IBD AND THE GUT MICROBIOTA

C. Mah¹, E. Bessède², N. Benech³,⁴, G.L. Hold¹, H. Sokol³,⁴,⁵

¹Microbiome Research Centre, St George & Sutherland Clinical School, University of New South Wales, Sydney, Australia
²INSERM, BaRITOn, U1053, Univ. Bordeaux, Bordeaux, France
³INSERM, Centre de Recherche Saint-Antoine, CRSA, AP-HP, Saint Antoine Hospital, Gastroenterology Department, Sorbonne Université, Paris, France
⁴Paris Center for Microbiome Medicine, Fédération Hospitalo-Universitaire, Paris, France
⁵INRA, UMR1319 Micalis & AgroParisTech, Jouy en Josas, France

CASSANDRA MAH, EMILIE BESSÈDE, AND NICOLAS BENECH EQUALLY CONTRIBUTED

Corresponding Author: Harry Sokol, MD; e-mail: harry.sokol@aphp.fr

Abstract – The current article is a review of the most important, accessible and relevant literature published between April 2020 and March 2021 on the gut microbiota and inflammatory bowel disease (IBD). The major areas of publication during this period were human clinical studies, as well as mechanistic insights from animal models. Most papers focused on the bacterial component of the gut microbiota although some papers described aspects of the virome and mycobiome. There were over 70 articles published in the reporting period.

Keywords: Inflammatory bowel disease, Microbiome, Microbiota, Colitis models.

IMPACT OF DIET ON IBD

A clinical trial aimed to compare the effects of a low-fat and high-fibre diet and the effects of an improved standard American diet on quality of life, markers of inflammation and faecal markers of intestinal dysbiosis in ulcerative colitis (UC) patients¹. In this cross-over study, both diets increased patients’ quality of life, decreased inflammatory markers and reduced intestinal dysbiosis, suggesting that dietary interventions might benefit patients in remission. Another study² focused on the effects of polysaccharides contained in flowers of tea on the gut microbiota. The authors reported that after fermentation of the polysaccharides from the tea flowers, significant changes in the composition of intestinal bacteria were observed simultaneously with their metabolites, including short-chain fatty acids (SCFAs). They concluded that polysaccharides from tea flowers have prebiotic effects but might have pro-inflammatory consequences in IBD based on the stimulated bacteria. A study aimed to determine whether seasonal serum vitamin D levels could influence the microbial composition of the lower gut of IBD patients living in the Central European region in relation to the seasonal change of sunlight³. The authors observed seasonal variation with a reduction in abundance of bacterial genera typically associated with inflammation during the summer/autumn period.

INTESTINAL PHYSIOLOGY

A study⁴ aimed to compare the bacterial diversity, composition and aerotolerance profiles across colonic and ileal regions, and Crohn’s disease (CD) phenotypes. The study showed that...
bacterial diversity decreased in the ileum and the colon of CD patients compared to healthy individuals. Furthermore, in healthy patients, the aerotolerance profiles of bacteria were significantly different between intestinal segments (although dominated by obligate anaerobes) whereas in CD patients, relatively high levels of obligate anaerobes were maintained in the colon and increased in the ileum, such as *Bacteroides fragilis*. They also observed that resected tissues of patients who developed postoperative disease recurrence had lower levels of *Bacteroides* and *Streptococcus*. The work shows that in CD patients, the mucosally adherent bacteria in the colon and ileum and their alterations are different from what is observed in faecal material.

The intestinal mucosal barrier function was evaluated in old and young UC patients and healthy controls. The expression of E-cadherin and occludin proteins were lower in the colon of older UC patients than in the younger UC patients. The authors used a dextran sulfate sodium (DSS) model in mice to explore these results further. The composition of gut microbiota differed between the young and aged mice. At baseline compared to young mice, aged mice had a lower abundance of beneficial bacteria (*Lactobacillus*) and a higher abundance of potentially harmful bacteria. However, a decrease in the biodiversity following DSS treatment was observed in both young and aged mice. Furthermore, this trend was more visible in the aged mice, which indicates a higher sensitivity to DSS-induced colitis in this group. This article demonstrated that aging human colon is characterized by an impairment of the intestinal barrier and that, in a mice model, age-related deteriorations of gastrointestinal barrier function and gut microbial dysbiosis may be involved in the colitis severity.

