy at weaning? Gut 51:51–55
7. Berer K, Mues M, Koutrolos M, Rasbi ZA, Boziki M, Johner C, Wekerle H, Krishnamoorthy G (2011) Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature 479:538–541
8. Finegold SM (2008) Therapy and epidemiology of autism–clostridial spores as key elements. Med Hypotheses 70:508–511
9. Wang F, Zhang P, Jiang H, Cheng S (2012) Gut bacterial translocation contributes to microinflammation in experimental uremia. Dig Dis Sci 57:2856–2862
10. Marchesi J, Holmes E, Khan F, Kochhar S, Scanlan P, Shanahan F, Wilson I, Wang Y (2007) Rapid and noninvasive metabonomic characterization of inflammatory bowel disease. J Proteome Res 6: 546–551
11. Scanlan PD, Shanahan F, Clune Y, Collins JK, O'Sullivan GC, O'Riordan M, Holmes E, Wang Y, Marchesi JR (2008) Culture-independent analysis of the gut microbiota in colorectal cancer and polyposis. Environ Microbiol 10:789–798
12. Clayton TA, Lindon JC, Cloarec O, Antti H, Charuel C, Hanton G, Provost JP, Le Net JL, Baker D, Walley RJ, Everett JR, Nicholson JK (2006) Pharmaco-metabonomic phenotyping and personalized drug treatment. Nature 440:1073–1077
13. Hooper LV (2001) Commensal host-bacterial relationships in the gut. Science 292:1115–1118
14. Macpherson AJ (2000) A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science 288:2222–2226
15. Kinross J, Alkhamesi N, Barton R, Silk D, Yap I, Darzi A, Holmes E, Nicholson J (2011) Global metabolic phenotyping in an experimental laparotomy model of surgical trauma. J Proteome Res 10: 277–287
16. Bianconi E, Piovesan A, Facchin F, Beraudi A, Casadei R, Frabetti F, Vitale L, Pelleri MC, Tassani S, Piva F, Perez-Amodio S, Strippoli P, Canaider S (2013) An estimation of the number of cells in the human body. Ann Hum Biol 40:463–471
17. Fukuda S, Ohno H (2014) Gut microbiome and metabolic diseases. Semin Immunopathol 36:103–114
18. Xu J, Mahowald M, Ley R, Lozupone C, Hamady M, Martens E, Henrissat B, Coutinho P, Minx P, Latreille P, Cordum H, Van Brunt A, Kim K, Fulton R, Fulton L, Clifton S, Wilson R, Knight R, Gordon J (2007) Evolution of symbiotic bacteria in the distal human intestine. PLoS Biol 5:1574–1586
19. Dettmer K, Aronov P, Hammock B (2007) Mass spectrometry based metabolomics. Mass Spectrom Rev 26:51–78
20. Wenk MR (2005) The emerging field of lipidomics. Nat Rev Drug Discov 4:594–610
21. Buescher J, Moco S, Sauer U, Zamboni N (2010) Ultrahigh performance liquid chromatography-tandem mass spectrometry method for fast and robust quantification of anionic and aromatic metabolites. Anal Chem 82:4403–4412
22. Moco S, Bino RJ, Vorst O, Verhoeven HA, de Groot J, van Beek TA, Vervoort J, de Vos CH (2006) A liquid chromatography-mass spectrometry-based metabolome database for tomato. Plant Physiol 141:1205–1218
23. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G (2009) Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci U S A 106:3698–3703
24. Martin FP, Wang Y, Sprenger N, Yap IK, Lundstedt T, Lek P, Rezzi S, Ramadan Z, van Bladeren P, Fay LB, Kochhar S, Lindon JC, Holmes E, Nicholson JK (2008) Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model. Mol Syst Biol 4:157
25. Nicholson J, Wilson I (2003) Understanding 'global' systems biology metabonomics and the continuum of metabolism. Nat Rev Drug Discov 2:668–676
26. Vance D (2008) Role of phosphatidylcholine biosynthesis in the regulation of lipoprotein homeostasis. Curr Opin Lipidol 19:229–234
27. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA (2012) Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature 482:179–185
28. Spencer MD, Hamp TJ, Reid RW, Fischer LM, Zeisel SH, Fodor AA (2011) Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. Gastroenterology 140:976–986
29. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, Hazen SL (2011) Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 472:57–63
30. Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, Fearnside J, Tatoud R, Blanc V, Lindon JC, Mitchell SC, Holmes E, McCarthy MI, Scott J, Gauguier D, Nicholson JK (2006) Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci U S A 103:12511–12516
31. Prentiss P, Rosen H, Brown N, Horowitz R, Malm O, Levenson S (1961) The metabolism of choline by the germfree rat. Arch Biochem Biophys 94:424–429
32. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL (2013) Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 368:1575–1584
33. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, Smith JD, DiDonato JA, Chen J, Li H, Wu GD, Lewis JD, Warrier M, Brown JM, Krauss RM, Tang WH, Bushman FD, Lusis AJ, Hazen SL (2013) Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 19:576–585
34. Swann JR, Want EJ, Geier FM, Spagou K, Wilson ID, Sidaway JE, Nicholson JK, Holmes E (2011) Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. Proc Natl Acad Sci U S A 108(Suppl 1):4523–4530
35. Ridlon JM, Kang DJ, Hylemon PB (2006) Bile salt biotransformations by human intestinal bacteria. J Lipid Res 47:241–259
36. Watanabe M, Houten SM, Mataki C, Christoffolete MA, Kim BW, Sato H, Messaddeq N, Harney JW, Ezaki O, Kodama T, Schoonjans K, Bianco AC, Auwerx J (2006) Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. Nature 439:484–489
37. Friedman SL (2008) Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 88:125–172
38. Orjalo AV, Bhaumik D, Gengler BK, Scott GK, Campisi J (2009) Cell surface-bound IL-1alpha is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. Proc Natl Acad Sci U S A 106:17031–17036
39. Coulouarn C, Corlu A, Glaise D, Guenon I, Thorgeirsson SS, Clement B (2012) Hepatocyte-stellate cell cross-talk in the liver

engenders a permissive inflammatory microenvironment that drives progression in hepatocellular carcinoma. Cancer Res 72:2533–2542
40. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, Iwakura Y, Oshima K, Morita H, Hattori M, Honda K, Ishikawa Y, Hara E, Ohtani N (2013) Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature 499: 97–101
41. Wostmann B (1973) Intestinal bile acids and cholesterol absorption in the germfree rat. J Nutr 103:982–990
42. Prawitt J, Abdelkarim M, Stroeve J, Popescu I, Duez H, Velagapudi V, Dumont J, Bouchaert E, van Dijk T, Lucas A, Dorchies E, Daoudi M, Lestavel S, Gonzalez F, Oresic M, Cariou B, Kuipers F, Caron S, Staels B (2011) Farnesoid X receptor deficiency improves glucose homeostasis in mouse models of obesity. Diabetes 60:1861–1871
43. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Mataki C, Pruzanski M, Pellicciari R, Auwerx J, Schoonjans K (2009) TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab 10:167–177
44. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ (2014) Diet rapidly and reproducibly alters the human gut microbiome. Nature 505:559–563
45. Jones BV, Begley M, Hill C, Gahan CG, Marchesi JR (2008) Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. Proc Natl Acad Sci U S A 105:13580–13585
46. Bone E, Tamm A, Hill M (1976) The production of urinary phenols by gut bacteria and their possible role in the causation of large bowel cancer. Am J Clin Nutr 29:1448–1457
47. Ott SJ (2004) Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. Gut 53:685–693
48. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI (2005) Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A 102:11070–11075
49. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M, Gordon JI (2008) Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc Natl Acad Sci U S A 105:16767–16772
50. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, Xavier RJ, Teixeira MM, Mackay CR (2009) Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature 461:1282–1286
51. Sina C, Gavrilova O, Forster M, Till A, Derer S, Hildebrand F, Raabe B, Chalaris A, Scheller J, Rehmann A, Franke A, Ott S, Hasler R, Nikolaus S, Folsch UR, Rose-John S, Jiang HP, Li J, Schreiber S, Rosenstiel P (2009) G protein-coupled receptor 43 is essential for neutrophil recruitment during intestinal inflammation. J Immunol 183:7514–7522
52. Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, Terasawa K, Kashihara D, Hirano K, Tani T, Takahashi T, Miyauchi S, Shioi G, Inoue H, Tsujimoto G (2013) The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. Nat Commun 4:1829
53. Tolhurst G, Heffron H, Lam Y, Parker H, Habib A, Diakogiannaki E, Cameron J, Grosse J, Reimann F, Gribble F (2012) Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes 61:364–371
54. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, Bultman SJ (2011) The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metab 13:517–526
55. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H (2013) Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 504:446–450
56. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, Taniguchi T, Takeda K, Hori S, Ivanov II, Umesaki Y, Itoh K, Honda K (2011) Induction of colonic regulatory T cells by indigenous Clostridium species. Science 331:337–341
57. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K (2013) Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 500:232–236
58. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, Blanchard C, Junt T, Nicod LP, Harris NL, Marsland BJ (2014) Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat Med 20:159–166
59. Tremaroli V, Backhed F (2012) Functional interactions between the gut microbiota and host metabolism. Nature 489:242–249
60. MacFabe DF, Cain NE, Boon F, Ossenkopp KP, Cain DP (2011) Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: relevance to autism spectrum disorder. Behav Brain Res 217:47–54
61. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, Backhed F, Mithieux G (2014) Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell 156:84–96
62. Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, Shi H, Thangaraju M, Prasad PD, Manicassamy S, Munn DH, Lee JR, Offermanns S, Ganapathy V (2014) Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity 40:128–139
63. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, Tobe T, Clarke JM, Topping DL, Suzuki T, Taylor TD, Itoh K, Kikuchi J, Morita H, Hattori M, Ohno H (2011) Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature 469:543–547
64. Okada T, Fukuda S, Hase K, Nishiumi S, Izumi Y, Yoshida M, Hagiwara T, Kawashima R, Yamazaki M, Oshio T, Otsubo T, Inagaki-Ohara K, Kakimoto K, Higuchi K, Kawamura YI, Ohno H, Dohi T (2013) Microbiota-derived lactate accelerates colon epithelial cell turnover in starvation-refed mice. Nat Commun 4:1654
65. Hawrelak J, Myers S (2004) The causes of intestinal dysbiosis: a review. Altern Med Rev 9:180–197
66. Ochoa-Reparaz J, Mielcarz DW, Ditrio LE, Burroughs AR, Foureau DM, Haque-Begum S, Kasper LH (2009) Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis. J Immunol 183:6041–6050
67. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK (2011) Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 108(Suppl 1):4615–4622
68. Buie T, Campbell DB, Fuchs GJ 3rd, Furuta GT, Levy J, Vandewater J, Whitaker AH, Atkins D, Bauman ML, Beaudet AL, Carr EG, Gershon MD, Hyman SL, Jirapinyo P, Jyonouchi H, Kooros K, Kushak R, Levitt P, Levy SE, Lewis JD, Murray KF, Natowicz MR, Sabra A, Wershil BK, Weston SC, Zeltzer L, Winter H (2010) Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. Pediatrics 125(Suppl 1):S1–S18

69. Coury DL, Ashwood P, Fasano A, Fuchs G, Geraghty M, Kaul A, Mawe G, Patterson P, Jones NE (2012) Gastrointestinal conditions in children with autism spectrum disorder: developing a research agenda. Pediatrics 130(Suppl 2):S160–S168
70. Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, Youn E, Summanen PH, Granpeesheh D, Dixon D, Liu M, Molitoris DR, Green JA 3rd (2010) Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe 16:444–453
71. Finegold SM, Downes J, Summanen PH (2012) Microbiology of regressive autism. Anaerobe 18:260–262
72. Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, Adams JB, Krajmalnik-Brown R (2013) Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. PLoS One 8:e68322
73. Parracho HM, Bingham MO, Gibson GR, McCartney AL (2005) Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J Med Microbiol 54:987–991
74. Williams BL, Hornig M, Buie T, Bauman ML, Cho Paik M, Wick I, Bennett A, Jabado O, Hirschberg DL, Lipkin WI (2011) Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. PLoS One 6:e24585
75. Williams BL, Hornig M, Parekh T, Lipkin WI (2012) Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of Sutterella species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. MBio 3
76. Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, Codelli JA, Chow J, Reisman SE, Petrosino JF, Patterson PH, Mazmanian SK (2013) Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155:1451–1463
77. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI (2009) A core gut microbiome in obese and lean twins. Nature 457:480–484
78. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, Sitaraman SV, Knight R, Ley RE, Gewirtz AT (2010) Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science 328:228–231
79. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI (2013) Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 341:1241214
80. Kalliomäki M, Collado M, Salminen S, Isolauri E (2008) Early differences in fecal microbiota composition in children may predict overweight. Am J Clin Nutr 87(3):534–538
81. Karlsson CL, Onnerfalt J, Xu J, Molin G, Ahrne S, Thorngren-Jerneck K (2012) The microbiota of the gut in preschool children with normal and excessive body weight. Obesity (Silver Spring) 20: 2257–2261
82. Belzer C, de Vos WM (2012) Microbes inside–from diversity to function: the case of Akkermansia. ISME J 6:1449–1458
83. Everard A, Belzer C, Geurts L, Ouwerkerk J, Druart C, Bindels L, Guiot Y, Derrien M, Muccioli G, Delzenne N, de Vos W, Cani P (2013) Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A 110:9066–9071
84. Everard A, Lazarevic V, Derrien M, Girard M, Muccioli G, Neyrinck A, Possemiers S, Van Holle A, François P, de Vos W, Delzenne N, Schrenzel J, Cani P (2011) Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. Diabetes 60:2775–2786
85. Collado M, Isolauri E, Laitinen K, Salminen S (2008) Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. Am J Clin Nutr 88:894–899
86. Santacruz A, Collado MC, Garcia-Valdes L, Segura MT, Martin-Lagos JA, Anjos T, Marti-Romero M, Lopez RM, Florido J, Campoy C, Sanz Y (2010) Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr 104:83–92
87. Hansen CH, Krych L, Nielsen DS, Vogensen FK, Hansen LH, Sorensen SJ, Buschard K, Hansen AK (2012) Early life treatment with vancomycin propagates Akkermansia muciniphila and reduces diabetes incidence in the NOD mouse. Diabetologia 55:2285–2294
88. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto JM, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R, Pedersen O, Kristiansen K, Wang J (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490:55–60
89. Fei N, Zhao L (2013) An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. ISME J 7: 880–884
90. Vrieze A, Van Nood E, Holleman F, Salojarvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, Derrien M, Druesne A, Van Hylckama Vlieg JE, Bloks VW, Groen AK, Heilig HG, Zoetendal EG, Stroes ES, de Vos WM, Hoekstra JB, Nieuwdorp M (2012) Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 143(913–916):e917
91. Ubeda C, Lipuma L, Gobourne A, Viale A, Leiner I, Equinda M, Khanin R, Pamer EG (2012) Familial transmission rather than defective innate immunity shapes the distinct intestinal microbiota of TLR-deficient mice. J Exp Med 209:1445–1456
92. Kriegel M, Sefik E, Hill J, Wu H, Benoist C, Mathis D (2011) Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice. Proc Natl Acad Sci U S A 108:11548–11553
93. Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, von Bergen M, McCoy KD, Macpherson AJ, Danska JS (2013) Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science 339: 1084–1088
94. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sorensen SJ, Hansen LH, Jakobsen M (2010) Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. PLoS One 5:e9085
95. Membrez M, Blancher F, Jaquet M, Bibiloni R, Cani PD, Burcelin RG, Corthesy I, Mace K, Chou CJ (2008) Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. FASEB J 22:2416–2426
96. Karlsson FH, Tremaroli V, Nookaew I, Bergstrom G, Behre CJ, Fagerberg B, Nielsen J, Backhed F (2013) Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 498:99–103
97. Chow J, Mazmanian SK (2010) A pathobiont of the microbiota balances host colonization and intestinal inflammation. Cell Host Microbe 7:265–276
98. Vaziri ND, Wong J, Pahl M, Piceno YM, Yuan J, DeSantis TZ, Ni Z, Nguyen TH, Andersen GL (2013) Chronic kidney disease alters intestinal microbial flora. Kidney Int 83:308–315
99. Schepers E, Glorieux G, Vanholder R (2010) The gut: the forgotten organ in uremia? Blood Purif 29:130–136

100. Kotanko P, Carter M, Levin NW (2006) Intestinal bacterial microflora–a potential source of chronic inflammation in patients with chronic kidney disease. Nephrol Dial Transplant 21:2057–2060
101. McIntyre CW, Harrison LE, Eldehni MT, Jefferies HJ, Szeto CC, John SG, Sigrist MK, Burton JO, Hothi D, Korsheed S, Owen PJ, Lai KB, Li PK (2011) Circulating endotoxemia: a novel factor in systemic inflammation and cardiovascular disease in chronic kidney disease. Clin J Am Soc Nephrol 6:133–141
