MICROBIOME

Microbial genes and pathways in inflammatory bowel disease

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Abstract | Perturbations in the intestinal microbiome are implicated in inflammatory bowel disease (IBD). Studies of treatment-naive patients have identified microbial taxa associated with disease course and treatment efficacy. To gain a mechanistic understanding of how the microbiome affects gastrointestinal health, we need to move from census to function. Bacteria, including those that adhere to epithelial cells as well as several *Clostridium* species, can alter differentiation of T helper 17 cells and regulatory T cells. Similarly, microbial products such as short-chain fatty acids and sphingolipids also influence immune responses. Metagenomics and culturomics have identified strains of *Ruminococcus gnavus* and adherent invasive *Escherichia coli* that are linked to IBD and gut inflammation. Integrated analysis of multiomics data, including metagenomics, metatranscriptomics and metabolomics, with measurements of host response and culturomics, have great potential in understanding the role of the microbiome in IBD. In this Review, we highlight current knowledge of gut microbial factors linked to IBD pathogenesis and discuss how multiomics data from large-scale population studies in health and disease have been used to identify specific microbial strains, transcriptional changes and metabolic alterations associated with IBD.

Microbiota
The collection of microorganisms in a particular environment.

Microbiome
The genes, genomes and products of the microbiota.

Barrier function
Epithelial cell–cell junctions plus the mucosal layer that permit nutrients and prevent luminal contents from accessing the rest of the body.

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The intestinal microbiota has a major role in human health, including the maturation and education of host immune responses, protection against enteric pathogen proliferation and response to or modification of specific drugs. Host physiology can be altered at the cellular level by microbiome-induced cell signalling, proliferation and neurotransmitter biosynthesis[1,2], leading to mucosal and systemic alterations and thereby affecting homeostasis, barrier function, innate and adaptive immune responses and metabolism[2–4]. Through microbial metabolites and their effect on dietary breakdown, the microbiota provides energy and vitamins for the host[5]. Furthermore, it alters therapeutic drug availability[6] and modifies bile salts, impacting various host functions[6]. With such a broad range of effects on host physiology and its role in the induction, education and function of the immune system, it is unsurprising that the microbiota is implicated in gut-related diseases such as inflammatory bowel disease (IBD) and metabolic disorders[7–10], as well as in diseases that affect other systems, including atherosclerosis[11–13], autism[14], asthma[15,16] and type I diabetes[17,18]. Although the microbiome is dysregulated in these conditions, causal roles have yet to be determined in most diseases, and whether the microbiome drives disease or is altered in response to disease pathology remains unclear.

IBD, which includes Crohn's disease (CD) and ulcerative colitis (UC), is used as a model for the study of microbiome-related diseases (BOX 1). IBD risk is linked to 200 host genetic loci, most of which are associated with key immunological pathways, including innate immunity (for example, the protein encoded by *NOD2* acts as a sensor to detect bacterial peptidoglycans), immune responses (for example, the *IL23R* allele rs11209026 provides a protective effect in CD) and autophagy (for example, the product of *Atg16L1* affects autophagy in Paneth cells and goblet cells)[19–21]. Since 1950, a drastic increase in IBD has been observed in Western countries. Incidence rates of IBD have been stable or falling since 1990; however, disease burden remains high, with prevalence surpassing 0.3% in the Western world. Additionally, IBD rates are also rising in many newly industrialized countries that are gradually becoming more westernized and urbanized[22]. These patterns suggest that other factors besides host genetics must be driving changes in disease prevalence. Environmental and gut microbial influences affect the host immune response and have been linked to IBD onset and disease progression. Recent studies found that host factors associated with industrialization, such as body mass index, glycaemic response, high-density lipoprotein cholesterol and lactose consumption, have dominant roles in shaping the microbiota[23,24]. Effects of specific environmental factors on IBD development have also been examined, including smoking, diet, medications, circadian rhythm

Box 1 | **Features of inflammatory bowel disease**

Inflammatory bowel disease (IBD) is a term used to describe chronic inflammatory disorders of the gastrointestinal (GI) tract that manifest in two major forms — Crohn's disease (CD) and ulcerative colitis (UC). Traditionally, CD and UC are defined by clinical, histological, endoscopic and radiological features[139]. The location and extent of inflammation along the GI tract distinguishes UC and CD: UC inflammation extends proximally from the rectum and is restricted to the colon, whereas CD may affect any site in the GI tract but commonly occurs in the terminal ileum and may be discontinuous. CD is histologically characterized by macrophage aggregates that often form non-caseating granulomas, whereas UC presents with micro-abscesses composed of neutrophils within the lamina propria and crypts. Macroscopically, extensive mucosal ulcerations develop in UC. Mucosal lesions in CD are often found over Peyer's patches (small masses of lymphatic tissue in the ileum). In addition to these features, main immune cell involvement differs between CD and UC. CD is dominated by a T helper 1 cell phenotype and production of interferon-γ and IL-2, whereas UC is primarily a T helper 2 cell phenotype with production of transforming growth factor-β and IL-5 but not IL-4. IBD treatment regimens vary based on disease type, extent and severity.

IBD onset and disease course are associated with a combination of genetic, host (BOX 2) and microbial (BOX 3) factors. However, precise pathophysiologies for IBD remain elusive. Although CD and UC are clinically distinct, it is unknown whether they are also pathologically distinct or are part of a singular spectrum. Recent multiomics studies, such as the ones highlighted in this Review, have greatly contributed to a more comprehensive understanding of the pathogenesis of IBD.

and stress. In particular, increased IBD risk was associated with early childhood exposure to antibiotics[25]. Interestingly, in some cases, disparate effects on CD and UC have been observed, suggesting unresolved heterogeneity in IBD.

In addition to these factors, changes in the gut microbiome influence IBD susceptibility. A lack of early childhood exposure to microorganisms due to cleaner living, urbanization and the widespread usage of antibiotics affects immune education and maturation (known as the hygiene hypothesis). This lack of early childhood exposure is hypothesized to lead to a loss of negative regulatory pathways, resulting in overactive immune responses to the commensal intestinal microbiota. Serological markers are associated with disease course and IBD phenotypes, providing further evidence for the microbiome's role. A combination of a mannan epitope of *Saccharomyces cerevisiae* (gASCA), the atypical perinuclear anti-neutrophil cytoplasmic antibody and laminaribioside (ALCA) can differentiate between healthy individuals, patients with IBD and patients with non-IBD gastrointestinal inflammation[26]. Furthermore, differences in gASCA and atypical perinuclear anti-neutrophil cytoplasmic antibody levels can distinguish between CD and UC, whereas increasing antibody responses against gASCA, ALCA, chitobioside, mannobioside and outer membrane porins are associated with more complicated disease behaviour and surgery requirement in CD[26,27]. In UC, anti-*S. cerevisiae* antibody (ASCA) IgA, anti-neutrophil cytoplasmic antibody, anti-flagellin antibodies and anti-outer membrane porin C are associated with disease severity. Additionally, ASCA IgA and anti-outer membrane porin C are associated with the later requirement of colectomy, and higher ASCA IgA levels are linked to refractory UC[28]. Further evidence supporting a causal role of the microbiome in IBD are the findings that transfer of faecal microbiota from mice with colitis to healthy mice is sufficient to induce colitis[29–34] and that many genetically susceptible mice do not spontaneously develop colitis in germ-free facilities[35–37].

Progress to date indicates that IBD is a polymicrobial disease with a combination of various gut microbial factors, abnormal immune responses and a weakened intestinal mucosal barrier leading to aberrant host–microbial interactions[38]. In this Review, we highlight current knowledge of gut microbial factors linked to IBD pathogenesis. We discuss how multiomics data from large-scale population studies in health and disease have been used to identify specific microbial strains from metagenomic data, transcriptional changes from metatranscriptomic data and metabolic alterations from metabolomic data. When integrated with host-derived data, analyses can link these microbial changes to the host to predict aberrant host–microbial interactions. Furthermore, much effort is currently being focused on creating large collections of strains isolated from individuals with altered mucosal ecosystems. These collections, together with culturomics techniques, will be invaluable to validate the colitogenic potential of disease-associated strains as well as to identify pathogenic factors. Thus, the gut microbiome's contribution to disease onset and activity, as well as the development of complications, needs to be taken into consideration to treat IBD effectively, achieve long-lasting remission and reset the host–microbial balance.

Microbial immunomodulation in IBD

IBD is believed to result from aberrant immune responses to commensal bacteria in genetically susceptible hosts that disrupt the host–microbial balance (FIG. 1). The symbiotic relationship between the gut microbiome and the host is foremost protected by the intestinal epithelial barrier: a mucus bilayer and cellular junctions within the intestinal epithelium that form a physical barrier to contain the microbiota. Moreover, secretion of antimicrobial peptides, such as defensins produced by Paneth cells, goblet cells and other types of epithelial cells, creates a chemical barrier against invading microorganisms (BOX 2). IgA also has a crucial role in maintaining homeostasis at mucosal surfaces. Two distinct types of humoral immunity were proposed to coexist in the gastrointestinal mucosa, where IgA can elicit high-affinity responses, for example, in the context of pathogens and vaccines, or be polyreactive and bind to a broad but taxonomically distinct subset of the microbiota[39]. IgA can mediate potent anti-inflammatory functions through interaction with the C-type lectin receptor SIGNR1 on dendritic cells, which induces immune tolerance via regulatory T (T_{reg}) cell expansion. IgA antibodies also have a crucial role in the prevention of tissue damage in autoimmune and inflammatory diseases[40]. The microbiome, in turn, influences IgA levels in the gut mucosa through degradation, potentially disrupting homeostasis[41]. In IBD, there is an increased coating of intestinal microbiota by IgA antibodies.

Defects of the epithelial barrier have been observed in IBD. Although host IBD-associated genes, such as *FUT2* and *C1orf106* (REF.[42]), have roles in mucosal barrier function, several mechanisms involve gut

Glycaemic response
The glycaemic response to food describes its effect on blood glucose levels after consumption.

Non-caseating granulomas
Granulomas are clusters of immune cells that form during infection, inflammation or in the presence of a foreign substance to prevent a systemic spread. The absence of necrosis is a defining feature of non-caseating granulomas. In Crohn's disease, non-caseating granulomas are formed during inflammation without an obvious infectious trigger.

Hygiene hypothesis
According to the hygiene hypothesis, a lack of early childhood microbial exposure affects the development of the immune system. This has been ascribed to the increase of allergic and autoimmune diseases in Western countries.

Mannan
A mannose polymer and component of fungal and plant cell walls.

