Review article

Toxicology Research and Application

Toxicology Research and Application
Volume 1: 1–37

DOI: 10.1177/2397847317741884
journals.sagepub.com/home/tor
SAGE

Exploring the microbiome in health and disease: Implications for toxicology

Elena Scotti[1], Stéphanie Boué[1], Giuseppe Lo Sasso[1], Filippo Zanetti[1], Vincenzo Belcastro[1], Carine Poussin[1], Nicolas Sierro[1], James Battey[1], Anne Gimalac[1], Nikolai V Ivanov[1], and Julia Hoeng[1]

Abstract

The analysis of human microbiome is an exciting and rapidly expanding field of research. In the past decade, the biological relevance of the microbiome for human health has become evident. Microbiome comprises a complex collection of microorganisms, with their genes and metabolites, colonizing different body niches. It is now well known that the microbiome interacts with its host, assisting in the bioconversion of nutrients and detoxification, supporting immunity, protecting against pathogenic microbes, and maintaining health. Remarkable new findings showed that our microbiome not only primarily affects the health and function of the gastrointestinal tract but also has a strong influence on general body health through its close interaction with the nervous system and the lung. Therefore, a perfect and sensitive balanced interaction of microbes with the host is required for a healthy body. In fact, growing evidence suggests that the dynamics and function of the indigenous microbiota can be influenced by many factors, including genetics, diet, age, and toxicological agents like cigarette smoke, environmental contaminants, and drugs. The disruption of this balance, that is called dysbiosis, is associated with a plethora of diseases, including metabolic diseases, inflammatory bowel disease, chronic obstructive pulmonary disease, periodontitis, skin diseases, and neurological disorders. The importance of the host microbiome for the human health has also led to the emergence of novel therapeutic approaches focused on the intentional manipulation of the microbiota, either by restoring missing functions or eliminating harmful roles. In the present review, we outline recent studies devoted to elucidate not only the role of microbiome in health conditions and the possible link with various types of diseases but also the influence of various toxicological factors on the microbial composition and function.

Keywords

Microbiome, microbiota, dysbiosis, lifestyle factors, environmental factors, metabolic diseases, inflammatory bowel disease, chronic obstructive pulmonary disease, periodontitis, skin diseases and neurological disorders, microbial axis

Date received: 21 September 2017; accepted: 22 October 2017

The microbiome and its importance in human health

We are close to the truth if we state that our bodies contain almost the same number of microbes, or even more based on an old measurement, as the number of human cells.[1,2] In fact, we live in symbiosis with a vast population of bacteria, archaea, eukaryotes, and viruses, collectively called microbiota, that colonize different bodily parts.[3] The

[1] PMI R&D, Philip Morris Products S.A., Neuchatel, Switzerland (Part of Philip Morris International group of companies)

Corresponding author:
Julia Hoeng, PMI R&D, Philip Morris Products S.A., Neuchatel, Switzerland (Part of Philip Morris International group of companies).
Email: Julia.Hoeng@pmi.com

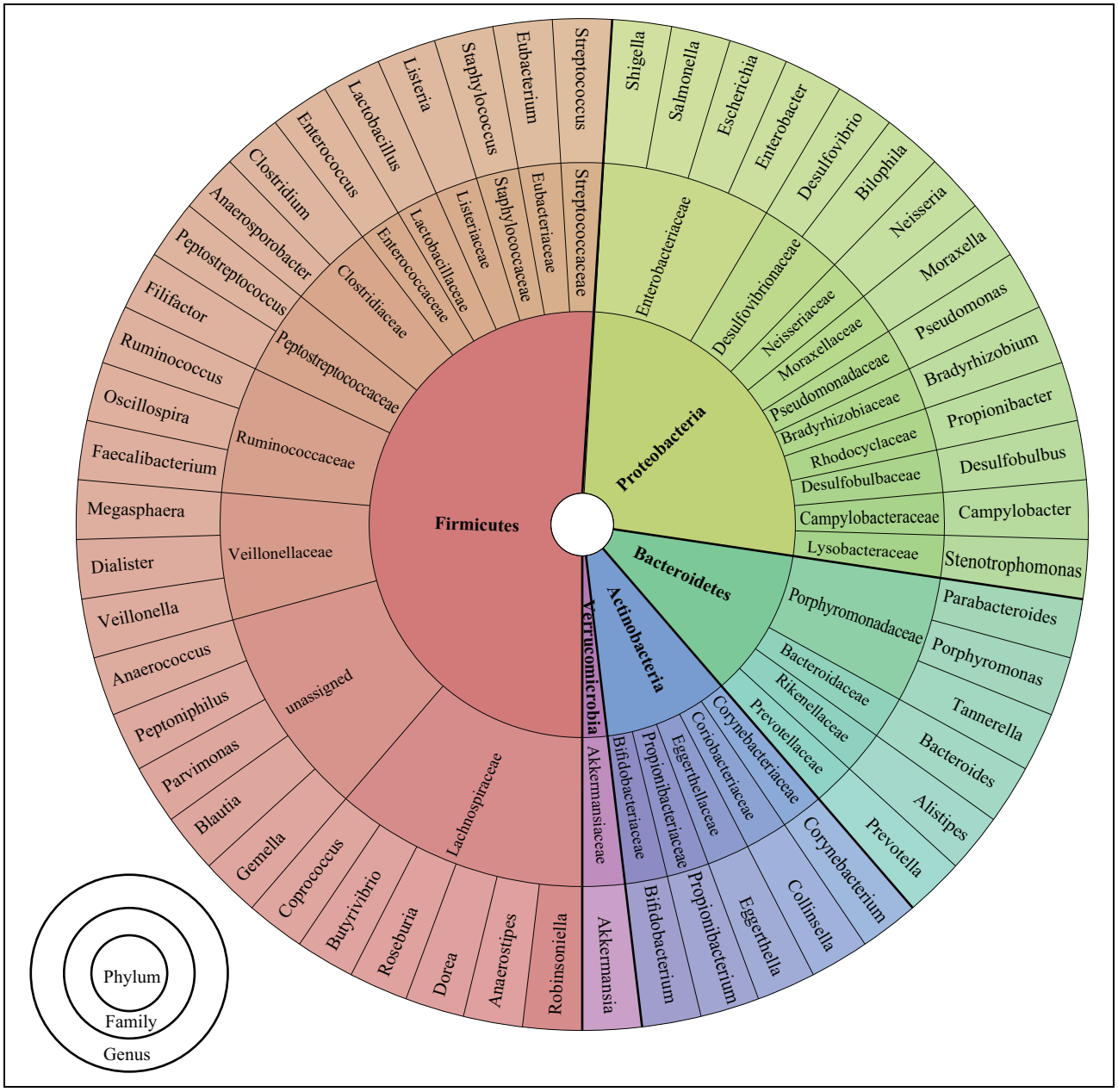

Figure 1. Taxonomical classification chart.[9] The pie chart reports the classification for all genus discussed in the review. The chart shows, for all genus (outer layer), the corresponding family (middle layer) and phylum (inner layer), omitting the class and the order classification rank. See Supplementary Figure 1 and Supplementary Table 1 for a more extensive interactive chart, and a table with the complete genus list and classification, respectively.

"microbiome" represents all the genes and genomes of the microbiota, as well as their metabolites and protein products and those of the host.[4]

Research has now started to focus on the roles played by fungal and viral members of the microbiota community in health and disease; however, scientific interest to date has mainly been focused on bacterial populations and their interactions with the human host. It is the latter area this review will focus on.

In the human body, the major niches colonized by microbes are the gut, mouth, genitals, skin, and airways. The composition of this bacterial population varies depending on the body part. For example, the human intestine is composed predominantly of *Bacteroidetes* and *Firmicutes* (90%), and complemented by *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*,[5–7] whereas the microbiome of the retroauricular skin crease is composed mainly of *Actinobacteria* followed by *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*[8] (see Figure 1, Supplementary Figure 1, and Supplementary Table 1 for general classification).

The microbiome can affect many physiological processes in our bodies, including immune system development, the

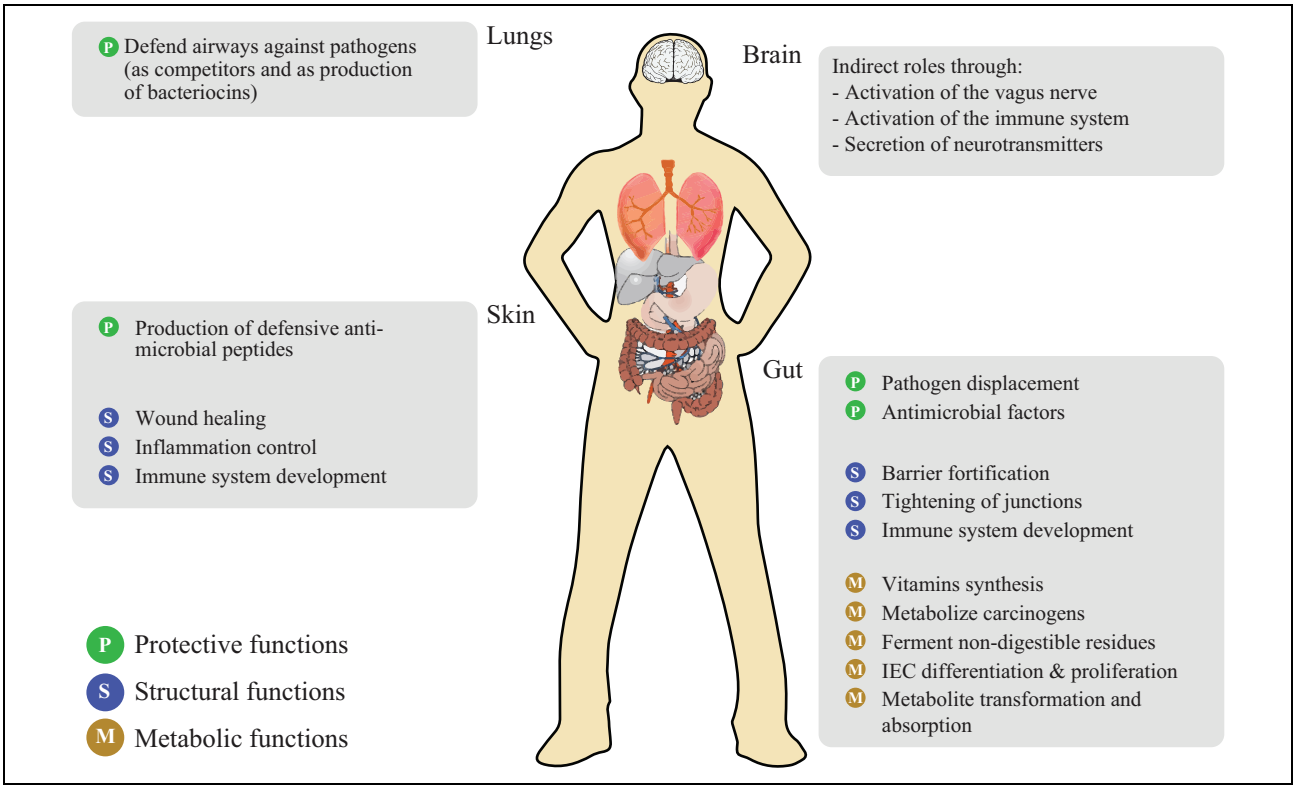

Figure 2. Main functions of bacteria in the human body. IEC: intestinal epithelial cell.

ability to process dietary polysaccharides, vitamin and hormone production, pH regulation, processing and detoxification of environmental chemicals, and maintenance of the skin and mucosal barrier function[10,11] (Figure 2).

In the intestine, for example, a healthy microbial population maintains local homeostatic immune responses via exposure to bacterial structural ligands (e.g. lipopolysaccharide, LPS, and/or peptidoglycan).[12–15] The importance of gut microbes in maintaining immune system homeostasis is highlighted by the fact that the complete absence of this population in mice leads to large defects in the development of gut-associated lymph tissues, low levels of intestinal secretory IgA antibodies, and fewer and smaller mesenteric lymph nodes.[13,16]

Intestinal bacteria are also essential for the breakdown of dietary fibers, such as inulin, pectin, xylans, and mannans. This process yields energy, which is important for the growth and maintenance of the microbial community and for the production of metabolic end products that are beneficial to the host.[17,18] The principal end products of carbohydrate processing are gases, such as CO_2, H_2, and CH_4, and short-chain fatty acids (SCFAs), such as acetate, butyrate, and propionate, which can serve as (1) important metabolites that create a direct energy source for intestinal epithelial cells, (2) inhibitors of inflammation, or (3) modulators of insulin secretion.[17,19–23] In fact, while butyrate is an important energy source for colonic epithelial cells and an inhibitor of the NF-κB signaling that supports mucosal barrier integrity, acetate and propionate can be utilized by the liver for lipogenesis and gluconeogenesis.[7,18,24]

Intestinal microbiota can also perform multiple metabolic activities ranging from the catabolism of certain oral drugs[25] to the synthesis of a wide range of compounds that have various effects on the host, such as several classical neurotransmitters like γ-aminobutyric acid (GABA) as well as bile acids. The latter compounds, synthesized in the liver, can undergo a second conversion in the intestine via microbiota processes to generate secondary bile acids. Ultimately, these acids can be taken up into the bloodstream, where they can potentially modulate host metabolism and other functions, including behavioral and neural functions.[26–30]

Thus, a complex symbiosis exists between the human body and its microbiome, the disruption of which can have detrimental effects on both. Indeed, several physiological changes (via antibiotic use and other lifestyle factors, including hygiene and diet)[8,31] can strongly impact microbial composition. The resulting dysbiosis (alteration of the microbial composition) may be unfavorable and associated with the development of the following diverse diseases[14] (Figures 3 and 4 and Table 1):

- Digestive pathologies (celiac disease, inflammatory bowel disease (IBD), and nonalcoholic hepatitis);
- Metabolic diseases (obesity, cardiovascular diseases, and type 2 diabetes (T2D));
- Neurological disorders (neurodegenerative diseases and other brain disorders);
- Skin problems (eczema, acne, and dermatitis);

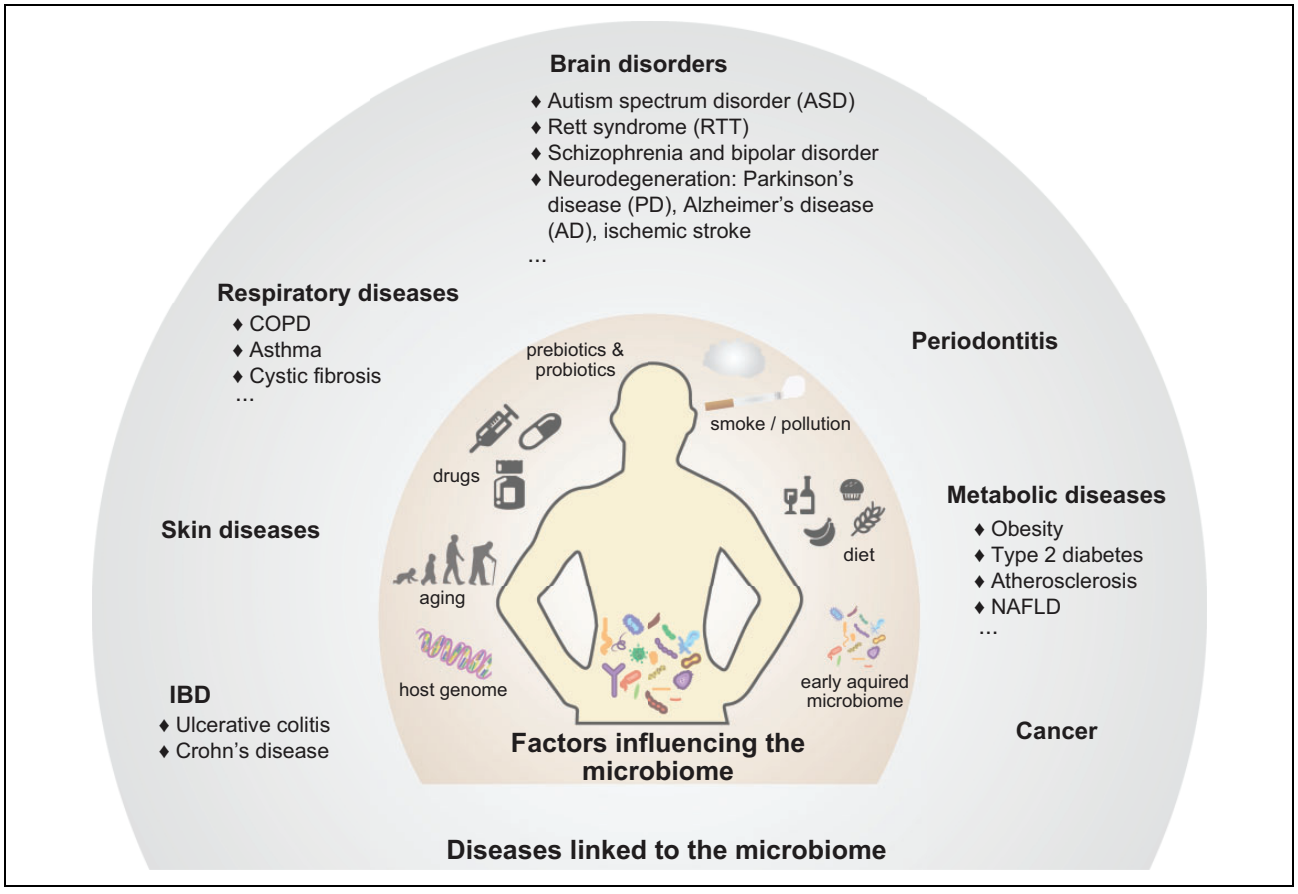

Figure 3. What can influence the microbiome? Factors influencing the intestinal microbial composition and the effects of dysbiosis on host health.

	Aging	Obesity	IBD	ASD	Smoking	Other
Ratio Firmicutes:Bacteroidetes	↓ elders vs. adults ↓ infants vs. adults	↑ ob/ob and HFD mice	↓ CD and UC patients	↑ ASD patients		↑ after cold exposure
Firmicutes		↑ ob/ob mice	↓ IBD patients	↓ ASD children	↑ after smoking cessation ↓ mice exposed to sidestream smoke ↑ in sputum of smokers	↓ in atherosclerotic plaques
Bacteroidetes		↓ ob/ob mice	↓ IBD patients	↑ ASD children ↓ ASD adults	↓ after smoking cessation	↑ after protein/animal fat diet

Figure 4. Summary of the main features of *Firmicutes* and *Bacteroidetes*.

- Oral diseases (caries and periodontitis);
- Pulmonary pathologies (chronic obstructive pulmonary disease (COPD), asthma, and cystic fibrosis (CF));[33]
- Cancers (e.g. colorectal cancer and colorectal adenoma, gastric and esophageal cancers, hepatobiliary cancers, pancreatic cancer, and lung cancer);[14,34–38] and
- Immune-related diseases (e.g. food allergies, asthma, celiac disease, and rheumatoid arthritis).[39–45]

The microbiome and lifestyle factors

In combination, multiple factors, such as genotype, dietary composition and mode of delivery, antibiotic therapy, pre

Table 1. Overview of selected potentially harmful and potentially beneficial bacteria present in our body.

Bacteria (Genus)	Basic features	Associated physiologic changes	Associated diseases states
Akkermansia muciniphila	Gram-negative obligate anaerobe	Anti-inflammatory effects	• Decreased in IBD • Decreased in obesity • Decreased in T2D (but increased after metformin treatment) • Increased in fish-oil-fed mice • Decreased after cold exposure
Bacteroides spp.	Gram-negative obligate anaerobe	Activate $CD4^+$ T cells	• Increased with animal-based diet • Increased in obesity • *Bacteroides vulgatus* positively correlates with IR
Bifidobacterium spp.	Gram-positive obligate anaerobe	SCFA production; improve gut mucosal barrier; lower intestinal LPS levels	• Decreased abundance in obesity • Decreased in smokers • Increased in RTT syndrome • Used as probiotic
Bilophila spp.	Gram-negative obligate anaerobe	Promote pro-inflammatory immunity	• Increased in colitis • Increased in lard-fed mice • Decreased in autism
Christensenella spp.	Gram-negative anaerobe		• Negative correlate with BMI • *Christensenella minuta* decreased weight gain after transplant
Clostridium spp.	Gram-positive obligate anaerobe	Promote generation T_H17 cells	• Several spp. are pathogenic causing botulism, tetanus, and so on. • Increased after sidestream smoke exposure • Decreased in IBD • Increased in autism and RTT syndrome • Positive correlation with plasma insulin and weight gain • Increased in T2D • *Clostridium perfringens* increased in old ages
Dialister spp.	Gram-positive obligate anaerobe		• Several spp. are pathogenic • Increased in obesity • Increased in periodontitis • Decreased in autism
Enterobacter spp.	Gram-negative facultative anaerobe		• Several spp. are pathogenic • Decreased after side-stream smoke exposure • *Enterobacter cloacae* induces obesity in germ-free mice
Escherichia coli	Gram-negative facultative anaerobe	TLR activation	• Increased in IBD • Increased in T2D
Eubacterium spp.	Gram-positive obligate anaerobe	SCFA and phenolic acids production	• Decreased in IBD • Decreased in atherosclerosis • Decreased in T2D • Decreased in IBD • *Eubacterium saphenum* increased in periodontitis
Faecalibacterium prausnitzii	Gram-positive obligate anaerobe	SCFA production and anti-inflammatory effects	• Decreased abundance in IBD • Decreased in obesity • Decreased in T2D • Decreased with overweight
Gemella spp.	Gram-positive facultative anaerobe	Sugar fermentation	• Decreased oral concentration after smoke and tobacco use • Decreased in oral cavity of smokers
Lachnospiraceae spp.	Gram-positive obligate anaerobe	Butyric acid production	• Increased after 24-week CS exposure in mice • Increased oral concentration after vitamin C administration
Lactobacillus spp.	Gram-positive facultative anaerobe	SCFA production; anti-inflammatory activity	• Attenuate IBD • Increased in fish oil-fed mice • Increased oral concentration after high-carbohydrate diet

(continued)

Table 1. (continued)

Bacteria (Genus)	Basic features	Associated physiologic changes	Associated diseases states
			• Decreased obesity (*Lactobacillus plantarum, Lactobacillus paracasei*) • Increased obesity (*Lactobacillus reuteri*) • *Lactobacillus casei* strengthen immune system • Used as probiotic: *L. reuteri* prevents tooth decay • *Lactobacillus farciminis* prevent gut leakiness • *Lactobacillus rhamnosus* decreases stress and depression • Increased in autism and RTT syndrome
Neisseria spp.	Gram-negative obligate aerobe	Sugar fermentation	• Only two species are pathogenic: *Neisseria meningitidis* and *Neisseria gonorrhoeae* • Decreased in oral cavity of smokers • Decreased after smoke and tobacco use
Porphyromonas spp.	Gram-negative obligate anaerobe		• Several spp. are pathogenic • Decreased in oral cavity of smokers • Increased in obesity • *Porphyromonas gingivalis* and *Porphyromonas endodontalis* increased in periodontitis
Prevotella spp.	Gram-negative obligate anaerobe		• Several spp. are pathogenic causing infections of the oral and respiratory tract • Increased with high-fiber diet • Increased in smokers with CD • *Prevotella copri* increased BCAA and insulin resistance • *Prevotella denticola* increased with periodontitis • Decreased in autism • Decreased in PD • Increased in UC
Roseburia spp.	Gram variable obligate anaerobe	SCFA production	• Decreased in IBD • *Roseburia intestinalis* decreased in obesity • *R. intestinalis* decreased in T2D • Decreased in atherosclerosis
Staphylococcus spp.	Gram-positive facultative anaerobe		• Pathogenic • Increased in obesity • *Staphylococcus aureus* increased in COPD and atopic dermatitis
Streptococcus spp.	Gram-positive facultative anaerobe		• Some spp. are pathogenic • *Streptococcus agalactiae* and *Streptococcus pyogenes* increased in COPD • *Streptococcus mutans* increased in oral cavity after high-carbohydrate diet and correlated with caries • *Streptococcus salivarius* used a probiotic for periodontitis
Veillonella spp.	Gram-negative obligate anaerobe	Fermentation of lactate to propionate and acetate	• Increased in oral cavity after smoking • Decreased in autism

Source: Adapted from "Influence of diet on the gut microbiome and implications for human health," 2017.[32]
BCAA: branched-chain amino acids; BMI: body mass index; CD: Crohn's disease; COPD: chronic obstructive pulmonary disease; CS: cigarette smoke; IBD: inflammatory bowel disease; IR: insulin resistance; PD: Parkinson's disease; RTT: Rett syndrome; SCFA: short-chain fatty acids; T2D: type 2 diabetes; TLR: Toll-like receptor; UC: ulcerative colitis.

and probiotic treatment, lifestyle (e.g. smoking and physical activity), social interactions, and environmental exposure to various xenobiotics, shape the gut microbiota, making every person microbially unique (Figure 3).