Arginase 1 (Arg1), which converts L-arginine into ornithine and urea, is involved in immune cell function and it has been shown that IBD patients have enhanced Arg1 activity. Arg1 deficiency could have consequences on the composition of intestinal microbiota since several microbes use the L-arginine pool of the host for their own survival. Conditional KO mice models showed that Arg1 expression in hematopoietic and endothelial cells is detrimental in DSS-induced colitis setting. The protection of mice lacking Arg1 in hematopoietic and endothelial cells was associated with compositional changes in the intestinal microbiota, intraluminal polyamine accumulation and altered permissiveness of the host to inflammatory microbial compounds. Furthermore, faecal microbiota transfer from Arg1 deficient mice donors into littermate recipients restores the protective, anti-inflammatory phenotype, demonstrating a causal effect of the Arg1-induced microbiota alterations. L-arginine metabolism may serve as a target for clinical intervention in IBD patients.

A study analysed the potential relationship between gut dysbiosis, stool miRNA composition and SCFA level in response to CD intestinal inflammation. Fifteen CD patients (8 with active disease and 7 in remission) and nine healthy controls were enrolled. Disease activity was determined by measuring the CD activity index. DNA, miRNA, and metabolites were extracted from the stool samples and microbial, miRNA and SCFA profiles were assessed. Beside alterations in microbiota composition, IBD was associated with reduced levels of SCFAs and secondary bile acids, but enhanced levels of primary bile acids. The study identified 13 miRNAs that differed between CD patients and healthy controls. miRNA profiles were also distinct between patients with active and inactive CD. Finally, the authors created multi-omics profiles characterizing the clinical status of CD patients based on gut dysbiosis, miRNA profiling and SCFA levels.

In another study, the mechanism linking Fut2 to IBD was explored. Colitis was induced by DSS in intestinal epithelium-specific Fut2 knockout (Fut2<sup>−/−</sup>) mice. Gut microbiota in Fut2<sup>−/−</sup> mice was altered structurally and functionally, promoting the generation of Lysophosphatidylcholine, which was shown to promote inflammation and induce epithelial barrier damage. Furthermore, Fut2 and α-1,2-fucosylation in the colon were decreased in CD and UC patients.

**CHARACTERIZATION OF THE NONBACTERIAL COMPONENT OF THE GUT MICROBIOTA IN IBD PATIENTS**

In accordance with previous results, overrepresentation of *Candida* species was reported in patients with active IBD, both in UC and CD. Moreover, in a small case-series, patients with pouchitis had lower mycobiota alpha diversity and a higher abundance of *Saccharomyces*
compared to patients with a normal pouch\textsuperscript{11}. Sarrabayrouse et al\textsuperscript{12} assessed the fungal and bacterial loads in IBD patient faecal samples. Authors performed quantitative PCR of the ITS2 region and the 16S rRNA gene in 206 participants including healthy controls, UC and CD patients from two independent cohorts. UC patients had a higher fungal/bacterial load ratio compared to controls or CD patients. In CD patients, bacterial and fungal loads were increased during flares, whereas no significant changes were found in UC patients according to disease activity. Surprisingly, no effect of immunosuppressants on microbial loads was reported. A random forest model to predict disease activity and diagnosis combining demographic characteristics and microbial loads was designed, reaching good diagnostic performance. However, these results require confirmation in a validation cohort.

Nishiyama et al\textsuperscript{13} explored the virome ecological structure in IBD patients and reported over-representation of bacteriophages infecting \textit{Bacteroides uniformis} and \textit{Bacteroides thetaiotaomicron}. In addition, the authors evidenced that many phages were infecting a phylogenetically broad range of bacteria. In early-onset IBD (before the age of 6 years), patients presented a specific viral signature with a higher \textit{Caudovirales/Microviridae} ratio compared to healthy controls. Beside, \textit{Anelloviridae}, which are not phage but infect human cells, were also increased in these patients\textsuperscript{14}.

\section*{STUDIES EXPLORING THE CROSSTALK BETWEEN THE GUT MICROBIOTA AND THE IMMUNE SYSTEM}

\subsection*{Antibody Response and the Gut Microbiota}

Immunoglobulins (Ig) are key features of intestinal homeostasis and there is mounting evidence that they play a significant role in regulating gut microbiota composition and the local immune landscape. Indeed, the microbiota of ATF3\textsuperscript{-/-} deficient mice with a defect in IgA secretion was described as having an increase in pro-inflammatory bacteria that can be corrected by adoptive transfer of IgA+ B cells, with a protective effect against colitis\textsuperscript{15}. Using Natural-Killer T lymphocytes (iNKT) deficient mice, iNKTs were also found to be important in IgA secretion and IgA-mediated protection against colitis\textsuperscript{16}.