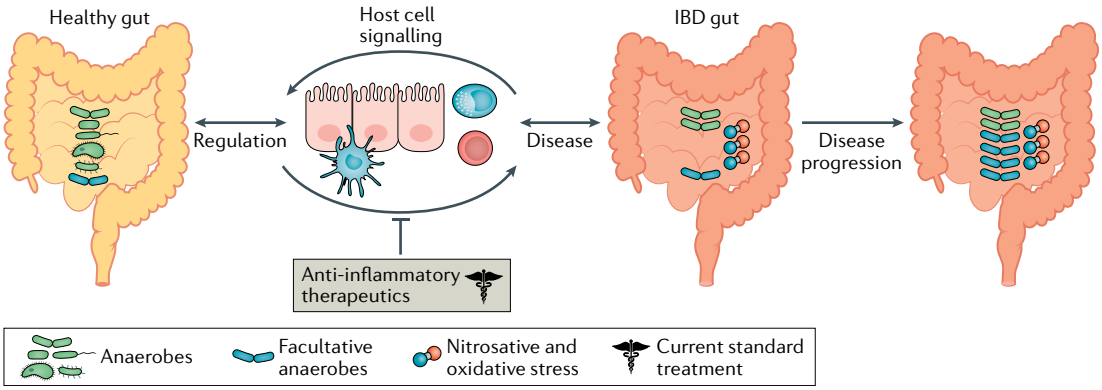

Fig. 1 | **Inflammatory bowel disease and the microbiota.** In a healthy gut, gut bacterial composition (anaerobes and facultative anaerobes) is maintained in balance with host cell physiology. Alterations in gut microbiome composition during disease include reduced microbial diversity and expansion of facultative anaerobes due to increased nitrosative and oxidative stress in the gut. Current standard treatments, such as 5-aminosalicylic acid, corticosteroids, immunomodulators and anti-tumour necrosis factor biologic therapy, focus on treating and controlling disease symptoms, in particular, chronic inflammation. IBD, inflammatory bowel disease.

microorganisms themselves. For example, short-chain fatty acids (SCFAs), particularly butyrate, produced by gut bacteria promote T_{reg} cell development and enhance mucus production from goblet cells to strengthen the mucosal barrier[43,44]. SCFAs activate cells via G-protein-coupled receptors, such as GPR41 and GPR43, leading to chemokine and cytokine production, which regulates protective immunity and tissue inflammation[45]. Bacterial indole metabolites such as indoleacrylic acid (IA) and indole 3-propionic acid, produced by *Peptostreptococcus* species and the intestinal commensal *Clostridium sporogenes*, respectively, regulate intestinal barrier function through the xenobiotic sensor pregnane X receptor[46]. IA promotes intestinal epithelial barrier function and mitigates inflammatory responses. The biosynthetic gene cluster for IA is decreased in the gut metagenomes of patients with IBD, perhaps contributing to barrier dysfunction[47]. Decreased tryptophan metabolism, reduced SCFA levels and a compromised epithelial barrier compound the detrimental effects of IBD.

Microbial communities have an important role in the maturation and education of the host immune system. Infant gut colonization and the early-life microbiome have long-lasting effects[48,49] and are strongly influenced by delivery mode and feeding. Lower diversity and delayed colonization of Bacteroidetes, for example, are linked to delivery by caesarean section and are associated with reduced T helper 1 (T_H1) cell responses in the first 2 years of life[50]. T cell subtypes have crucial roles in sensing inflammation and ensuring appropriately timed and localized immune responses. The importance of the microbiota for T cell development and immune tolerance has been studied in mouse models. Germ-free mice have fewer $CD4^+$ T_{reg} cells in their colons than conventional mice[51]. $CD4^+$ T_{reg} cells express the Foxp3 transcription factor and have a crucial role in the maintenance of immune homeostasis. Furthermore, early life exposure to microorganisms is required in mice to prevent an accumulation of invariant natural killer T (iNKT) cells, which predispose mice to increased morbidity in models of IBD and allergic asthma[52]. Specifically, either colonization of neonatal mice with conventional microbiota from specific pathogen-free mice[52] or mono-colonization with a sphingolipid-expressing strain of *Bacteroides fragilis*[53] is sufficient to prevent the accumulation of iNKT cells. Exposure of adult germ-free mice to these microorganisms is insufficient to decrease the number of iNKT cells[52,53], supporting a body of literature that suggests early-life exposure to microorganisms regulates adult immune functions and disease susceptibility[54,55]. Colonizing germ-free mice with faecal microbiota from individuals with IBD increases the number of intestinal T_H17 cells and decreases the number of $ROR\gamma t^+$ T_{reg} cells, providing evidence for disease mechanisms involving the gut microbiome in humans. Furthermore, the induced proportions of T_H17 and $ROR\gamma t^+$ T_{reg} cells accounted for colitis severity in *Rag1*$^{-/-}$ mice lacking adaptive immunity[56] and were predictive of human disease status.

In mouse models, segmented filamentous bacteria (SFB) are primary drivers of T_H17 responses[34,57]. *Rag1*$^{-/-}$ mice have higher levels of SFB than immunocompetent mice[58]. SFB levels in these immunocompromised mice further increase when the signal transducer and activator of transcription 3-dependent innate immune response is compromised, indicating that the murine immune system continues to restrict these closely associated bacteria even in the absence of a functional adaptive immune response[58]. These findings provided a unique framework to translate mouse studies to human disease. One study isolated 20 bacterial strains from a patient with UC based on their ability to induce T_H17 cells in mice and showed that adhesion to epithelial cells is a common mechanism used by intestinal microorganisms to activate host T_H17 responses[59]. One host mechanism that restricts SFB independently of the adaptive immune system is the production of α-defensins by Paneth cells, a cell type whose function is diminished in CD. SFB abundances are increased in mouse models of autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy, with the host also being seropositive for autoantibodies against an enteric

Atypical perinuclear anti-neutrophil cytoplasmic antibody
Anti-neutrophil cytoplasmic antibodies are classified based on staining patterns. Cytoplasmic anti-neutrophil cytoplasmic antibody (ANCA) refers to staining of the entire cytoplasm, and perinuclear ANCA refers to staining of the area around the nucleus. Perinuclear ANCAs have been implicated in inflammatory bowel disease; however, their target antigens are unknown and they are therefore described as atypical perinuclear ANCA.

Laminaribioside
A glucose disaccharide building block of laminarin and a component of the cell walls of fungi and algae.

Chitobioside
A building block of the *N*-acetylglucosamine-based glycan chitin and a component of the cell walls of microorganisms.

Mannobioside
A disaccharide of mannose.

Colectomy
A surgical procedure removing all or part of the colon.

Strains
The classical microbiological definition of strain is a single bacterial isolate. In the context of metagenomic data, it refers to a combination of single-nucleotide polymorphisms that are computationally predicted to be linked and originating from an individual strain genome.

Metatranscriptome
All of the RNA in an environment.

Culturomics
The process of using classical microbiological techniques to culture and identify unknown bacteria that inhabit a given environment.

Indole metabolites
Indole metabolites derive from microbial metabolism of tryptophan and can be recognized by several host receptors that regulate host–microbial homeostasis.

Box 2 | **Host physiological factors associated with inflammatory bowel disease**

Local factors

Changes in host physiology are observed within the gut during inflammatory bowel disease (IBD).

Barrier function. The intestinal barrier in IBD is leaky due to changes in epithelial tight junctions, increased rates of apoptosis, erosion events and ulcerations in the intestinal lining[140].

Paneth cells. These specialized intestinal epithelial cells produce antimicrobial peptides, which regulate the gut microbiota. Changes in the numbers and function of Paneth cells have been noted in patients with Crohn's disease (CD), and functional consequences of these abnormalities have been demonstrated in mice[110,141–143]. Paneth cell dysfunction is associated with mutations in the IBD-associated host genes *NOD2* and *Atg16L1*[142].

Goblet cells. Goblet cells, which are specialized intestinal epithelial cells that secrete mucin, are commonly depleted in IBD[144]. The mucin layer is thinner in IBD, and mouse mutants lacking *Muc2* develop chronic colitis[145]. Goblet cells also secrete cytokines, deliver antigens to dendritic cells and affect barrier function[146].

Faecal calprotectin. Quantification of this abundant neutrophil protein in stool serves as a readout of gut inflammation. Upon activation or cell death, neutrophils release calprotectin, which is then stable in the stool for several days. Faecal calprotectin levels correlate with histological examination and disease severity, particularly in ulcerative colitis (UC).

Systematic factors

Evidence of a host response to IBD is also found systemically such as in serum.

Microbial antibodies. One of the most commonly measured antibodies, anti-*Saccharomyces cerevisiae* antibody, is used to distinguish patients with CD from patients with UC during diagnosis, with those with CD being more likely to have higher levels of anti-*Saccharomyces cerevisiae* antibody than those with UC[105].

Inflammatory markers. The most commonly used inflammatory marker is C-reactive protein (CRP). CRP is produced by the liver and released into the blood in response to inflammatory cytokines accumulating at the site of infection. Due to a short half-life, serum CRP levels rapidly decrease upon the cessation of inflammation. CRP correlates well with disease activity for most patients with UC or CD, although patients with CD tend to have higher CRP levels relative to patients with UC[147].

Cytokines. Inflammation associated with IBD is largely driven by an imbalance between pro-inflammatory and anti-inflammatory cytokines. The role of cytokines in IBD pathogenesis has been recently reviewed[148].

Metagenomes
All the genetic material present in an environment, consisting of the genomes of numerous organisms.

RORγt$^+$ T_{reg} cells
Regulatory T cells that express the transcription factor RORγt, a nuclear hormone receptor and critical regulator of antimicrobial immunity.

Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy
An autoimmune disease characterized by destruction of endocrine tissues, chronic mucocutaneous candidiasis and ectodermal disorders.

16S ribosomal RNA (rRNA) gene
A gene conserved among bacteria often used for taxonomic classification.

Keystone taxa
Species with high connectivity in microbial networks (built based on statistical associations), suggesting that they are a key component of the ecosystem and their removal would result in drastic changes to the microbial ecosystem.

Anti-tumour necrosis factor therapy
Drugs that target tumour necrosis factor to decrease inflammation are often used to treat autoimmune diseases and inflammatory bowel disease.

α-defensin[60]. SFB are typically only found in mice and the specific human counterpart is unknown.

Microbial factors also affect T_{reg} cell differentiation. T_{reg} cells are negative regulators of inflammation, and commensal species such as *B. fragilis*[61] and species from *Clostridium* clusters XIVa, IV and XVIII[51,62] can stimulate the differentiation of T_{reg} cells. Alterations of T cell subtypes can have long-lasting effects, in which T_{reg} cell depletion leads to non-remitting destructive disease even after restoration of normal T_{reg} cell numbers in mouse models of arthritis. Microbial imbalances in IBD probably disrupt regulatory processes that suppress inflammation. For example, a pronounced depletion of Clostridiales organisms was observed in treatment-naive patients with new-onset CD and UC[28,63].

These studies present evidence that the gut microbiome is a crucial component for a healthy immune system; however, a comprehensive understanding of mechanisms mediating host–microbial interactions is currently incomplete. To this end, several extensive longitudinal IBD studies were initiated with the aim of linking taxonomic and functional changes in the gut microbiome to IBD pathogenesis.

The gut microbiome in IBD pathology

Early studies determined differences in the microbiota of patients with IBD based on 16S ribosomal RNA (rRNA) gene amplicon sequencing of stool and biopsy samples[28,63,64]. Collectively, these studies found a decrease in gut microbial diversity in patients with IBD[65,66], including a decrease in the abundance of Firmicutes with a depletion of *Clostridium* cluster IV and XIV species[65,67–69] and an increase in the abundance of Enterobacteriaceae species[70–72] (FIG. 1). Some studies identified changes in *Bacteroides, Bifidobacterium* or *Lactobacillus* species[65,67–70,72,73]. In paediatric patients with IBD, microbial shifts were detected at earlier time points in biopsy samples than in stool samples[63]. Studies examining bacteria associated with tissue biopsies from patients with IBD found more mucosa-associated bacteria in IBD than in controls[69,70,73,74] and significant differences in the abundances of tissue-associated bacteria between inflamed and non-inflamed sites[67,75]. Defining distinct association networks of taxa from intestinal biopsies of patients with CD and UC, one study identified *Blautia*, *Faecalibacterium* and *Ruminococcus* species as probable keystone taxa in CD and UC[76]. They further linked disturbances of Lachnospiraceae and Ruminococcaceae species with relapsing disease, poor response to anti-tumour necrosis factor therapy (anti-TNF therapy) and disease recurrence after surgical interventions in patients with CD.