Diet. Diet plays an important role in shaping the gut microbiome. An increasing body of evidence from humans and mouse models suggests that diet supersedes host genetics in terms of the gut microbiota composition.[46,47] Studies on the influence of diet on the human gut microbiome that have

involved the intake of specific dietary components (e.g. proteins, fats, carbohydrates, polyphenols) have shown how certain bacteria respond to nutrient-specific challenges.[32,48] A shift in the microbiome composition may have secondary effects on host immunologic and metabolic markers. For instance, a plant-based diet is reported to be associated with a higher abundance of *Bacteroides* (*Bacteroidetes*) and *Firmicutes*, an animal-based diet has been linked with a higher abundance of *Bacteroides*, and a high fiber diet is linked with higher levels of *Prevotella (Bacteroidetes)*.[49]

Diets rich in saturated fat or in polyunsaturated fat seem to profoundly affect the gut microbiome composition and the host immune system,[50] and in one study, mice fed fish oil showed increased levels of *Lactobacillus* and *Akkermansia* while those fed on lard had increased levels of *Bilophila* (which has been shown to be correlated with colitis[51]). The high level of white adipose tissue inflammation observed in the lard-fed mice could be caused in part by gut–microbiome metabolism, whereby bacterial products, like LPS, can activate inflammatory immune processes, such as the Toll-like receptor (TLR) signaling.

Different diets can also affect microbial gene richness (determined simply by gene count) and microbial gene diversity (which also takes into account the degree to which the numbers of genes are evenly distributed). A comparison of three different diets revealed that the healthiest diet (with the lowest consumption of sugary drinks and confectionary and the highest consumption of fruit, yogurts, and soups) is associated with the highest microbial diversity in overweight or obese people, whereas a high-fat diet (HFD) is linked to lower microbial gene richness.[52]

Diet-induced microbial changes in the gut can be gender-specific[53] and affected by the dietary history (any effect due to diet change depends on the initial individual's microbiota composition[46,54]) or the timing of the diet change because the composition of the gut microbiota appears to be stable up to 10 days after switching to a new diet.[55] Some of the results in this area have been summarized in recent reviews.[31,32,49,56]

Smoking. Smoking is addictive and a major risk factor for several diseases, like cardiovascular disease (CVD), lung cancer, and COPD.[57–59] Two studies have highlighted the strong impact of smoking on gastrointestinal microbiota.[60,61] Significant changes were also observed in the fecal microbiota of healthy individuals undergoing smoking cessation that included an increase in the relative abundance of *Firmicutes* and *Actinobacteria* and a reduction of *Bacteroidetes* and *Proteobacteria*.[60,61] These changes were similar to the gut microbial changes observed in obese people compared with leaner people, suggesting a potential link between smoking cessation and weight gain. Currently, only a few studies in nonhuman animals have been published, but they do align with observations in humans, thereby highlighting the cigarette smoke (CS)-dependent shift in the gut microbiota structure.[62–65] Four weeks of CS exposure induced a decrease of the *Bifidobacterium* population in the rat cecum.[64] In mice, sidestream smoke exposure increased *Clostridium* spp. but decreased the *Firmicutes* phylum, the *Enterobacteriaceae* family, and the segmented filamentous bacteria in the cecum.[63]

The first comprehensive analysis of bacterial colonization of the upper respiratory tract of healthy adult cigarette smokers[66] found that the microbial communities in this tract differed significantly from those of nonsmokers, suggesting that degradation of normal community structure in the smokers had occurred. In nonsmoking subjects, the nasopharynx bacterial population mainly comprised *Firmicutes* (73%), *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* phyla. However, smokers also had greatly increased levels of *Megasphaera* spp. (of *Firmicutes* phylum), which are known to reside in the oral cavity and to be associated with periodontitis.[67] Moreover, studies analyzing the oral and lung microbiome of smokers and nonsmokers have shown significant differences between them.[68] In particular, in the oral cavity of smokers, the abundance of *Neisseria*, *Porphyromonas*, and *Gemella* species decreased, while no significant differences were found in the lungs of smokers versus nonsmokers.

Physical activity. There is new evidence suggesting that physical activity may modify the microbiota of mice[69] and humans.[70,71] In humans, it has been shown that athletes have a higher diversity of gut microbiota (with higher proportions of the genus *Akkermansia* levels), which in turn is positively correlated with improved protein consumption and higher creatine kinase levels.[70]

Drugs. Widespread antibiotics use has led to deleterious consequences for microbiome diversity in humans.[8] For example, a longitudinal study found that ciprofloxacin use in adults led to decreased bacterial diversity in the gut, which had not recovered 6 months after treatment.[72] Similar results have been seen in mice where antibiotic treatment depleted the gut microbiota.[73] In humans, obesity development is also associated with antibiotic treatment (see under paragraph "Metabolic diseases and the microbiome").

In addition to the disruption of the ecology of the human microbiome, inappropriate use of antibiotics has led to heavy selective pressure and, consequently, to the development of human pathogens able to survive antibiotic treatment. Antibiotic resistance is quickly becoming a significant health-care problem and we refer the reader to recent reviews on this important topic.[74–76]

Prebiotics and probiotics. Prebiotics are food supplements ingested by the host but metabolized by the host's gut bacteria. These supplements favor specific changes in the activity and composition of the gut microbiome, which in turn benefits the host's health and wellness. The ability of prebiotics to promote human health has been extensively studied in animals and clinical studies.[48] Prebiotics are

mainly short-chain nondigestible carbohydrates like inulin-type fructans, fructo-oligosaccharides, and galacto-oligosaccharides. Interestingly, one of the first prebiotics a human receives is in the form of oligosaccharides from mother's milk (human milk oligosaccharides, HMO). HMOs are a group of complex and diverse glycans that are resistant to gastrointestinal digestion[77] and are used as energy sources for the development of *Bifidobacteria*.[49] Furthermore, HMOs act as antiadhesive molecules that block the attachment of viral, bacterial, or protozoan parasite pathogens to epithelial cells, thereby preventing infectious diseases. HMOs also have bacteriostatic and bactericidal activities and they alter host epithelial and immune cell responses with benefits for the neonate.[78]

In general, the prebiotics are only nutrients for *Bifidobacterium* and *Lactobacillus*, and these genera are not the only important microbial contributors to human health. Recently, discussion around the development of new prebiotics with wider ranges of action and the capacity to have positive effects on the growth of other potentially beneficial bacteria like *Ruminococcus bromii*, *Roseburia intestinalis*, *Eubacterium rectale*, and *Faecalibacterium prausnitzii*[48] has been ongoing.

Unlike prebiotics, which are nutrients for the microbes already present in the host, probiotics are specific bacterial strains administered with the aim of improving the health of the recipient.[79–81] The most commonly used probiotics are *Lactobacillus* and *Bifidobacterium*, although other genera like *Bacillus*, *Enterococcus*, and *Streptococcus*, as well as yeast, such as *Saccharomyces*, have also been included in the probiotics category.[82]

The principal roles for probiotics in promoting health are as follows: (1) reinforcing mucosal barrier functioning, (2) reducing the mucosal transfer of luminal organisms and metabolites to the host, (3) improving the mucosal antibody production, and (4) the direct antagonism of pathogens.

The results from human studies on prebiotics are variable, and this probably relates to the methodological differences employed (e.g. dosing, duration of administration, sample collection) and differences in the chosen cohorts (age, health status).[48] For instance, in the treatment of allergic diseases, while a few clinical trials show outstanding support for prebiotic use, some studies also report no effects.[83] Other studies have reported on the significant cholesterol-lowering effect of probiotic treatment, while others have found no effects.[84]

Both prebiotics and probiotics target or augment specific genera (*Lactobacillus* and *Bifidobacterium*), but the overall compositional change only occurs over the treatment duration. Definitive proof linking transient compositional alterations with improved health in the host remains elusive.

Age. The host's age has a significant effect on the microbiota composition. Emerging evidence shows that, unlike what was once believed, the in utero environment is not sterile. Indeed, traces of *Enterococcus faecalis*, *Staphylococcus epidermis*, and *Escherichia coli* have been found in the newborn meconium.[85] Wider microbiota colonization appears immediately after birth and varies depending on the route of delivery (vaginal or caesarian).[86,87] In fact, the neonate gut microbiome is colonized first by the mother's vaginal or skin bacteria population based on the route of childbirth.[85] Later on, the microbes present in the colostrum add to this community, thereby increasing the child's microbiota complexity.[49] The first bacteria to appear in the intestines are aerobic strains, such as *Proteobacteria.* These bacteria decrease the oxygen concentration and allow colonization by anaerobic strains, such as *Bacteroides*, *Actinobacteria*, and *Firmicutes*.

During the first year, the microbiota composition continues to change and starts to resemble that of the adult at 1 to 2 years of age.[14] The composition of gut microbiota appears to be more stable during adulthood with small changes occurring in adolescents.[85] Later on, a final set of changes in gut microbiota composition and function occurs, as characterized by a shift in the ratio of *Firmicutes* to *Bacteroidetes*, a decrease in *Bifidobacteria*, an increase in anaerobic species, an increase in the potentially toxic *Clostridium perfringens*, and production changes in the gut-related metabolites of choline.[48,81,85,88] The reasons for these microbial changes have been proposed to include increased frailty, decreased diet diversity and health, and increased inflammatory marker levels.[89]

Various studies have shown that ageing is correlated with a shift in the intestinal microbe composition in Drosophila and mice also.[90] Very recently, a study by Thevaranjan and co-workers reported that ageing induces intestinal permeability changes in mice.[91] This type of disruption results in microbes colonizing nonpermissive areas with consequent inflammation, mostly via tumor necrosis factor (TNF) involvement. This inflammation status can also affect macrophage functions, and antipneumococcal immunity, in particular. This could explain why elderly people are often hospitalized with pneumonia.[92]

Environmental factors. Different environmental factors can affect our bodily microbial composition. High humidity and low temperature are associated with higher gram-negative bacteria in the skin's microbiome, in particular on the back and the feet.[87] Our ethnicity and cultural habits are additional environmental factors influencing the gut microbiome.[49] Not surprisingly, different studies have reported changes in taxonomic/phylogenetic composition of fecal samples from different geographical areas.[93,94] There are also well-documented disparities in the genetic background, body mass index (BMI), diet, sanitary and hygiene (including the use of antibiotics and vaccines), variations in the incidences and concentrations of foreign (xenobiotic) metabolites, and differences in the socioeconomic status between geographically dispersed populations.[10] Even traveling overseas can alter the composition

of the microbiome, and contracting gastrointestinal tract infections while abroad can also affect the microflora content.[48]

A variety of environmental chemicals are metabolized by the enzymatic activities of the gut microbiota. They are classifiable into five core enzymatic families (azoreductases, nitroreductases, β-glucuronidases, sulfatases, and β-lyases) that are capable of metabolizing 430 environmental contaminants. Conversely, environmental contaminants can alter the composition and/or the metabolic activities of gastrointestinal bacteria, thereby probably contributing to their toxicities. Such contaminants include pesticides, heavy metals (e.g. cadmium), persistent organic pollutants, such as the aryl hydrocarbon receptor ligand 2,3,7,8-tetrachlorodibenzofuran, and artificial sweeteners.[95]

Laboratory animal studies have revealed a correlation between xenobiotic exposure and gut microbiota composition changes. Arsenic-treated mice experienced reductions in *Firmicutes* but not *Bacteroidetes* in the gut.[96] The same mice also exhibited bidirectional changes in key metabolites, including those related to bile acids, lipids, amino acids, and isoflavones.[97] Further evidence in the same mice pointed to a reduction in the gut bacterial abundance after ingesting polychlorinated biphenyls.[96] Similar results have been obtained in studies on humans.[98,99] A recent longitudinal agricultural community cohort study investigated the effect of pesticides on the oral microbiome. In this study, the farmworkers in whom organophosphate pesticide azinphos-methyl was detected in the blood showed a significantly reduced abundance of seven common taxa of oral bacteria, including *Streptococcus* and *Halomonas*. This pesticide-associated spring/summer general reduction in bacterial diversity lasted until the winter season.[100]

Metabolic diseases and the microbiome

Metabolic diseases, such as obesity, CVD, and T2D, are multifactorial in their etiologies and chronically persistent. The prevalence of two closely linked metabolic disorders, obesity and T2D, is increasing worldwide. These disorders pose a major public health risk in European countries and in the United States, and also in rapidly developing countries such as China and India.[101–104] Their rise has been attributed mainly to socioeconomic factors, dietary changes, and sedentary lifestyles. Recent evidence suggests that the gut microbiome also influences the whole metabolic activity of the body and its immune function and could, therefore, play a role in these disorders. The complex metabolic interplay between the gut microbiome and the host has encouraged a detailed analysis of the potential role of the microbial population in metabolic conditions to be undertaken by many researchers. Below, we provide an overview of the most recent reviews on gut microbiota and metabolic diseases.[31,56,105–110]

Obesity

Gut microbial composition alterations have been extensively described as either potentially causal or protective toward weight gain[111,112] both in human and mouse studies. An analysis of the metabolic phenotypes of eight genetically distinct inbred mouse strains in response to a HFD revealed several variations in their metabolic-related phenotypes and gut microbiota.[113] *Clostridiaceae* show the strongest positive correlation with plasma insulin levels and weight gain, whereas *Bacteroidaceae* are negatively associated with these parameters. In a follow-up microbiota transplant experiment, the composition of the murine gut microbiome was shown to be able to modify the host's susceptibility to diet-induced metabolic diseases,[113] a finding that is possibly related to an enhanced capacity to process dietary sugar and to produce hydrophobic bile acids. Furthermore, genetically obese mice (ob/ob mice) had an altered microbial composition with increased cecal levels of *Firmicutes* and reduced levels of *Bacteroidetes* when compared with their lean wild-type, heterozygous littermates.[114] An increase in the *Firmicutes* to *Bacteroidetes* ratio was also observed in obese rats[115] and pigs,[116] when compared with lean animals. However, a more recent paper reported that these changes, in mice, are caused primarily by high-fat feeding rather than genetically induced obesity.[117] Therefore, high-fat feeding, independent of the genetic background, seems to be the primary determinant of gut microbial alterations.

The important role of the gut microbiome in obesity development was also confirmed by a study done with germ-free HFD-fed mice. In this study, germ-free mice fed an HFD displayed reduced adiposity compared with conventionally raised animals that were also fed an HFD, despite the increased caloric intake and decreased energy expenditure. After colonization of germ-free mice with the fecal content from conventionally raised mice, the originally germ-free mice rapidly gained weight and had an increased fat mass without any change in caloric intake,[118–120] thus illustrating a cross talk between gut microbiota and host tissue homeostasis. This finding also indicates that the gut microbial ecosystem is transmissible and that gut microbiota composition can affect obesity in mammals.

Interestingly, weight gain in germ-free mice can also be affected if they are colonized with the fecal microbiota from adult humans placed on a Western diet.[121] Indeed, when feces from human twin pairs discordant for obesity were transferred to germ-free mice,[122] different results were obtained. The mice that received feces from the obese twin developed metabolic alterations and gained more weight than those that received feces from the lean twin. The authors of this study showed that the microbiota from the lean twin had a higher SCFA fermenting efficiency, which promoted good metabolic health. Moreover, when the two groups of mice were co-housed, microbial transfer

occurred by coprophagia; consequently, the mice that received the microbiota from the obese twin showed changes in their microbiota profile that were accompanied by improvements in their metabolic phenotype. The improved phenotype in the obese mice was, however, only achieved upon feeding them with a healthy diet that was high in fiber and low in saturated fat. Additionally, following consumption of a low-fat, high-fiber diet, the "obese-microbiota" failed to colonize lean mice as efficiently as they had in mice that had consumed an HFD. This emphasizes, once again, the importance to health of dietary components that may favor the growth of certain bacterial strains.[123,124]

An interesting line of research has shown that the pharmacological removal of gut microbiota using broad-spectrum antibiotic cocktails prevented fat accumulation even in ob/ob mice, highlighting the importance of the gut microbiome in mediating diet-induced obesity.[125] However, such antibiotic administration was shown to have the opposite effect if administered in a different situation. As discussed in the paragraph "The microbiome and lifestyle factors," for instance, the antibiotic treatment in early life can induce disruption of the normal microbial balance and increase the risk of developing obesity.[126]

Analyses of the gut microbiota composition in humans and mice have shown that the presence of *Akkermansia muciniphila* and *F. prausnitzii* is inversely correlated with weight gain, obesity, and diabetes.[127,128] In addition, inoculation with *Enterobacter cloacae* can induce fully developed obesity phenotypes in germ-free mice.[129] The following compositional differences have been found in obese people relative to lean people: *Bacteroides*, *Parabacteroides*, *Ruminococcus*, *Campylobacter*, *Dialister*, *Porphyromonas*, *Staphylococcus*, and *Anaerostipes* are more prevalent in people with obese phenotypes, while *Faecalibacterium*, *Bifidobacterium*, *Lactobacillus*, *Butyrivibrio*, *Alistipes*, *Akkermansia*, *Coprococcus*, and *Methanobrevibacter* are more prevalent in people with lean phenotypes.[130] A positive association has also been found between weight gain and the presence of *Lactobacillus reuteri*, whereas the opposite has been shown for *R. intestinalis*.[109] Elsewhere, a strong correlation has been shown to exist between a low BMI in humans and an increased abundance of the *Christensenellaceae* family.[131] This finding was supported by an association study involving 416 twin pairs where the *Christensenellaceae* family showed an increased abundance in individuals with low BMI. After being transplanted to germ-free mice, *Christensenella minuta* (DSM22607) was able to reduce weight gain and alter the microbiome of the recipient mice.[132] An association has been found between weight gain and the presence of *L. reuteri*,[133] whereas the opposite has been found for *R. intestinalis*.[109]

When advocating the positive or negative health benefits of different bacterial phyla, one should be careful, as a recent study has revealed that *Bifidobacteria* and *Lactobacillus* may have different properties according to their species. For example, *Bifidobacterium animalis*, *Lactobacillus plantarum*, and *Lactobacillus paracasei* are associated with lean phenotypes, whereas, as previously mentioned, *L. reuteri* is associated with obesity in humans and other animals.[133,134]

In a prospective study of a population of children that were followed for several years, the authors found that microbiota alteration preceded weight gain and that *Staphylococcus aureus* could have an important role in triggering the low-grade inflammation that contributes to obesity development.[135] A new concept is now emerging: rather than the loss of a specific microorganism, it is the loss in microbial richness that is related to the development of obesity and other metabolic diseases.[123,129]

Beyond pure association studies, recent work has elucidated some of the mechanisms by which microbiota may influence obesity development. Although the main cause of obesity remains an excess calorific intake over energy expenditure, it has been shown recently that differences in gut microbial composition may be an important factor affecting host energy homeostasis and lipid storage. In particular, gut microbiota composition has been linked with the low-grade inflammatory status present in obesity, which may be caused by bacterial LPS entering the systemic circulation via gut barrier dysfunction (i.e. disruption of the tight-junction proteins that link epithelial cells together).[136] Several factors can perturb gut permeability: for example, genetic factors can create a weaker mucosal barrier, as can gastroenteritis, detergent ingestion, and emotional stress.[137,138] Furthermore, recent evidence from animal models suggests that mucolytic bacterial species, such as *A. muciniphila* and *Bacteroides thetaiotaomicron*, can degrade the mucosal layer, thus reducing the barrier function of the gut.[139]

Another important mechanism by which the gut microbiome can promote excessive fat accumulation in obesity is dysregulation of the important genes involved in host lipid metabolism (e.g. acetyl-CoA carboxylase 1 and fatty acid synthase), which is mediated by suppressing the intestinal expression of a circulating lipoprotein lipase inhibitor.[120]

Other lines of research have investigated the effects of microbial metabolism on energy balance.[36,119,120,122,140] For example, in a recent paper, Trajkovski et al. showed that the microbiota plays a key role in mediating tight control of energy homeostasis by helping the host to withstand periods of high energy demand.[141] They found that exposure to cold temperature leads to dramatic changes in the microbiota composition, referred to as "cold microbiota," and this was characterized by an increase in the *Firmicutes* to *Bacteroidetes* ratio with almost complete depletion of the *Verrucomicrobia* phylum. These changes favored enhanced energy extraction during cold and were mediated by an increase in the intestinal absorptive surface via a marked increase in the number of intestinal villi and microvilli length. This increased absorptive surface is a

general adaptive mechanism that promotes caloric uptake when food is available. Transplantation of cold microbiota to germ-free mice was sufficient to induce cold tolerance and increase insulin sensitivity, which is in part mediated by browning of their white fat depots. This effect was diminished by co-transplantation with *A. muciniphila*, the most abundant *Verrucomicrobia* species and the phylum most negatively affected by cold exposures.

