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Dysbiosis of the gut microbiota in disease

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There is growing evidence that dysbiosis of the gut microbiota is associated with the pathogenesis of both intestinal and extra-intestinal disorders. Intestinal disorders include inflammatory bowel disease, irritable bowel syndrome (IBS), and coeliac disease, while extra-intestinal disorders include allergy, asthma, metabolic syndrome, cardiovascular disease, and obesity.

In many of these conditions, the mechanisms leading to disease development involves the pivotal mutualistic relationship between the colonic microbiota, their metabolic products, and the host immune system. The establishment of a 'healthy' relationship early in life appears to be critical to maintaining intestinal homeostasis. Whilst we do not yet have a clear understanding of what constitutes a 'healthy' colonic microbiota, a picture is emerging from many recent studies identifying particular bacterial species associated with a healthy microbiota. In particular, the bacterial species residing within the mucus layer of the colon, either through direct contact with host cells, or through indirect communication via bacterial metabolites, may influence whether host cellular homeostasis is maintained or whether inflammatory mechanisms are triggered. In addition to inflammation, there is some evidence that perturbations in the gut microbiota is involved with the development of colorectal cancer. In this case, dysbiosis may not be the most important factor, rather the products of interaction between diet and the microbiome. High-protein diets are thought to result in the production of carcinogenic metabolites from the colonic microbiota that may result in the induction of neoplasia in the colonic epithelium.

Ever more sensitive metabolomics methodologies reveal a suite of small molecules produced in the microbiome which mimic or act as neurosignallers or neurotransmitters. Coupled with evidence that probiotic interventions may alter psychological endpoints in both humans and in rodent models, these data suggest that CNS-related co-morbidities frequently associated with GI disease may originate in the intestine as a result of microbial dysbiosis. This review outlines the current evidence showing the extent to which the gut microbiota contributes to the development of disease. Based on evidence to date, we can assess the potential to positively modulate the composition of the colonic microbiota and ameliorate disease activity through bacterial intervention.

Keywords: *Microbiome*; *short-chain fatty acids*; *gut health*; *colonic metabolome*; *gut–brain–axis*; *inflammation*

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The human intestinal microbiota is made up of trillions of microorganisms most of which are of bacterial and viral origin that are considered to be non-pathogenic (1, 2). The microbiota functions in tandem with the host's defences and the immune system to protect against pathogen colonisation and invasion. It also performs an essential metabolic function, acting as a source of essential nutrients and vitamins and aiding in the extraction of energy and nutrients, such as short-chain fatty acids (SCFA) and amino acids, from food. Ultimately, the host depends on its intestinal microbiota for a number of vital functions and thus the intestinal microbiota may contribute to health. It is, however, difficult to describe the precise impact of the intestinal microbiota on human health and the involvement in human disease.

Alterations in the microbiota can result from exposure to various environmental factors, including diet, toxins, drugs, and pathogens. Of these, enteric pathogens have the greatest potential to cause microbial dysbiosis as seen in experimental animal models, where foodborne viral

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pathogens can trigger both local and systemic inflammation altering the composition of the microbiota and barrier function, as a mechanism for developing autoimmunity, as shown in type 1 diabetes and T-cell mediated destruction of insulin-producing pancreatic β-cells (3–5). Documenting dysbiosis has traditionally relied on classical microbiological techniques and the ability to culture pure isolates for identification and classification, which is necessarily limited to 'culturable' microorganisms. The advent of high-throughput DNA based pyrosequencing technology to classify bacteria and archaea according to individual 16S rRNA sequences directly from human samples (usually faecal in origin) with no need for culturing now provides a rapid and detailed means of profiling complex communities of microorganisms. Since the first application of this technology, it has been shown that the composition of the intestinal microbiota varies substantially amongst individuals (6). This can in part be explained by genetic differences amongst hosts with positive relationships between similarity in dominant faecal microbial communities and genetic relatedness of the host being observed (7). At the phylum level, *Bacteroidetes* and *Firmicutes* dominate with *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, *Spriochaetes*, *Verrucomicrobia*, and *Lentisphaerae* also present (8, 9). Using metagenomic analysis to investigate the functional capability of the intestinal microbiota genome (microbiome), it has been shown that almost 40% of the microbial genes present in each individual are shared with at least half the general population providing evidence for the existence of a functional core, or core microbiome (10). The main approach to studying changes in composition of the intestinal microbiota in relation to disease has relied primarily on the phylogenetic characterisation of the microbiota of diseased individuals in comparison with apparently healthy individuals. However, since there are substantial inter-individual and intra-individual variations in addition to age-related changes in the composition of the intestinal microbiota, it is difficult to establish precise relations between human health and the presence and relative abundance of specific microbial communities. It may be possible in the future to use specific changes in compositional diversity, or even functional diversity, as biomarkers for health or specific diseases. It is important to note, however, that it is questionable whether changes in phylogenetic composition are a cause or consequence of a given disease.