16S rRNA amplicon studies have limited taxonomic resolution and predominantly identify family-level or genus-level associations but rarely those at the species level. Although taxonomic imbalances in IBD have been described, functional disruptions may have a greater impact. However, amplicon studies do not reveal information about the metabolic pathways encoded by the microbial community. To address this possibility and circumvent the limitations described above, metagenomic sequencing of stool samples showed that metabolic

Table 1 | **Summary of human stool inflammatory bowel disease metagenomic studies to date**

Title of paper	Year published	Number of metagenomic (and metatranscriptomic) data sets	Sequence Read Archive or BioProject identifier	Ref.
Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment	2012	11	PRJNA175224	77
The treatment-naive microbiome in new-onset Crohn's disease	2014	43	• PRJNA237362 • PRJNA205152	63
Inflammation, antibiotics, and diet as environmental stressors of the gut microbiome in paediatric Crohn's disease	2015	369	SRP057027	80
Increased intestinal microbial diversity following faecal microbiota transplant for active Crohn's disease	2016	53	PRJNA321058	162
A novel *Ruminococcus gnavus* clade enriched in inflammatory bowel disease patients	2017	267	PRJNA385949	81
Gut microbiome function predicts response to anti-integrin biologic therapy in inflammatory bowel diseases	2017	175	PRJNA384246	115
Dynamics of metatranscription in the inflammatory bowel disease gut microbiome	2018	300 (78)	PRJNA389280	95
Gut microbiota composition and functional changes in inflammatory bowel disease and irritable bowel syndrome	2018	1,792	• LifeLines DEEP (on request): European Genome-Phenome Archive EGAS00001001704 • UMCG IBD (on request): EGAD00001004194 • Maastricht IBS (MIBS): EGAS00001001924	78
Gut microbiome structure and metabolic activity in inflammatory bowel disease	2019	222	PRJNA400072	79
Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases[a]	2019	1,638 (835)	PRJNA398089	83

[a]The collection and data generation methods as well as the processed and raw data for this project are all available at the Inflammatory Bowel Disease Multi'omics Database website.

pathway abundances were more consistently perturbed. The microbiomes of patients with IBD encode more oxidative stress and nutrient transport pathways and fewer pathways related to carbohydrate metabolism and amino acid synthesis[63,77]. Metagenomic studies have been largely limited to stool samples due to the high host to microbial DNA ratio present in biopsies that makes sequencing total DNA an inefficient method to profile microbial communities. Nevertheless, these early studies indicate that important insights can be gained through metagenomic data.

Several large cohort studies (TABLE 1) have now used metagenomic sequencing on human stool samples to characterize species-level and strain-level differences as well as functional alterations in IBD. A recent study[78] analysed metagenomic profiles of stool samples from 1,792 individuals, including 355 patients with IBD from the NLIBD cohort (of whom 208 patients were diagnosed with CD, 126 patients with UC and 21 patients with IBD unclassified or indeterminate), 412 patients with irritable bowel syndrome and 1,025 controls from the LifeLines DEEP cohort. Overall, 219 taxa from various taxonomic levels (including 152 species) were associated with CD and 102 taxa (including 93 species) were associated with UC. Profiles were similar between both IBD subtypes, with 87 of the UC-associated taxa also associated with CD. Families with the highest number of decreased taxa in CD were Lachnospiraceae (n = 21 taxa) and Ruminococcaceae (n = 17), whereas the highest number of increased taxa belonged to the Enterobacteriaceae family (n = 8). Taxa decreased in UC were largely from the Bacteroidaceae family (n = 5) and taxa increased in UC were from the Lachnospiraceae family (n = 11). Furthermore, a significantly reduced strain diversity in beneficial species such as *Faecalibacterium prausnitzii* and *Roseburia intestinalis* was observed, and bacterial growth rates of *B. fragilis* and *Escherichia coli* were increased in patients with CD compared with controls. The abundance of antibiotic resistance genes was increased in IBD and correlated with *Escherichia* and *Bacteroides* species abundance. Functional differences were observed in the microbiome of patients with IBD, in particular, in the synthesis of amino acids, neurotransmitters and vitamins, as well as the regulation of mineral absorption and the degradation of complex carbohydrates. Pathways related to SCFA and L-arginine synthesis, which have important roles in maintaining intestinal barrier function and inflammation-associated immunosuppression, were perturbed in IBD.

In a study investigating changes in metabolic activity of the IBD microbiota[79], the authors performed metagenomic and untargeted metabolomics profiling of 220 samples from an American discovery cohort (PRISM) and a Dutch validation cohort (LifeLines DEEP and NLIBD). Metagenomic analysis revealed a pronounced separation of patients with CD and non-IBD, whereas patients with UC were more heterogeneous.

Irritable bowel syndrome
A chronic condition, which affects the large intestine and causes abdominal pain, cramping and shifts in bowel movement patterns. In contrast to inflammatory bowel disease, irritable bowel syndrome is not associated with mucosal inflammation, ulcers or other damage to the bowel.

In total, 50 species were differentially abundant in IBD, of which 35 were elevated in controls, indicating a general loss of diversity in patients with IBD. In particular, *Roseburia hominis*, *Dorea formicigenerans* and *Ruminococcus obeum* were depleted in IBD. In patients with CD, an enrichment of *R. gnavus, E. coli* and *Clostridium clostridioforme* was observed, whereas *Bifidobacterium breve* and *Clostridium symbiosum* were uniquely enriched in UC. Functional metagenomic analysis identified 568 differentially abundant enzymes, the majority of which could be ascribed to a single species. For example, *E. coli* dominated 200 of the differentially abundant enzymes, owing in part to the species' strong enrichment in IBD and exceptionally thorough functional annotations. Whereas some predicted functions may indicate mechanistic links between the microbiome and IBD, others may simply indicate changes in the abundances of genomes encoding those functions. Overall, 246 differentially abundant enzymes were not dominated by a single species, suggesting that their enrichment is the result of a community-level shift in functional potential and therefore is of greater mechanistic significance. Examples include a magnesium-importing ATPase and an ethanolamine ammonia lyase. As metabolites constitute a direct measurement of functional activity, their quantification may be more effective in identifying putative mechanistic associations. Metabolic alterations identified in this and other studies will be discussed further in a later section.

In addition to species-level and functional characterizations of the gut microbiome in patients with IBD, temporal changes are important to monitor. Interindividual differences account for ~50% of the variation observed in taxonomic community composition[28]. Furthermore, less than half of patients with IBD respond to conventional treatment strategies and disease is characterized by periodic bouts of inflammation separated by periods of remission. Therefore, it is particularly important to conduct longitudinal studies that capture changes of microbial features and loss of response to treatment over time. To date, there have been only a handful of longitudinal, metagenomic studies associating microbial species with IBD, as described in more detail below.

One study focused on a treatment-naive paediatric CD cohort and monitored patients over the first 8 weeks of treatment with either anti-TNF therapy or enteral nutrition[80]. Enteral nutrition consists of nutritionally complete liquid diets presenting simpler, often predigested amino acids, fatty acids and oligosaccharides that can be absorbed quickly by the host and reduce the metabolic activity of the microbiota. Using metagenomic sequencing of gut microbiota from healthy individuals and patients with CD, the authors found that changes in microbial genes distinguished between patients with CD: decreases in selenocompound metabolism pathways and increases in microbial genes encoding glycerophospholipid metabolism, aminobenzoate degradation, sulfur relay systems and glutathione metabolism were predictive of more profound microbial shifts within patients with CD. By sequencing the entire genetic content of the samples instead of amplifying a specific bacterial gene (such as the 16S rRNA gene), they further showed that IBD was correlated with higher levels of fungal and human DNA. After 1 week of therapy, the authors were able to predict which patients would achieve remission based on their gut microbiome; however, the gut microbial composition of patients with CD remained altered over the course of the study[80].

Another study examined the microbiome of patients with established CD or UC in a meta-analysis of two studies with longitudinal data[80,81]. In addition to examining taxonomic differences, they binned bacteria by their oxygen utilization capabilities as facultative or obligate anaerobes, according to whether they can or cannot utilize oxygen as a terminal electron acceptor. The authors found that facultative anaerobes are overrepresented in patients with IBD and that oxygen utilization explained eight out of nine species that were consistently differentially abundant between the two studies. This supports previously published hypotheses that the microbial shifts associated with IBD are, in part, due to increased aerobicity in the inflamed gut[63,77]. The only species that was not explained by oxygen utilization was *R. gnavus*, an aerotolerant obligate anaerobe that transiently dominates the IBD microbiota during periods of increased disease severity. Such dynamics of bacterial abundances can only be captured in longitudinal studies. Furthermore, metagenomic data allowed the researchers to distinguish between two strain-specific clades of *R. gnavus*, one of which is found in both controls and patients with IBD, whereas the other is only present in the IBD gut[81]. As the field transitions from amplicon studies towards metagenomic studies, our knowledge of strain diversity within a given species will increase.

Although these studies identified differentially abundant species in IBD, many microorganisms identified across studies differ regarding their presence or absence, or abundance, making it challenging to identify common patterns. FIGURE 2 is a phylogenetic representation summarizing all species associations identified in the PRISM cohort[79], a longitudinal stool study[81], Lewis et al.[80,81] and the NLIBD cohort in combination with LifeLines DEEP controls[78]. Coloured leaves of the phylogenetic tree represent differentially abundant species identified in at least two of the studies. The outer rings summarize study-specific results. While this visualization highlights the broad spectrum of IBD-associated bacterial species from all major taxonomic groups typically found in the gut, it also reveals phylogenetic patterns. Species from the Proteobacteria phylum, such as *E. coli*, are generally increased in IBD. Actinobacteria are generally decreased, with the majority of these associations identified in the NLIBD cohort. Actinobacteria have been observed to be highly prevalent in the gut microbiomes of healthy Dutch individuals compared with American individuals[23,82]. Most Bacteroidetes species identified in at least two studies were depleted in IBD, in particular, several *Alistipes* species. Although Firmicutes include increased and decreased species abundance in IBD, the phylogenetic analysis highlights that patterns are largely clade specific, with the abundance of *Eubacterium* species and Ruminococcaceae consistently decreased and *Streptococcus* and *Lactobacillus* species increased. Furthermore, Lachnospiraceae species,