Type 2 diabetes

T2D is a chronic metabolic disorder where the body either does not produce enough insulin or cannot effectively metabolize glucose despite insulin production. Alterations in the intestinal microbiota composition have been shown to modulate insulin sensitivity and thus play a role in diabetes susceptibility.[142] The fact that germ-free mice, after receiving microbiota from conventionally raised mice, increased their body fat content and became insulin resistant highlights the importance of the microbiome in the regulation of insulin and glucose homeostasis.[120]

Two recent quantitative gut metagenomics studies on patients with T2D (unstratified for treatment) yielded divergent conclusions about gut microbial dysbiosis. The first metagenome-wide association study on a large cohort of Chinese patients with T2D revealed a decrease in butyrate-producing bacteria like *R. intestinalis* and *F. prausnitzii* in them, together with an increased prevalence of opportunistic pathogens, such as *Bacteroides caccae*, various Clostridiales and *E. coli*, and decreases in the known mucin degrading species *A. muciniphila* and in the sulfate-reducing genus *Desulfovibrio*.[143] In the second study, a similar analysis was conducted in a cohort of Scandinavian women with T2D, but the authors instead reported on an enrichment of several *Lactobacilli* species;[127] this apparent contradiction may be explained by the fact that the antidiabetic medication (metformin) used by this group confounded the results. The following year, the role of *A. muciniphila* in mediating an improvement of the metabolic profile of T2D mice was elucidated in a published study showing that oral administration of *A. muciniphila* to mice reversed metabolic disorders, including insulin resistance (IR).[128]

As previously mentioned, metformin, one of the most widely prescribed anti-diabetic drugs, can have a profound effect on the microbiome composition, leading to an improvement in the gut microbial profile. In patients with T2D taking the drug, an increase in SCFA production, butyrate and propionate production, as well as increased *Escherichia* abundance (associated with known side effects of metformin, such as bloating) were observed. A similar effect was shown in HFD-fed mice that exhibited a higher abundance of the mucin-degrading bacterium *Akkermansia* spp. after antidiabetic treatment.[144,145] Other studies have found that the IR-associated metabolome is linked with gut microbiome-encoded functions, such as production of LPS and branched-chain amino acids (BCAAs). Positive correlations between these microbial functions and IR are largely driven by *Prevotella copri* and *Bacteroides vulgatus*, suggesting that they may directly impact host metabolism. In mice, *P. copri* led to increased serum levels of BCAAs and IR.[146]

Despite these studies, understanding the precise mechanisms underlying the association between microbiome dysbiosis and T2D remains elusive. Multiple mechanisms of action have been proposed in mediating the T2D-associated inflammation such as gut permeability, metabolic LPS-mediated endotoxemia, and modifications to incretin secretion and butyrate production.[145]

Cardiovascular disease

Some studies have suggested a linkage between the gut, oral microbiota, and several facets of CVD such as atherosclerotic plaque formation, myocardial infarction, and heart failure. Patients with atherosclerosis have a reduced fecal abundance of butyrate-producing *Roseburia* and *Eubacterium*, which are also known to be decreased in patients with T2D.[143,147,148] The gut microbiome from CVD patients is enriched in genes encoding peptidoglycan synthesis and depleted in phytoene dehydrogenase, which contributes to a pro-inflammatory status.[148]

Interestingly, bacterial DNA was also discovered in atherosclerotic plaques and some of the species identified were similar to those found in the microbiota of the oral cavity, with high levels of *Proteobacteria* and low levels of *Firmicutes* being identified.[149] Moreover, the abundance of bacterial DNA in the atherosclerotic plaques was correlated with CVD risk factors,[150] such as serum LDL-cholesterol.[149]

The metabolic activity of gut microbiota can also have a direct effect on atherosclerosis development, and a key example of this is the metabolism of dietary phosphatidylcholine. This phospholipid (which is present mostly in meat, eggs, and fish) is hydrolyzed by gut bacteria into trimethylamine, which is further oxidized in the liver by flavin monooxygenase into circulating trimethylamine *N*-oxide (TMAO),[151–153] whose levels are associated with cardiovascular risk.[154–156] Intriguingly, it has also been found that inhibiting gut microbial TMAO production using nonlethal inhibitors may serve as a potential therapeutic treatment for atherosclerosis.[157] This study's findings may explain the link between certain dietary habits and CVD development.

Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD), as characterized by lipid deposition in the hepatocytes, is considered the hepatic manifestation of metabolic syndrome. NAFLD is initiated by hepatic steatosis and may progress to

nonalcoholic steatohepatitis (NASH). While most patients with NAFLD remain asymptomatic, 20% develop chronic hepatic inflammation, which in turn can lead to cirrhosis, portal hypertension, and hepatocellularcarcinoma.[158]

During the last decades, researchers have characterized multiple processes that play crucial roles in the so-called gut–liver axis. These mechanisms may explain the multiple processes that occur from fatty liver accumulation to inflammation and fibrosis. In fact, the gut–liver axis is the route by which bacteria and their potential hepatotoxic products, like LPS, can easily reach the liver.[159–161] Ultimately, pro-inflammatory cytokine (e.g. interleukin: IL-1β and IL-8) production plays a pivotal role in the induction and progression of nonalcoholic liver disease to NASH and cirrhosis.

In this context, the microbiome plays an important role in the development of NAFLD, and multiple molecular pathways have been postulated to explain the relationship between NAFLD and dysbiosis.[162] Several human and animal studies that have shown a link between the gut microbiome, NAFLD and pathogenesis have been reviewed in some recent papers.[107,158,163–165] Zhu et al. showed that children and adolescents with NASH have different microbiome patterns respect to the healthy subjects. In fact they have increased *Bacteroidetes* (*Prevotellaceae* (*Prevotella, Porphyromonas*)) and *Proteobacteria* (*Enterobacteriaceae* (*Escherichia*), *Alcaligenaceae*) numbers, and decreased *Firmicutes* (*Lachnospiraceae* (*Blautia, Coprococcus, Eubacterium, Roseburia*), *Ruminococcaceae* (*Faecalibacterium, Oscillospira, Ruminococcus*)), and *Actinobacteria* (*Bifidobacteriaceae* (*Bifidobacterium*)) numbers in their feces.[166] Supporting these studies, pediatric patients with NAFLD had increased numbers of *Bradyrhizobium, Anaerococcus, Peptoniphilus, Propionibacterium acnes, Dorea,* and *Ruminococcus*, and decreased numbers of *Oscillospira* and *Rikenellaceae*.[167]

In another study with adults, patients with NASH harbored a lower abundance of *Faecalibacterium* and *Firmicutes* (*Clostridiales* family, *Anaerosporobacter*) but a higher abundance of *Parabacteroides* and *Allisonella* in their fecal microbiomes.[168] Crucially, it has been reported that improved intrahepatic triglyceride content is related to a lower abundance of *Firmicutes* and a higher abundance of *Bacteroidetes*.[168] In contrast with this, obese individuals with NAFLD have increased members of the *Firmicutes* phylum (*Lachnospiraceae* (*Dorea*, *Robinsoniella*, and *Roseburia*)).[169]

Conversely, a recent study showed a reduction in *Bacteroidetes* in NASH patients compared with the other groups,[170] while Qin et al, when characterizing the gut microbiome in liver cirrhosis, showed that at the phylum level, patients with this disease had fewer *Bacteroidetes* but more *Proteobacteria* and *Fusobacteria* than the controls[171] (without distinguishing patients with NASH or virus-related cirrhosis). Similar to other microbiome studies, discrepancies can be caused by variations in the study design (factors such as age, concomitant medications, health status, and lifestyle are heterogeneous among all studies). An exhaustive table of the intestinal microbiota composition in NAFLD patients can be found in the review by Mokhtari et al.[164]

Possible explanations for the correlation found between the microbiome and NAFLD could relate to changes in SCFA metabolism, release of LPS from gut microbiota, endogenous ethanol production, or a decrease in choline and trimethylamine levels. Dysbiosis might also promote de novo hepatic lipogenesis, increase intestinal permeability, and lead to translocation of both bacteria and endotoxins from the intestinal lumen to extraintestinal sites.[158] More recently, it has been shown that diet fructose can induce alterations of the tight junction proteins, altering the gut permeability, which contributes to an inflammatory status promoting the development of NAFLD.[172,173]

The use of microbiome-based therapies as possible treatments for NAFLD is still under investigation. However, a recent study showed that fecal microbiota transplantation (FMT) can alleviate the steatohepatitis induced in mice by a HFD, which was accompanied by an observed increased in beneficial bacteria like *Christensenellaceae* and *Lactobacillus*, decreased hepatocyte lipid accumulation, and decreased pro-inflammatory marker levels.[174]

Links between metabolic diseases, the microbiome, and lifestyle factors

Both genetic susceptibility and environmental factors (e.g. intrauterine conditions, physical inactivity, smoking, and unhealthy dietary habits) are involved in the pathogenesis of metabolic disorders.[110] Recently, studies have suggested that the environmental component in the development of metabolic syndrome is partially mediated through an altered gut microbial structure and function.

Diet. An individual's diet shapes the diversity of their gut microbial community. As mentioned previously, an example of this is where HFD-fed mice experienced an increased *Firmicutes* to *Bacteroidetes* ratio. However, although human studies have not been equally consistent on the link between diet and microbial content,[110,175–177] it is clear that the composition and the functional capabilities of human and rodent gut microbiota rapidly adapt to changes in the macronutrient content of the diet.[110]

Western-style diets have a strong impact on gut microbiota, particularly on its structure and function.[178] When trying to understand the impact of diet in determining the influence of the microbiome in the development of metabolic diseases, the following points should be considered: (1) the amount, the type (e.g. unsaturated vs. saturated fatty acids), and the mixture of dietary fats can affect the gut microbial composition; (2) the effect of a HFD on the gut microbiome can be rapid (occurring within 24–48 h) and sustained if the dietary habits persist, and may not be

recoverable without a change in diet, reintroduction of the extinguished microbial strains, or dietary supplementation.

A HFD induces complex interactions between microbes and the host via various mechanisms such as those mediated by angiopoietin-like 4 (Angptl4), AMP-activated protein kinase, and TLRs.

Physical activity. Physical activity has been shown to affect gut microbiota composition and diversity in murine models of obesity and hypertension, with some results indicating that the effects demonstrated are independent of diet.[110,179,180] Following exercise, obese and hypertensive rats both experienced increased microbiota diversity, and this was associated with an increase in the relative abundance at the genus level in all the rat models that were studied.

Drugs. Broad-spectrum antibiotic use has been proposed to possibly contribute to the obesity epidemic and to a decreased gut microbiome diversity. This theory is supported by a few epidemiological studies showing that antibiotic intake in infancy or exposure during prenatal life increases the risk of being overweight in childhood.[181–183]

Additionally, a few clinical studies in adults have identified an increase in BMI following antibiotic treatment, especially after *Helicobacter pylori* eradication in patients with gastric ulcers. In contrast, individuals with metabolic syndrome when treated with vancomycin have reduced peripheral insulin sensitivity, while amoxicillin treatment has no such effect.[184] This probably results from the preferential targeting of gram-positive butyrate-producing bacteria by vancomycin. However, the decrease was modest, and the study did not include a control group. In a more recent study, researchers analyzed the effects of vancomycin and amoxicillin (compared with a placebo-treated group) on gut microbiota and metabolism in obese people.[185] Here, Reijnders et al. reported that in contrast to amoxicillin, vancomycin decreased bacterial diversity in the subjects; however, neither antibiotic had an effect on insulin sensitivity, calorie expenditure, or body weight.

In experimental animal studies, the effect of antibiotic treatment on metabolic features has led to controversial results. An increased SCFA content and lower caloric output have been observed in the feces from antibiotic-treated newborn mice, despite having a similar caloric intake as the control mice, which suggests a possible link between obesity and antibiotic treatment.[126] By contrast, another mouse study has shown improved glucose tolerance that was independent of weight changes, along with lower LPS levels and a lower bacterial count following antibiotic treatment, thereby suggesting an improved metabolic state.[186,187] In an animal model of NAFLD, chronic oral antibiotic administration was able to attenuate hepatic inflammation and fibrosis.[188] This difference could probably be ascribed to the different age, genetic background, and diet used in the two studies.

Prebiotics and probiotics. In a series of studies in rodents and humans, prebiotics have been shown to reduce energy intake and body weight, concomitantly reducing IR and hyperglycemia.[110,178] These effects appear to be mediated by

- Increased release of anorexigenic gut hormones such as glucagon-like peptide (GLP)-1, GLP-2, and peptide tyrosine;
- Reduced release of ghrelin (an orexigenic peptide);
- Improved mucosal barrier function with consequent reduced levels of inflammatory markers and decreased endotoxemia; and
- Increased butyrate production

Several studies in murine models of obesity and diabetes have demonstrated an improved metabolic profile following probiotic administration, with administration of *Bifidobacterium adolescentis*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *L. plantarum* LG42, *Lactobacillus gasseri* BNR17, and *Lactobacillus rhamnosus*.[56,110,178]

Most human studies have reported the beneficial metabolic effect of probiotics, with only a few smaller studies failing to show an improvement in cardio-metabolic variables.[110] Probiotics have been also used in NAFLD animal models. Several animal studies have reported on the profound effect of probiotics on NASH, showing reduced liver damage and de novo fatty acid synthesis, decreased metabolic endotoxemia and inflammation,[189–192] as well as improved aminotransferase concentrations.[193–196] Despite these findings, the potential effectiveness of probiotics for patients with NAFLD is still unclear. Loguercio et al. provided the first evidence that probiotic treatment with "VSL#3" (a mixture containing 450 billion bacteria (including *Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *L. acidophilus*, *L. plantarum, L. casei*, and *Lactobacillus bulgaricus*) could reduce the serum transaminase level and improve some liver function parameters in a group of patients with different types of chronic liver disease.[197] Other studies have reported an improvement of biochemical parameters in patients with NAFLD after probiotic treatments (in particular, *B. longum* or *Lactobacillus* spp.).[193,198] However, few randomized controlled trials have found support for the therapeutic use of probiotics in humans. Some recent reviews have summarized the findings of studies showing that probiotic supplementation in animal models and humans improves the inflammatory status and clinical manifestations of NAFLD.[164,199]

IBD and the microbiome

IBD is a group of disorders characterized by chronic inflammation of the gastrointestinal tract, of which the two main disease manifestations, ulcerative colitis (UC) and Crohn's disease (CD), each have distinctive clinical and

pathological features.[200–203] Both CD and UC result from a complex interplay among environmental factors, genetics, and intestinal microbiota composition.[204,205]

The important role of the microbiome in IBD pathogenesis has been elucidated by a study where germ-free animals showed less inflammation than controls in response to dextran sulfate sodium (DSS)-induced colitis.[13,206] The fact that the gut microbiota shapes intestinal immune responses has been demonstrated in germ-free mice that showed impaired gut immunity. Evidence for the involvement of the microbiome in IBD comes from experiments where FMT of the microbiota from mice with colitis induced colitis in healthy mice.[207]

Another study has found that antibiotic administration in wild-type mice may protect them against colitis.[208] Antibiotics can induce changes in T-cell subpopulations, including reductions in colonic lamina propria, and T cell and $CD4^+$ T cell numbers. Antibiotic treatment conferred protection against DSS-induced colitis, and this effect was transferable by FMT indicating the protective role of the microbial community.

Multiple clinical studies have shown that the intestinal microbiome composition of IBD patients differs from that of people without IBD. Dysbiosis in IBD is characterized by a depletion of *Bacteroidetes* and *Lachnospiraceae* (phylum *Firmicutes*), an increase in *Actinobacteria* and *Proteobacteria*,[209] and decreased microbial diversity overall.[209,210] Moreover, gut samples from patients with IBD were depleted of *Lachnospiraceae* members, in particular, group IV and XIVa *Clostridia*.[210] At the species level, in patients with CD postsurgery, a lower proportion of *F. prausnitzii* bacteria were found in their ileum samples,[211,212] while the feces samples from UC patients were associated with an abundance of butyrate-producing *Roseburia hominis* (phylum *Firmicutes*).[213] It is important to realize that most of these studies were cross-sectional, which makes it difficult to determine a cause and effect relationship between disease and microbial composition. However, a more recent longitudinal study that analyzed the microbiome in patients at the initial occurrence of disease found an increased abundance of *Enterobacteriaceae* and *Pasteurellaceae* (*Proteobacteria*), *Veillonellaceae (Firmicutes)*, and *Fusobacteriaceae*, and a decreased abundance in *Erysipelotrichales* (*Firmicutes*) and *Bacteroidales*, and the presence of *Clostridia* was strongly correlated with the disease status.[214]

Discordant results have been found in IBD microbiome studies when different sample types and diverse collection sites (e.g. fecal or surgical sample, remission or inflamed condition, or different portions of the gastrointestinal tract) are taken into account. Indeed, the composition and abundance of both lumen and mucosa-associated microbiota vary along the gastrointestinal tract and both can be significantly impacted by intestinal inflammation in a number of different ways. Ultimately, this could represent a confounding factor for data interpretation.

FMTs are being extensively investigated as therapies for treating IBD.[215] The availability of different mouse models of IBD has led to multiple studies focusing on discerning the mechanisms underlying linkage between the microbiome and IBD. The following immunoinflammatory mechanisms have been proposed:

- An increased polysaccharide A production and suppressed production of pro-inflammatory cytokine IL-17 and IL-10-producing $CD4^+$ T cell induction;[216]
- The complexity of the interplay between gut microbiota and luminal IgA responses;[217–219]
- An increase in glycerophospholipid and lipopolysaccharide metabolism;[220]
- A decreased SCFA production with compromised intestinal and immune homeostasis;[213,221]
- An increase in sulfur-reducing bacteria (observed in UC patients) with consequent production of hydrogen sulfide, a known genotoxic substance able to modulate gene expression in the cell cycle and induce inflammatory responses;[220] and
- An increased representation of genes involved in cell wall degradation and the exotoxins that facilitate the passage of inflammatory gut mediators into the blood circulation.

Recently, Chu et al. found new gene–microbiota interactions that can contribute to IBD development.[222] In their study, the human gut microbe *Bacteroides fragilis* was found to produce immunomodulatory molecules that are released via outer membrane vesicles (OMVs). The OMVs triggered the ATG16L1-mediated and the nucleotide-binding oligomerization domain-containing protein 2-mediated noncanonical autophagy pathway in the host dendritic cells (DCs). The OMV-primed the DCs and, in turn, induced the intestinal regulatory T cells that protect against colitis. Immune cells from humans at high risk of IBD have polymorphisms in ATG16L1 and they are defective in their response to OMVs; consequently, this promotes disease through defects in "sensing" the protective signals from the gut microbiome.

IBD, microbiome, and lifestyle factors

Diet. A person's diet can influence intestinal inflammation (see paragraphs "The microbiome and lifestyle factors" and "Metabolic diseases and the microbiome") and increase their risk of developing IBD. In human cohort studies, this risk is positively correlated with dietary fat intake. Different mouse models of intestinal inflammation (i.e. TNFdeltaARE, CEABAC10, and IL-$10^{-/-}$ mice) have also identified the same risk. Moreover, the presence of iron in the drinking water, detergents, and artificial sweeteners in drinks and processed foods has also been associated with IBD.[223] Additionally, different diet modification strategies

have been shown to have positive effects in IBD patients, including exclusive enteral nutrition (where a precisely defined liquid diet is used exclusively for nutrition), partial enteral nutrition, and semi-vegetarian diets. In addition, probiotic and prebiotic administration showed beneficial effects in treating IBD.[224]

Smoking. Extensive research has shown the strong impact of environmental factors on gut microbiota, and smoking has recently been studied as a potential factor involved in shaping microbiota communities. Interestingly, in the two main subtypes of IBD, CD and UC, smoking has divergent effects on the disease course. While CS is the most prominent environmental risk factor for developing CD, it exerts a protective role in UC.[225,226]

The molecular and cellular mechanisms by which CS interferes with the pathogenesis of CD and UC are poorly understood. Several potential mechanisms have been proposed and, most probably, the causes of CS-associated changes in the composition of the gut microbiota are a combination of environmental, host, and microbial changes. Among them, the following should be mentioned: epigenetic susceptibility, modulation of mucosal immune response, alterations in intestinal cytokine and eicosanoid levels, modification of gut permeability with consequent impaired clearance of pathogen alteration in the H^+/K^+-ATPase pump with consequential acidification of gastric contents, and ingestion of the bacteria present in cigarettes.[227–230,231,232]

Human studies targeting selected bacterial groups have reported that smoking patients with active CD have increased abundances of *Bacteroides* and *Prevotella*[233] (both *Bacteroidetes*) and decreased *Firmicutes* to *Bacteroidetes* ratios.[60] Similar results were obtained in healthy CS controls, suggesting that the association may not be related to intestinal inflammation but may instead reflect the direct impact of CS on the microbiota. Smokers also display a decreased abundance of *Bifidobacterium* spp.,[60] and hence may lose the anti-inflammatory effects that are often associated with this genus. Despite its small sample size, a recent study partially confirmed and further expanded on the aforementioned studies using a more comprehensive approach (metagenomics) in which the researchers were able to sequence and evaluate the whole gut microbiota of CD patients.[234]

Despite the species level differences between human and rodent microbiota,[114,235] a few studies with rats and mice have aligned with the observations from humans, highlighting the CS-dependent shift of the gut microbiota composition.[62–64] A recent paper reported that 24 weeks of CS exposure increased the activity of *Lachnospiraceae* spp. (*Firmicutes*) in the colon, with alteration in inflammatory gene expression.[231] These data highlight a possible role for CS in gut microbiome shaping with unknown consequences in the evolution of inflammation-related disorders such as IBD.

COPD and the microbiome

COPD is characterized by a slow, progressive, irreversible airflow obstruction, and loss of lung tissue leading to emphysema and remodeling of the tissue (fibrosis), both of which contribute further to lung function decline, reduced quality of life, and high mortality.[236,237] Lung infections triggered by pathogenic bacteria and viruses can lead to acute worsening of COPD (exacerbation). Exacerbations are an additional major factor in the morbidity and mortality caused by COPD, and the major source of healthcare costs associated with the disease.[238,239]

Recent studies, using high-throughput next-generation sequencing (NGS) techniques, have reported that the airways are not sterile (even in healthy hosts) and harbor diverse microbial communities.[240] Nevertheless, the enclosed nature of the lungs presents difficulties for microbiome sampling. Biofluid sampling is possible via bronchoalveolar lavage (BAL) or sputum collection. Recently, it has been suggested that sputum and BAL samples offer spatially distinct representations of the lung microbiome: BAL samples appear to represent the lower bronchial mucosal flora and sputum samples the upper bronchial tract.[241] Therefore, this should be taken into consideration when interpreting COPD microbiome studies in view of the differences between spatially distinct regions of the lungs.