How immunoglobulins bind to bacteria and whether there is a link with IBD disease activity remains poorly understood. Several works were published in 2020 to explore these questions. Kabbert et al\textsuperscript{17} studied antigen specificity of both IgA and IgG in IBD patients and healthy individuals. They showed that IgG and IgA can bind numerous different bacteria with a cross-species reactivity both in a normal or inflamed condition. Another study explored the repertoire of carbohydrate-specific antibodies (IgG and IgM) in the serum of CD or UC patients. Authors reported an increased affinity for fucosylated oligosaccharides and fucose-carrying \textit{Bacteroides} species in CD but not in UC suggesting a potential role for bacteria carrying such molecular unit in CD\textsuperscript{18}. Similarly, stressing the importance of specific IgG response in IBD patients, single-cell analysis of immune cells from UC patients revealed a specific increase of IgG1+ plasma cells in the gut of IBD patients compared to healthy individuals\textsuperscript{19}. Rengarajan et al\textsuperscript{20} analyzed IgA and IgG-bound bacteria from faecal samples of healthy controls, UC and CD patients using FACS-sorting and 16S rRNA sequencing. They found that IBD patients have an increased percentage of IgA and IgG-bound bacteria. Moreover, disease activity was correlated with increased IgA- and IgG-bound bacteria in CD and an increase of only IgG-bound bacteria in UC. Several Ig-bound bacteria were identified as usual member of the oral microbiota suggesting a potential link between the oral microbiota and IBD.

\section*{THE ORAL MICROBIOTA, A NEW ACTOR OF IMMUNE REGULATION AND IBD PATHOPHYSIOLOGY}

New data have been recently reported linking periodontal inflammation and gut inflammation. A seminal paper published in \textit{Cell}\textsuperscript{21} explored the underlying mechanisms. In a murine model of oral periodontitis, periodontal inflammation favoured expansion of pro-in-
Inflammatory Enterobacteriaceae that translocated to the gut while favoring expansion of inflammatory Th17 cells both in the gut and the oral mucosa, contributing to colitis through inflammasome activation in genetically susceptible mice (IL10^{-/-})^{21}. Along the same lines, salivary microbiota dysbiosis was described; *Saccharibacteria*, *Absconditabacteria*, *Leptotrichia*, *Prevotella*, *Bulleidia*, and *Atopobium* were found to significantly increase in the oral cavity of IBD patients and associated with systemic inflammatory markers for *Saccharibacteria* and *Absconditabacteria*^{22}.

**NEW ROLES FOR “OLD” IMMUNE CELLS IN IBD IN RESPONSE TO THE GUT MICROBIOTA**

Two recent reports suggest a significant role for tissue-resident CD8 memory T-cells (CD8_{TM}) in IBD pathogenesis. First, Boland et al^{19}, with single-cell analysis of intestinal FACS-sorted immune cells, identified a subset of CD8TM with a specific inflammatory transcriptional pattern in UC. Noble et al^{23} also reported a reduced number of mucosal CD8TM with regulatory T cells (Treg) characteristics in IBD patients associated with an increase in IgA secretion against gut bacteria. Regarding the innate immune system, striking findings from Bessman et al^{24} revealed for the first time that local intestinal iron regulation by conventional dendritic cells (DC) through hepcidin secretion was under the dependence of the gut microbiota, which promoted intestinal repair and microbiota regulation. Durant et al^{25} reported a loss of regulatory DC in the colon of UC and CD patients associated with the loss of IL-10 secretion in response to nanosized outer membrane vesicles produced by *Bacteroides thetaiotaomicron*. These results suggest that the regulation of the innate immune compartment by these bacterial products may play a significant role in gut homeostasis and immune regulation.

Macrophage response to lipopolysaccharide (LPS), a bacterial component of Gram-negative bacteria may also play a significant role in intestinal inflammation. Indeed, excessive Toll-like receptor 4 (TLR4) signaling in response to LPS was found in macrophages where synbindin, a canonical membrane tethering factor, favoring TLR4 degradation is deleted. Synbindin deletion was associated with exacerbated DSS-induced intestinal inflammation in mice while it was increased in mucosal macrophages of patients with active IBD. These results suggest that TLR4 regulation in human macrophages does play a significant role in mucosal inflammation.