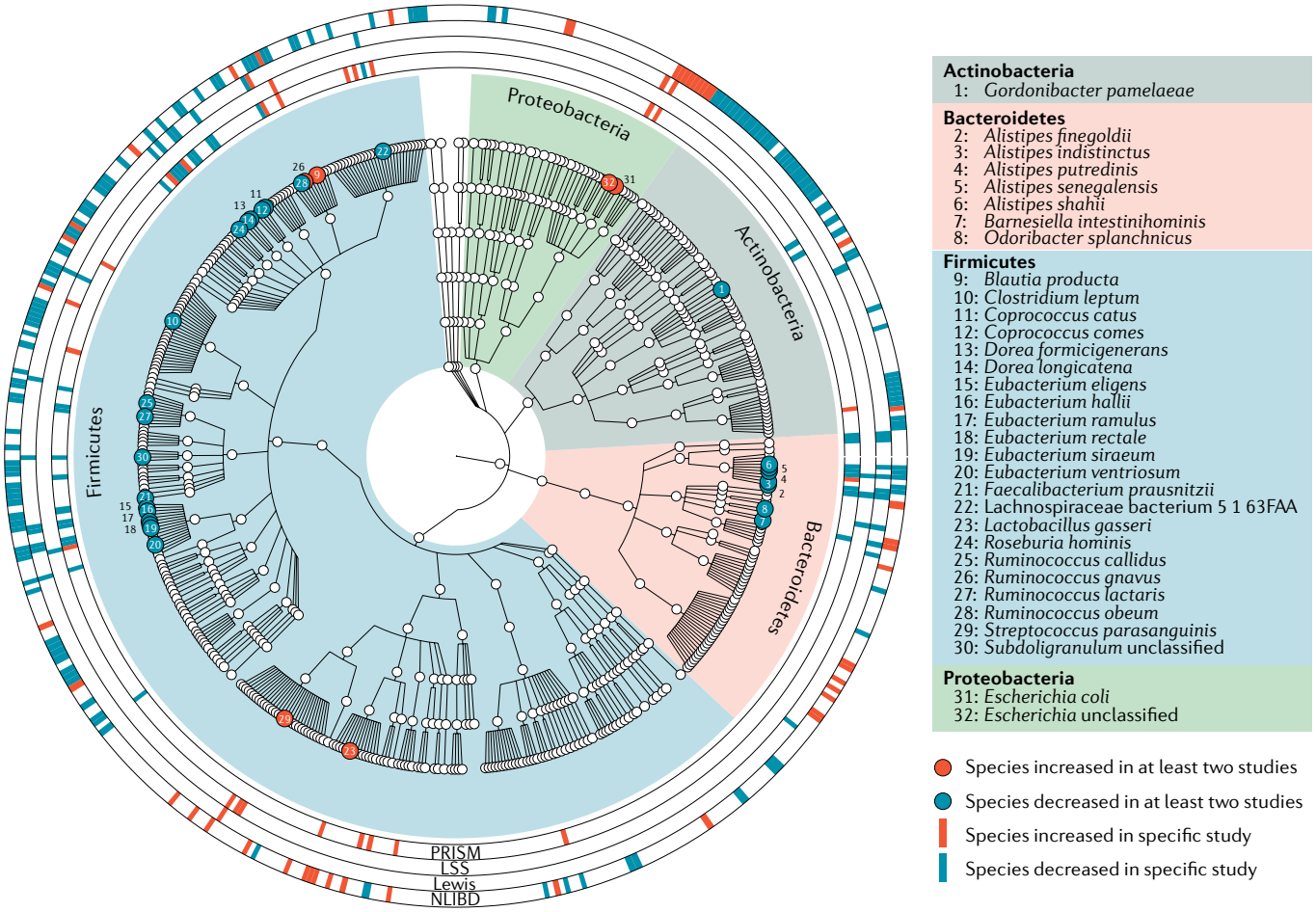

Fig. 2 | **Phylogenetic tree of bacterial species associated with inflammatory bowel disease.** Multiple studies have implicated bacterial species in inflammatory bowel disease; however, results differ between studies. The association results from metagenomic studies have been summarized in a phylogenetic tree to highlight common patterns across studies. The phylogenetic tree (generated using the software GraPhlAn)[161] was constructed based on all bacterial species identified in samples from the PRISM cohort[79] (PRISM; $n = 159$), a longitudinal stool study[81] (LSS; $n = 271$), Lewis et al.[80,81] (Lewis; $n = 368$) and the NLIBD cohort in combination with LifeLines DEEP controls[78] (NLIBD; $n = 1,380$), and includes all species that were detected in at least 20 samples ($n_{species} = 726$). Coloured tree leaves indicate species that were differentially abundant (false discovery rate of <0.1) in at least 2 studies, with 6 increased (red circles) and 26 decreased (blue circles) species. The outer rings indicate study-specific results and highlight all microorganisms identified in the respective study (Supplementary Table 1 lists the differentially abundant species for each study, respectively). Background colours indicate all species that belong to the same phylum.

including *Clostridium* species, showed opposing trends, suggesting species-specific interactions with the host immune system.

A large collection of longitudinal multiomics data sets became available during the production of this Review as part of the integrative Human Microbiome Project (iHMP)[83]. This second phase of the Human Microbiome Project followed 132 individuals with and without IBD for one year, collecting bi-weekly stool samples as well as biopsy samples, blood specimens and detailed health questionnaires. The resulting molecular profiles of host and microbial activity during disease provide a comprehensive view of functional imbalances associated with the gut microbiome during IBD. Complementary molecular components of longitudinal dysbioses were identified by different microbial measurements such as stool metagenomics, metatranscriptomics and metabolomics. These include taxonomic shifts in favour of aerotolerant, pro-inflammatory microbial taxa, greater gene expression by Clostridia during disease, disruption of metabolite pools (for example, acylcarnitines, bile acids and SCFAs) and differences in temporal stability across IBD phenotypes and disease activity. Importantly, the collection and data generation methods as well as the processed and raw data for this project are all available at the Inflammatory Bowel Disease Multi'omics Database website for the community to use.

Communication between microorganisms and the host is not unidirectional. Just as microbial products can influence host cell physiology, host factors such as chromogranins and secretogranins appear to influence gut microbial composition[84]. Overall, large cohort analyses have provided a detailed characterization and indicate substantial changes in gut microbiota composition during IBD based on metagenomic data. Integrating functional microbial measurements, such as

Chromogranins and secretogranins
A family of water-soluble acidic glycoproteins that are mainly produced by endocrine cells, such as the enteroendocrine cells of the gut. Also known as granins, they are precursors of biologically active peptides involved in inflammation.

metatranscriptomic and metabolomic data, and measurements of host responses will be crucial to generate testable hypotheses and gain mechanistic insights into the role of the microbiome in disease.

Microbial strains implicated in IBD

As highlighted by the example of *R. gnavus* in the previous section, strain-level variation within a species can be important. One of the difficulties in drawing conclusions from amplicon sequencing data is that diversity within a bacterial species is not fully captured. Organisms of the same species are defined to have 70% DNA–DNA hybridization or 95% average nucleotide identity[85]; therefore, strains of the same species can possess important functional differences, including antibiotic resistance or pathogenicity. Most *E. coli* strains are harmless and part of a healthy gut microbiota, but some are pathogenic and can cause serious and fatal diseases by producing, for example, Shiga toxin. Furthermore, specific *E. coli* strains such as adhesive invasive *E. coli* (AIEC) are implicated in IBD. AIEC is a pathobiont and its role in IBD was recently reviewed in detail[86]. Briefly, AIEC is able to evade the immune system and to adhere and invade intestinal epithelial cells and macrophages in genetically susceptible hosts, including patients with IBD. In addition to taking advantage of IBD host genetics that impair autophagy mechanisms, AIEC can suppress autophagic processes, enabling it to survive and thrive inside cells. First, bacterial translocation allows AIEC to gain access to the lamina propria, where it is engulfed by macrophages but escapes autophagy. Continuous replication within macrophages results in the secretion of high levels of TNF without inducing host cell death, leading to gut inflammation and AIEC overcolonization. Hypermotility and increased acetate consumption are associated with strains of *E. coli* from patients with CD compared with those from healthy individuals, suggesting AIEC adaptation within the host[87]. Various strategies for targeting AIEC strains and/or inhibiting AIEC adhesion, including bacteriophages and small-molecule drugs, are being investigated as potential therapies for IBD[88].

In the absence of metagenomic sequencing, studies rely on culture-based methods to distinguish pathogenic strains. Culture-based methods were instrumental in identifying the ability of human oral *Klebsiella* strains to drive inflammation in the colon of genetically susceptible mice (that is, *Il10*$^{-/-}$). Although some oral isolates of *Klebsiella* species from both controls and patients with IBD induced murine colon inflammation, this was not consistent across the tested human, mouse and environmental strains[89]. In particular, the *Klebsiella pneumoniae* 2H7 strain strongly induced accumulation of interferon-γ^+ CD4$^+$ T_H1 cells in the intestinal lamina propria, although only in genetically susceptible mice. Strain-specific genes associated with T_H1 cell induction are predicted to encode haemolysin co-regulated protein and enzymes involved in fructose-, galactitol-, mannose- and long-chain fatty acid-related uptake and metabolic pathways. Furthermore, the ability of pathobionts to outgrow other bacteria in the inflamed gut is supported by sequencing data, in which *Klebsiella* species and other bacteria associated with the oral cavity are more abundant in the gut of patients with IBD than in the gut of controls[28,89,90]. *Klebsiella* species are also more abundant in mouse models and human cohort studies of other gut diseases[91,92]. However, the specific mechanisms involved in bacterial translocation and inflammatory responses remain to be elucidated.

Beneficial species that elicit positive effects and provide protection for the host can also be diverse, as evidenced by *F. prausnitzii*, which contains numerous subspecies or phylogroups. Targeted quantitative PCR studies suggested that the presence or absence of *F. prausnitzii* phylogroups may be indicative of disease[93]. However, the implication of the presence or absence of different *F. prausnitzii* phylogroups on disease is currently unclear as the full diversity of this group is not apparent in the short hypervariable regions sequenced in most amplicon-based studies. Even when the full 16S rRNA gene is sequenced, many *F. prausnitzii* strains do not fall into any of the identified phylogroups[93].

Studies of strain-specific phenotypes, such as those outlined here, will probably become more common and will enable a deeper understanding of the role of microorganisms in IBD pathogenesis. As the number of genomes associated with pathogenesis-promoting strains increases, fewer genes will be shared exclusively among these disease-causing strains. This will eventually facilitate the identification of virulence factors (either presence or absence) and enable the comparison of potential virulence genes (including single-nucleotide polymorphism identification) across species to identify commonalities.

Insights from metatranscriptomics

Metagenomics is limited to revealing the functional potential of microorganisms, rather than the actual functional activity. The presence of a gene or pathway does not necessarily mean that it is expressed. In addition to isolating DNA for metagenomics, RNA can be extracted from a sample, reversed transcribed into cDNA and sequenced for metatranscriptomics, which measures actual microbial gene expression. To date, few studies have investigated the functional activity of the gut microbiome. One study established the feasibility of metatranscriptomics for faecal samples and found that metatranscriptional profiles varied more between individuals than metagenome functional profiles, highlighting the importance of measuring actual gene expression in addition to functional potential inferred from gene presence to understand disease-related microbiota changes. This subject-specific, whole-community regulation suggests that bacteria interact with their host in a very specific, individualistic manner[94].

Another study further showed that directly measuring functional activity reveals important insight into gut microbial community dynamics, including IBD-specific transcriptional activity that was either more pronounced or only detectable at the RNA level[95]. The authors observed that the distribution of microbial species encoding a pathway can remain fairly constant in a patient over time, whereas pathway transcription can change. For example, in the context of the

Box 3 | **Microbial immunomodulatory molecules**

Microbial metabolites are crucial for host–microbial interactions and regulate host immune responses.

Short-chain fatty acids, including butyrate, propionate and acetate, are by-products of bacterial breakdown of dietary fibres that can affect gene expression and cell proliferation during immune responses by modulating histone deacetylases[149]. Butyrate and butyrate-producing bacteria are less abundant in the stool of patients with inflammatory bowel disease (IBD)[21].

Tryptophan can be converted into bioactive indole-containing metabolites by gut bacteria. Significant differences in tryptophan metabolite levels have been observed in the serum of germ-free mice compared with conventional mice[150]. Indole derivatives can affect the host by activating the aryl hydrocarbon receptor, which regulates inflammation[151]. Furthermore, indoleacrylic acid, a specific indole derivative produced by the mucus-utilizing bacterium *Peptostreptococcus*, induces mucin gene expression and activates the NRF2 pathway[47]. This further affects host tryptophan metabolism by decreasing tryptophan availability, which reduces host metabolism products such as serotonin[152]. In patients with IBD, bacterial metabolism of tryptophan is reduced.

Bile acid metabolites are generated by bacteria from host-produced bile acids. These secondary bile acids are sensed by host receptors, including FXR and TGR5, and can regulate genes involved in immune cell maturation, cytokine release and microbial defence[153]. Production of secondary bile acids is reduced in patients with IBD. Taurine, which is cleaved from certain classes of primary bile acids, is an inflammatory metabolite that activates IL-18 expression[100]. Additionally, conjugated bile acids cause $CD4^+$ T effector cell-mediated upregulation of the xenobiotic transporter Mdr1 to maintain homeostasis. Mdr1 function in patients with IBD is reduced relative to controls[154].

Succinate is produced by *Bacteroides* species through the breakdown of dietary fibre, which in turn is metabolized by *Clostridium* species to produce butyrate and ATP[155]. Succinate increases IL-1β production by stabilizing hypoxia-inducible factor-1α in macrophages and stimulating dendritic cells via succinate receptor 1 (REF.[156]). Serum succinate levels are increased in hypertension, ischaemic heart disease, type 2 diabetes and obesity[157]. Murine colonic succinate levels are affected by dietary fibre concentrations, antibiotics and chemically induced intestinal motility disturbances[158,159].