Several investigations have shown that changes in the composition of the airway microbiota are associated with developing chronic lung diseases, such as asthma, COPD, and CF.[242–244] The heterogeneous nature of COPD implies that for this disease the concept of a "core" lung microbiome is difficult to establish. Candidate genera that constitute the lung core microbiome from a number of COPD lung microbiome studies include *Pseudomonas*, *Streptococcus*, *Prevotella*, and *Fusobacteria*.[245]

Studies have found that the lung microbiome plays an important role in COPD severity.[246,247] Initial studies have suggested that the lung microbiome of patients with moderate and severe COPD is less diverse than those of healthy controls,[244] although other work has suggested that this conclusion may have been based on an underestimation of bacterial diversity.[248] Thus, using a larger cohort of moderate and severe COPD patients, more recent work has suggested that there is increased microbial diversity in more severe COPD cases.[249]

Metagenomic sequencing of the sputum microbiome of COPD patients and healthy smokers (considered as controls), which was conducted to elucidate its taxonomic composition, revealed an increase in four bacterial species, all pathogens (*S. aureus*, *Stenotrophomonas maltophilia*, *Streptococcus agalactiae*, and *Streptococcus pyogenes*).[250] Cameron et al. analyzed the lung microbiome in a large COPD patient cohort and found interesting changes that were associated with multiple characteristics of COPD, including specific exacerbation phenotypes, treatment regimen differences, and differences in the levels of key

sputum and serum mediators. The COPD exacerbation events appeared to be associated with decreased microbial diversity and an increased proportion of *Proteobacteria*. There was also a marked proliferation of *Moraxella* in a subgroup of subjects during their disease exacerbations. A reduction in microbial diversity and an increased *Proteobacteria* to *Firmicutes* ratio toward recovery were observed in the subjects treated with steroids alone, whereas the trend was reversed in the subjects who received antibiotics. However, this study, despite its large cohort size, was focused exclusively on COPD patients undergoing exacerbations. Healthy control subjects and those who did not experience exacerbations were excluded.[250]

Links between COPD, the microbiome, and lifestyle factors

Smoking. Smoking is the leading risk factor for COPD. Changes in the immune system, triggered by the noxious particles present in tobacco smoke, lead to an inflammatory cellular infiltrate and to a pronounced and chronic lung inflammation. This, in turn, induces other pathological changes, including chronic obstructive bronchitis with fibrosis and obstruction of the small airways, emphysema, destruction of lung parenchyma, and the loss of lung elasticity.[251,252] CS also leads to lung infections and to consequential exacerbations.

Opinion on the effect of smoking on the COPD microbiome is controversial. Erb-Downward et al. recently failed to report any difference in the bacterial communities of the lower respiratory tract microbiome from a very small cohort of smokers, nonsmokers, and COPD patients through analysis of their BAL fluids.[244] Contrastingly, an analysis of sputum microbial composition revealed that CS is a major environmental factor that not only drives the difference in microbial community structure between samples but also affects the abundance of specific microbial taxa.[253] In particular, smokers showed an increased abundance of specific members of sputum microbiota, such as *Veillonella* and *Megasphaera* (both *Firmicutes*) and all the taxa to which these genera belong (*Firmicutes*, *Clostridia*, *Clostridiales*, and *Veillonellaceae*). This respiratory microbiota alteration can lead to an inflammatory condition or an environment in which pathogenic bacteria can thrive and can also subsequently contribute to the development of smoking-related lung diseases, such as COPD. Similar to what occurs in the gastrointestinal tract, an interesting paper has reported that CS can induce the loss of lung barrier integrity, and this allows bacterial translocation and increased tumor-associated inflammation.[254]

Probiotics. Cigarette smoking impairs human natural killer (NK)-cell cytotoxic activity and cytokine release.[255] However, it has been found that the daily intake of the *L. casei* Shirota strain increases NK cell activity in smokers. This suggests that probiotics may be useful for patients with COPD, particularly those experiencing frequent viral infections.[256]

Drugs. Currently, there is no cure for COPD. Therefore, the goals of COPD treatment are mainly focused on relieving the symptoms and preventing or treating the complications of it. The main drugs used are bronchodilators that can be used in combination with glucocorticosteroids and antibiotics in cases with complications. These treatments can also affect the composition of the gut microbiota. With disease exacerbations, antibiotic treatment induces a reduction in *Proteobacteria*, and prolonged suppression of some microbiota members has been observed.[257] Conversely, corticosteroid treatment increases the abundance of *Proteobacteria* and other phyla members.

Environmental factors. Research on the alterations in lung microbiota resulting from environmental exposure to a range of pollutants is a nascent field with the potential to explain the pathophysiological mechanisms of lung disease. A clinical study in a healthy adult population in Malawi revealed a correlation between high levels of particulates from the inhalation of smoke from biomass fuel and changes in their lung microbiomes. Exposure to other environmental sources of particulates caused a higher abundance of potentially pathogenic bacteria (*Streptococcus*, *Neisseria*) within their lung microbiomes.[258]

Periodontitis and the microbiome

Periodontitis, a chronic inflammatory disease affecting both soft (gingiva) and hard (alveolar bone) tissues,[259,260] can destroy periodontal tissues and cause tooth loss. Around 40% of people in low-income countries are affected by periodontal diseases, and the percentage remains high even in high-income countries, where 30% of the people are reported to have oral health problems.[261] Periodontitis development is associated with an increase in microbial load and dramatic shifts in the microbial community structure, with the primary health-associated species remaining part of the periodontal communities, albeit in low proportions, and a diverse range of periodontitis-associated taxa becoming numerically dominant.[262–264]

The human oral cavity contains a number of different habitats, including the teeth and oral mucosa, which are colonized by bacteria. Saliva, which is constantly in contact with the bacterial flora attached to the surfaces, contains the whole representative population of the oral cavity.[265] The commensal populations (normal microflora) may keep pathogenic species in check by creating a biofilm and not allowing them to adhere to mucosal surfaces. In effect, the bacteria do not become pathogenic and cause infection and disease until they breach the commensal barrier.[266] The bacterial tropism and distribution in the oral cavity varies depending on the physical location, oral cavity physiology, immunity, metabolism, and habits.[267–270] Disruption of

bacterial plaque homeostasis and an increased bacterial load are associated with pathological outcomes such as dental caries, gingivitis, and periodontitis.[271,272]

The oral microbial composition in patients with periodontitis undergoes complex changes and more than 400 microbial phenotypes are associated with periodontal pockets.[272] Different bacterial species combinations are associated with different pathogenicity grades. For example, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* (all *Bacteroidetes*) are considered among the most pathogenic when complexed together.[273,274] Other *Bacteroidetes* species, like *Eubacterium saphenum*, *Porphyromonas endodontalis*, *Prevotella denticola*, *Parvimonas micra*, *Peptostreptococcus* species, *Filifactor alocis*, *Desulfobulbus* species, *Dialister* species, and *Synergistetes* are also associated with periodontitis.[67,275–277]

Links between periodontitis, the microbiome, and lifestyle factors

Diet. Until now, very little information has been available about the association between diet and the oral microbiome. Individuals fed on high-carbohydrate diets have higher abundances in their oral cavities of acidogenic and aciduric bacteria such as *Lactobacilli* and *Streptococcus mutans* (both *Firmicutes*) whose metabolites are the primary cause of dental caries.[278] Consistent with these findings, it has been found that high levels of salivary glucose (derived from an unbalanced diet) are associated with a reduced bacterial count and modified bacterial frequencies.[279] Fatty acid and vitamin intake can also modify the oral microbiome, with saturated fats being associated positively with a high *Betaproteobacteria* and *Fusobacteria* abundance, whereas vitamin C supplementation is associated with the presence of *Fusobacteria*, *Leptotrichiaceae*, and *Lachnospiraceae*; however, these findings involve quite modest relative changes.[278]

Oral bacteria can activate alcohol and convert it to the genotoxic and carcinogenic compound, acetaldehyde. For this reason, oral exposure to acetaldehyde can lead to carcinogenic effects in the oral and gastrointestinal tract. Moreover, researchers have found that pretreatment with the antibacterial chlorhexidine prior to ethanol exposure can reduce acetaldehyde levels in the saliva.[280] The continued use of alcohol and tobacco is known to lead to reduced bacterial richness (decrease in *Neisseria*, *Fusobacteria*, *Granulicatella*, *Peptostreptococcus*, and *Gemella* abundance), with possible consequences for human oral diseases.[281]

Smoking. Several conditions are risk factors for periodontitis, with smoking identified as a major contributor.[282,283] Depending on the definition of disease and the exposure level to smoking, the risk of contracting a destructive periodontal disease is 5- to 20-fold higher for a smoker than for someone who has never smoked.[284] Ge et al.[285] reported that smoking was accountable for bacterial variations in both healthy and diseased pockets in patient with periodontitis.[285] They also found that periodontal disease may be associated with a consortium of bacteria rather than few specific species. However, the composition of oral bacteria in smokers versus nonsmokers varies largely among studies.[66,285–288] This might be related to different sample sizes, different sampling sites in the mouth, and/or the use of different methodologies and analysis tools. Generally, smokers have a variable, pathogen-rich, and commensal-poor anaerobic microbiome, resembling more closely a disease-associated state than a clinically healthy community.[66,288–290]

Smoking-induced perturbations may, for example, be attributed to oxygen deprivation, the effects of antibiotics, decreased saliva pH, and impaired host immunity.[291–293] Several studies have investigated the changes occurring in the oral bacterial composition in smokers, nonsmokers, and quitters. Higher abundances of species belonging to *Bacteroides*, *Campylobacter*, *Fusobacterium*, *Parvimonas*, and *Treponema*, and lower abundances of *Veillonella* and *Streptococcus* (both *Firmicutes*) as well as *Neisseria*, have been detected in smokers with periodontitis compared with those who had never smoked.[294] *Streptococcus* prevents pathogen colonization[295,296] but its commensalistic relationship with *Parvimonas* is impaired by CS.[297]

A longitudinal 12-month smoking cessation study in patients with periodontitis showed that the changes occurring in the microbiome of quitters were related mainly to shifts in the relative proportions of bacterial species rather than in the number of species.[297] In a similar study, Delima et al. observed a decreased presence of *P. endodontalis*, *Dialister pneumosintes*, *P. micra, F. alocis*, and *T. denticola*, and a re-colonization with healthy bacterial species, such as *Veillonella parvula*, in patients with periodontitis who quit smoking.[291] Interestingly, members of the *Veillonella* genus (*Firmicutes*) form a major component of the subgingival microbiome when the periodontal area is healthy. Finally, Wu et al. showed that the oral microbiome of smokers versus nonsmokers and former smokers differed substantially and that smoking cessation reverted the microbiome to a composition similar to that of nonsmokers.[298]

The impact of electronic nicotine delivery systems (ENDS) on the microbial physiology is poorly understood. Recently, Kumar et al. compared the effects of ENDS and CS, reporting that the oral microbial composition of ENDS users differs from smokers and those who had never smoked, with its composition sharing no more than 15% of functionally annotated genes with controls and current smokers (abstract from IADR 2017—Using e-cigarettes to quit smoking is not helping your microbiome—Kumar et al.).

Drugs. Antibiotic use in dentistry has a profound impact in the clinical setting, as antibiotic-resistant bacteria cause

difficult to treat oral infections or cause treatment failures. Metagenomics insights have provided evidence of multiple antibiotic resistance genes in the human oral microbiome. This type of antibiotic resistance is acquired through horizontal gene transfer and the oral biofilm is probably the perfect environment for this transfer through the close physical contact between phylogenetically distant bacteria.[299] Recently, a metagenomic approach was used to develop "genome-inspired personalized medicine," whereby analyzing the oral microbiome after antibiotic treatment would allow the prescription of an antibiotic with an appropriate dosage and spectrum of activity to be tailored to the targeted bacteria. For instance, in a clinical study on a healthy population, Zaura et al. found that after one treatment with antibiotics the oral microbiome became more ecologically stable than the gut microbiome in terms of its species composition.[299,300]

Probiotics. Diet supplementation with *Lactobacilli* has negative effects on the growth of salivary *S. mutans*, which is a major contributor to tooth decay.[301] Similar results have been found with people who have a daily intake of yoghurt containing *Bifidobacteria* and tablets containing *L. reuteri*. One recent model for probiotic treatment is represented by the ingestion of *Streptococcus salivarius*, a nonpathogenic oral species that produces broad-spectrum bacteriocins (antimicrobial peptides produced by bacteria) and also harbors mega-plasmids containing bacteriocin-encoding genes.[302]

Environmental factors. Personal hygiene products, such as soaps and toothpastes, that contain the antibiotic triclosan do not seem to have a major influence on microbial communities or endocrine function, according to a small, randomized trial.[303]

Brain disorders and the microbiome

Mental health problems such as anxiety and psychotic disorders are not just diseases caused by psychological stressors added to genetic vulnerability, but rather full-body, inflammatory conditions related to the immune state.[304–307] Similarly, even neurodegenerative diseases, such as Alzheimer's disease (AD), are marked not only by age-related brain changes but also by disturbed immune function and increased oxidative stress. In rodent models, these factors have been shown to be influenced by diet and the gut microbiota.[308] Over the past few years, accumulating evidence has pointed to the critical role played by bidirectional communication between the gut and the brain in neurological disorders.

The gut microbiota makes a critical contribution to the control of the central nervous system (CNS) activities through neural, endocrine, and immune pathways,[309] including a direct interaction between the gut microbiota and enteric neurons,[310,311] regulation of the hypothalamic–pituitary–adrenal axis (HPA axis),[312] growth and function of CNS cell populations,[313] and the production of many chemicals important for normal brain functioning (e.g. serotonin, dopamine, kynurenine, γ-aminobutyric acid, SCFAs, *p*-cresol).[314,315] This gut–brain axis will be discussed further in the paragraph "Microbial community cross talk."

Alterations in the gut microbiome are also associated with neuropsychiatric disorders, such as schizophrenia, bipolar disorder, autism, major depressive disorder, chronic fatigue syndrome, anxiety, and stress,[316–319] as well as neurodegenerative diseases (Parkinson's disease (PD), AD, dementia, and stroke).[313]

Autistic spectrum disorders

Autistic spectrum disorders (ASDs) are neurodevelopmental disorders characterized by alterations in the social interactions associated with communication as well as behavioral impairment. It has been found that ASD subjects frequently have problems related to a dysfunctional bowel with an aberrant intestinal barrier function.[320,321] While analyzing the microbiota of children with ASDs, it was found that they usually have a higher abundance of *Proteobacteria* and *Bacteroidetes* and a lower abundance of *Firmicutes* and *Bifidobacteria*, when compared with the healthy controls.[322–324] Interestingly, of the many classes of bacteria within the *Firmicutes* phylum, one class, in particular, the *Clostridia*, are present in higher numbers in autistic children with a history of gastrointestinal problems.[325] Moreover, despite the overall abundance of *Bacteroidetes* in autistic subjects, lower counts of *Prevotella* are seen.[49]

A recent study analyzed a cohort of autistic individuals and the authors found a different composition of bacterial gut microbiota compared with the controls,[326] typified by a significant increase in the *Firmicutes* to *Bacteroidetes* ratio in these subjects via a reduction the relative abundance of *Bacteroidetes*. At the genus level, there was a decreased relative abundance of *Alistipes*, *Bilophila*, *Dialister*, *Parabacteroides*, and *Veillonella* in the ASD cohort, while *Collinsella*, *Corynebacterium*, *Dorea*, and *Lactobacillus* levels were significantly increased. Additionally, the same authors analyzed the ASD subjects with constipation, one of the common gastrointestinal problems in this group and found high levels of bacterial taxa belonging to *Escherichia*, *Shigella*, and *Clostridium* cluster XVIII. In a recent small study of 18 children with autism, positive changes in gastrointestinal symptoms and neurological symptoms have been noted after microbiota transfer therapy.[327]

Rett syndrome

Rett syndrome (RTT), a severe and progressive neurological disorder, is linked primarily with a mutation in the gene encoding methyl-CpG-binding protein 2, which is a

fundamental mediator of synaptic development and plasticity. This syndrome is commonly associated with gastrointestinal disorders such as constipation. Indeed, RTT subjects are characterized by a reduction in gut microbial richness and an increase in microbial taxa belonging to *Bifidobacterium*, several *Clostridia* (*Anaerostipes*, *Clostridium* XIVa, and *Clostridium* XIVb) as well as *Erysipelotrichaceae*, *Actinomyces*, *Lactobacillus*, *Enterococcus*, *Eggerthella*, *Escherichia*, and *Shigella*.[328]

Schizophrenia and bipolar disorder

Schizophrenia and bipolar disorder are two human psychiatric disorders with uncertain etiologies. To date, few studies that have analyzed a possible link between the microbiome and these two mental illnesses have been published, but the topic has been reviewed very recently by Dickerson et al.[311] Dysbiosis, increased gastrointestinal inflammation, and the association between antibiotic treatment and the incidence of psychiatric disorders are the main features found in the different clinical studies that have analyzed patients with psychiatric disorders.[311]

Neurodegeneration

Parkinson's disease. PD is the second most common adult neurodegenerative disorder. Its clinical signs include abnormal movement, tremor at rest, rigidity, slowness or absence of voluntary movement, postural instability, and movement freezing, all of which are caused by defects in motor control, but nonmotor symptoms are also common (depression, lack of motivation, passivity, and dementia).[329] Despite improvements in treatment, the etiology of PD remains unclear and there are no therapies to delay or prevent it.

The role of the gut microbiome in the pathogenesis of PD is beginning to emerge. Braak et al. hypothesized that the disease begins in the gut and spreads to the brain via the gut–brain axis (i.e. the vagus nerve and spinal cord). Indeed, the parasympathetic fibers of the vagus nerve that innervate the intestine among other regions arise from the dorsal motor nucleus. Lewy bodies (aggregated proteins, mainly alpha-synuclein and ubiquitin), which are the hallmark of PD, were found in the enteric nervous system in postmortem cases of early PD.[330]

In one study, PD was reported to be associated with alterations in *Prevotella* and *Enterobacteria* populations.[331] *Prevotella* is known to break down complex carbohydrates, providing SCFAs as well as thiamine and folate as by-products that promote a healthy intestinal environment. Decreased *Prevotella* numbers are likely to result in reduced production of these important micronutrients, and this might lead to reduced production of essential vitamins and impaired secretion of gut hormones. However, the study did not evaluate whether the patients had a history of gastrointestinal disturbances or significant inflammation.[332]

Several papers published by Sampson et al. over the last few years showed that in animal studies the connection between the gut microbiota and the pathogenesis of PD seems to be mediated by the regulation of the activation status of microglia[333] and that gut microbiota could affect the disease severity.[334,335] It is clear that identifying the gut microbiota-generated compounds that can affect the immune response of microglia in the brain and the development of PD will be important in future investigations.

Alzheimer's disease. The microbiome may play a role in the formation of beta-amyloid plaques in the mouse brain during AD development.[336] Germ-free AD transgenic mice have significantly lower levels of beta-amyloid in their brains than conventionally raised transgenic mice. Moreover, AD transgenic mice have a significantly different microbiome than that of wild-type mice and, at the phylum level, *Firmicutes* and *Bacteroidetes* levels were significantly altered, whereas at the genus level, *Allobaculum* and *Akkermansia* levels were lowered, while unclassified *Rikenellaceae* and *S24-7* genera levels increased. Furthermore, fecal transplants from transgenic mice were found to induce significant upregulation of beta-amyloid production in the brains of the germ-free AD transgenic mice.[336]

Ischemic stroke. In three separate mouse models of microbiota disruption, the microbiome was shown to impact the outcome of ischemic stroke. Depletion of the microbiota with a cocktail of antibiotics decreased survival in a middle cerebral artery occlusion model of murine stroke and severe colitis in mice.[337] In another model in which the mouse microbiota was altered, but not depleted by antibiotic treatment, the infarct volume was significantly reduced compared with mice with an unaltered microbiota.[338] This microbiota-stroke effect may be bidirectional, as several changes in the microbiome have been identified in different experimental stroke models (reviewed in literature[339]). For instance, Singh et al. found that particularly large infarcts can cause gut dysbiosis, possibly potentiating the neuroinflammatory effects within the CNS.[340] Interestingly, Benakis et al. reported that gut microbiota confers a neuroprotective effect by modulating immune cells in the small intestine.[338] After antibiotic treatment, the authors found that the consequent dysbiosis induced an altered DC activity associated with an expansion of regulatory T cells which secrete IL-10. This cytokine is able to suppress the differentiation of $\gamma\delta$ T cells into IL-17-producing $\gamma\delta$ T cells. IL-17$^+$ $\gamma\delta$ T cells are known to have a deleterious effect after stroke because they can migrate to the meninges and aggravate ischemic brain injury by secreting IL-17 and promoting neutrophil infiltration. The decrease in IL-17$^+$ $\gamma\delta$ T cells, observed after antibiotic treatment, is essential for the reduction of post-ischemic chemokines (Cxcl1 and Cxcl2) leading to a decrease in ischemic brain injury.[338] A recent study discovered the molecular mechanism in endothelial cells that underlies the formation of cerebral

cavernous malformations (CCMs), which are clusters of dilated, thin-walled blood vessels in the brain that can cause strokes and seizures. This molecular pathway is activated by TLR4, a receptor for the bacterial molecule LPS, which is present on brain endothelial cells and is capable of vastly accelerating CCM formation.[341]

Links between brain disorders, the microbiome, and lifestyle factors

Prebiotics and probiotics. Recently, the term "psychobiotics" has been introduced into the scientific vocabulary. They are a family of probiotics and prebiotics that are capable of modulating the gut–brain axis and have a positive mental health benefit. In animal models, it has been extensively shown that psychobiotics can alleviate neuropsychiatric disorders via different neurochemical and humoral mechanisms. These mechanisms include modulation of the expression of brain-derived neurotrophic factor, increased hippocampal neurotrophin levels, regulation of GABA transcription, activation and/or inhibition of specific neurons, and regulation of the immune system in CNS autoimmunity.[316] Prolonged administration of *L. rhamnosus* reduced the stress-induced corticosterone levels and signs of depression and anxiety in mice.[342] These effects were probably induced by alterations in GABA (B1b) receptor gene expression in the brain. However, these effects disappeared in mice in which the vagus nerve had been removed, suggesting once again that the vagus nerve is a key mediator of gut–brain communication. In contrast, similar administration of a combination of *L. rhamnosus* and *B. animalis* in patients diagnosed with schizophrenia saw no difference in the psychiatric symptom severity between the placebo and probiotics groups.[311] Contradictory results have emerged from depressed patients who consumed probiotics; while in some studies the use of probiotics seemed not to be associated with lower rates of depression,[318] in other studies the probiotic treatment produced a reduction in network-level neural reactivity to negative emotions, anxiolytic activity, suppression of psychological stress, and reduction of the negative thoughts associated with low mood.[343]

Skin diseases and the microbiome

The skin is the largest organ in the human body and forms an immense interface between the host and its environment. As such, it is an important site for interactions between the immune system and its microbial inhabitants. Skin symbiotic organisms play essential roles in lipid metabolism and colonization resistance to transient organisms.[86,344]

Generally, the four dominant bacterial phyla residing on the skin are *Actinobacteria*, *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*,[345] whereas, in terms of the different genera, the most abundant are *Staphylococcus*, *Propionibacterium*, and *Corynebacterium*. A feature of the skin is the topographical diversity of the bacterial populations, with the composition and diversity of the bacteria colonizing the skin depending on the microenvironment. Compared with other sites in the human microbiome, variability between individuals is similarly high.[346,347] Recently, it has been shown that despite the skin's exposure to the external environment, its bacterial community is stable at the strain level. The nature and degree of this stability is highly individual and site-specific and is driven primarily by the maintenance of individual strains over time.[348]