Arguably the strongest evidence of the direct involvement in or requirement for the intestinal microbiota in disease pathogenesis comes from studies using germ-free mouse models of human autoimmune disease in which the requirement for exposure to and colonisation by environmental microorganisms on disease initiation and progression can be determined (Table 1). In most but not all of the disease models, the severity and/or incidence of disease is reduced under germ-free conditions consistent with the microbiota being a 'trigger' for disease progression. However, attempts to identify the members of the 'pathogenic' microbiota (pathobionts) that can reproduce the effect of the microbiota as a whole have to date failed.

It is perhaps not surprising that intestinal dysbiosis is most often associated with GI-related diseases in which alterations in the interaction of the host (immune system) with lumen-derived stimuli and antigens initiate and/or perpetuate uncontrolled inflammation in the intestinal mucosa, and in some cases beyond.

Metabolomic impact of the interaction between diet and the microbiome on human health

Food components that escape digestion in the small intestine, as well as endogenous compounds such as digestive enzymes and shed epithelial cells and associated mucus, enter the colon and become available for fermentation by the colonic microbiota. Bacterial conversion of these compounds results in a wide variety of metabolites that are in close contact with host's cells. In this way, these metabolites can affect the metabolic phenotype of the host and influence the risk of disease (11).

Undigested carbohydrates and proteins constitute the major substrates at the disposal of the microbiota. Fermentation of these substrates results in the production of a range of metabolites including SCFA, branched chain fatty acids, ammonia, amines, phenolic compounds, and gases, including hydrogen, methane, and hydrogen sulphide. In addition, the intestinal microbiota is involved in the production of vitamins, the activation or inactivation of bioactive food components such as isoflavanoids

Table 1. The intestinal microbiota and autoimmunity

Disease	Microbiota status	Disease impact
Inflammatory bowel disease	Germ free, antibiotics or probiotics	No disease or reduced severity
Spontaneous arthritis	Germ free	No disease
Autoimmune arthritis	Germ free	No disease
Autoimmune encephalomyelitis	Germ-free	Weak severity
Systemic lupus erythematosus	Germ free	No change
Type 1 diabetes	Germ free	No disease
Spontaneous ankylosing enteropathy	Germ free or probiotics	No disease

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and plant lignans, the conversion of prodrugs to their bioactive forms, and the transformation of bile acids and xenobiotics (12, 13).

Mechanistic effect of metabolites on host health

The SCFA acetate, propionate, and butyrate are the major anions in the colon and are mainly produced by bacterial fermentation of undigested carbohydrates. Up to 95% of produced SCFA are readily absorbed by the colonocytes for use as energy substrates. As colonocytes derive up to 60–70% of their energy needs from SCFA oxidation (14), SCFA provide about 10% of the daily caloric requirements in humans (15). The fraction that is not consumed by the colonocytes is transported across the basolateral membrane to the liver via the portal blood stream. Besides their local role as energy substrates within the colon, SCFA act as signalling molecules involved in systemic lipid metabolism and glucose/insulin regulation (16). These effects are, at least partly, mediated through interaction with two specific G-protein-coupled receptors – GPR41 and GPR43 (later renamed to FFAR3 and FFAR2, respectively) (17) that are widely distributed throughout the human body, including the small intestine and colon (18). Within the cells, SCFA can act as inhibitors of histone deacetylases to induce hyperacetylation of histones which affects gene expression and results in anti-inflammatory properties, induction of growth arrest, and apoptosis (19). However, an integrated understanding of the impact of SCFA on host metabolism requires more quantitative data on fluxes of SCFA in different body compartments. Due to its inaccessibility, little information is available on *in vivo* production rates of SCFA and kinetics of absorption in the large intestine.