Sphingolipids, including sphingosine, ceramide and sphingomyelin, are a class of plasma membrane-associated lipids that are produced by both the host and specific bacteria. Sphingolipids, most prominently ceramide, are closely tied to metabolic, apoptotic and inflammatory pathways in host cells. In IBD, the cellular levels and distribution of different sphingolipids is significantly different between inflamed and non-inflamed intestinal tissue[53,160].

methylerythritol phosphate pathway, increases in disease severity for a patient co-occurred with *Alistipes putredinis* domination of pathway transcription, while no DNA-level changes were observed. Species-specific transcriptional biases in metabolic pathways were also observed. Furthermore, *F. prausnitzii* dominated the transcription of many pathways, including the dTDP-L-rhamnose biosynthesis I pathway, which produces deoxysugar β-L-rhamnopyranose — a building block of surface glycans that are often targets of the immune system.

Microbial transcriptional programmes can respond rapidly to environmental cues, such as changes in inflammation and oxygen levels, which may not necessarily be reflected at the DNA level. However, some caveats apply to faecal metatranscriptomics, such as variation due to subject-specific transit times, and the fact that it only captures extractable, non-degraded RNA restricted to organisms that are present in stool. Additionally, owing to a high ratio of host to microbial DNA and RNA, performing metagenomics or metatranscriptomics on biopsy samples is not yet cost effective. In the absence of metatranscriptomic data, the ratio of reads located near the chromosomal origin of replication compared with those near the terminus for a given bacteria can be used to estimate growth dynamics from metagenomic data[96]. Although it does not answer questions about gene expression, this approach cleverly enables the determination of which bacteria are actively dividing and presumably transcribing their genes.

Metabolites associated with health and IBD

Several classes of small molecules produced or modified by the gut microbiota can modulate immune and epithelial cell function[97,98] (BOX 3). Comparisons between conventional and germ-free or specific pathogen-free mice show drastic differences in serum and tissue metabolites, underscoring the importance of the microbiome for host metabolism system wide (reviewed in REF.[99]). In mouse models, intestinal inflammation and clinical response to dextran sulfate sodium-induced colitis can be altered by postbiotically modulating levels of the microbial metabolites taurine, histamine and spermine, highlighting the potential clinical relevance of microbial metabolites[100]. A combination of microbial-derived and diet-derived metabolites probably contributes to inflammatory diseases such as IBD[97].

Several human studies identified metabolite differences in the stool[79,101,102], serum[102,103] or mucosa of patients with IBD compared with controls. Taurine and cadaverine levels are increased in UC, and carnosine, ribose and choline levels correlate with inflammation as measured by faecal calprotectin[102]. In a twin-pair study of healthy individuals and patients with IBD, increases in tryptophan, bile acids and unsaturated fatty acids were linked to ileal CD[104]. Strong associations between disease-associated microorganisms and metabolites were found by pairing metabolomics with microbial taxonomic analyses, showing increased levels of bile acids, sphingolipids and tryptophan[79,101,104]. Patients with IBD with inactive disease displayed similar microbiomes and metabotypes to their healthy first-degree relatives[101]. Although the mechanistic relationship between host disease, microorganisms and metabolites is becoming clearer, key questions about disease-associated metabolites remain to be answered, including whether they are bacterially produced or metabolized, whether they affect bacteria directly or indirectly by altering host physiology, or are a combination of these possibilities.

Studies often perform a combination of targeted (known) and untargeted (unknown) metabolomics, but subsequent analyses, such as those described above, have focused on the small subset of known molecules. The majority of the gut metabolome is uncharacterized, and untargeted metabolomics has great potential to identify novel disease-associated molecules. Computational approaches will have a crucial role in prioritizing microorganism–metabolite associations for experimental validations. This knowledge can be further used to develop therapeutic approaches that either inhibit disease-associated microbial metabolism and the corresponding microorganisms (for example, tungstate treatment) or augment beneficial metabolites and their respective species (for example, probiotic and postbiotic treatments; FIG. 3).

Metabolome
All of the metabolites in an environment.

Probiotic
An organism or multiple organisms that confer beneficial effects to the host.

Postbiotic
A bacterial metabolic product that mediates benefits to the host.

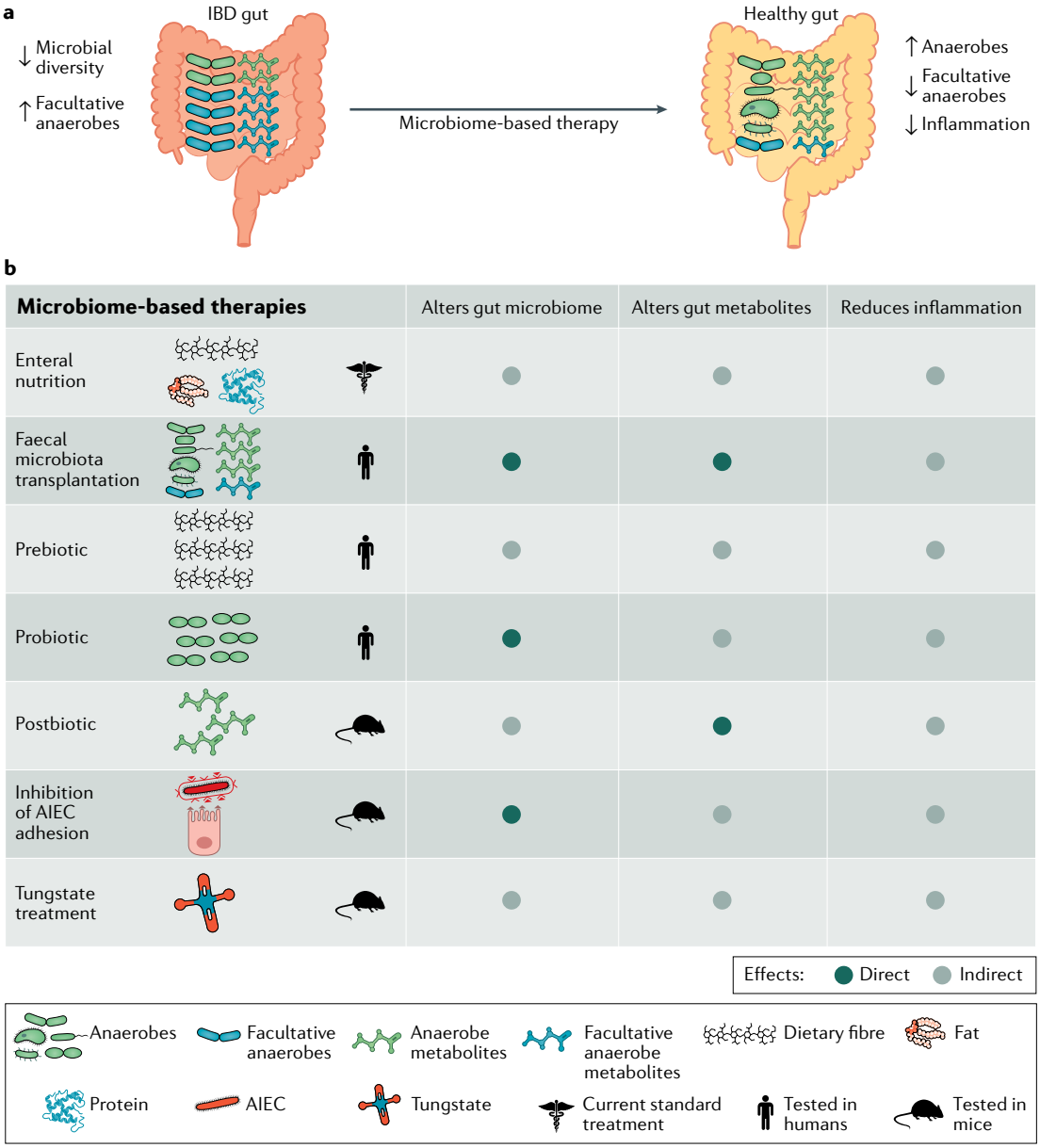

Fig. 3 | **Microbiome-based therapies for inflammatory bowel disease.** Microbiome-based therapies for inflammatory bowel disease aim to restore the gut microbial balance, which includes increasing microbial diversity, in particular, anaerobic bacteria, reducing facultative anaerobes and reducing gut inflammation (part **a**). Current and developing treatments either alter nutrition, administer microbial organisms and/or metabolites, or directly target microorganisms and/or pathways (part **b**). The effects of these treatments, including reduction of gut inflammation and alterations of the gut microbial communities and metabolites, are either direct or indirect. AIEC, adhesive invasive *Escherichia coli*.

Fungi and viruses in IBD

Most efforts to date have focused on bacterial alterations of the IBD gut microbiome, in part due to the popularity of 16S rRNA amplicon sequencing. However, the gut also harbours a diverse community of fungi and viruses that may have roles in IBD pathogenesis, directly or indirectly influencing the host by affecting bacterial members of the gut microbiota (for example, bacteriophages). Although relatively few studies have investigated IBD-associated fungi, ASCA levels are elevated in IBD, suggesting a role for fungi in disease pathogenesis[105] (BOX 2). One study observed fungal taxonomic shifts in IBD with an increased *Basidiomycota* to *Ascomycota* species ratio, a decreased proportion of *S. cerevisiae* and an increased proportion of *Candida albicans* compared with healthy controls[106]. Similarly, another study found that disease severity was positively correlated with fungal representation[80] and hypothesized that the CD-specific gut environment may favour fungi at the expense of bacteria or that antibiotic treatment creates a niche for fungal expansion. Recent studies have started to investigate the interactions between intestinal fungi and immune cells; for example, CX3CR1^{+} mononuclear phagocytes are essential for the initiation of immune responses to intestinal fungi, and a missense mutation in the gene encoding CX3CR1 was associated

with impaired antifungal responses in patients with CD[107]. Furthermore, *S. cerevisiae* enhanced host purine metabolism in murine colitis models, leading to an increase in uric acid production. Treatment with uric acid in turn increased gut permeability in mice[108]. In addition, *Malassezia restricta*, a fungus typically found on human skin, is enriched in the colonic mucosa of patients with CD with a disease-linked polymorphism in *CARD9*, which encodes a signalling adaptor important for anti-fungal defence. In mouse models, *M. restricta* exacerbated colitis through mechanisms requiring *Card9* (REF.[109]).

Enteric viruses are known to trigger disease onset in genetically susceptible mice. An interaction between murine norovirus infection and a mutation in the autophagy gene *Atg16L1*, a known CD susceptibility allele, induces abnormalities in granule packaging and unique patterns of gene expression in Paneth cells, although Paneth cells are not infected by the virus[110]. CD-like pathologies were observed in response to dextran sulfate sodium-induced colitis but were dependent on this virus-plus-susceptibility gene interaction, as mice carrying either alone did not exhibit similar pathologies. Furthermore, pathologies were TNF dependent and interferon-γ dependent, and preventable with broad-spectrum antibiotics, implicating the commensal gut microbiota. Thus, a combination of environmental factors, commensal bacteria and a virus-plus-susceptibility gene interaction led to an IBD phenotype. Other studies linked *FUT2* mutations, which lead to asymptomatic norovirus infections, with the pathogenesis of ileal CD[111]. Recent findings revealed that noroviruses target tuft cells within the intestinal epithelium and that tuft cell-specific gene expression in the colon decreases with broad-spectrum antibiotic treatment[112]. In antibiotic-treated mice, IL-4 and IL-25 stimulation induced tuft cell hyperplasia in the ileum but not in the colon, indicating that both intestinal bacteria and type 2 cytokines regulate tuft cells in a tissue-specific manner.