Emerging evidence has established a link between the development of the resident immune system of the skin and its microbiota by identifying the direct contact between the two (reviewed in Belkaid and Segre[344]). A clinical study showed that patients with primary immunodeficiency suffer from atopic dermatitis-like eczema and increased risk of skin infections.[349] Moreover, a study in mice has shown that complement signaling antagonism (through the C5a receptor) resulted in a shift in skin microbial composition and diversity.[350] A different study found that a molecule released by staphylococcal bacteria, lipoteichoic acid, inhibits skin inflammation through a TLR2-dependent pathway after injury.[351] Collectively, these findings suggest that the microbiota modulates cutaneous inflammation and immunity, thereby maintaining the delicate balance between the host and microorganisms. Disrupting this balance can cause skin disorders and infections. Classical microbiological and dermatological studies have reproducibly pointed to the strong association between *P. acnes* and acne vulgaris,[352] between *S. aureus* and atopic dermatitis, and between *Malassezia* species and seborrheic dermatitis.[86,352]

The advent of NGS technology, as applied to research on the microbial communities of the skin, has led to further information about the microbial biofilm nature of certain skin diseases that were difficult to analyze using classical microbial techniques. In atopic dermatitis, for example, a decrease in microbial diversity was discovered in the lesions.[353]

Links between skin diseases, the microbiome, and lifestyle factors

Diet. Acne vulgaris is known to be fueled by the high glycemic load typical of a Western diet, which stimulates lipid production in hair follicle sebaceous glands, leading to overgrowth of *P. acnes*.[354] In people with acne, the activity of the transcription factor FoxO1 and the kinase mTORC1 have been found to be aberrant, leading to an overproduction of monounsaturated fatty acids and triglycerides in the sebum, thereby allowing the growth and colonization of *P. acnes*.[355–357] Treatment with metformin, an mTORC1 inhibitor, produced positive results in male subjects who did not respond to common acne treatments.[358]

Prebiotics and probiotics. The beneficial effects of pre and probiotics are achievable either by ingestion or by topical application. Prebiotics applied topically can enhance the growth of beneficial "normal" resident skin microbiota. A cosmetic product with specific prebiotic extracts including ginseng, black currant, and pine was shown to reduce colonization by *P. acnes* in patients with acne.[359] Ingested probiotics can be effective at reducing acne and atopic dermatitis, probably by inhibiting pro-inflammatory cytokine production. Topical applications of probiotics have a direct result at the application site and improve the skin's natural barrier defenses. In fact, probiotics can produce antimicrobial peptides that enhance the skin's immune responses thereby eliminating pathogens.[82]

Topical probiotics and their lysates have been shown to be of value in treating acne. A topical product containing a 5% extract of *L. plantarum* was found to reduce erythema, acne lesion size and improve the skin barrier of patients with acne.[360] A lactic acid bacterial strain, *E. faecalis* SL-5, seems to have antimicrobial activity against *P. acnes*.[361] Furthermore, innovative nutritional products called synbiotics, which contain prebiotics and probiotics, have positive therapeutic results for atopic diseases.[82]

Microbial community cross talk

In this ever-expanding field, research has focused particularly on how local microbiota influences immunity at distal sites, especially on how gut microbiota influences other organs, such as the brain, liver, or lungs. This has led to the coining of terms such as "gut–lung axis," "gut–brain axis," "gut–brain–β-cell axis," and "gut-skin axis."

The gut–lung axis

Chronic lung diseases, such as asthma and COPD, often occur together with chronic gastrointestinal tract diseases, such as IBD or IBS.[362,363] Almost 50 years ago, the first evidence of pulmonary-intestinal cross talk was reported by Turner-Warwick.[364] In 1976, Kraft et al.[365] also noted the development of severe, chronic bronchopulmonary disease in patients with IBD, years after being diagnosed. In a large population-based Canadian study performed 15 years ago, it was reported that pulmonary complications were the most common concomitant chronic disorder in patients with IBD[366] and that pulmonary involvement was more pronounced in patients with active disease compared with those in remission.[367]

Today, it is believed that almost half of patients with UC[368,369] and half of those with CD[369,370] have subclinical pulmonary abnormalities, and COPD is now even considered to be a significant mortality factor among patients with CD.[371,372] Evidently, it seems that a healthy gut microbiota is beneficial for lung health and that the existence of co-morbidities in many diseases should not be ignored.

From a mechanistic perspective, we now know that cross-regulation of gut–lung immunity exists and that intestinal microbiota have been shown to directly modulate the pulmonary immune responses to invading pathogens.[373,374] When the gut microbiota is disturbed during infection or antibiotic exposure, for example, the normal microbiota-derived signals are altered, which changes in the immune response. This means that dysbiosis of the gut microbiota, for example, through exposure to CS, can cause systemic inflammation with outgrowth and secretion in the systemic circulation of opportunistic pathogens, which in turn can further negatively perturb the chronic inflammation already present at distal sites (e.g. the lungs). Considerable evidence suggests that host epithelia and other structural and immune cells assimilate information directly from microorganisms and from the concomitant local cytokine response to adjust inflammatory responses and that this shapes immune responses at distal sites, such as in the lungs.[375,376] It is not clear if there is a direct transfer of microorganisms between sites, although the translocation of bacteria from the gastrointestinal tract to the lungs has been observed in sepsis and acute respiratory distress syndrome, where the barrier integrity has been compromised.[377]

Since 2015, few clinical studies have tested whether altering the gut microbiota with probiotics or antibiotics can influence lung health.[378] Research conducted by Benoit Guery (Lausanne University Hospital, Switzerland) is now exploring "narrow-spectrum" antibiotics to treat lung infection without damaging the gut microbiota.[378]

Thus far, in gut–lung microbiota studies, it is difficult to ascertain whether changes in the gut microbiota are a cause or a consequence of COPD. Most likely, both are true and operate simultaneously or at different stages of the disease. Clearly, longitudinal studies in humans and other animals are now required to determine whether changes in the gut or lung microbiota are associated with the development and severity of COPD or IBD, respectively. Undoubtedly, diseases should no longer be studied in isolation, but rather all comorbidities should be taken into account so that treatments are adapted to avoid a negative impact on overall health.

The gut–brain axis

The role of the gut–brain axis is to monitor and integrate gut functions as well as to link the emotional and cognitive centers of the brain with the peripheral intestinal functions and mechanisms such as immune activation, intestinal permeability, enteric reflex, and enteroendocrine signaling. The mechanisms involve neuro-immuno-endocrine mediators, including also the glucose produced in the liver through gluconeogenesis.[379–382] This bidirectional communication network includes the CNS, the brain and spinal cord, the autonomic nervous system, the enteric nervous system, and the HPA axis. Recent advances in research have described the importance of gut microbiota in influencing these interactions.

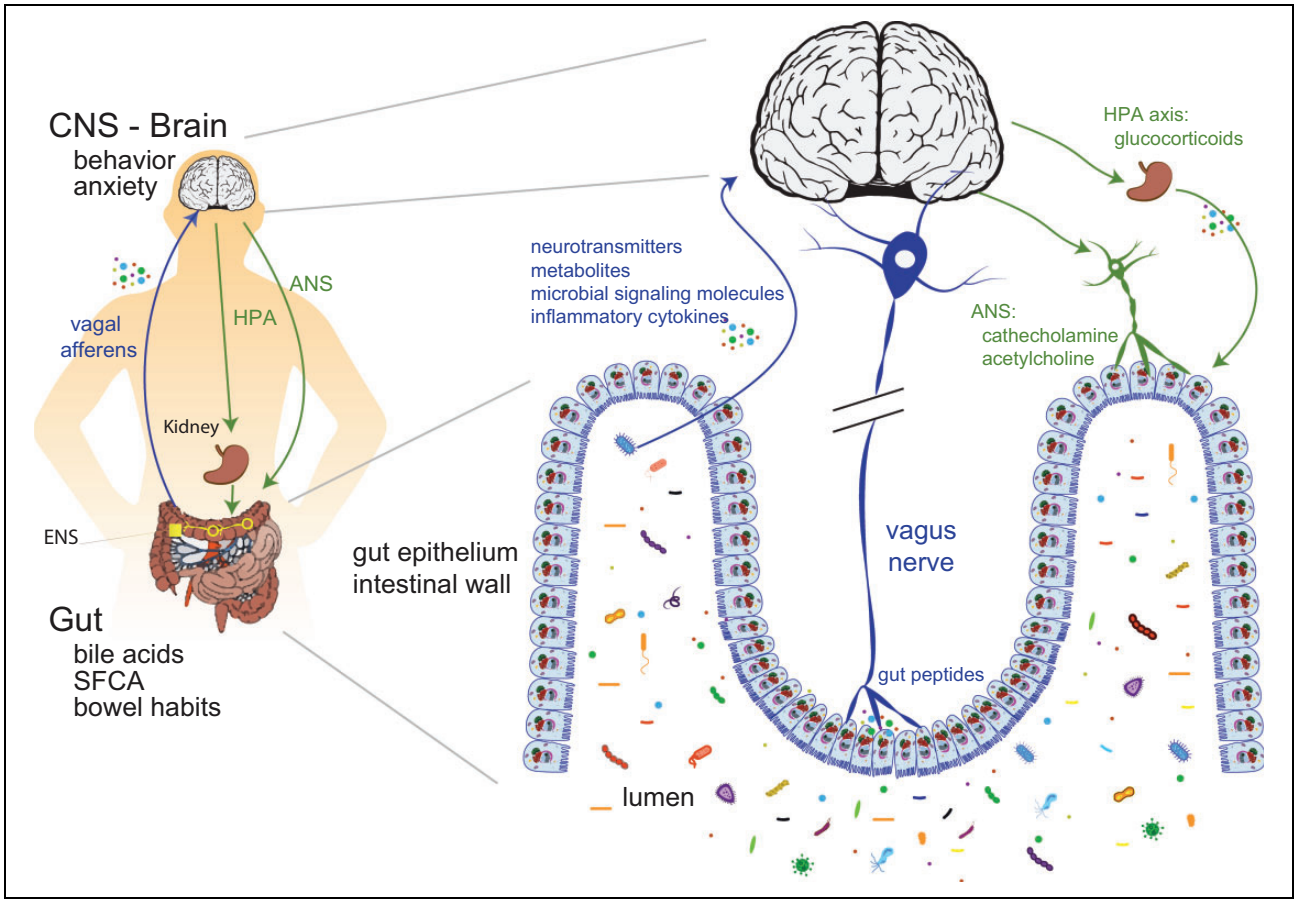

Figure 5. Gut–brain axis. The principal components of the gut–brain axis: endocrine, neural, immunological, and metabolic pathways by which the microbiota influences the brain and vice versa. The afferent arm pathways are represented in blue. (1) inflammatory cytokines released by lymphocytes in the gut lumen may have endocrine or paracrine actions, (2) vagus nerve can be activated by gut peptides released by enteroendocrine cells, (3) gut microbiota can also release neurotransmitters or its precursors with endocrine or paracrine actions, (4) microbial metabolites like SCFA could influence directly or indirectly brain function and behavior. On the opposite direction, there is the efferent arm (in green), (5) glucocorticoids, released as results of the HPA axis activation, modulates gut microbiota composition, (6) neuronal efferent activation of ANS may include the release of acetylcholine and catecholamine. These pathways are so-called "anti-inflammatory cholinergic reflex" and sympathetic activation respectively and they can alter the gut microbiota composition. Adapted from "The microbiota–gut–brain axis: neurobehavioral correlates, health and sociality," 2013.[391] SCFA: short-chain fatty acids; HPA: hypothalamic–pituitary–adrenal; ANS: autonomous nervous system.

The gut–brain microbiota axis is a bidirectional communication system enabling gut microbes to communicate with the brain and vice versa.[383] Different studies have pointed out the correlation between gastrointestinal and brain disorders. For example, IBD has been shown to be a risk factor for PD,[384] for dementia[385] and for AD.[386] Moreover, IBD was shown to precede glaucoma, a progressive neurodegeneration of the optic nerve, in two different cohorts.[387]

The mechanisms of signal transmission are complex and not fully elucidated, but we are aware of the involvement of endocrine-, neurocrine-, and inflammation-related signals generated by the gut microbiota that can affect the brain. In turn, the brain can influence the microbial composition and function via endocrine and neural mechanisms[388–390] (Figure 5). An intestinal change in the symbiont (commensal bacteria with beneficial potential) balance and pathobionts (commensal bacteria with pathogenic potential), favoring pathobiont overgrowth, results in dysbiosis, which can induce inflammation. During inflammatory responses, macrophages contribute to pathogenesis via their inappropriate responses to enteric microbial stimuli, their inefficient clearance of microbes from host tissues, their impaired pro- and anti-inflammatory responses, and the loss of barrier function (leaky gut). This promotes the increased translocation of pathogenic bacterial components from the intestinal mucosa to the systemic circulation, where they activate innate immunity, as characterized by the production of pro-inflammatory cytokines, and resulting in systemic inflammation and abnormal gut function. These mechanisms potentially lead to impaired CNS function such as alterations in neurochemistry, cognition, behavior, stress responses, and visceral pain. Conversely, pathological stress at the level of the CNS can affect the

gut microbiota function and lead to perturbations of the barrier function and gut motility.

Several animal studies have elucidated various details about the gut–brain axis. For example, the prevention of gut leakiness with *Lactobacillus farciminis* and blocking LPS translocation using a specific myosin light chain kinase inhibitor have been found to attenuate the HPA response to stress.[392] Animal models have also shown that chronic mild stress alters the microbiota profile associated with stress-induced despair behavior[393] and that in germ-free mice there is an exacerbation of the HPA axis response to acute stress.[394] *In vivo* studies have opened new avenues to future therapeutic approaches. In mice, a specific probiotic therapy attenuated intestinal dysbiosis and its consequent visceral hypersensitivity.[395] Similarly, the ingestion of *L. rhamnosus* (JB-1) in mice reduced stress-induced anxiety and depression-like behavior through a vagal pathway.[396] The effect of probiotics on depressive clinical signs has been extensively studied in clinical studies and is reviewed by Wallace and Milev.[397] From a mechanistic standpoint, preclinical studies have implicated the vagus nerve as a key route of neural communication between gut microbes and centrally mediated behavioral effects. As mentioned above, this connection was revealed by the elimination of the CNS effects of *L. rhamnosus* after vagotomy[396] and by a decreased risk of certain neurologic disorders in humans who underwent vagotomy.[398]

The gut microbiota also regulates key neurotransmitters, such as serotonin, by altering the precursor levels; for example, *B. infantis* has been shown to elevate plasma tryptophan levels and thus influence central serotonin transmission.[399] Intriguingly, the synthesis and release of neurotransmitters from bacteria has been reported. *Lactobacillus* and *Bifidobacterium* spp. can produce GABA, *Escherichia*, *Bacillus*, and *Saccharomyces* spp. can generate noradrenaline, *Candida*, *Streptococcus*, *Escherichia*, and *Enterococcus* spp. can produce serotonin, *Bacillus* can produce dopamine, and *Lactobacillus* acetylcholine.[400,401] These microbial-synthesized neurotransmitters can cross the mucosal layer of the intestine. However, it is highly unlikely that they directly influence brain function because they are incapable of crossing the blood–brain barrier (BBB). Instead, their impact on brain function is likely to be indirect, acting on the enteric nervous system. Inflammatory cytokines produced in the gut can travel via the bloodstream to the brain. Under normal physiological circumstances, they cannot signal across the BBB, but they can influence brain areas, such as the hypothalamus, where the BBB is more permeable. It is through the latter mechanism that IL-1 and IL-6 cytokines activate the HPA axis, leading to the release of cortisol, which is the most potent activator of the stress system. In conclusion, the HPA axis, immune system, and vagus nerve represent key players in the neuroendocrine system of the gut and have a significant impact on the gut–brain–microbiota axis.[316,402]

The gut–brain–β cell axis

Recent publications have highlighted the previously unknown role for gut microbiota in stimulating insulin secretion by signaling to the brain, through an axis called the "gut–brain–β-cell axis."[22,403,404] Upon exposure of rodents to a high-calorie diet, the microbiota increases the acetate turnover. The resulting augmentation of acetate production leads to activation of the parasympathetic nervous system. This neurological activity triggers in turn both secretion of the hormone ghrelin, which increases food intake, and augments insulin levels, thereby promoting calorie storage and fat gain. This generates a positive feedback loop, resulting in hyperphagia (via increased ghrelin secretion), increased energy storage as fat (via increased glucose-stimulated insulin secretion) and liver and muscle IR. Consequently, the gut microbiota–brain–β cell axis promotes obesity and concomitant hyperlipidemia, fatty liver disease, and IR. Thus, a high-fat enriched diet can induce obesity not only by providing excess nutrients but also by increasing the microbiota-mediated acetate production that stimulates the brain to promote hyperinsulinemia and energy storage.

The gut–skin axis and gut–brain–skin axis

Recently emerging evidence from interdisciplinary research groups supports the existence of a gut–skin axis that communicates via metabolites, the neuroendocrine system, diet, and the central nervous system.[354] In healthy state, different types of interactions occur between the gut and skin. Gut microbiota produce metabolites, hormones, and neurotransmitters that can enter the blood circulation and affect the skin function. Dietary components can also have access to the skin either directly or via processing by the microbiota. On the other hand, the skin is able to synthesize vitamin D, which can modulate the gut function.[405]

In a disease state, under the conditions of dysbiosis, the toxins produced by the gut microbiota can reach the skin through a leaky gut barrier. The inefficient handling of gut commensal bacteria and bacterial components by the liver can also cause the induction of pro-inflammatory cytokines that can reach the skin. This alteration in the skin's immune environment, together with toxin production, can then contribute to the development of skin pathologies.

Only recently has more attention been focused on the interaction of the gut–skin axis with the CNS, the so-called gut–brain–skin axis.[406,407] Several studies have demonstrated a correlation among these organs such as pathological changes in the visceral organs that can lead to "zones of referred pain" (Head's zones) in the skin.[408] Arck et al. found that when mice ingested a *Lactobacillus* strain, reduced neurogenic skin inflammation and hair growth inhibition was induced by a perceived stress.[406,407] Several questions remain open for the scientific community to

answer, including the nature and the effects of the molecules present in the gut on the skin and the response of the skin microbiota to gut changes.

Conclusions and outlook

According to the current scientific literature, the composition and metabolic activity of the microbiota has an association with many diseases, including the disorders associated with obesity, chronic inflammatory diseases, CVD, cancers, stress, and even neurodegenerative disorders. Our biology seems to be a complex interplay between the gene products encoded in our DNA and those originating from the microbes within us.

During the past decade, microbiome research has expanded massively. Innovative sequencing techniques now allow us to detect bacteria and other organisms that are unable to grow in the laboratory and to reveal the nature of the microbial communities present on every bodily surface. Studies involving germ-free mice or antibiotic-treated mice have also uncovered connections between microbiota and human health.

The increasing need for population-scale data to better understand the role of these bacterial communities has led to the creation of different global projects. The US NIH-funded Human Microbiome Project Consortium has brought together several scientific experts to evaluate the role of the microbiome in health and disease and its relationship with the human host.[409] Similarly, the American Gut Project aims to analyze the human gut microbiome to determine what makes a healthy or sick gut.[410]

One important factor emerging from these research advances is the importance of microbial diversity. The gut microbiota of an individual is more diverse under healthy conditions than during disease when the diversity is reduced. In fact, low microbiome richness is associated with metabolic dysfunction, skin disorders, gastrointestinal disorders, and low-grade inflammation.[130,411,412] It is believed that for pathology to develop, the ratio of potentially pathogenic to beneficial commensal microbes determines outcome, rather than the presence of a specific organism or group[413] (Table 1 and Figure 2). Microbial diversity is influenced not only by unhealthy conditions but also by diet and exercise, with diversity levels found to be decreased in overweight and obese patients.[130,412]

Promising results are also emerging from metabolomics analyses in various biological matrices (plasma, feces, urines, or tissues). The metabolite profiles obtained from the microbiota, the host, or their co-metabolites have a high-resolution power that enables discrimination of healthy from diseased subjects.[414,415]

- IBD: A complete list of the altered metabolites can be found in the review by Smirnov et al.[414] The most significantly affected metabolites are certain amino acids (alanine, isoleucine, leucine, lysine, valine, and phenylalanine), SCFAs, and some acids (propanoic, butanoic, oleic, stearic, palmitic, linoleic, and arachidonic). However, most of these effects are a consequence of the inflammation status during IBD with malnutrition and decreased nutrient absorption.
- Obesity: Different studies have discovered altered levels for urine hippurate, 4-hydroxylphenylacetic acid and phenylacetylglycine, acetate, lactate, and branched chain amino acids.[415]
- NAFLD: Metabolites like 1-butanol, 2-butanone, and 4-methyl-2-pentanone have been found to be perturbed in people with NAFLD;[415]
- Neuropathology: Modified levels of free amino acids and SCFAs, tryptophan-nicotinic acid, sulfur metabolic pathways, and urinary metabolites have been recorded in autistic children.[415]

Microbial metabolites should be adequately quantified using different methods in order to cover as much as possible of the metabolome and be further correlated against the gene expression levels and the corresponding enzymatic activity of each gene. Preferably, these metabolomics results should be combined and integrated with different "omics" techniques applied to DNA, RNA, and protein to provide a full microbiome analysis.

Despite the growing interest and exponential number of studies on this topic, many questions, including those that follow, require clarification:

- What is the precise composition of the different microbial communities living in our bodies? We still do not really know what a healthy microbiota composition looks like and how much variation exists between people. The microbiota are constantly under the influence of many factors, such as the delivery route during birth, genetics, metabolism, immunity (innate and adaptive), nutrition, and many different lifestyle factors. These factors have made it difficult to define the core healthy microbiome associated with a "normal" healthy individual.
- What are the causative and correlative effects between the microbiome and disease? It is typically unclear whether the changes observed in the microbiota are the cause or the effect of different diseases.
- Comparing health and disease situations without clearly demonstrating causality, or at least having some strong evidence of any link, is a major issue. In most studies, the overall assumption that the gut microbiota are causally linked with the onset or progression of a disease is often made based on a unique analysis of the composition of the fecal microbiota at a specific time point. Hence, being able to determine whether microbiome alterations precede or are caused by a particular disease would be essential.

However, in terms of what came first, a disease or a change in the microbiome, there probably will not be a single answer, but it would be very informative to be able to characterize the disease-associated changes in the microbiome before and after the disease diagnosis. Thus, variations in scientific results may be reduced by performing long-term longitudinal studies monitoring the disease progression and by characterizing the changes in the taxonomic and functional composition of the microbiome, thereby defining the disease state. The further investigations that follow would need to confirm that *in vivo* replacement of the missing microorganisms could at least mitigate and/or cure the disease, which is the ultimate objective to reach.