**WHAT IS NEW IN THE PATHOPHYSIOLOGY OF COLITIS-INDUCED CANCER IN RELATION TO THE GUT MICROBIOTA?**

Only a few relevant original articles were published on this topic last year. TLR4 activation in epithelial cells was found to be linked with colorectal cancer. Recently, Burgueño et al^{27} dissected the biological process underlying such an effect. They showed that TLR4 activation by the gut microbiota triggered the activation of NADPH oxidases DUOX2 and NOX1, leading to increased production of H_{2}O_{2} and a TLR-4 dependent enhanced tumorigenesis in mouse models of colitis-induced cancer. *In silico* analysis of available human gene expression dataset suggests that these mechanisms are also present in sporadic and IBD related CRC.

Longitudinal analysis of metabolomic and microbial landscapes along the tumorigenesis process in a rat model of colitis-induced cancer pointed out the potential role of the linoleic acid metabolism in pathogenesis while tumour formation was associated with a gradual decrease of *Lactobacillus* and a marginal increase of *Escherichia-Shigella* in the gut microbiota^{28}. Lastly, many probiotics are now evaluated as antitumor agents. *Lactobacillus bulgaricus* has been found to reduce tumorigenesis in a mouse model of colitis-induced cancer^{29}.

**BRAIN/GUT AXIS AND IBD**

Significant data were published supporting a direct functional link between the brain and the gut microenvironment, including the gut microbiota. New pathways that allow the central nervous system to sense and modulate the gut niche have been unveiled last year. Re-
ently, a new reflex arc control was described linking the central nervous system and the gut immune niche through vagal sensory afferents from the liver. In this work, the colonic pTReg cell pool was decreased after surgical and pharmacological modulation of the vagal sensory afferents inducing an increased susceptibility to colitis. Similarly, Ye et al. demonstrated that tryptophan-derived catabolites from the gut microbiota can trigger vagal neuronal activation through the TrpA1 receptor of enteroendocrine cells.

In clinical practice, a prospective Swiss study assessed potential association between the gut microbiota structure and psychological disorders in 171 IBD patients in clinical remission of the Swiss IBD cohort. Psychometric evaluations of anxiety, depression and quality of life were performed while the gut microbiota composition was determined through 16S rRNA sequencing from intestinal biopsies. The authors reported significant associations between anxiety and depressive symptoms and quality of life and various bacterial families both in CD and UC with distinct patterns according to the disease and the psychological symptom. No underlying mechanism was explored and further studies are needed to confirm these associations and evaluate potential causality.

HUMAN STUDIES

Structural and Functional Microbial Changes in IBD

Studies published in this reporting period analysed IBD microbiota profiles compared to healthy controls using stool and/or mucosal samples. The studies’ findings of decreased alpha-diversity, lower abundance of phylum Firmicutes and higher abundance of phylum Proteobacteria in IBD patients compared to healthy controls is consistent with existing literature. An extensive unbiased meta-analysis of gut microbiome data from five different IBD patient cohorts of varying countries was performed by Sankarasubramanian et al. The authors established that significant disease-specific changes can be observed only at or below the order level in the taxonomic rank. Specifically, species that showed significant association with healthy controls included *Coprococcus catus*, *Coprococcus eutatus*, *Ruminococcus bromii*, and *Gemmiger formicilis*. CD-specific organisms included *Clostridium ramosum*, *Ruminococcus lactaris*, and *Clostridium clostridioforme* and *Clostridium bolteae*, two species that belonged to the genus *Clostridium* and family Lachnospiraceae. Similarly, the four differentiating microbial species that showed significant association with UC included *Ruminococcus albus*, *Ruminococcus callidus*, *Faecalibacterium prausnitzii*, and *Clostridium celatum*. The authors also identified unique differences in CD and UC in metabolic pathways including amino acid and glycan biosynthesis and metabolism. The findings concur with previous studies that disease specific influences of microbial profiles will impact the host metabolic pathways in unique ways.

Results from previous faecal microbiota studies of IBD patients compared to their healthy siblings/relatives have been inconclusive about whether dysbiosis in IBD is a cause or consequence of intestinal inflammation. To investigate further, Sila et al. compared the gut microbiota in newly diagnosed, treatment naïve IBD paediatric patients with that of healthy siblings and unrelated healthy controls. Microbial diversity and composition were significantly reduced in IBD patients compared to both control groups. Notably, reduced abundance of *Eubacterium*, *Lactobacillus*, *Enterobacter* and *Clostridium*, and increased abundance of *Streptococcus*, *Prevotella* and *Escherichia* genera were observed in IBD patients.