Several studies have recently investigated the role of the enteric virome in human IBD using virus-like particle enrichment[113,114]. A significant expansion of *Caudovirales* bacteriophages was detected in patients with CD or UC; however, this expansion was cohort specific and highlights the need for further studies. Analysis of viral sequencing data is currently challenging as the large majority of reads are of unknown origin and cannot be assigned to reference genomes. Viral classification is further complicated by integration of many virus genomes into the host genome. As more tools are developed and viral reference genomes are sequenced, we will be better able to determine the role of the virome in IBD.

Microbiome-based IBD therapies

Several treatments for IBD are available; however, most of these treatments have remission rates of less than 50%. In some cases, knowledge of a patient's microbiome composition can predict response to specific IBD treatments. For example, the response to anti-integrin treatment[115], anti-TNF therapy[116] or ustekinumab therapy can be predicted based on a combination of the gut microbiome and other clinical factors[117]. Another study examined treatment-naive paediatric patients, whose treatment was not standardized in the study protocol, and was able to predict a patient treatment response with 76.5% accuracy based on the abundance of six bacterial genera (*Faecalibacterium*, *Veillonella*, *Fusobacterium*, *Coprococcus*, *Akkermansia* and *Adlercreutzia*) in pre-treatment samples[118]. Although additional replications of these results are required, they highlight the therapeutic potential of the gut microbiome in choosing optimal treatment strategies for patients with IBD.

IBD treatments targeting host factors and microorganisms (including microbial physiology and metabolites) that influence disease are in various stages of development (FIGS 1,3). Current standard treatments suppress the immune system and alter the diet, with dietary modification being one of the most common behavioural interventions for IBD. These dietary modifications include prebiotic effects that shift the microbial composition as a result of changes in nutrient availability; however, the scientific evidence for many dietary modifications is lacking (reviewed in REF.[119]). To date, the strongest clinical evidence was observed with enteral nutrition treatment for CD. Although not effective for patients with UC, enteral nutrition has comparable efficacy to corticosteroids in paediatric patients with CD; the effect of enteral nutrition is less compelling in adults, which may in part be due to poor compliance[80,120,121].

Diet has a strong influence on gut microbial communities[122] and dietary changes have rapid effects on gut microbiota composition independent of inflammation and antibiotics[80]. Moreover, dietary patterns have been associated with IBD risk (recently reviewed in REF.[123]). Nevertheless, many challenges arise when evaluating dietary effects on disease, including accuracy of information on dietary intake, the complex interactions between foods consumed and differences in food metabolism among individuals[123]. Despite these challenges, clinical studies have shown that certain dietary components can promote or prevent intestinal inflammation and influence IBD risk. A prospective study following 170,776 women monitored long-term intake of dietary fibre. Intake of the highest quintile (median of 24.3 g per day) was associated with a 40% reduction of CD risk compared with the lowest quintile (median of 12.7 g per day)[124]. The protective effect of dietary fibre on CD risk may be mediated through gut microorganisms[124] that metabolize fibre into SCFAs, which leads to an increased mucosal immune tolerance through the activation of G-protein-coupled receptors and the subsequent activation of T_{reg} cells[122]. In addition, interactions between the gut microbiota and dietary concentrations of proteins and fibre can change intestinal permeability and severity of intestinal inflammation in mice[125]. However, a reduced consumption of red and processed meat did not decrease the rate of CD flares in a separate study[126]. In the future, engineered diets that restrict deleterious components but supplement beneficial nutrients may be used alone or in combination with other therapies to maintain or prevent disease.

Faecal microbiota transplantation (FMT), in which the stool of a healthy donor is transferred to the intestinal tract of a patient, has been highly effective in treating

Prebiotic
A certain food or food component that confers a beneficial effect by providing a competitive advantage to beneficial commensal bacteria capable of metabolizing these substrates or by augmenting the production of metabolic products that result from their fermentation.

Clostridioides difficile (formerly known as *Clostridium difficile*) infections and has been assessed for the treatment of IBD[127]. Many FMT studies have been limited by small sample numbers and have employed different methods for administering FMT, making results across studies difficult to compare. Despite these challenges, evidence supports that FMT induces clinical remission in UC, particularly when patients received multiple lower gastrointestinal infusions[127]. Clinical remission was achieved for 28% of patients with UC across four randomized controlled trials[128]. Variable response rates to FMT in UC are probably due to the heterogeneity of the disease. The emerging consensus in the field, however, is that FMT has potential as a treatment for UC if (1) antibiotics are administered before FMT treatment; (2) inflammation can be controlled, which would probably result in higher efficacy rates; and (3) a designed cocktail is used that replaces missing and boosts beneficial organisms. Encouragingly, engraftment of species after FMT can be predicted based on the abundance and phylogeny of the bacteria in the donor and pre-FMT patient samples, with donor strains engrafting in an all-or-nothing manner[129]. Although there is evidence that FMT may induce clinical remission in CD, fewer studies of FMT in CD have been conducted and the confidence interval is broad[127]. Only one study reported endoscopic outcomes, which may not correlate with clinical outcomes in CD described in other studies. In this study, no patient achieved endoscopic remission[127,130]. The long-term effects of FMT and the ability to use it as a maintenance therapy have not yet been examined in IBD.

The microbiome alters host immune function and provides several therapeutic leads and targets[34,41,47,51,57–59,61,62,131,132]. Little evidence supports the efficacy of prebiotic or probiotic treatment for IBD; however, the possibility remains that the most efficacious probiotic bacterial strains are yet to be identified. The adaptive immune response could be modulated by administrating microbially derived metabolites or enzymes in postbiotic therapies. For example, nitrogen scavenging pathways of Proteobacteria are a potential therapeutic target, as Proteobacteria gain a growth advantage from host-derived nitrogen, which exacerbates colitis in mouse models[133,134]. Host nitrogen sources include nitric oxide produced by immune cells or urea produced as a by-product of host metabolism. In fact, one study showed that tungstate treatment, which specifically targets the molybdenum cofactor-requiring enzymes needed to use nitric oxide for anaerobic respiration, can inhibit Proteobacteria replication and ameliorate colitis in mice[135]. These types of therapies are still in early stages of development; however, increasing our knowledge of mechanisms by which specific microorganisms interact with the immune system will increase our ability to develop directed microbiome-based therapies.

Conclusions and future perspectives

IBD is a complex disease involving host, microbial and environmental factors. Adopting multidisciplinary approaches that connect genetic risk factors, microorganisms and microbial metabolites with altered immune responses and epithelial cell functions will be essential to fully understand the underlying mechanisms of host–microbiota interactions in disease[136,137]. Gut commensals contribute important functions for human health, including developing and maintaining a robust immune system, providing colonization resistance against pathogens, maintaining the intestinal mucosal barrier and regulating host immunity. There is no single causative organism and IBD is a polymicrobial disease, with more severe disease linked to reduced gut microbial diversity and blooms of bacteria such as *R. gnavus* and *E. coli*. At the same time, the gut microbiome is required for disease onset, as mouse models in germ-free conditions rarely develop IBD-like phenotypes, and broad-spectrum antibiotics can prevent disease onset in mice. In humans, antibiotics can lead to remission in severe cases, and enteral nutrition reliably induces remission in paediatric CD, albeit reducing gut diversity. However, neither of these approaches results in lasting remission or curing the disease. This paradox highlights that, to develop effective therapies and eventually cure IBD, we need to understand the mechanisms underlying aberrant interactions between the host immune system and the gut microbiome.

For future research in the field it will be particularly important to develop an understanding of how microorganisms and microbial products affect the immune status of the host in both health and disease. Functional differences between strains (for example, *R. gnavus* and inflammation-inducing oral *Klebsiella* strains) can provide important insights and opportunities for mechanistic studies. Multiomics technologies have potential to predict disease-relevant host–microbial mechanisms by identifying transcriptional alterations and metabolic changes in IBD. However, the large proportion of microbial genes with unknown function and microorganisms without a sequenced genome need to be taken into account to fully characterize the impact of the microbiome. Fungal and viral communities in the gut have also been implicated in IBD, but are currently largely unexplored. The ability of norovirus to trigger IBD-like phenotypes and the tropism of norovirus for tuft cells suggest that tuft cells may have a yet-unknown role in IBD. Furthermore, because gut microbial composition is highly individualized, we must focus clinical studies on longitudinal tracking of unique patient subsets to understand how normal host–microbiota interactions are shifted in IBD and in response to treatments. Investigating the initial microbiome state of treatment-naive patients and identifying changes over time implicated in disease progression, such as the development of complications[136], disease course and treatment efficacy[28], will be particularly illuminating. Finally, rather than identifying microorganisms and microbial products that cause inflammation in mice and exploring their relevance in humans, it will be important for future functional studies to focus on human disease-relevant microbial factors and test their effects in mouse models[138].

All of these research avenues have high clinical value and will reveal important factors implicated in IBD development. One of the greatest limitations of the current standard of care is that it treats disease symptoms,

such as chronic inflammation, instead of correcting the host–microbiota imbalance that is linked to IBD. Microbiome-based therapeutic interventions could augment beneficial bacteria, target pathogenic organisms, use synthetic organisms or leverage microbial bioactive metabolites to reverse specific defects in IBD by restoring community structure and promoting barrier restitution, immune tolerance and tissue healing. Importantly, IBD is a heterogeneous disease with distinct clinical manifestations. As findings are translated to the clinic, significant microbiome-related differences between disease subtypes will be crucial to consider in order to develop targeted therapies and improve treatment efficacy rates.