- What are the functional pathways by which the gut, lungs, brain, and skin microbial populations can communicate inside the human body? The ability to modify the function of one organ by manipulation of the other now depends on concerted interdisciplinary efforts focused on better understanding of the targetable pathways by which the gut, skin, lungs, oral cavity, and brain communicate with each other.

Evidently, an enormously complex interplay exists among our different bodily organs and the different microbial communities we harbor. We are just at the beginning of our understanding of the microbiome and interdisciplinary effort will be essential for us to answer some of the open questions about the role of the microbiome in health and disease.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: All authors are employees of, or contracted and paid by, Philip Morris International.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Philip Morris International is the sole source of funding and sponsor of this article.

Supplemental material

Supplementary material for this article is available online.

References

1. Sender R, Fuchs S and Milo R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* 2016; **164**: 337–340.
2. Sender R, Fuchs S and Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLOS Biol* 2016; **14**: e1002533.
3. Maczulak A. *Allies and enemies: How the world depends on bacteria*. Upper Saddle River, New Jersey: Pearson Education, 2011.
4. Whiteside SA, Razvi H, Dave S, et al. The microbiome of the urinary tract-a role beyond infection. *Nat Rev Urol* 2015; **12**: 81–90.
5. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. *Science* 2005; **308**: 1635–1638.
6. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59–65.
7. D'Aversa F, Tortora A, Ianiro G, et al. Gut microbiota and metabolic syndrome. *Int Emerg Med* 2013; **8**(Suppl 1): S11–S15.
8. Grice EA and Segre JA. The human microbiome: our second genome. *Ann Rev Genom Human Genet* 2012; **13**: 151–170.
9. Ondov BD, Bergman NH and Phillippy AM. Interactive metagenomic visualization in a Web browser. *BMC Bioinform* 2011; **12**: 385.
10. Lloyd-Price J, Abu-Ali G and Huttenhower C. The healthy human microbiome. *Genom Med* 2016; **8**: 51.
11. Koppel N, Maini Rekdal V and Balskus EP. Chemical transformation of xenobiotics by the human gut microbiota. *Science* 2017; **356**.
12. Smith K, McCoy KD and Macpherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semi Immunol* 2007; **19**: 59–69.
13. Round JL and Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nature Rev Immunol* 2009; **9**: 313–323.
14. Sommer F and Backhed F. The gut microbiota–masters of host development and physiology. *Nature Rev Microbiol* 2013; **11**: 227–238.
15. Postler TS and Ghosh S. Understanding the holobiont: how microbial metabolites affect human health and shape the immune system. *Cell Metab* 2017; **26**: 110–130.
16. Eberl G and Lochner M. The development of intestinal lymphoid tissues at the interface of self and microbiota. *Mucosal Immunol* 2009; **2**: 478–485.
17. Chassard C and Lacroix C. Carbohydrates and the human gut microbiota. *Curr Opini Clin Nutrit Metab Care* 2013; **16**: 453–460.
18. Wong JM, Esfahani A, Singh N, et al. Gut microbiota, diet, and heart disease. *J AOAC Int* 2012; **95**: 24–30.
19. Louis P, Scott KP, Duncan SH, et al. Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol* 2007; **102**: 1197–1208.
20. Tan J, McKenzie C, Potamitis M, et al. The role of short-chain fatty acids in health and disease. *Adv Immunol* 2014; **121**: 91–119.
21. Kelly CJ, Zheng L, Campbell EL, et al. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Micro* 2015; **17**: 662–671.

22. Perry RJ, Peng L, Barry NA, et al. Acetate mediates a microbiome-brain-beta-cell axis to promote metabolic syndrome. *Nature* 2016; **534**: 213–217.
23. Rios-Covian D, Ruas-Madiedo P, Margolles A, et al. Intestinal short chain fatty acids and their link with diet and human health. *Front Microbiol* 2016; **7**: 185.
24. Tremaroli V and Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012; **489**: 242–249.
25. Young VB. The role of the microbiome in human health and disease: an introduction for clinicians. *BMJ* 2017; **356**: j831.
26. Wahlstrom A, Sayin SI, Marschall HU, et al. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. *Cell Metab* 2016; **24**: 41–50.
27. Sayin SI, Wahlstrom A, Felin J, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab* 2013; **17**: 225–235.
28. Ryan KK, Tremaroli V, Clemmensen C, et al. FXR is a molecular target for the effects of vertical sleeve gastrectomy. *Nature* 2014; **509**: 183–188.
29. Gacias M, Gaspari S, Santos PM, et al. Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior. *eLife* 2016; **5**.
30. Buffington SA, Di Prisco GV, Auchtung TA, et al. Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. *Cell* 2016; **165**: 1762–1775.
31. Janssen AW and Kersten S. The role of the gut microbiota in metabolic health. *FASEB J* 2015; **29**: 3111–3123.
32. Singh RK, Chang HW, Yan D, et al. Influence of diet on the gut microbiome and implications for human health. *J Trans Med* 2017; **15**: 73.
33. Shukla SD, Budden KF, Neal R, et al. Microbiome effects on immunity, health and disease in the lung. *Clin Trans Immunol* 2017; **6**: e133.
34. Turnbaugh PJ, Ley RE, Hamady M, et al. The human microbiome project. *Nature* 2007; **449**: 804–810.
35. Benson AK, Kelly SA, Legge R, et al. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc Natl Acad Sci USA* 2010; **107**: 18933–18938.
36. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012; **150**: 470–480.
37. Roy S and Trinchieri G. Microbiota: a key orchestrator of cancer therapy. *Nat Rev Cancer* 2017; **17**: 271–285.
38. Vogtmann E and Goedert JJ. Epidemiologic studies of the human microbiome and cancer. *Br J Cancer* 2016; **114**: 237–242.
39. Vatanen T, Kostic AD, d'Hennezel E, et al. Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. *Cell* 2016; **165**: 1551.
40. Schuppan D, Junker Y and Barisani D. Celiac disease: from pathogenesis to novel therapies. *Gastroenterology* 2009; **137**: 1912–1933.
41. Galipeau HJ, McCarville JL, Huebener S, et al. Intestinal microbiota modulates gluten-induced immunopathology in humanized mice. *Am J Pathol* 2015; **185**: 2969–2982.
42. Caminero A, Galipeau HJ, McCarville JL, et al. Duodenal bacteria from patients with celiac disease and healthy subjects distinctly affect gluten breakdown and immunogenicity. *Gastroenterology* 2016; **151**: 670–683.
43. Scher JU, Littman DR and Abramson SB. Microbiome in inflammatory arthritis and human rheumatic diseases. *Art Rheumatol (Hoboken, NJ)* 2016; **68**: 35–45.
44. Blázquez AB and Berin MC. Microbiome and food allergy. *Trans Res* 2017; **179**: 199–203.
45. Riiser A. The human microbiome, asthma, and allergy. *Allerg Asthm Clin Immunol* 2015; **11**: 35.
46. Carmody Rachel N, Gerber Georg K, Luevano Jesus M Jr, et al. Diet dominates host genotype in shaping the murine gut microbiota. *Cell Host Micro* 2015; **17**: 72–84.
47. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480–484.
48. Conlon MA and Bird AR. The impact of diet and lifestyle on gut microbiota and human health. *Nutrients* 2014; **7**: 17–44.
49. Ghaisas S, Maher J and Kanthasamy A. Gut microbiome in health and disease: linking the microbiome-gut-brain axis and environmental factors in the pathogenesis of systemic and neurodegenerative diseases. *Pharmacol Therap* 2016; **158**: 52–62.
50. Caesar R, Tremaroli V, Kovatcheva-Datchary P, et al. Crosstalk between gut microbiota and dietary lipids aggravates WAT inflammation through TLR signaling. *Cell Metab* 2015; **22**: 658–668.
51. Devkota S, Wang Y, Musch MW, et al. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. *Nature* 2012; **487**: 104–108.
52. Kong LC, Holmes BA, Cotillard A, et al. Dietary patterns differently associate with inflammation and gut microbiota in overweight and obese subjects. *PLOS One* 2014; **9**: e109434.
53. Bolnick DI, Snowberg LK, Hirsch PE, et al. Individual diet has sex-dependent effects on vertebrate gut microbiota. *Nature Commun* 2014; **5**: 4500.
54. Walker AW, Ince J, Duncan SH, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J* 2011; **5**: 220–230.
55. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011; **334**: 105–108.
56. Patterson E, Ryan PM, Cryan JF, et al. Gut microbiota, obesity and diabetes. *Post Med J* 2016; **92**: 286–300.
57. Phillips B, Veljkovic E, Boue S, et al. An 8-month systems toxicology inhalation/cessation study in apoe-/- mice to investigate cardiovascular and respiratory exposure effects of a candidate modified risk tobacco product, ths 2.2, compared with conventional cigarettes. *Toxicol Sci* 2016; **151**: 462–464.
58. Phillips B, Veljkovic E, Peck MJ, et al. A 7-month cigarette smoke inhalation study in C57BL/6 mice demonstrates

reduced lung inflammation and emphysema following smoking cessation or aerosol exposure from a prototypic modified risk tobacco product. *Food Chem Toxicol* 2015; **80**: 328–345.
59. Luettich K, Xiang Y, Iskandar A, et al. Systems toxicology approaches enable mechanistic comparison of spontaneous and cigarette smoke-related lung tumor development in the A/J mouse model. *Int Toxicol* 2014; **7**: 73–84.
60. Biedermann L, Brulisauer K, Zeitz J, et al. Smoking cessation alters intestinal microbiota: insights from quantitative investigations on human fecal samples using FISH. *Inflam Bowel Dis* 2014; **20**: 1496–1501.
61. Biedermann L, Zeitz J, Mwinyi J, et al. Smoking cessation induces profound changes in the composition of the intestinal microbiota in humans. *PLOS One* 2013; **8**: e59260.
62. Allais L, De Smet R, Verschuere S, et al. Transient receptor potential channels in intestinal inflammation: what is the impact of cigarette smoking? *Pathobiol J Immunopathol Mole Cell Biol* 2017; **84**: 1–15.
63. Wang H, Zhao JX, Hu N, et al. Side-stream smoking reduces intestinal inflammation and increases expression of tight junction proteins. *World J Gastroenterol* 2012; **18**: 2180–2187.
64. Tomoda K, Kubo K, Asahara T, et al. Cigarette smoke decreases organic acids levels and population of bifidobacterium in the caecum of rats. *J Toxicol Sci* 2011; **36**: 261–266.
65. Wang T, Cai G, Qiu Y, et al. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. *ISME J* 2012; **6**: 320–329.
66. Charlson ES, Chen J, Custers-Allen R, et al. Disordered microbial communities in the upper respiratory tract of cigarette smokers. *PLOS One* 2010; **5**: e15216.
67. Kumar PS, Griffen AL, Moeschberger ML, et al. Identification of candidate periodontal pathogens and beneficial species by quantitative 16 S clonal analysis. *J Clin Microbiol* 2005; **43**: 3944–3955.
68. Morris A, Beck JM, Schloss PD, et al. Comparison of the respiratory microbiome in healthy nonsmokers and smokers. *Am J Res Crit Care Med* 2013; **187**: 1067–1075.
69. Queipo-Ortuño MI, Seoane LM, Murri M, et al. Gut microbiota composition in male rat models under different nutritional status and physical activity and its association with serum leptin and ghrelin levels. *PLOS One* 2013; **8**: e65465.
70. Clarke SF, Murphy EF, O'Sullivan O, et al. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* 2014; **63**: 1913–1920.
71. Whisner CM and Bruening M. Associations between physical activity and the intestinal microbiome of college freshmen. *FASEB J* 2016; **30**: 146.2.
72. Dethlefsen L, Huse S, Sogin ML, et al. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16 S rRNA sequencing. *PLOS Biol* 2008; **6**: e280.
73. Ubeda C, Taur Y, Jenq RR, et al. Vancomycin-resistant enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *J Clin Invest* 2010; **120**: 4332–4341.
74. Sommer MO, Munck C, Toft-Kehler RV, et al. Prediction of antibiotic resistance: time for a new preclinical paradigm? *Nature Rev Microbiol* 2017; **15**: 689–696.
75. Blair JM, Webber MA, Baylay AJ, et al. Molecular mechanisms of antibiotic resistance. *Nature Rev Microbiol* 2015; **13**: 42.
76. Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance—the need for global solutions. *Lancet Infect Dis* 2013; **13**: 1057–1098.
77. Barile D and Rastall RA. Human milk and related oligosaccharides as prebiotics. *Curr Opin Biotechnol* 2013; **24**: 214–219.
78. Bode L. The functional biology of human milk oligosaccharides. *Early Human Develop* 2015; **91**: 619–622.
79. Maslowski KM and Mackay CR. Diet, gut microbiota and immune responses. *Nat Immunol* 2011; **12**: 5–9.
80. Gibson GR, Probert HM, Loo JV, et al. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr Res Rev* 2004; **17**: 259–275.
81. Valle Gottlieb MG, Closs VE, Junges VM, et al. Impact of human aging and modern lifestyle on microbiota. *Crit Rev Food Sci Nutr* 2017; **13**: 1–8.
82. Al-Ghazzewi FH and Tester RF. Impact of prebiotics and probiotics on skin health. *Benef Micro* 2014; **5**: 99–107.
83. Kalliomaki M, Antoine JM, Herz U, et al. Guidance for substantiating the evidence for beneficial effects of probiotics: prevention and management of allergic diseases by probiotics. *J Nutr* 2010; **140**: 713S–721S.
84. Zhuang G, Liu X, Zhang Q, et al. Research advances with regards to clinical outcome and potential mechanisms of the cholesterol-lowering effects of probiotics. *Clin Lipidol* 2012; **7**: 501–507.
85. Nicholson JK, Holmes E, Kinross J, et al. Host-gut microbiota metabolic interactions. *Science* 2012; **336**: 1262–1267.
86. Grice EA and Segre JA. The skin microbiome. *Nature Rev Microbiol* 2011; **9**: 244–253.
87. McBride ME, Duncan WC and Knox JM. The environment and the microbial ecology of human skin. *Appl Environ Microbiol* 1977; **33**: 603–608.
88. Duncan SH and Flint HJ. Probiotics and prebiotics and health in ageing populations. *Maturitas* 2013; **75**: 44–50.
89. Saraswati S and Sitaraman R. Aging and the human gut microbiota-from correlation to causality. *Front Microbiol* 2014; **5**: 764.
90. Keebaugh ES and Ja WW. Breaking down walls: microbiota and the aging gut. *Cell Host Micro* 2017; **21**: 417–418.
91. Thevaranjan N, Puchta A, Schulz C, et al. Age-associated microbial dysbiosis promotes intestinal permeability, systemic inflammation, and macrophage dysfunction. *Cell Host Micro* 2017; **21**: 455–466.e4.
92. Yende S, Alvarez K, Loehr L, et al. Epidemiology and long-term clinical and biologic risk factors for pneumonia in community-dwelling older Americans: analysis of three cohorts. *Chest* 2013; **144**: 1008–1017.
93. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature* 2012; **486**: 222–227.

94. De Filippo C, Cavalieri D, Di Paola M, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc National Acad Sci USA* 2010; **107**: 14691–14696.
95. Claus SP, Guillou H and Ellero-Simatos S. The gut microbiota: a major player in the toxicity of environmental pollutants? *NPJ Biofilms Microbiomes* 2016; **2**: 16003.
96. Choi JJ, Eum SY, Rampersaud E, et al. Exercise attenuates PCB-induced changes in the mouse gut microbiome. *Environ Health Perspect* 2013; **121**: 725–730.
97. Lu K, Abo RP, Schlieper KA, et al. Arsenic exposure perturbs the gut microbiome and its metabolic profile in mice: an integrated metagenomics and metabolomics analysis. *Environ Health Perspect* 2014; **122**: 284–291.
98. Spanogiannopoulos P, Bess EN, Carmody RN, et al. The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism. *Nat Rev Micro* 2016; **14**: 273–287.
99. Maurice Corinne F, Haiser Henry J and Turnbaugh Peter J. Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *Cell* 2013; **152**: 39–50.
100. Stanaway IB, Wallace JC, Shojaie A, et al. Human oral buccal microbiomes are associated with farmworker status and azinphos-methyl agricultural pesticide exposure. *Appl Environ Microbiol* 2017; **83**.
101. Cuschieri S and Mamo J. Getting to grips with the obesity epidemic in Europe. *SAGE Open Med* 2016; **4**: 2050312116670406.
102. Misra A and Khurana L. Obesity and the metabolic syndrome in developing countries. *J Clin Endocrinol Metab* 2008; **93**: S9–S30.
103. Wang Y, Mi J, Shan XY, et al. Is China facing an obesity epidemic and the consequences? The trends in obesity and chronic disease in China. *Int J Obesit* 2007; **31**: 177–188.
104. Dietz WH. The response of the US centers for disease control and prevention to the obesity epidemic. *Ann Rev Pub Health* 2015; **36**: 575–596.
105. Aron-Wisnewsky J and Clement K. The gut microbiome, diet, and links to cardiometabolic and chronic disorders. *Nature Rev Nephrol* 2016; **12**: 169–181.
106. Zhang C and Zhao L. Strain-level dissection of the contribution of the gut microbiome to human metabolic disease. *Genome Med* 2016; **8**: 41.
107. Sinha-Hikim AP, Sinha-Hikim I and Friedman TC. Connection of nicotine to diet-induced obesity and non-alcoholic fatty liver disease: cellular and mechanistic insights. *Front Endocrinol* 2017; **8**: 23.
108. Johnson EL, Heaver SL, Walters WA, et al. Microbiome and metabolic disease: revisiting the bacterial phylum Bacteroidetes. *J Mole Med* 2017; **95**: 1–8.
109. Ussar S, Fujisaka S and Kahn CR. Interactions between host genetics and gut microbiome in diabetes and metabolic syndrome. *Mole Metab* 2016; **5**: 795–803.
110. Hansen TH, Gøbel RJ, Hansen T, et al. The gut microbiome in cardio-metabolic health. *Genome Med* 2015; **7**: 33.
111. Benoit B, Laugerette F, Plaisancie P, et al. Increasing fat content from 20 to 45 wt% in a complex diet induces lower endotoxemia in parallel with an increased number of intestinal goblet cells in mice. *Nutr Res* 2015; **35**: 346–356.
112. de Wit N, Derrien M, Bosch-Vermeulen H, et al. Saturated fat stimulates obesity and hepatic steatosis and affects gut microbiota composition by an enhanced overflow of dietary fat to the distal intestine. *Am J Physiol Gastro Liver Physiol* 2012; **303**: G589–G599.
113. Kreznar JH, Keller MP, Traeger LL, et al. Host genotype and gut microbiome modulate insulin secretion and diet-induced metabolic phenotypes. *Cell Rep* 2017; **18**: 1739–1750.
114. Ley RE, Backhed F, Turnbaugh P, et al. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* 2005; **102**: 11070–11075.
115. Mozes S, Bujnakova D, Sefcikova Z, et al. Intestinal microflora and obesity in rats. *Folia Microbiol* 2008; **53**: 225–228.
116. Guo X, Xia X, Tang R, et al. Development of a real-time PCR method for Firmicutes and Bacteroidetes in faeces and its application to quantify intestinal population of obese and lean pigs. *Lett Appl Microbiol* 2008; **47**: 367–373.
117. Murphy EF, Cotter PD, Healy S, et al. Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. *Gut* 2010; **59**: 1635–1642.
118. Turnbaugh PJ, Backhed F, Fulton L, et al. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Micro* 2008; **3**: 213–223.
119. Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027–1031.
120. Backhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004; **101**: 15718–15723.
121. Turnbaugh PJ, Ridaura VK, Faith JJ, et al. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Trans Med* 2009; **1**: 6ra14.
122. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 2013; **341**: 1241214.
123. Psichas A, Sleeth ML, Murphy KG, et al. The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int J Obesity* 2015; **39**: 424–429.
124. Chambers ES, Viardot A, Psichas A, et al. Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut* 2015; **64**: 1744–1754.
125. Cani PD, Bibiloni R, Knauf C, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008; **57**: 1470–1481.
126. Cho I, Yamanishi S, Cox L, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature* 2012; **488**: 621–626.

127. Karlsson CL, Onnerfalt J, Xu J, et al. The microbiota of the gut in preschool children with normal and excessive body weight. *Obesity* 2012; **20**: 2257–2261.
128. Everard A, Belzer C, Geurts L, et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci USA* 2013; **110**: 9066–9071.
129. Fei N and Zhao L. An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. *ISME J* 2013; **7**: 880–884.
130. Le Chatelier E, Nielsen T, Qin J, et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013; **500**: 541–546.
131. Goodrich JK, Waters JL, Poole AC, et al. Human genetics shape the gut microbiome. *Cell* 2014; **159**: 789–799.
132. Goodrich Julia K, Waters Jillian L, Poole Angela C, et al. Human genetics shape the gut microbiome. *Cell* 2014; **159**: 789–799.
133. Million M, Maraninchi M, Henry M, et al. Obesity-associated gut microbiota is enriched in Lactobacillus reuteri and depleted in Bifidobacterium animalis and Methanobrevibacter smithii. *Int J Obesity* 2012; **36**: 817–825.
134. Million M, Angelakis E, Paul M, et al. Comparative meta-analysis of the effect of Lactobacillus species on weight gain in humans and animals. *Micro Pathog* 2012; **53**: 100–108.
135. Kalliomäki M, Collado MC, Salminen S, et al. Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr* 2008; **87**: 534.
136. Boulangé CL, Neves AL, Chilloux J, et al. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. *Genome Med* 2016; **8**: 42.
137. Merga Y, Campbell BJ and Rhodes JM. Mucosal barrier, bacteria and inflammatory bowel disease: possibilities for therapy. *Digest Dise* 2014; **32**: 475–483.
138. Kelly JR, Kennedy PJ, Cryan JF, et al. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Front Cell Neurosci* 2015; **9**: 392.
139. Desai MS, Seekatz AM, Koropatkin NM, et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 2016; **167**: 1339–1353.e21.
140. Chou CJ, Membrez M and Blancher F. Gut decontamination with norfloxacin and ampicillin enhances insulin sensitivity in mice. *Nestle Nutr Workshop Ser Pediatr Program* 2008; **62**: 127–137.
141. Chevalier C, Stojanovic O, Colin DJ, et al. Gut microbiota orchestrates energy homeostasis during cold. *Cell* 2015; **163**: 1360–1374.
142. Vrieze A, Holleman F, Zoetendal EG, et al. The environment within: how gut microbiota may influence metabolism and body composition. *Diabetologia* 2010; **53**: 606–613.
143. Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012; **490**: 55–60.
144. Shin NR, Lee JC, Lee HY, et al. An increase in the akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut* 2014; **63**: 727–735.
145. Forslund K, Hildebrand F, Nielsen T, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* 2015; **528**: 262–266.
146. Pedersen HK, Gudmundsdottir V, Nielsen HB, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* 2016; **535**: 376–381.
147. Karlsson FH, Tremaroli V, Nookaew I, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 2013; **498**: 99–103.
148. Karlsson FH, Fak F, Nookaew I, et al. Symptomatic atherosclerosis is associated with an altered gut metagenome. *Nature Commun* 2012; **3**: 1245.
149. Koren O, Spor A, Felin J, et al. Human oral, gut, and plaque microbiota in patients with atherosclerosis. *Proc Natl Acad Sci USA* 2011; **108**(Suppl 1): 4592–4598.
150. Spinler SA, Cziraky MJ, Willey VJ, et al. Frequency of attainment of low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol goals in cardiovascular clinical practice (from the national cardiovascular data registry PINNACLE registry). *Am J Cardiol* 2015; **116**: 547–553.
151. Lang DH, Yeung CK, Peter RM, et al. Isoform specificity of trimethylamine N-oxygenation by human flavin-containing monooxygenase (FMO) and P450 enzymes: selective catalysis by FMO3. *Biochem Pharmacol* 1998; **56**: 1005–1012.
152. Stock J. Gut microbiota: an environmental risk factor for cardiovascular disease. *Atherosclerosis* 2013; **229**: 440–442.
153. Bennett BJ, de Aguiar Vallim TQ, Wang Z, et al. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metab* 2013; **17**: 49–60.
154. Randrianarisoa E, Lehn-Stefan A, Wang X, et al. Relationship of serum trimethylamine N-Oxide (TMAO) levels with early atherosclerosis in humans. *Sci Rep* 2016; **6**: 26745.
155. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011; **472**: 57–63.
156. Tang WH, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *New Engl J Med* 2013; **368**: 1575–1584.
157. Wang Z, Roberts Adam B, Buffa Jennifer A, et al. Nonlethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell* 2015; **163**: 1585–1595.
158. Brandi G, De Lorenzo S, Candela M, et al. Microbiota, NASH, HCC and the potential role of probiotics. *Carcinogenesis* 2017; **38**: 231–240.
159. Patrice DC and Nathalie MD. The role of the gut microbiota in energy metabolism and metabolic disease. *Curr Pharm Des* 2009; **15**: 1546–1558.