Plant polyphenols have been associated with health benefits including anti-inflammatory, antiestrogenic, cardioprotective, chemoprotective, and neuroprotective effects (10). However, the mechanistic evidence *in vivo* is not yet fully understood. The majority of plant polyphenols require metabolic transformation (including deglycation and hydrolysis) to render them biologically active. Within the colon, they are broken down by the microbiota to a variety of small phenolic compounds of which the physiological relevance is not well known (20). In addition, recent studies indicate a selective modulation of the microbiota composition after polyphenol consumption (21). For instance, consumption of red wine polyphenols significantly increases *Enterococcus*, *Prevotella*, *Bacteroides*, *Bifidobacterium*, *Bacteroides uniformis*, *Eggerthella lenta*, and *Blautia coccoides-Eubacterium rectale* numbers in healthy humans (22). Therefore, the health benefits associated with polyphenols should not only be attributed to their bioactive metabolites but also to the modulation of the intestinal microbiota.

Other products of bacterial metabolism have been associated with diseases affecting the liver, cardiovascular system and the kidneys.

In recent years, the gut–liver axis and the impact of the intestinal microbiota on liver function has gained increasing attention. The liver is extensively exposed to metabolites produced at intracolonic fermentation as it receives 70% of its blood supply from the intestine through the portal vein (23). In the early 1980s, a possible causative role of the microbiota in the development of non-alcoholic fatty liver disease (NAFLD) was suggested. In patients that underwent intestinal bypass surgery, hepatic steatosis developed in parallel with bacterial overgrowth. Interestingly, the steatosis regressed after treatment with the antibiotic, metronidazole (24). One of the mechanisms relating the microbiota to NAFLD is bacterial metabolism of choline. In mice susceptible to NAFLD and fed a high-fat diet, choline was increasingly metabolised to methylamines resulting in high urinary excretion of dimethylamine (DMA) and trimethylamine (TMA) and correspondingly low levels of serum phosphatidylcholine (25).

Due to conversion of choline into methylamines by the microbiota, the bioavailability of choline is reduced, resulting in the inability to synthesise phosphatidylcholine with subsequent accumulation of triglycerides in the liver. This mimics choline-deficient diets which have been consistently associated with hepatic steatosis (26).

The bacterial metabolite TMA is consequently absorbed by the intestinal mucosa and transported to the liver via the portal vein where it is oxidised to trimethylamine *N*-oxide (TMAO) by the flavin mono-oxygenase (FMO) enzyme complex. In a metabolomics study profiling the plasma of patients undergoing elective cardiac evaluation, TMAO was identified and confirmed as a predictor of cardiovascular disease (CVD). Subsequent mice experiments confirmed the obligate role of the intestinal microbiota in the formation of TMAO and indicated the pro-atherogenic nature of TMAO by augmentation of cholesterol loaded macrophages and foam cell formation (27). Similarly, metabolism by the intestinal microbiota of dietary L-carnitine, a TMA abundant in red meat, also produced TMAO and accelerated atherosclerosis in mice (28).

Dysbiosis in disease

Dysbiosis and GI-tract-related disorders

Inflammatory bowel disease

Crohn's disease (CD) and ulcerative colitis (UC) are the most prevalent forms of inflammatory bowel disease (IBD), characterised by chronic relapsing inflammation affecting the intestinal mucosa. Although the aetiology of both diseases is unknown, there is increasing evidence that intestinal microbial dysbiosis has a role in the pathogenesis of IBD (29). Overall, patients exhibit a decrease in microbial population and functional diversity and stability of