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1. Ijssennagger, N. et al. Gut microbiota facilitates dietary heme-induced epithelial hyperproliferation by opening the mucus barrier in colon. *Proc. Natl Acad. Sci. USA* **112**, 10038–10043 (2015).
2. Yano, J. M. et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* **161**, 264–276 (2015).
3. Reinhardt, C. et al. Tissue factor and PAR1 promote microbiota-induced intestinal vascular remodelling. *Nature* **483**, 627–631 (2012).
4. Cho, I. et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature* **488**, 621–626 (2012).
5. Lee, W. J. & Hase, K. Gut microbiota-generated metabolites in animal health and disease. *Nat. Chem. Biol.* **10**, 416–424 (2014).
6. Fischbach, M. A. & Segre, J. A. Signaling in host-associated microbial communities. *Cell* **164**, 1288–1300 (2016).
7. Turnbaugh, P. J. et al. A core gut microbiome in obese and lean twins. *Nature* **457**, 480–484 (2009).
8. Le Chatelier, E. et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* **500**, 541–546 (2013).
9. Qin, J. et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* **490**, 55–60 (2012).
10. Karlsson, F. H. et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* **498**, 99–103 (2013).
11. Wang, Z. et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **472**, 57–63 (2011).
12. Koeth, R. A. et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat. Med.* **19**, 576–585 (2013).
13. Tang, W. H. et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N. Engl. J. Med.* **368**, 1575–1584 (2013).
14. Hsiao, E. Y. et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* **155**, 1451–1463 (2013).
15. Fujimura, K. E. et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat. Med.* **22**, 1187–1191 (2016).
16. Arrieta, M. C. et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci. Transl Med.* **7**, 307ra152 (2015).
17. Vatanen, T. et al. Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. *Cell* **165**, 842–853 (2016).
18. Zhao, G. et al. Intestinal virome changes precede autoimmunity in type I diabetes-susceptible children. *Proc. Natl Acad. Sci. USA* **114**, E6166–E6175 (2017).
19. Huang, H. et al. Fine-mapping inflammatory bowel disease loci to single-variant resolution. *Nature* **547**, 173–178 (2017).
20. Lamas, B. et al. CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nat. Med.* **22**, 598–605 (2016).
21. Imhann, F. et al. Interplay of host genetics and gut microbiota underlying the onset and clinical presentation of inflammatory bowel disease. *Gut* **67**, 108–119 (2018).
22. Ng, S. C. et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet* **390**, 2769–2778 (2018).
23. Zhernakova, A. et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science* **352**, 565–569 (2016).
24. Rothschild, D. et al. Environment dominates over host genetics in shaping human gut microbiota. *Nature* **555**, 210–215 (2018).
25. Kronman, M. P., Zaoutis, T. E., Haynes, K., Feng, R. & Coffin, S. E. Antibiotic exposure and IBD development among children: a population-based cohort study. *Pediatrics* **130**, e794–e803 (2012).
26. Ferrante, M. et al. New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. *Gut* **56**, 1394–1403 (2007).
27. Dotan, I. et al. Antibodies against laminaribioside and chitobioside are novel serologic markers in Crohn's disease. *Gastroenterology* **131**, 366–378 (2006).
28. Schirmer, M. et al. Compositional and temporal changes in the gut microbiome of pediatric ulcerative colitis patients are linked to disease course. *Cell Host Microbe* **24**, 600–610 (2018).
29. Schaubeck, M. et al. Dysbiotic gut microbiota causes transmissible Crohn's disease-like ileitis independent of failure in antimicrobial defence. *Gut* **65**, 225–237 (2016).
30. Couturier-Maillard, A. et al. NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J. Clin. Invest.* **123**, 700–711 (2013).
31. Elinav, E. et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* **145**, 745–757 (2011).
32. Garrett, W. S. et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell* **131**, 33–45 (2007).
33. Henao-Mejia, J. et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* **482**, 179–185 (2012).
34. Ivanov, I. I. et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* **139**, 485–498 (2009).
35. Sellon, R. K. et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect. Immun.* **66**, 5224–5231 (1998).
36. Schultz, M. et al. IL-2-deficient mice raised under germfree conditions develop delayed mild focal intestinal inflammation. *Am. J. Physiol.* **276**, G1461–G1472 (1999).
37. Rath, H. C., Wilson, K. H. & Sartor, R. B. Differential induction of colitis and gastritis in HLA-B27 transgenic rats selectively colonized with *Bacteroides vulgatus* or *Escherichia coli*. *Infect. Immun.* **67**, 2969–2974 (1999).
38. Xavier, R. J. & Podolsky, D. K. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* **448**, 427–434 (2007).
39. Bunker, J. J. & Bendelac, A. IgA responses to microbiota. *Immunity* **49**, 211–224 (2018).
40. Mkaddem, S. B. et al. IgA, IgA receptors, and their anti-inflammatory properties. *Curr. Top. Microbiol. Immunol.* **382**, 221–235 (2014).
41. Moon, C. et al. Vertically transmitted faecal IgA levels determine extra-chromosomal phenotypic variation. *Nature* **521**, 90–93 (2015).
42. Mohanan, V. et al. C1orf106 is a colitis risk gene that regulates stability of epithelial adherens junctions. *Science* **359**, 1161–1166 (2018).
43. Gaudier, E. et al. Butyrate specifically modulates MUC gene expression in intestinal epithelial goblet cells deprived of glucose. *Am. J. Physiol. Gastrointest. Liver Physiol.* **287**, G1168–G1174 (2004).
44. Furusawa, Y. et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* **504**, 446–450 (2013).
45. Kim, M. H., Kang, S. G., Park, J. H., Yanagisawa, M. & Kim, C. H. Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology* **145**, 396–406 (2013).
46. Venkatesh, M. et al. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. *Immunity* **41**, 296–310 (2014).
47. Wlodarska, M. et al. Indoleacrylic acid produced by commensal *Peptostreptococcus* species suppresses inflammation. *Cell Host Microbe* **22**, 25–37 (2017). **This work identifies a tryptophan derivative produced by gut bacteria that increases mucus production and decreases inflammatory cytokine production**.
48. Dominguez-Bello, M. G. et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl Acad. Sci. USA* **107**, 11971–11975 (2010).
49. Backhed, F. et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* **17**, 690–703 (2015).
50. Jakobsson, H. E. et al. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. *Gut* **63**, 559–566 (2014).
51. Atarashi, K. et al. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* **331**, 337–341 (2011).
52. Olszak, T. et al. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* **336**, 489–493 (2012).
53. An, D. et al. Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells. *Cell* **156**, 123–133 (2014). **This paper shows that sphingolipids produced by intestinal bacteria have a role in colonic iNKT cell homeostasis**.
54. Lopez-Serrano, P. et al. Environmental risk factors in inflammatory bowel diseases. Investigating the hygiene hypothesis: a Spanish case–control study. *Scand. J. Gastroenterol.* **45**, 1464–1471 (2010).
55. von Mutius, E. Allergies, infections and the hygiene hypothesis—the epidemiological evidence. *Immunobiology* **212**, 433–439 (2007).
56. Britton, G. J. et al. Microbiotas from humans with inflammatory bowel disease alter the balance of gut Th17 and RORγt$^+$ regulatory T cells and exacerbate colitis in mice. *Immunity* **50**, 212–224 (2019).
57. Gaboriau-Routhiau, V. et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* **31**, 677–689 (2009).
58. Mao, K. et al. Innate and adaptive lymphocytes sequentially shape the gut microbiota and lipid metabolism. *Nature* **554**, 255–259 (2018).
59. Atarashi, K. et al. Th17 cell induction by adhesion of microbes to intestinal epithelial cells. *Cell* **163**, 367–380 (2015).
60. Dobes, J. et al. Gastrointestinal autoimmunity associated with loss of central tolerance to enteric α-defensins. *Gastroenterology* **149**, 139–150 (2015).
61. Round, J. L. & Mazmanian, S. K. Inducible Foxp3$^+$ regulatory T cell development by a commensal bacterium of the intestinal microbiota. *Proc. Natl Acad. Sci. USA* **107**, 12204–12209 (2010).
62. Atarashi, K. et al. T_{reg} induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* **500**, 232–236 (2013).
63. Gevers, D. et al. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* **15**, 382–392 (2014).
64. Sokol, H., Lay, C., Seksik, P. & Tannock, G. W. Analysis of bacterial bowel communities of IBD patients: what has it revealed? *Inflamm. Bowel Dis.* **14**, 858–867 (2008).
65. Scanlan, P. D., Shanahan, F., O'Mahony, C. & Marchesi, J. R. Culture-independent analyses of temporal variation of the dominant fecal microbiota and targeted bacterial subgroups in Crohn's disease. *J. Clin. Microbiol.* **44**, 3980–3988 (2006).
66. Ott, S. J. et al. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut* **53**, 685–693 (2004).