160. Schaffert CS, Duryee MJ, Hunter CD, et al. Alcohol metabolites and lipopolysaccharide: roles in the development and/or progression of alcoholic liver disease. *World J Gastroenterol WJG* 2009; **15**: 1209–1218.
161. Alisi A, Ceccarelli S, Panera N, et al. Causative role of gut microbiota in non-alcoholic fatty liver disease pathogenesis. *Front Cell Infect Microbiol* 2012; **2**: 132.
162. Loguercio C, De Simone T, Federico A, et al. Gut-liver axis: a new point of attack to treat chronic liver damage? *Am J Gastroenterol* 2002; **97**: 2144–2146.
163. LaRusso NF, Tabibian JH and O'Hara SP. Role of the intestinal microbiome in cholestatic liver disease. *Digest Dise* 2017; **35**: 166–168.
164. Mokhtari Z, Gibson DL and Hekmatdoost A. Nonalcoholic fatty liver disease, the gut microbiome, and diet. *Adv Nutr Int Rev J* 2017; **8**: 240–252.
165. Doulberis M, Kotronis G, Gialamprinou D, et al. Non-alcoholic fatty liver disease: an update with special focus on the role of gut microbiota. *Metabolism* 2017; **71**: 182–197.
166. Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology* 2013; **57**: 601–609.
167. Del Chierico F, Nobili V, Vernocchi P, et al. Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology* 2017; **65**: 451–464.
168. Wong VWS, Tse CH, Lam TTY, et al. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis – a longitudinal study. *PLOS one* 2013; **8**: e62885.
169. Raman M, Ahmed I, Gillevet PM, et al. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2013; **11**: 868–875.e3.
170. Mouzaki M, Comelli EM, Arendt BM, et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology* 2013; **58**: 120–127.
171. Qin N, Yang F, Li A, et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014; **513**: 59–64.
172. Lambertz J, Weiskirchen S, Landert S, et al. Fructose: a dietary sugar in crosstalk with microbiota contributing to the development and progression of non-alcoholic liver disease. *Front Immunol* 2017; **8**: 1159.
173. Vos MB. Nutrition, nonalcoholic fatty liver disease and the microbiome: recent progress in the field. *Curr Opin Lipidol* 2014; **25**: 61–66.
174. Zhou D, Pan Q, Shen F, et al. Total fecal microbiota transplantation alleviates high-fat diet-induced steatohepatitis in mice via beneficial regulation of gut microbiota. *Sci Rep* 2017; **7**: 1529.
175. Arumugam M, Raes J, Pelletier E, et al. Enterotypes of the human gut microbiome. *Nature* 2011; **473**: 174–180.
176. Ley RE, Turnbaugh PJ, Klein S, et al. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**: 1022–1023.
177. Duncan SH, Belenguer A, Holtrop G, et al. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Appl Environ Microbiol* 2007; **73**: 1073–1078.
178. Martinez KB, Leone V and Chang EB. Western diets, gut dysbiosis, and metabolic diseases: are they linked? *Gut Micro* 2017; **8**: 130–142.
179. Evans CC, LePard KJ, Kwak JW, et al. Exercise prevents weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. *PLOS One* 2014; **9**: e92193.
180. Kang SS, Jeraldo PR, Kurti A, et al. Diet and exercise orthogonally alter the gut microbiome and reveal independent associations with anxiety and cognition. *Mole Neurodegenerat* 2014; **9**: 36.
181. Mueller NT, Whyatt R, Hoepner L, et al. Prenatal exposure to antibiotics, cesarean section and risk of childhood obesity. *Int J Obesity* 2015; **39**: 665–670.
182. Trasande L, Blustein J, Liu M, et al. Infant antibiotic exposures and early-life body mass. *Int J Obesity* 2013; **37**: 16–23.
183. Ajslev T, Andersen C, Gamborg M, et al. Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics. *Int J Obesity* 2011; **35**: 522–529.
184. Vrieze A, Out C, Fuentes S, et al. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. *J Hepatol* 2014; **60**: 824–831.
185. Reijnders D, Goossens Gijs H, Hermes Gerben DA, et al. Effects of gut microbiota manipulation by antibiotics on host metabolism in obese humans: a randomized double-blind placebo-controlled trial. *Cell Metab* 2016; **24**: 63–74.
186. Carvalho B, Guadagnini D, Tsukumo D, et al. Modulation of gut microbiota by antibiotics improves insulin signalling in high-fat fed mice. *Diabetologia* 2012; **55**: 2823–2834.
187. Membrez M, Blancher F, Jaquet M, et al. Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. *FASEB J* 2008; **22**: 2416–2426.
188. Janssen AWF, Houben T, Katiraei S, et al. Modulation of the gut microbiota impacts nonalcoholic fatty liver disease: a potential role for bile acids. *J Lipid Res* 2017; **58**: 1399–1416.
189. Xu RY, Wan YP, Fang QY, et al. Supplementation with probiotics modifies gut flora and attenuates liver fat accumulation in rat nonalcoholic fatty liver disease model. *J Clin Biochem Nutr* 2012; **50**: 72–77.
190. Endo H, Niioka M, Kobayashi N, et al. Butyrate-producing probiotics reduce nonalcoholic fatty liver disease progression in rats: new insight into the probiotics for the gut-liver axis. *PLOS One* 2013; **8**: e63388.
191. Mattace Raso G, Simeoli R, Iacono A, et al. Effects of a Lactobacillus paracasei B21060 based synbiotic on steatosis, insulin signaling and toll-like receptor expression in rats fed a high-fat diet. *J Nutr Biochem* 2014; **25**: 81–90.
192. Wagnerberger S, Spruss A, Kanuri G, et al. Lactobacillus casei Shirota protects from fructose-induced liver steatosis: a mouse model. *J Nutr Biochem* 2013; **24**: 531–538.

193. Wong VW, Won GL, Chim AM, et al. Treatment of non-alcoholic steatohepatitis with probiotics. A proof-of-concept study. *Annal Hepatol* 2013; **12**: 256–262.
194. Compare D, Coccoli P, Rocco A, et al. Gut–liver axis: the impact of gut microbiota on non alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dise* 2012; **22**: 471–476.
195. Nardone G, Compare D, Liguori E, et al. Protective effects of *Lactobacillus paracasei* F19 in a rat model of oxidative and metabolic hepatic injury. *Am J Physiol Gastro Liver Physiol* 2010; **299**: G669–G676.
196. Everard A, Matamoros S, Geurts L, et al. Saccharomyces boulardii administration changes gut microbiota and reduces hepatic steatosis, low-grade inflammation, and fat mass in obese and type 2 diabetic db/db Mice. *mBio* 2014; **5**: e01011–e01014.
197. Loguercio C, Federico A, Tuccillo C, et al. Beneficial effects of a probiotic VSL#3 on parameters of liver dysfunction in chronic liver diseases. *J Clin Gastroenterol* 2005; **39**: 540–543.
198. Malaguarnera M, Vacante M, Antic T, et al. Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. *Digest Dise Sci* 2012; **57**: 545–553.
199. Eslamparast T, Eghtesad S, Hekmatdoost A, et al. Probiotics and nonalcoholic fatty liver disease. *Middle East J Digest Dise* 2013; **5**: 129–136.
200. Bernstein CN, Fried M, Krabshuis JH, et al. World gastroenterology organization practice guidelines for the diagnosis and management of IBD in 2010. *Inflam Bowel Dise* 2010; **16**: 112–124.
201. Ponder A and Long MD. A clinical review of recent findings in the epidemiology of inflammatory bowel disease. *Clin Epidemiol* 2013; **5**: 237–247.
202. Montbarbon M, Pichavant M, Langlois A, et al. Colonic inflammation in mice is improved by cigarette smoke through iNKT cells recruitment. *PLOS One* 2013; **8**: e62208.
203. Uhlig HH. Monogenic diseases associated with intestinal inflammation: implications for the understanding of inflammatory bowel disease. *Gut* 2013; **62**: 1795–1805.
204. Neurath MF. Cytokines in inflammatory bowel disease. *Nature Rev Immunol* 2014; **14**: 329–342.
205. Spor A, Koren O and Ley R. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Rev Microbiol* 2011; **9**: 279–290.
206. Hernandez-Chirlaque C, Aranda CJ, Ocon B, et al. Germ-free and antibiotic-treated mice are highly susceptible to epithelial injury in DSS colitis. *J Crohn's Colit* 2016; **10**: 1324–1335.
207. Rigoni R, Fontana E, Guglielmetti S, et al. Intestinal microbiota sustains inflammation and autoimmunity induced by hypomorphic RAG defects. *J Exp Med* 2016; **213**: 355–375.
208. Ward NL, Phillips CD, Nguyen DD, et al. Antibiotic treatment induces long-lasting changes in the fecal microbiota that protect against colitis. *Inflam Bowel Dise* 2016; **22**: 2328–2340.
209. Sartor RB and Mazmanian SK. Intestinal microbes in inflammatory bowel diseases. *Am J Gastroenterol Suppl* 2012; **1**: 15–21.
210. Frank DN, Amand AL, Feldman RA, et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc National Acad Sci* 2007; **104**: 13780–13785.
211. Sokol H, Pigneur B, Watterlot L, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc National Acad Sci* 2008; **105**: 16731–16736.
212. Sokol H, Seksik P, Furet JP, et al. Low counts of Faecalibacterium prausnitzii in colitis microbiota. *Inflam Bowel Dis* 2009; **15**: 1183–1189.
213. Machiels K, Joossens M, Sabino J, et al. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. *Gut* 2014; **63**: 1275–1283.
214. Gevers D, Kugathasan S, Denson Lee A, et al. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Micro* 2014; **15**: 382–392.
215. Pigneur B and Sokol H. Fecal microbiota transplantation in inflammatory bowel disease: the quest for the Holy Grail. *Mucosal Immunol* 2016; **9**: 1360–1365.
216. Mazmanian SK, Round JL and Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 2008; **453**: 620–625.
217. Palm Noah W, de Zoete Marcel R, Cullen Thomas W, et al. Immunoglobulin a coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell* 2014; **158**: 1000–1010.
218. Kau AL, Planer JD, Liu J, et al. Functional characterization of IgA-targeted bacterial taxa from undernourished Malawian children that produce diet-dependent enteropathy. *Sci Trans Med* 2015; **7**: 276ra24.
219. Moon C, Baldridge MT, Wallace MA, et al. Vertically transmitted faecal IgA levels determine extra-chromosomal phenotypic variation. *Nature* 2015; **521**: 90–93.
220. Dalal SR and Chang EB. The microbial basis of inflammatory bowel diseases. *J Clin Invest* 2014; **124**: 4190–4196.
221. Vital M, Penton CR, Wang Q, et al. A gene-targeted approach to investigate the intestinal butyrate-producing bacterial community. *Micro* 2013; **1**: 8.
222. Chu H, Khosravi A, Kusumawardhani IP, et al. Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. *Sci* 2016; **352**: 1116–1120.
223. Schaubeck M and Haller D. Reciprocal interaction of diet and microbiome in inflammatory bowel diseases. *Curr Opin Gastroenterol* 2015; **31**: 464–470.
224. Dolan KT and Chang EB. Diet, gut microbes, and the pathogenesis of inflammatory bowel diseases. *Mole Nutr Food Res* 2017; **61**: 1600129–n/a.
225. Ananthakrishnan AN. Environmental risk factors for inflammatory bowel disease. *Gastroenterol Hepatol* 2013; **9**: 367–374.

226. Persson PG, Ahlbom A and Hellers G. Inflammatory bowel disease and tobacco smoke–a case-control study. *Gut* 1990; **31**: 1377–1381.
227. Birrenbach T and Bocker U. Inflammatory bowel disease and smoking: a review of epidemiology, pathophysiology, and therapeutic implications. *Inflam Bowel Dise* 2004; **10**: 848–859.
228. Parkes GC, Whelan K and Lindsay JO. Smoking in inflammatory bowel disease: impact on disease course and insights into the aetiology of its effect. *J Crohn Colit* 2014; **8**: 717–725.
229. Allais L, Kerckhof FM, Verschuere S, et al. Chronic cigarette smoke exposure induces microbial and inflammatory shifts and mucin changes in the murine gut. *Environ Microbiol* 2016; **18**: 1352–1363.
230. Verschuere S, Bracke KR, Demoor T, et al. Cigarette smoking alters epithelial apoptosis and immune composition in murine GALT. *Lab Invest J Tech Method Pathol* 2011; **91**: 1056–1067.
231. Hammadi M, Adi M, John R, et al. Dysregulation of gastric H, K-ATPase by cigarette smoke extract. *World J Gastroenterol* 2009; **15**: 4016–4022.
232. Sapkota AR, Berger S and Vogel TM. Human pathogens abundant in the bacterial metagenome of cigarettes. *Environ Health Perspect* 2010; **118**: 351–356.
233. Benjamin JL, Hedin CR, Koutsoumpas A, et al. Smokers with active Crohn's disease have a clinically relevant dysbiosis of the gastrointestinal microbiota. *Inflam Bowel Dise* 2012; **18**: 1092–1100.
234. Opstelten JL, Plassais J, van Mil SW, et al. Gut microbial diversity is reduced in smokers with Crohn's disease. *Inflam Bowel Dise* 2016; **22**: 2070–2077.
235. Dethlefsen L, McFall-Ngai M and Relman DA. An ecological and evolutionary perspective on human–microbe mutualism and disease. *Nature* 2007; **449**: 811–818.
236. MacNee W. Pathogenesis of chronic obstructive pulmonary disease. *Proc Am Thor Soc* 2005; **2**: 258–266; discussion 90–91.
237. Taylor JD. COPD and the response of the lung to tobacco smoke exposure. *Pulm Pharm Therap* 2010; **23**: 376–383.
238. Barnes PJ and Celli BR. Systemic manifestations and comorbidities of COPD. *Eur Res J* 2009; **33**: 1165–1185.
239. Celli BR and Barnes PJ. Exacerbations of chronic obstructive pulmonary disease. *Eur Res J* 2007; **29**: 1224–1238.
240. Charlson ES, Bittinger K, Haas AR, et al. Topographical continuity of bacterial populations in the healthy human respiratory tract. *Am J Res Crit Care Med* 2011; **184**: 957–963.
241. Cabrera-Rubio R, Garcia-Nunez M, Seto L, et al. Microbiome diversity in the bronchial tracts of patients with chronic obstructive pulmonary disease. *J Clin Microbiol* 2012; **50**: 3562–3568.
242. van der Gast CJ, Walker AW, Stressmann FA, et al. Partitioning core and satellite taxa from within cystic fibrosis lung bacterial communities. *ISME J* 2011; **5**: 780–791.
243. Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. *PLOS One* 2010; **5**: e8578.
244. Erb-Downward JR, Thompson DL, Han MK, et al. Analysis of the lung microbiome in the "healthy" smoker and in COPD. *PLOS One* 2011; **6**: e16384.
245. Han MK, Huang YJ, Lipuma JJ, et al. Significance of the microbiome in obstructive lung disease. *Thorax* 2012; **67**: 456–463.
246. Soler N, Torres A, Ewig S, et al. Bronchial microbial patterns in severe exacerbations of chronic obstructive pulmonary disease (COPD) requiring mechanical ventilation. *Am J Res Crit Care Med* 1998; **157**: 1498–1505.
247. Miravitlles M, Espinosa C, Fernandez-Laso E, et al. Relationship between bacterial flora in sputum and functional impairment in patients with acute exacerbations of COPD. Study group of bacterial infection in COPD. *Chest* 1999; **116**: 40–46.
248. Huang YJ, Kim E, Cox MJ, et al. A persistent and diverse airway microbiota present during chronic obstructive pulmonary disease exacerbations. *Omics J Integrat Biol* 2010; **14**: 9–59.
249. Pragman AA, Kim HB, Reilly CS, et al. The lung microbiome in moderate and severe chronic obstructive pulmonary disease. *PLOS One* 2012; **7**: e47305.
250. Cameron SJ, Lewis KE, Huws SA, et al. Metagenomic sequencing of the chronic obstructive pulmonary disease upper bronchial tract microbiome reveals functional changes associated with disease severity. *PLOS One* 2016; **11**: e0149095.
251. Barnes PJ. Mediators of chronic obstructive pulmonary disease. *Pharm Rev* 2004; **56**: 515–548.
252. Cosio MG, Saetta M and Agusti A. Immunologic aspects of chronic obstructive pulmonary disease. *New Engl J Med* 2009; **360**: 2445–2454.
253. Lim MY, Yoon HS, Rho M, et al. Analysis of the association between host genetics, smoking, and sputum microbiota in healthy humans. *Sci Rep* 2016; **6**: 23745.
254. Jungnickel C, Wonnenberg B, Karabiber O, et al. Cigarette smoke-induced disruption of pulmonary barrier and bacterial translocation drive tumor-associated inflammation and growth. *Am J Physiol Lung Cell Mole Physiol* 2015; **309**: L605–L613.
255. Mian MF, Lauzon NM, Stampfli MR, et al. Impairment of human NK cell cytotoxic activity and cytokine release by cigarette smoke. *J Leuk Biol* 2008; **83**: 774–784.
256. Morimoto K, Takeshita T, Nanno M, et al. Modulation of natural killer cell activity by supplementation of fermented milk containing Lactobacillus casei in habitual smokers. *Prevent Med* 2005; **40**: 589–594.
257. Huang YJ, Sethi S, Murphy T, et al. Airway microbiome dynamics in exacerbations of chronic obstructive pulmonary disease. *J Clin Microbiol* 2014; **52**: 2813–2823.
258. Rylance J, Kankwatira A, Nelson DE, et al. Household air pollution and the lung microbiome of healthy adults in Malawi: a cross-sectional study. *BMC Microbiol* 2016; **16**: 182.

259. Pihlstrom BL, Michalowicz BS and Johnson NW. Periodontal diseases. *Lancet* 2005; **366**: 1809–1820.
260. Tumolo AT. Effects of periodontitis. *J Am Dent Assoc* 2013; **144**: 1100.
261. Petersen PE and Ogawa H. The global burden of periodontal disease: towards integration with chronic disease prevention and control. *Periodontol 2000* 2012; **60**: 15–39.
262. Abusleme L, Dupuy AK, Dutzan N, et al. The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. *ISME J* 2013; **7**: 1016–1025.
263. Griffen AL, Beall CJ, Campbell JH, et al. Distinct and complex bacterial profiles in human periodontitis and health revealed by 16 S pyrosequencing. *ISME J* 2012; **6**: 1176–1185.
264. Costalonga M and Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. *Immunol Lett* 2014; **162**: 22–38.
265. Fabian TK, Fejerdy P and Csermely P. Salivary genomics, transcriptomics and proteomics: the emerging concept of the oral ecosystem and their use in the early diagnosis of cancer and other diseases. *Curr Genomics* 2008; **9**: 11–21.
266. Jenkinson HF and Lamont RJ. Oral microbial communities in sickness and in health. *Trends Microbiol* 2005; **13**: 589–595.
267. Cho I and Blaser MJ. The human microbiome: at the interface of health and disease. *Nature Rev Genet* 2012; **13**: 260–270.
268. Madupu R, Szpakowski S and Nelson KE. Microbiome in human health and disease. *Sci Prog* 2013; **96**: 153–170.
269. Pflughoeft KJ and Versalovic J. Human microbiome in health and disease. *Annu Rev Pathol* 2012; **7**: 99–122.
270. Mager DL, Ximenez-Fyvie LA, Haffajee AD, et al. Distribution of selected bacterial species on intraoral surfaces. *J Clin Periodontol* 2003; **30**: 644–654.
271. Marsh PD. Dental plaque as a biofilm and a microbial community - implications for health and disease. *BMC Oral Health* 2006; **6**(Suppl 1): S14.
272. Wade WG. The oral microbiome in health and disease. *Pharmacol Res* 2013; **69**: 137–143.
273. Socransky SS and Haffajee AD. Periodontal microbial ecology. *Periodontol 2000* 2005; **38**: 135–187.
274. Socransky SS, Haffajee AD, Cugini MA, et al. Microbial complexes in subgingival plaque. *J Clin Periodontol* 1998; **25**: 134–144.
275. Dahlen G and Leonhardt A. A new checkerboard panel for testing bacterial markers in periodontal disease. *Oral Microbiol Immunol* 2006; **21**: 6–11.
276. Kumar PS, Griffen AL, Barton JA, et al. New bacterial species associated with chronic periodontitis. *J Dent Res* 2003; **82**: 338–344.
277. Paster BJ, Olsen I, Aas JA, et al. The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Periodontol 2000* 2006; **42**: 80–87.
278. Kato I, Vasquez A, Moyerbrailean G, et al. Nutritional correlates of human oral microbiome. *J Am College Nutr* 2017; **36**: 88–98.
279. Goodson JM, Hartman ML, Shi P, et al. The salivary microbiome is altered in the presence of a high salivary glucose concentration. *PLOS One* 2017; **12**: e0170437.
280. Ahn J, Chen CY and Hayes RB. Oral microbiome and oral and gastrointestinal cancer risk. *Cancer Cause Control CCC* 2012; **23**: 399–404.
281. Thomas AM, Gleber-Netto FO, Fernandes GR, et al. Alcohol and tobacco consumption affects bacterial richness in oral cavity mucosa biofilms. *BMC Microbiol* 2014; **14**: 250.
282. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71–96.
283. Gomes SC, Piccinin FB, Oppermann RV, et al. Periodontal status in smokers and never-smokers: clinical findings and real-time polymerase chain reaction quantification of putative periodontal pathogens. *J Periodontol* 2006; **77**: 1483–1490.
284. Bergstrom J. Tobacco smoking and chronic destructive periodontal disease. *Odontology* 2004; **92**: 1–8.
285. Ge X, Rodriguez R, Trinh M, et al. Oral microbiome of deep and shallow dental pockets in chronic periodontitis. *PLOS One* 2013; **8**: e65520.
286. Belstrom D, Holmstrup P, Nielsen CH, et al. Bacterial profiles of saliva in relation to diet, lifestyle factors, and socioeconomic status. *J Oral Microbiol* 2014; **6**.
287. Kumar PS, Matthews CR, Joshi V, et al. Tobacco smoking affects bacterial acquisition and colonization in oral biofilms. *Infect Immun* 2011; **79**: 4730–4738.
288. Mason MR, Preshaw PM, Nagaraja HN, et al. The subgingival microbiome of clinically healthy current and never smokers. *ISME J* 2015; **9**: 268–272.
289. Brook I and Gober AE. Recovery of potential pathogens and interfering bacteria in the nasopharynx of smokers and nonsmokers. *Chest* 2005; **127**: 2072–2075.
290. Delima SL, McBride RK, Preshaw PM, et al. Response of subgingival bacteria to smoking cessation. *J Clin Microbiol* 2010; **48**: 2344–2349.
291. Macgregor ID.Effects of smoking on oral ecology. A review of the literature. *Clin Prev Dent* 1989; **11**: 3–7.
292. Parvinen T. Stimulated salivary flow rate, pH and lactobacillus and yeast concentrations in persons with different types of dentition. *Scand J Dent Res* 1984; **92**: 412–418.
293. Sopori M. Effects of cigarette smoke on the immune system. *Nature Rev Immunol* 2002; **2**: 372–377.
294. Shchipkova AY, Nagaraja HN and Kumar PS. Subgingival microbial profiles of smokers with periodontitis. *J Dent Res* 2010; **89**: 1247–1253.
295. Stingu CS, Eschrich K, Rodloff AC, et al. Periodontitis is associated with a loss of colonization by Streptococcus sanguinis. *J Med Microbiol* 2008; **57**: 495–499.
296. Van Hoogmoed CG, Geertsema-Doornbusch GI, Teughels W, et al. Reduction of periodontal pathogens adhesion by antagonistic strains. *Oral Microbiol Immunol* 2008; **23**: 43–48.
297. Fullmer SC, Preshaw PM, Heasman PA, et al. Smoking cessation alters subgingival microbial recolonization. *J Dent Res* 2009; **88**: 524–528.