their intestinal microbiota with decreases in specific *Firmicutes* and a concomitant increase in *Bacteroidetes* and facultative anaerobes such as *Enterobacteriaceae* (30). Significant differences in the microbiota of CD versus UC patients have also been noted (31, 32). In CD, the predominant dysbiosis has been described to be associated with five bacterial species amongst which alterations in the abundance of *Faecalibacterium prausnitzii* is associated with the prolongation of disease remission (32, 33), with this bacterium having a therapeutic effect in experimental models of colitis (34). Conversely, adherent-invasive *E. coli* and *Mycobacterium paratuberculosis* have been implicated in CD pathogenesis although a causal relationship is yet to be demonstrated (35, 36). Indeed, up to now, it is still unclear whether intestinal microbial dysbiosis is a direct cause for the inflammation in IBD, or merely the result of a disturbed environment in the GI-tract. One study that has sought to determine the status of the microbiota in early-diagnosis CD cases is that of Gevers et al. (Cell Host Microbe 2014) (37). This study analysed the microbiota of a large cohort of newly diagnosed paediatric CD patients and found clear differences in bacterial populations between CD and healthy control patients. CD patients had increased abundance of *Enterobacteriaceae*, *Pasteurellaceae*, *Veillonellaceae*, and *Fusobacteriaceae*, and decreased abundance in *Erysipelotrichales*, *Bacteroidales*, and *Clostridiales* compared to healthy control patients. Interestingly, these differences were only revealed when analysing mucosal samples (rather than faecal samples), indicating that the bacteria resident in the mucosal layer may be more significant for disease aetiology.

Dysbiosis and other GI-tract disorders

In addition to IBD, metabolic disorders, obesity, and type 2 diabetes (T2D), the intestinal microbiota has also been implicated in several other (chronic) GI-related diseases and disorders, such as irritable bowel syndrome (IBS), coeliac disease, and colorectal cancer (CRC). In IBS, changes in microbiota composition have been described in the different subtypes of disease compared to healthy individuals (38, 39) although the changes are not uniform (40). Coeliac disease and CRC have also been associated with alterations in microbiota composition with increased diversity and richness observed compared to control subjects (41, 42). In all of these diseases, however, no consistent pattern of microbiota changes has yet been observed. In the case of coeliac disease, however, a recent study has shed light on the interaction between host genetics and microbiota composition in relation to disease development. Expression of the leukocyte antigen DQ2 is a strong risk factor for the development of coeliac disease. Children with this haplotype have an altered microbiota composition (compared to non-HLA DQ2 individuals) prior to clinically apparent disease (43). Coeliac disease results from CD4 T-cell reactivity to dietary gliadin, with some bacterial species being able to digest gliadin and perhaps therefore reduce the immunopathogenicity of ingested gliadin.

Dysbiosis in systemic disease

Metabolic disorders

An increase in the relative abundance of *Firmicutes* and a reduction in the level of *Bacteroidetes* have been observed in both obese mice (44) and humans (45) although these findings have not been replicated in all studies (46–52). Of note, intestinal dysbiosis is not currently used as a factor in diagnosing or predicting onset of a metabolic disease such as obesity or T2D. More subtle changes in the composition of the intestinal microbiota have been described in obese individuals with a reduced compositional microbial diversity compared with lean individuals (7). Additional evidence implicating the intestinal microbiota in obesity originates from obese (*ob*/*ob*) mice that lack expression of the gene encoding leptin, the product of which promotes satiety. In support of the involvement of the microbiota in the development of obesity in these mice, antibiotic treatment conferred changes in the gut microbiota, reducing the incidence of metabolic endotoxemia, inflammation, and several obesity-linked parameters (53). In human populations, it is evident that a high-fat diet and overconsumption of food are responsible for the greater prevalence of obesity and T2D in the West, thus conspiring to alter host metabolism and immune homeostasis via diet-induced changes in the intestinal microbiota. Indeed, the role of the microbiota in metabolism, and notably its ability to harvest energy from food, highlight a significant environmental factor impacting the risk of metabolic disease. A direct link between intestinal microbiota composition and body weight comes from studies using germ-free mice to show that the absence of intestinal microbes protects against diet-induced obesity, and that the intestinal microbiota is involved in the regulation of fat storage (54–56). These and similar studies have led to the proposal that obese individuals are more efficient in converting food into useable energy and in storing this energy in fat than lean individuals, which is related to, and may be a consequence of, the functionality of the intestinal microbiota. Major insights into differences between various physiological states of the host, such as in obese versus lean individuals, should therefore be obtained by studying the functional microbial diversity in addition to phylogenetic diversity. Indeed, an altered representation of bacterial genes and metabolic pathways, including those involved in nutrient harvest, has been found to be related to obesity (7). Also, the amount of SCFA produced by the intestinal microbiota, rather than the changes in the composition of the microbiota, is important in the development of obesity (51). Perhaps unsurprisingly, shifts