67. Gophna, U., Sommerfeld, K., Gophna, S., Doolittle, W. F. & Veldhuyzen van Zanten, S. J. Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. *J. Clin. Microbiol.* **44**, 4136–4141 (2006).
68. Frank, D. N. et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl Acad. Sci. USA* **104**, 13780–13785 (2007).
69. Swidsinski, A., Weber, J., Loening-Baucke, V., Hale, L. P. & Lochs, H. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. *J. Clin. Microbiol.* **43**, 3380–3389 (2005).
70. Swidsinski, A. et al. Mucosal flora in inflammatory bowel disease. *Gastroenterology* **122**, 44–54 (2002).
71. Kotlowski, R., Bernstein, C. N., Sepehri, S. & Krause, D. O. High prevalence of *Escherichia coli* belonging to the B2+D phylogenetic group in inflammatory bowel disease. *Gut* **56**, 669–675 (2007).
72. Mylonaki, M., Rayment, N. B., Rampton, D. S., Hudspith, B. N. & Brostoff, J. Molecular characterization of rectal mucosa-associated bacterial flora in inflammatory bowel disease. *Inflamm. Bowel Dis.* **11**, 481–487 (2005).
73. Conte, M. P. et al. Gut-associated bacterial microbiota in paediatric patients with inflammatory bowel disease. *Gut* **55**, 1760–1767 (2006).
74. Schultsz, C., Van Den Berg, F. M., Ten Kate, F. W., Tytgat, G. N. & Dankert, J. The intestinal mucus layer from patients with inflammatory bowel disease harbors high numbers of bacteria compared with controls. *Gastroenterology* **117**, 1089–1097 (1999).
75. Prindiville, T., Cantrell, M. & Wilson, K. H. Ribosomal DNA sequence analysis of mucosa-associated bacteria in Crohn's disease. *Inflamm. Bowel Dis.* **10**, 824–833 (2004).
76. Yilmaz, B. et al. Microbial network disturbances in relapsing refractory Crohn's disease. *Nat. Med.* **25**, 323–336 (2019).
77. Morgan, X. C. et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol.* **13**, R79 (2012).
78. Vich Vila, A. et al. Gut microbiota composition and functional changes in inflammatory bowel disease and irritable bowel syndrome. *Sci. Transl Med.* **10**, eaap8914 (2018).
79. Franzosa, E. A. et al. Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat. Microbiol.* **4**, 293–305 (2019).
This work pairs metagenomic and metabolomic data from a cross-sectional IBD cohort to identify associations between gut bacteria and metabolites that are predictive of IBD status.
80. Lewis, J. D. et al. Inflammation, antibiotics, and diet as environmental stressors of the gut microbiome in pediatric Crohn's disease. *Cell Host Microbe* **18**, 489–500 (2015).
This study shows that microbial dysbiosis results from independent effects of inflammation, diet and antibiotics and is an important factor in the context of enteral nutrition and anti-TNF treatment in paediatric patients with CD.
81. Hall, A. B. et al. A novel *Ruminococcus gnavus* clade enriched in inflammatory bowel disease patients. *Genome Med.* **9**, 103 (2017).
82. The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214 (2012).
83. Lloyd-Price, J. et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* **569**, 655–662 (2019)
84. Sundin, J. et al. Fecal chromogranins and secretogranins are linked to the fecal and mucosal intestinal bacterial composition of IBS patients and healthy subjects. *Sci. Rep.* **8**, 16821 (2018).
85. Konstantinidis, K. T., Rossello-Mora, R. & Amann, R. Uncultivated microbes in need of their own taxonomy. *ISME J.* **11**, 2399–2406 (2017).
86. Martinez-Medina, M. & Garcia-Gil, L. J. *Escherichia coli* in chronic inflammatory bowel diseases: an update on adherent invasive *Escherichia coli* pathogenicity. *World J. Gastrointest. Pathophysiol.* **5**, 213–227 (2014).
87. Elhenawy, W., Tsai, C. N. & Coombes, B. K. Host-specific adaptive diversification of Crohn's disease-associated adherent-invasive *Escherichia coli*. *Cell Host Microbe* **25**, 301–312 (2019).
88. Palmela, C. et al. Adherent-invasive *Escherichia coli* in inflammatory bowel disease. *Gut* **67**, 574–587 (2018).
89. Atarashi, K. et al. Ectopic colonization of oral bacteria in the intestine drives T_H1 cell induction and inflammation. *Science* **358**, 359–365 (2017).
This study links specific strains of oral *Klebsiella* species that are increased in IBD with T_H1 cell induction and inflammation.
90. Rashid, T., Ebringer, A. & Wilson, C. The role of *Klebsiella* in Crohn's disease with a potential for the use of antimicrobial measures. *Int. J. Rheumatol.* **2013**, 610393 (2013).
91. Garrett, W. S. et al. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe* **8**, 292–300 (2010).
92. Carvalho, F. A. et al. Crohn's disease adherent-invasive *Escherichia coli* colonize and induce strong gut inflammation in transgenic mice expressing human CEACAM. *J. Exp. Med.* **206**, 2179–2189 (2009).
93. Lopez-Siles, M., Duncan, S. H., Garcia-Gil, L. J. & Martinez-Medina, M. *Faecalibacterium prausnitzii*: from microbiology to diagnostics and prognostics. *ISME J.* **11**, 841–852 (2017).
94. Franzosa, E. A. et al. Relating the metatranscriptome and metagenome of the human gut. *Proc. Natl Acad. Sci. USA* **111**, E2329–E2338 (2014).
95. Schirmer, M. et al. Dynamics of metatranscription in the inflammatory bowel disease gut microbiome. *Nat. Microbiol.* **3**, 337–346 (2018).
This work demonstrates that metatranscriptomic profiling can reveal species-specific biases in transcriptional activity of gut bacteria, including IBD-specific microbial characteristics, providing new insight into the potential mechanisms of host–microbial dysbiosis in disease.
96. Korem, T. et al. Growth dynamics of gut microbiota in health and disease inferred from single metagenomic samples. *Science* **349**, 1101–1106 (2015).
97. Thorburn, A. N., Macia, L. & Mackay, C. R. Diet, metabolites, and "western-lifestyle" inflammatory diseases. *Immunity* **40**, 833–842 (2014).
98. Donia, M. S. & Fischbach, M. A. Human microbiota. Small molecules from the human microbiota. *Science* **349**, 1254766 (2015).
99. Ursell, L. K. et al. The intestinal metabolome: an intersection between microbiota and host. *Gastroenterology* **146**, 1470–1476 (2014).
100. Levy, M. et al. Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. *Cell* **163**, 1428–1443 (2015).
101. Jacobs, J. P. et al. A disease-associated microbial and metabolomics state in relatives of pediatric inflammatory bowel disease patients. *Cell. Mol. Gastroenterol. Hepatol.* **2**, 750–766 (2016).
102. Kolho, K. L., Pessia, A., Jaakkola, T., de Vos, W. M. & Velagapudi, V. Faecal and serum metabolomics in paediatric inflammatory bowel disease. *J. Crohns Colitis* **11**, 321–334 (2017).
103. Scoville, E. A. et al. Alterations in lipid, amino acid, and energy metabolism distinguish Crohn's disease from ulcerative colitis and control subjects by serum metabolomic profiling. *Metabolomics* **14**, 17 (2018).
104. Jansson, J. et al. Metabolomics reveals metabolic biomarkers of Crohn's disease. *PLOS ONE* **4**, e6386 (2009).
105. Mitsuyama, K. et al. Antibody markers in the diagnosis of inflammatory bowel disease. *World J. Gastroenterol.* **22**, 1304–1310 (2016).
106. Sokol, H. et al. Fungal microbiota dysbiosis in IBD. *Gut* **66**, 1039–1048 (2017).
107. Leonardi, I. et al. $CX3CR1^+$ mononuclear phagocytes control immunity to intestinal fungi. *Science* **359**, 232–236 (2018).
108. Chiaro, T. R. et al. A member of the gut mycobiota modulates host purine metabolism exacerbating colitis in mice. *Sci. Transl Med.* **9**, eaaf9044 (2017).
109. Limon, J. J. et al. Malassezia is associated with Crohn's disease and exacerbates colitis in mouse models. *Cell Host Microbe* **25**, 377–388 (2019).
110. Cadwell, K. et al. Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. *Cell* **141**, 1135–1145 (2010).
111. McGovern, D. P. et al. Fucosyltransferase 2 (FUT2) non-secretor status is associated with Crohn's disease. *Hum. Mol. Genet.* **19**, 3468–3476 (2010).
112. Wilen, C. B. et al. Tropism for tuft cells determines immune promotion of norovirus pathogenesis. *Science* **360**, 204–208 (2018).
113. Norman, J. M. et al. Disease-specific alterations in the enteric virome in inflammatory bowel disease. *Cell* **160**, 447–460 (2015).
114. Lopetuso, L. R., Ianiro, G., Scaldaferri, F., Cammarota, G. & Gasbarrini, A. Gut virome and inflammatory bowel disease. *Inflamm. Bowel Dis.* **22**, 1708–1712 (2016).
115. Ananthakrishnan, A. N. et al. Gut microbiome function predicts response to anti-integrin biologic therapy in inflammatory bowel diseases. *Cell Host Microbe* **21**, 603–610 (2017).
116. Kolho, K. L. et al. Fecal microbiota in pediatric inflammatory bowel disease and its relation to inflammation. *Am. J. Gastroenterol.* **110**, 921–930 (2015).
117. Doherty, M. K. et al. Fecal microbiota signatures are associated with response to ustekinumab therapy among Crohn's disease patients. *mBio* **9**, e02120–17 (2018).
118. Shaw, K. A. et al. Dysbiosis, inflammation, and response to treatment: a longitudinal study of pediatric subjects with newly diagnosed inflammatory bowel disease. *Genome Med.* **8**, 75 (2016).
119. Halmos, E. P. & Gibson, P. R. Dietary management of IBD—insights and advice. *Nat. Rev. Gastroenterol. Hepatol.* **12**, 133–146 (2015).
120. Narula, N. et al. Enteral nutritional therapy for induction of remission in Crohn's disease. *Cochrane Database Syst. Rev.* **4**, CD000542 (2018).
121. Dziechciarz, P., Horvath, A., Shamir, R. & Szajewska, H. Meta-analysis: enteral nutrition in active Crohn's disease in children. *Aliment. Pharmacol. Ther.* **26**, 795–806 (2007).
122. Albenberg, L. G. & Wu, G. D. Diet and the intestinal microbiome: associations, functions, and implications for health and disease. *Gastroenterology* **146**, 1564–1572 (2014).
123. Lee, D. et al. Diet in the pathogenesis and treatment of inflammatory bowel diseases. *Gastroenterology* **148**, 1087–1106 (2015).
124. Ananthakrishnan, A. N. et al. A prospective study of long-term intake of dietary fiber and risk of Crohn's disease and ulcerative colitis. *Gastroenterology* **145**, 970–977 (2013).
125. Llewellyn, S. R. et al. Interactions between diet and the intestinal microbiota alter intestinal permeability and colitis severity in mice. *Gastroenterology* **154**, 1037–1046 (2018).
126. Albenberg, L. et al. A diet low in red and processed meat does not reduce rate of Crohn's disease flares. *Gastroenterology* https://doi.org/10.1053/j.gastro.2019.03.015 (2019).
127. Paramsothy, S. et al. Faecal microbiota transplantation for inflammatory bowel disease: a systematic review and meta-analysis. *J. Crohns Colitis* **11**, 1180–1199 (2017).
128. Levy, A. N. & Allegretti, J. R. Insights into the role of fecal microbiota transplantation for the treatment of inflammatory bowel disease. *Therap. Adv. Gastroenterol.* **12**, 1756284819836893 (2019).
129. Smillie, C. S. et al. Strain tracking reveals the determinants of bacterial engraftment in the human gut following fecal microbiota transplantation. *Cell Host Microbe* **23**, 229–240 (2018).
130. Vermeire, S. et al. Donor species richness determines faecal microbiota transplantation success in inflammatory bowel disease. *J. Crohns Colitis* **10**, 387–394 (2016).
131. Palm, N. W. et al. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell* **158**, 1000–1010 (2014).
This study pioneered the use of IgA coating to identify inflammatory members of the IBD gut microbiota.
132. Larmonier, C. B., Shehab, K. W., Ghishan, F. K. & Kiela, P. R. T. Lymphocyte dynamics in inflammatory bowel diseases: role of the microbiome. *Biomed. Res. Int.* **2015**, 504638 (2015).
133. Ni, J. et al. A role for bacterial urease in gut dysbiosis and Crohn's disease. *Sci. Transl Med.* **9**, eaah6888 (2017).
134. Winter, S. E. et al. Host-derived nitrate boosts growth of *E. coli* in the inflamed gut. *Science* **339**, 708–711 (2013).
135. Zhu, W. et al. Precision editing of the gut microbiota ameliorates colitis. *Nature* **553**, 208–211 (2018).
This paper shows that tungstate treatment inhibits molybdenum-cofactor-dependent microbial respiratory pathways in the mammalian gut and prevents the dysbiotic expansion of Enterobacteriaceae during gut inflammation.
136. Kugathasan, S. et al. Prediction of complicated disease course for children newly diagnosed with Crohn's disease: a multicentre inception cohort study. *Lancet* **389**, 1710–1718 (2017).
137. Hyams, J. S. et al. Clinical and biological predictors of response to standardised paediatric colitis therapy (PROTECT): a multicentre inception cohort study. *Lancet* **393**, 1708–1720 (2019).

138. Tanoue, T. et al. A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. *Nature* **565**, 600–605 (2019).
139. Podolsky, D. K. Inflammatory bowel disease. *N. Engl. J. Med.* **347**, 417–429 (2002).
140. Hering, N. A., Fromm, M. & Schulzke, J. D. Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics. *J. Physiol.* **590**, 1035–1044 (2012).
141. Salzman, N. H. et al. Enteric defensins are essential regulators of intestinal microbial ecology. *Nat. Immunol.* **11**, 76–83 (2010).
142. VanDussen, K. L. et al. Genetic variants synthesize to produce Paneth cell phenotypes that define subtypes of Crohn's disease. *Gastroenterology* **146**, 200–209 (2014).
143. Adolph, T. E. et al. Paneth cells as a site of origin for intestinal inflammation. *Nature* **503**, 272–276 (2013).
144. Surawicz, C. M., Haggitt, R. C., Husseman, M. & McFarland, L. V. Mucosal biopsy diagnosis of colitis: acute self-limited colitis and idiopathic inflammatory bowel disease. *Gastroenterology* **107**, 755–763 (1994).
145. Van der Sluis, M. et al. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology* **131**, 117–129 (2006).
146. McDole, J. R. et al. Goblet cells deliver luminal antigen to CD103^{+} dendritic cells in the small intestine. *Nature* **483**, 345–349 (2012).
147. Desai, D., Faubion, W. A. & Sandborn, W. J. Review article: biological activity markers in inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **25**, 247–255 (2007).
148. Francescone, R., Hou, V. & Grivennikov, S. I. Cytokines, IBD, and colitis-associated cancer. *Inflamm. Bowel Dis.* **21**, 409–418 (2015).
149. Fernando, M. R., Saxena, A., Reyes, J. L. & McKay, D. M. Butyrate enhances antibacterial effects while suppressing other features of alternative activation in IL-4-induced macrophages. *Am. J. Physiol. Gastrointest. Liver Physiol.* **310**, G822–G831 (2016).
150. Wikoff, W. R. et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc. Natl Acad. Sci. USA* **106**, 3698–3703 (2009).
151. Zelante, T. et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity* **39**, 372–385 (2013).
152. Williams, B. B. et al. Discovery and characterization of gut microbiota decarboxylases that can produce the neurotransmitter tryptamine. *Cell Host Microbe* **16**, 495–503 (2014).
153. Devlin, A. S. & Fischbach, M. A. A biosynthetic pathway for a prominent class of microbiota-derived bile acids. *Nat. Chem. Biol.* **11**, 685–690 (2015).
154. Cao, W. et al. The xenobiotic transporter Mdr1 enforces T cell homeostasis in the presence of intestinal bile acids. *Immunity* **47**, 1182–1196 (2017).
155. Fischbach, M. A. & Sonnenburg, J. L. Eating for two: how metabolism establishes interspecies interactions in the gut. *Cell Host Microbe* **10**, 336–347 (2011).
156. Tannahill, G. M. et al. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* **496**, 238–242 (2013).
157. Serena, C. et al. Elevated circulating levels of succinate in human obesity are linked to specific gut microbiota. *ISME J.* **12**, 1642–1657 (2018).
158. Ferreyra, J. A. et al. Gut microbiota-produced succinate promotes *C. difficile* infection after antibiotic treatment or motility disturbance. *Cell Host Microbe* **16**, 770–777 (2014).
159. De Vadder, F. et al. Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis. *Cell Metab.* **24**, 151–157 (2016).
160. Abdel Hadi, L., Di Vito, C. & Riboni, L. Fostering inflammatory bowel disease: sphingolipid strategies to join forces. *Mediators Inflamm.* **2016**, 3827684 (2016).
161. Segata, N. GraPhlAn. *Bitbucket* https://bitbucket.org/nsegata/graphlan/wiki/Home (2018).
162. Vaughn, B. P. et al. Increased intestinal microbial diversity following fecal microbiota transplant for active Crohn's disease. *Inflamm. Bowel Dis.* **22**, 2182–2190 (2016).

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