Other studies investigated the gut microbiota profiles in IBD stratified by lifestyle and environmental variables. A Russian study used metagenomic sequencing to evaluate IBD-associated biomarkers and gut microbial profiles/functions in 42 healthy controls, 41 CD and 43 UC Kazan subjects. The classic dysbiotic features of increased abundance of Proteobacteria, Actinobacteria, and Fusobacteria and decreased abundance of Firmicutes, Bacteroidetes, and Verrucomicrobia were observed in patients with IBD compared to healthy controls. The study observed that hydrogen releasing and hydrogenotrophic microbes are altered in IBD. For instance, *Ruminococcus* levels were decreased in IBD patients, whilst *Methanobrevibacter* levels, whilst not statistically significant, tended to be overabundant in UC patients. Sulphate-reducing bacteria consisting of *Veillonella*, *Streptococcus*, *Leptotrichia*, and *Desulfovibrio* were...
increased in IBD patients. In another Russian study, Gryaznova and colleagues\textsuperscript{36} compared the microbial profiles of UC patients and healthy controls. Using high throughput sequencing, they observed a higher abundance of Haemophilus, Olsenella, Prevotella, Cedecea, Peptostreptococcus, Faecalibacterium, Lachnospira, Negativibacillus, Butyrivibrio genera and Bacteroides coprocola, Phascolarctobacterium succinatutens, Dialister succinatiphilus, Sutterella wadsworthensis, F. prausnitzii in UC patient faecal samples. Similarly, they found a decrease abundance of Fusicatenibacter, Butyrivibrio, Lactococcus, Eisenbergiella, Coprobacter, Cutibacterium, Falsochrobactrum, Brevundimonas, Yersinia, Leuconostoc and Fusicatenibacter saccharivorans. In a Chinese study involving 72 CD, 51 UC and 73 healthy controls, Zhou et al\textsuperscript{37} investigated the potential of using gut microbiota signatures to differentiate between IBD phenotypes (UC, colonic CD and non-colonic CD) using Random forest approach. The use of total gut microbiota at the genus level was 76.3% accurate in distinguishing UC from colonic CD, while the accuracy was as high as 88.6% when 10 taxa, including *Fusobacterium*, *Gardnerella*, *Odoribacter*, *Holdemania*, *Ruminococcus*, *Sneathia*, *Paraprevotella*, *Lactobacillus*, *Fusobacterium*, *Gardnerella*, Bacteroidales_S24-7, were used. The AUC was 70.7% when using the top 3 taxa containing *Gardnerella* and *Fusobacterium*. These findings demonstrate key characteristics of the gut microbiota, such as *Gardnerella* and *Fusobacterium*, that are potentially useful in identifying IBD disease location. Additionally, the authors also found that gut microbial profiles are distinctly different from their Western counterparts (Human Microbiome Project).

In a large longitudinal intercontinental study published in Gut\textsuperscript{38}, authors aimed to evaluate the relative contribution of different lifestyle and environmental factors to the compositional variability of the gut microbiota. Using 16S rRNA gene sequencing, faecal samples from 303 CD, 228 UC and 161 healthy controls collected at three time points over 16 weeks were assessed. The greatest microbial variance was associated with the presence of CD, followed by geographical location, history of surgery, with most of the compositional variance (90.3%) remaining unexplained. Temporal stability was assessed by comparing within-subject Bray-Curtis distances across multiple time points, which showed that IBD patients had less microbiota stability compared to controls. It was highlighted that inter-individual variance was greater than intra-individual variance, with disturbances in faecal microbial composition being greatest during active disease, especially in CD. The findings emphasize that longitudinal sampling is key to assessing intra-individual variance and afford increased diagnostic potential.

Two studies evaluated the small intestinal microbiota in IBD. Faecal samples were collected from 57 IBD patients who had either an ileostomy or ileoanal pouch. Small intestinal metagenomes were then compared with colonic microbiota metagenomes of 1178 subjects from the general population and 478 IBD patients. Ruigrok et al\textsuperscript{39} observed that colonic microbiota profiles of IBD patients, particularly those with intestinal resections, showed the closest resemblance to that of the small intestine. *Veillonella atypica*, *Streptococcus salivarius* and *Actinomyces graevenitzii* were found to be significantly enriched in the small intestine. These findings highlight the potential implication of the small intestinal microbiome in IBD pathogenesis. In a first of its kind study, Olaisen et al\textsuperscript{40} investigated the ileal bacterial microbiome of adult CD patients by comparing bacterial mucosa-associated microbial signatures in paired inflamed and proximal non-inflamed ileal mucosa. Despite CD specific alterations in ileal mucosa-associated microbiota, including a significant overrepresentation of Tyzzerella compared to healthy controls, there were no differences in alpha or beta diversity and no bacterial taxa were differentially expressed between inflamed and noninflamed mucosa. This suggests that CD-altered ileal mucosal microbiota is independent from ileal sublocation and inflammation status, however, further assessment is required to extend the findings to defining potential functional impact as well as microbiota community composition. Functional consequences of microbiota changes remain relatively under-explored.