298. Wu J, Peters BA, Dominianni C, et al. Cigarette smoking and the oral microbiome in a large study of American adults. *The ISME Journal* 2016; **10**: 2435–2446.
299. Sukumar S, Roberts AP, Martin FE, et al. Metagenomic insights into transferable antibiotic resistance in oral bacteria. *J Dent Res* 2016; **95**: 969–976.
300. Zaura E, Brandt BW, Teixeira de Mattos MJ, et al. Same exposure but two radically different responses to antibiotics: resilience of the salivary microbiome versus long-term microbial shifts in feces. *MBio* 2015; **6**: e01693–15.
301. Caglar E, Kavaloglu SC, Kuscu OO, et al. Effect of chewing gums containing xylitol or probiotic bacteria on salivary mutans streptococci and lactobacilli. *Clin Oral Invest* 2007; **11**: 425–429.
302. Wescombe PA, Heng NC, Burton JP, et al. Streptococcal bacteriocins and the case for Streptococcus salivarius as model oral probiotics. *Future Microbiol* 2009; **4**: 819–835.
303. Poole AC, Pischel L, Ley C, et al. Crossover control study of the effect of personal care products containing triclosan on the microbiome. *mSphere* 2016; **1**: e00056–15.
304. Noto C, Ota VK, Santoro ML, et al. Depression, cytokine, and cytokine by treatment interactions modulate gene expression in antipsychotic naïve first episode psychosis. *Mole Neurobiol* 2016; **53**: 5701–5709.
305. Kapczinski F, Dal-Pizzol F, Teixeira AL, et al. Peripheral biomarkers and illness activity in bipolar disorder. *J Psych Res* 2011; **45**: 156–161.
306. Leonard B and Maes M. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. *Neurosci Bio Rev* 2012; **36**: 764–785.
307. Berk M, Williams LJ, Jacka FN, et al. So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med* 2013; **11**: 200.
308. Rogers GB, Keating DJ, Young RL, et al. From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways. *Mole Psyc* 2016; **21**: 738–748.
309. Sampson TR and Mazmanian SK. Control of brain development, function, and behavior by the microbiome. *Cell Host Micro* 2015; **17**: 565–576.
310. Hyland NP and Cryan JF. Microbe-host interactions: influence of the gut microbiota on the enteric nervous system. *Develop Biol* 2016; **417**: 182–187.
311. Dickerson F, Severance E and Yolken R. The microbiome, immunity, and schizophrenia and bipolar disorder. *Brain Behav Immun* 2017; **62**: 46–52.
312. Sudo N. Role of microbiome in regulating the HPA axis and its relevance to allergy. *Chem Immunol Allerg* 2012; **98**: 163–175.
313. Lee HU, McPherson ZE, Tan B, et al. Host-microbiome interactions: the aryl hydrocarbon receptor and the central nervous system. *J Mole Med* 2017; **95**: 29–39.
314. Ray K. Gut microbiota: microbial metabolites feed into the gut-brain-gut circuit during host metabolism. *Nature Rev Gastroenterol Hepatol* 2014; **11**: 76.
315. De Vadder F, Kovatcheva-Datchary P, Goncalves D, et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* 2014; **156**: 84–96.
316. Wang Y and Kasper LH. The role of microbiome in central nervous system disorders. *Brain Behav Immun* 2014; **38**: 1–12.
317. Slyepchenko A, Maes M, Jacka FN, et al. Gut microbiota, bacterial translocation, and interactions with diet: pathophysiological links between major depressive disorder and non-communicable medical comorbidities. *Psychother Psychosom* 2017; **86**: 31–46.
318. Cepeda MS, Katz EG and Blacketer C. Microbiome-gut-brain axis: probiotics and their association with depression. *J Neuropsy Clin Neurosci* 2017; **29**: 39–44.
319. Nagy-Szakal D, Williams BL, Mishra N, et al. Fecal metagenomic profiles in subgroups of patients with myalgic encephalomyelitis/chronic fatigue syndrome. *Microbiome* 2017; **5**: 44.
320. Hsiao EY. Gastrointestinal issues in autism spectrum disorder. *Harvard Rev Psych* 2014; **22**: 104–111.
321. Rosenfeld CS. Microbiome disturbances and autism spectrum disorders. *Drug Metab Dis Biol Fate Chem* 2015; **43**: 1557–1571.
322. Mezzelani A, Landini M, Facchiano F, et al. Environment, dysbiosis, immunity and sex-specific susceptibility: a translational hypothesis for regressive autism pathogenesis. *Nutr Neurosci* 2015; **18**: 145–161.
323. Finegold SM, Dowd SE, Gontcharova V, et al. Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe* 2010; **16**: 444–453.
324. Finegold SM, Downes J and Summanen PH. Microbiology of regressive autism. *Anaerobe* 2012; **18**: 260–262.
325. Song Y, Liu C and Finegold SM. Real-time PCR quantitation of clostridia in feces of autistic children. *Appl Environ Microbiol* 2004; **70**: 6459–6465.
326. Strati F, Cavalieri D, Albanese D, et al. New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome* 2017; **5**: 24.
327. Kang DW, Adams JB, Gregory AC, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome* 2017; **5**: 10.
328. Strati F, Cavalieri D, Albanese D, et al. Altered gut microbiota in Rett syndrome. *Microbiome* 2016; **4**: 41.
329. de Lau LML and Breteler MMB. Epidemiology of Parkinson's disease. *Lancet Neurol* 2006; **5**: 525–535.
330. Braak H, de Vos RA, Bohl J, et al. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neurosci Lett* 2006; **396**: 67–72.
331. Scheperjans F, Aho V, Pereira PA, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Move Dis Off J Move Dis Soc* 2015; **30**: 350–358.
332. Derkinderen P, Shannon KM and Brundin P. Gut feelings about smoking and coffee in Parkinson's disease. *Move Dis Off J Move Dis Soc* 2014; **29**: 976–979.

333. Erny D and Prinz M. Microbiology: gut microbes augment neurodegeneration. *Nature* 2017; **544**: 304–305.
334. Sharon G, Sampson TR, Geschwind DH, et al. The central nervous system and the gut microbiome. *Cell* 2016; **167**: 915–932.
335. Sampson TR, Debelius JW, Thron T, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell* 2016; **167**: 1469–1480 e12.
336. Harach T, Marungruang N, Duthilleul N, et al. Reduction of abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. *Sci Rep* 2017; **7**: 41802.
337. Winek K, Engel O, Koduah P, et al. Depletion of cultivatable gut microbiota by broad-spectrum antibiotic pretreatment worsens outcome after murine stroke. *Stroke* 2016; **47**: 1354–1363.
338. Benakis C, Brea D, Caballero S, et al. Commensal microbiota affects ischemic stroke outcome by regulating intestinal gammadelta T cells. *Nature Med* 2016; **22**: 516–523.
339. Winek K, Dirnagl U and Meisel A. The gut microbiome as therapeutic target in central nervous system diseases: implications for stroke. *Neurotherapeutics* 2016; **13**: 762–774.
340. Singh V, Roth S, Llovera G, et al. Microbiota dysbiosis controls the neuroinflammatory response after stroke. *J Neurosci Off J Soc Neurosci* 2016; **36**: 7428–7440.
341. Tang AT, Choi JP, Kotzin JJ, et al. Endothelial TLR4 and the microbiome drive cerebral cavernous malformations. *Nature* 2017; **545**: 305–310.
342. Daulatzai MA. Chronic functional bowel syndrome enhances gut-brain axis dysfunction, neuroinflammation, cognitive impairment, and vulnerability to dementia. *Neurochem Res* 2014; **39**: 624–644.
343. Dinan TG and Cryan JF. The microbiome-gut-brain axis in health and disease. *Gastroenterol Clin North Am* 2017; **46**: 77–89.
344. Belkaid Y and Segre JA. Dialogue between skin microbiota and immunity. *Science* 2014; **346**: 954–959.
345. Belizario JE and Napolitano M. Human microbiomes and their roles in dysbiosis, common diseases, and novel therapeutic approaches. *Front Microbiol* 2015; **6**: 1050.
346. Stacy A, McNally L, Darch SE, et al. The biogeography of polymicrobial infection. *Nature Rev Microbiol* 2016; **14**: 93–105.
347. Donlan RM and Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev* 2002; **15**: 167–193.
348. Oh J, Byrd AL, Park M, et al. Temporal stability of the human skin microbiome. *Cell* 2016; **165**: 854–866.
349. Smeekens SP, Huttenhower C, Riza A, et al. Skin microbiome imbalance in patients with STAT1/STAT3 defects impairs innate host defense responses. *J Innate Immun* 2014; **6**: 253–262.
350. Hannigan GD and Grice EA. Microbial ecology of the skin in the era of metagenomics and molecular microbiology. *Cold Spring Harbor Perspect Med* 2013; **3**: a015362.
351. Lai Y, Di Nardo A, Nakatsuji T, et al. Commensal bacteria regulate TLR3-dependent inflammation following skin injury. *Nature Med* 2009; **15**: 1377–1382.
352. Brandwein M, Steinberg D and Meshner S. Microbial biofilms and the human skin microbiome. *NPJ Biofilm Micro* 2016; **2**: 3.
353. Kong HH, Oh J, Deming C, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res* 2012; **22**: 850–859.
354. O'Neill CA, Monteleone G, McLaughlin JT, et al. The gut-skin axis in health and disease: a paradigm with therapeutic implications. *BioEssays* 2016; **38**: 1167–1176.
355. Jahns AC, Lundskog B, Ganceviciene R, et al. An increased incidence of propionibacterium acnes biofilms in acne vulgaris: a case–control study. *British J Dermatol* 2012; **167**: 50–58.
356. McGinley KJ, Webster GF, Ruggieri MR, et al. Regional variations in density of cutaneous propionibacteria: correlation of propionibacterium acnes populations with sebaceous secretion. *J Clin Microbiol* 1980; **12**: 672–675.
357. Smith TM, Gilliland K, Clawson GA, et al. IGF-1 induces SREBP-1 expression and lipogenesis in SEB-1 sebocytes via activation of the phosphoinositide 3-kinase/akt pathway. *J Invest Dermatol* 2008; **128**: 1286–1293.
358. Fabbrocini G, Izzo R, Faggiano A, et al. Low glycaemic diet and metformin therapy: a new approach in male subjects with acne resistant to common treatments. *Clin Exp Dermatol* 2016; **41**: 38–42.
359. Bockmühl D, Jassoy C, Nieveler S, et al. Prebiotic cosmetics: an alternative to antibacterial products. *Int J Cosmet Sci* 2007; **29**: 63–64.
360. Muizzuddin N, Maher W, Sullivan M, et al. Physiological effect of a probiotic on skin. *J Cosmet Sci* 2012; **63**: 385–395.
361. Kang BS, Seo JG, Lee GS, et al. Antimicrobial activity of enterocins from Enterococcus faecalis SL-5 against Propionibacterium acnes, the causative agent in acne vulgaris, and its therapeutic effect. *J Microbiol* 2009; **47**: 101–109.
362. Rutten EP, Lenaerts K, Buurman WA, et al. Disturbed intestinal integrity in patients with COPD: effects of activities of daily living. *Chest* 2014; **145**: 245–252.
363. Roussos A, Koursarakos P, Patsopoulos D, et al. Increased prevalence of irritable bowel syndrome in patients with bronchial asthma. *Res Med* 2003; **97**: 75–79.
364. Turner-Warwick M. Fibrosing alveolitis and chronic liver disease. *Quarter J Med* 1968; **37**: 133–149.
365. Kraft SC, Earle RH, Roesler M, et al. Unexplained bronchopulmonary disease with inflammatory bowel disease. *Arch Int Med* 1976; **136**: 454–459.
366. Bernstein CN, Blanchard JF, Rawsthorne P, et al. The prevalence of extraintestinal diseases in inflammatory bowel disease: a population-based study. *Am J Gastroenterol* 2001; **96**: 1116–1122.
367. Rothfuss KS, Stange EF and Herrlinger KR. Extraintestinal manifestations and complications in inflammatory bowel diseases. *World J Gastroenterol* 2006; **12**: 4819–4831.

368. Mohamed-Hussein AA, Mohamed NA and Ibrahim ME. Changes in pulmonary function in patients with ulcerative colitis. *Res Med* 2007; **101**: 977–982.
369. Kuzela L, Vavrecka A, Prikazska M, et al. Pulmonary complications in patients with inflammatory bowel disease. *Hepato Gastroenterol* 1999; **46**: 1714–1719.
370. Duricova D, Pedersen N, Elkjaer M, et al. Overall and cause-specific mortality in Crohn's disease: a meta-analysis of population-based studies. *Inflam Bowel Dise* 2010; **16**: 347–353.
371. Ekbom A, Brandt L, Granath F, et al. Increased risk of both ulcerative colitis and Crohn's disease in a population suffering from COPD. *Lung* 2008; **186**: 167–172.
372. Jess T, Loftus EV Jr, Harmsen WS, et al. Survival and cause specific mortality in patients with inflammatory bowel disease: a long term outcome study in Olmsted County, Minnesota, 1940-2004. *Gut* 2006; **55**: 1248–1254.
373. Schuijt TJ, Lankelma JM, Scicluna BP, et al. The gut microbiota plays a protective role in the host defence against pneumococcal pneumonia. *Gut* 2016; **65**: 575–583.
374. Samuelson DR, Welsh DA and Shellito JE. Regulation of lung immunity and host defense by the intestinal microbiota. *Front Microbiol* 2015; **6**: 1085.
375. Trompette A, Gollwitzer ES, Yadava K, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nature Med* 2014; **20**: 159–166.
376. Marsland BJ, Trompette A and Gollwitzer ES. The gut-lung axis in respiratory disease. *Annal Am Thor Soc* 2015; **12**(Suppl 2): S150–S156.
377. Dickson RP, Singer BH, Newstead MW, et al. Enrichment of the lung microbiome with gut bacteria in sepsis and the acute respiratory distress syndrome. *Nature Microbiol* 2016; **1**: 16113.
378. Chakradhar S. A curious connection: teasing apart the link between gut microbes and lung disease. *Nature Med* 2017; **23**: 402–404.
379. Carabotti M, Scirocco A, Maselli MA, et al. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Annal Gastroenterol* 2015; **28**: 203–209.
380. Xiyue C, Shabnam E, Luoyun F, et al. Maintenance of gastrointestinal glucose homeostasis by the gut-brain axis. *Curr Prot Pept Sci* 2017; **18**: 541–547.
381. Migrenne S, Marsollier N, Cruciani-Guglielmacci C, et al. Importance of the gut–brain axis in the control of glucose homeostasis. *Curr Opin Pharmacol* 2006; **6**: 592–597.
382. Soty M, Gautier-Stein A, Rajas F, et al. Gut-brain glucose signaling in energy homeostasis. *Cell Metab* 2017; **25**: 1231–1242.
383. Rhee SH, Pothoulakis C and Mayer EA. Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nature Rev Gastroenterol Hepatol* 2009; **6**: 306–314.
384. Lai SW, Liao KF, Lin CL, et al. Irritable bowel syndrome correlates with increased risk of Parkinson's disease in Taiwan. *Eur J Epidemiol* 2014; **29**: 57–62.
385. Chen CH, Lin CL and Kao CH. Irritable bowel syndrome is associated with an increased risk of dementia: a nationwide population-based study. *PLOS One* 2016; **11**: e0144589.
386. Hu X, Wang T and Jin F. Alzheimer's disease and gut microbiota. *Sci Chin Life Sci* 2016; **59**: 1006–1023.
387. McPherson Z, Talley NJ, Walker MM, et al. Su1088 A novel predictive association between irritable bowel syndrome and glaucomatous optic neuropathy. *Gastroenterology* 2015; **148**: S–404.
388. Grenham S, Clarke G, Cryan JF, et al. Brain-gut-microbe communication in health and disease. *Front Physiol* 2011; **2**: 94.
389. El Aidy S, Dinan TG and Cryan JF. Gut Microbiota: the conductor in the orchestra of immune-neuroendocrine communication. *Clin Therap* 2015; **37**: 954–967.
390. Cryan JF and Dinan TG. More than a gut feeling: the microbiota regulates neurodevelopment and behavior. *Neuropsychopharmacology* 2015; **40**: 241–242.
391. Montiel-Castro AJ, González-Cervantes RM, Bravo-Ruiseco G, et al. The microbiota-gut-brain axis: neurobehavioral correlates, health and sociality. *Front Integ Neurosci* 2013; **7**: 70.
392. Ait-Belgnaoui A, Durand H, Cartier C, et al. Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. *Psychoneuroendocrinology* 2012; **37**: 1885–1895.
393. Marin IA, Goertz JE, Ren T, et al. Microbiota alteration is associated with the development of stress-induced despair behavior. *Sci Rep* 2017; **7**: 43859.
394. Sudo N, Chida Y, Aiba Y, et al. Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *J Physiol* 2004; **558**: 263–275.
395. Verdú EF, Bercik P, Verma-Gandhu M, et al. Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut* 2006; **55**: 182–190.
396. Bravo JA, Forsythe P, Chew MV, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc National Acad Sci* 2011; **108**: 16050–16055.
397. Wallace CJK and Milev R. The effects of probiotics on depressive symptoms in humans: a systematic review. *Annal General Psych* 2017; **16**: 14.
398. Svensson E, Horvath-Puho E, Thomsen RW, et al. Vagotomy and subsequent risk of Parkinson's disease. *Annal Neurol* 2015; **78**: 522–529.
399. Desbonnet L, Garrett L, Clarke G, et al. Effects of the probiotic bifidobacterium infantis in the maternal separation model of depression. *Neuroscience* 2010; **170**: 1179–1188.
400. Lyte M. Microbial endocrinology and the microbiota-gut-brain axis. In: Lyte M and Cryan JF (eds) *Microbial endocrinology: the microbiota-gut-brain axis in health and disease.* New York, NY: Springer New York, 2014, pp. 3–24.
401. Lyte M. Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. *PLOS Patho* 2013; **9**: e1003726.

402. Foster JA, Rinaman L and Cryan JF. Stress & the gut-brain axis: regulation by the microbiome. *Neurobiol Stress* 2017; in press.
403. VanHook AM. Bugs–blood–brain–β cell signaling axis. *Sci Signal* 2016; **9**: ec140–ec.
404. Trajkovski M and Wollheim CB. Physiology: microbial signals to the brain control weight. *Nature* 2016; **534**: 185–187.
405. Clark A and Mach N. Role of vitamin D in the hygiene hypothesis: the interplay between vitamin D, vitamin D receptors, gut microbiota, and immune response. *Front Immunol* 2016; **7**: 627.
406. Bowe WP and Logan AC. Acne vulgaris, probiotics and the gut-brain-skin axis - back to the future? *Gut Patho* 2011; **3**: 1.
407. Arck P, Handjiski B, Hagen E, et al. Is there a 'gut-brain-skin axis'? *Exp Dermatol* 2010; **19**: 401–405.
408. Henke C and Beissner F. Illustrations of visceral referred pain. "Head-less" Head's zones. *Schmerz* 2011; **25**: 132–136, 138–139.
409. Human Microbiome Project C. A framework for human microbiome research. *Nature* 2012; **486**: 215–221.
410. McDonald D, Birmingham A and Knight R. Context and the human microbiome. *Microbiome* 2015; **3**: 52.
411. Alekseyenko AV, Perez-Perez GI, De Souza A, et al. Community differentiation of the cutaneous microbiota in psoriasis. *Microbiome* 2013; **1**: 31.
412. Cotillard A, Kennedy SP, Kong LC, et al. Dietary intervention impact on gut microbial gene richness. *Nature* 2013; **500**: 585–588.
413. Lupp C, Robertson ML, Wickham ME, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. *Cell Host Micro* 2007; **2**: 204.
414. Smirnov KS, Maier TV, Walker A, et al. Challenges of metabolomics in human gut microbiota research. *Int J Med Microbiol* 2016; **306**: 266–279.
415. Vernocchi P, Del Chierico F and Putignani L. Gut microbiota profiling: metabolomics based approach to unravel compounds affecting human health. *Front Microbiol* 2016; **7**.