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in microbiota phyla have also been described in T2D (57), with metagenomics-based studies identifying discriminant metagenomic markers that may differ between different ethnicities of patients (58, 59). The question remains whether dysbiosis of the intestinal microbiota is a direct cause for any metabolism-related disorder, or whether changes in the intestinal microbial communities in affected and obese individuals are an adaptation to a change in the host's diet. Two observations relevant to answering this question are one, that the transfer of microbiota from lean donors into individuals with metabolic syndrome can increase insulin sensitivity and overall amelioration of symptoms of metabolic disease (60) and two, dietary changes in humans leads to rapid and reversible changes in the relative abundance of dominant members of the intestinal microbiota (61).

The potential interaction between host physiology, behaviour, the microbiome, and diet is evidenced in both animal and human studies showing rapid changes in microbiota composition after Roux-en-Y gastric bypass surgery (RYGB) (52, 62) although the impact on metabolite levels has been less explored. Nevertheless, in a non-obese rat model, RYGB surgery resulted in profound metabolic perturbations (63). Besides lower concentrations of oligosaccharides and higher concentrations of SCFA, increased levels of colonic protein fermentation metabolites were found in faecal samples obtained after surgery. These results might point at an incomplete digestion of proteins in the small intestine as a result of the bypass leading to an increased supply of protein to the colon with increased protein fermentation. Interestingly, faecal water samples obtained 2 and 8 weeks after the operation, displayed significantly more cytotoxicity compared to the samples obtained from sham-operated animals (64). It needs to be investigated whether the observed association between increased levels of amino acid fermentation metabolites and increased cytotoxicity also involves a causal relationship. In healthy, normal weight subjects, increased protein fermentation after a high-protein diet was not associated with increased faecal water cytotoxicity (65).

Also, between the large intestine and the kidney, a bi-directional functional relationship exists. Uremia influences the colonic microbial metabolism whereas microbial-related metabolites are involved in the progression of the kidney disease (66). p-Cresyl sulphate and indoxyl sulphate have been most extensively studied and are considered as prototypes of the so-called uremic toxins. They are derived from bacterial fermentation of the aromatic amino acids tyrosine and tryptophan, respectively, followed by sulphation in the colonic mucosa or the liver. Within the plasma, they are highly protein-bound and accumulate when kidney function fails. The free, unbound levels of these solutes increase more than their total plasma levels due to competition for binding sites on plasma proteins (67). In patients with chronic kidney disease, both p-cresyl sulphate and indoxyl sulphate levels have been linked to overall mortality, CVD and progression of the kidney disease (68).

Dysbiosis and CNS-related disorders

Intestinal microbial dysbiosis has also been observed in extra-intestinal diseases and in particular those that may impact on the 'gut–brain–axis' to affect the CNS and behaviour and cognitive function.

Several studies have focused on the possibility that the intestinal microbiota may influence cognitive function and behaviour by direct reprogramming of the hypothalamus–pituitary–adrenal (HPA) axis, a common pathway activated in response to infection and perturbed by psychological stressors. It is known that enteric infections can cause anxiety, depression, and cognitive dysfunction; germ-free mice that have no intestinal microbiota display alterations in stress-responsivity, central neurochemistry, and behaviour indicative of a reduction in anxiety in comparison to conventionalised mice (43). For example, in germ-free mice, increased anxiety-like behaviour has been associated with changes in the production of neurotrophic factors and hormones and expression of their receptors (69). In pathogen-infected mice (70–73), *Campylobacter jejuni* (a common cause of gastroenteritis) can induce anxiety-like behaviour in mice and brainstem activation (the nucleus tractus solitarius and lateral parabrachial nucleus). Commensal bacteria may affect brain changes through GABA, which can directly influence receptors both immune and neural within the ENS and CNS (74, 75). GABA is the main CNS inhibitory neurotransmitter and is involved in regulating physiological and psychological processes. Alterations in central GABA receptor expression are implicated in the pathogenesis of anxiety and depression (76).

Early colonisation of the intestinal tract by microbes is known to be important for the post-natal development of the enteric nervous system (77). Accordingly, intestinal microbiota may have implications on the development and function of the CNS (78, 79).