102. Aronov PA, Luo FJ, Plummer NS, Quan Z, Holmes S, Hostetter TH, Meyer TW (2011) Colonic contribution to uremic solutes. J Am Soc Nephrol 22:1769–1776
103. Ranganathan N, Patel BG, Ranganathan P, Marczely J, Dheer R, Pechenyak B, Dunn SR, Verstraete W, Decroos K, Mehta R, Friedman EA (2006) In vitro and in vivo assessment of intraintestinal bacteriotherapy in chronic kidney disease. ASAIO J 52:70–79
104. Niwa T (2011) Role of indoxyl sulfate in the progression of chronic kidney disease and cardiovascular disease: experimental and clinical effects of oral sorbent AST-120. Ther Apher Dial 15:120–124
105. Borody T, Warren E, Leis S, Surace R, Ashman O, Siarakas S (2004) Bacteriotherapy using fecal flora toying with human motions. J Clin Gastroenterol 38:475–483
106. Gibson G, Roberfroid M (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412
107. Holmes E, Kinross J, Gibson GR, Burcelin R, Jia W, Pettersson S, Nicholson JK (2012) Therapeutic modulation of microbiota-host metabolic interactions. Sci Transl Med 4, 137rv136
108. Smits L, Bouter K, de Vos W, Borody T, Nieuwdorp M (2013) Therapeutic Potential of Fecal Microbiota Transplantation. Gastroenterology 145:946–953
109. Borody T (2000) "Flora Power"– fecal bacteria cure chronic C. difficile diarrhea. Am J Gastroenterol 95:3028–3029
110. Khoruts A, Dicksved J, Jansson J, Sadowsky M (2010) Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent Clostridium difficile-associated diarrhea. J Clin Gastroenterol 44:354–360
111. Guarner F (2009) Prebiotics, probiotics and helminths: the 'natural' solution? Dig Dis 27:412–417
112. Fukuda S, Furuya H, Suzuki Y, Asanuma N, Hino T (2005) A new strain of Butyrivibrio fibrisolvens that has high ability to isomerize linoleic acid to conjugated linoleic acid. J Gen Appl Microbiol 51: 105–113
113. West D, Delany J, Camet P, Blohm F, Truett A, Scimeca J (1998) Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. Am J Physiol 275(3 Pt 2):R667-72
114. Fukuda S, Suzuki Y, Murai M, Asanuma N, Hino T (2006) Isolation of a novel strain of Butyrivibrio fibrisolvens that isomerizes linoleic acid to conjugated linoleic acid without hydrogenation, and its utilization as a probiotic for animals. J Appl Microbiol 100:787–794
115. Ji YS, Kim HN, Park HJ, Lee JE, Yeo SY, Yang JS, Park SY, Yoon HS, Cho GS, Franz CM, Bomba A, Shin HK, Holzapfel WH (2012) Modulation of the murine microbiome with a concomitant anti-obesity effect by Lactobacillus rhamnosus GG and Lactobacillus sakei NR28. Benefic Microbes 3:13–22
116. Arora T, Anastasovska J, Gibson G, Tuohy K, Sharma RK, Bell J, Frost G (2012) Effect of Lactobacillus acidophilus NCDC 13 supplementation on the progression of obesity in diet-induced obese mice. Br J Nutr 108:1382–1389
117. Vendt N, Grünberg H, Tuure T, Malminiemi O, Wuolijoki E, Tillmann V, Sepp E, Korpela R (2006) Growth during the first 6 months of life in infants using formula enriched with Lactobacillus rhamnosus GG: double-blind, randomized trial. J Hum Nutr Diet 19:51–58
118. Luoto R, Kalliomaki M, Laitinen K, Isolauri E (2010) The impact of perinatal probiotic intervention on the development of overweight and obesity: follow-up study from birth to 10 years. Int J Obes (Lond) 34:1531–1537
119. Parnell JA, Reimer RA (2012) Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats. Br J Nutr 107:601–613
120. Neyrinck AM, Possemiers S, Verstraete W, De Backer F, Cani PD, Delzenne NM (2012) Dietary modulation of clostridial cluster XIVa gut bacteria (Roseburia spp.) by chitin-glucan fiber improves host metabolic alterations induced by high-fat diet in mice. J Nutr Biochem 23:51–59
121. Neyrinck AM, Possemiers S, Druart C, Van de Wiele T, De Backer F, Cani PD, Larondelle Y, Delzenne NM (2011) Prebiotic effects of wheat arabinoxylan related to the increase in bifidobacteria, Roseburia and Bacteroides/Prevotella in diet-induced obese mice. PLoS One 6:e20944
122. Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PG, Neyrinck AM, Bindels LB, de Vos WM, Gibson GR, Thissen JP, Delzenne NM (2013) Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. Gut 62: 1112–1121
123. Davis LM, Martinez I, Walter J, Goin C, Hutkins RW (2011) Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. PLoS One 6:e25200