Evidence of a possible causal role of the intestinal microbiota in the development of autism spectrum disorder (ASD) comes from a maternal immune activation (MIA) mouse model in which pregnant animals after being administered the viral mimetic, ploy(I:C), display increased intestinal permeability and develop stereotypical abnormalities in behaviour, social ability, and communication that resemble ASD (80). MIA offspring display intestinal dysbiosis and an altered serum metabolomic profile, characterised by excessive levels of microbiota-derived 4-ethylphenylsulphate (4EPS), compared to control offspring, with intestinal barrier function being restored and ASD-like symptoms being alleviated after

administering probiotic bacteria. Of particular note, exogenously administered 4EPS, which is structurally related to the toxic sulphated form of *p*-cresol, resulted in an anxiety-like behaviour in naïve mice, suggesting that autism, and maybe other behavioural conditions, involve the GI-tract eventually impacting on the immune, metabolic, and nervous systems.

With the emerging preclinical data and indications in developmental disorders, it is perhaps no coincidence that GI-tract disorders including IBD and IBS are common co-morbidities in debilitating stress-related disorders, including depression and anxiety (81, 82). Recent research suggested that intestinal permeability and bacterial translocation may drive immuno-inflammatory and oxidative and nitrosative stress (IO&NS) pathways in depression and thus play a role in its pathophysiology. Chronic depression in humans was shown to be accompanied by increased immune response (serum IgM and IgA responses) directed against lipopolysaccharide (LPS) products of gram negative gut enterobacteria, that is, *Hafnia alvei*, *Pseudomonas aeruginosa*, Morganella morganii, *Pseudomonas putida*, *Citrobacter koseri*, and *Klebsiella pneumonia* (83). Attempts have been made to examine the potential CNS and behavioural impact of bacteriotherapy in germ-free and pathogen-infected rodents. Germ-free mice exhibit hyper-responsive HPA axis activity following stress as compared to specific-pathogen free mice (78) and this hyper-response of the HPA axis was reversed by *Bifidobacterium infantis* (84). *B. infantis* increased plasma tryptophan levels, decreased serotonin metabolite concentrations in the frontal cortex and dopamine metabolite concentrations in the amygdaloid cortex (85), both of which are implicated in depression (86, 87). In humans, the efficacy of probiotics for mood regulation was suggested in a trial of *Lactobacillus casei* that showed subjects with the lowest scores in the depressed/elated dimension at baseline had significant improvement in mood scores after taking the probiotic compared to the placebo group (88). The combination of *L. helveticus* and *B. longum* reduced anxiety and had beneficial psychological effects with decreased serum cortisol in healthy human volunteers (89).

Functional brain activity measured by functional magnetic resonance (fMRI) showed that a probiotic formulation reduced brain intrinsic connectivity and response to emotive stimuli and changes in midbrain connectivity (90).

However, it should be noted that several studies have failed to observe an effect of probiotic supplementation on anxiety measures in clinical populations, including IBS (91, 92), schizophrenia (93), and rheumatoid arthritis (94). This may be explained in part by the spectrum of doses, species (and combinations thereof), and timings used in probiotic interventions and the lack of a standard trial design.

Future approaches: restoration of the intestinal microbiota through bacteriotherapy

There is huge potential for manipulating the microbiota to sustain, improve, or restore the microbiota in at risk or diseased individuals.

An important pre-requisite for bacteria-based therapy (bacteriotherapy) is defining what constitutes a 'healthy' microbiota during and throughout life, which may be defined differently at the population and individual level. More research is needed to examine species and strain diversity in the GI-tract, the diversity of microbial genes (microbiome), and what their functionality is in the GI-tract throughout human development – from the cradle to the grave! Therapeutically, probiotic-based approaches have been used with some success for centuries (95, 96), as have the more drastic and cruder approach of wholesale microbiota replacement strategies based upon faecal transplantation (97). The application of these procedures is discussed in more detail in a separate review in this supplement – Manipulating the gut microbiota to maintain health and treat disease. The development and use of these and other more refined approaches using chemically defined bacterial products in the clinic will rely on understanding their molecular mechanisms of action and the particular host features requiring personalisation of approach in order to enable bacterial/probiotic therapies to yield their full potential in the treatment and management of human health.

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