Published in Nature Communication, Chen et al\textsuperscript{41} evaluated and compared microbial co-abundance networks using a large metagenomics dataset comprising 2379 participants from four cohorts: IBD, obesity and 2 population-based cohorts. The study showed that microbial dysbiosis can be reflected in alterations in microbial co-abundance. Several species (*E. coli* and *Oxalobacter formigenes*, *Actinomyces graevenitzii*) and functional pathways were identified as key players in IBD co-abundance networks. *E. coli* was shown to have a strong positive co-abundance with other inflammation-inducing species, including *Streptococcus* species (*S. mutans*, *S. vestibularis* and *S. infantis*). While the most significant functional
pathways implicated in IBD was the reductive tricarboxylic acid cycle and tetrahydrofolate pathways. Lysine acetylation was also an important post-translational modification function which is widely distributed in gut microbial metabolic pathways including anaerobic fermentation to produce short chain fatty acids (SCFAs). Zhang et al\(^42\) analysed lysine acetylation in the gut microbiota of patients with CD (limited dataset). Alterations in lysine acetylation, in particular decreased levels of proteins of butyrate/acetate producing bacteria were identified, although further studies are required to assess whether this is a cause or consequence of microbiota community composition changes.

During this reporting period, the protocols of longitudinal observational prospective studies tracking microbiota changes in IBD patients in Australia and Sub-Saharan Africa were also published\(^43,44\).

**MICROBIOTA CHANGES AND DISEASE ACTIVITY/PROGNOSIS**

Several articles investigated the potential for the gut microbiota as a biomarker to complement current clinical practices in diagnostics, disease activity, subtype classification or disease prognosis of IBD\(^41,45-56\). In the field of diagnostics, Galipeau et al\(^45\) conducted a prospective study involving a unique cohort of asymptomatic individuals at risk for IBD to evaluate for specific changes that occur prior to disease onset. Sixty-one first-degree relatives of individuals with CD were recruited in 2008 and as of December 2018, 13 of them subsequently developed UC. Microbiota composition and functions were elucidated using faecal samples collected at enrolment and diagnosis for those who developed UC. Faecal samples from matched subjects that did not develop UC were used as controls. Interestingly, functional analysis revealed increased faecal proteolytic and elastase activity before a clinical diagnosis of UC. Elastase activity was inversely correlated with the abundance of *Adlercreutzia* and directly correlated with proteolytic taxa, including *Bacteroides vulgatus*. High elastase activity was demonstrated in *Bacteroides* isolates from faecal samples. This finding was replicated in germ-free adult mice colonised with pre-UC colonised dams. This unique proteolytic signature may be a non-invasive biomarker to monitor at-risk populations.

Lopez-Siles et al\(^46\) evaluated the potential of two extensively researched microbial biomarkers implicated in IBD-associated dysbiosis, *E. coli* and *F. prausnitzii*, to distinguish between gastrointestinal diseases. Using both collected biopsies and faecal samples, this Spanish retrospective study evaluated 45 CD, 25 UC, 10 Irritable bowel syndrome (IBS), 20 colorectal cancer (CRC) and 31 controls. Relative abundances of *F. prausnitzii* (FP), its phylogroups I and II (PHG1, PHGII) and *E.coli* (E) copy numbers were used to formulate FP-E, PHGII-E and PHGII-E indexes. In biopsies, FP-E index was able to differentiate IBD from CD, while PHGII-E index accurately discriminated IBS from UC. The former index was found to differentiate IBD, especially UC, from CRC regardless of disease activity and patient health status. The PHGII-E index can discriminate within IBD subtypes and establish progression from proctitis and left-sided colitis to ulcerative pancolitis. Further investigation of the key intestinal microbiota changes in healthy subjects, patients with IBD and patients with CRC was undertaken by Ma et al\(^47\). With data from European Nucleotide Archive and Integrated Human Microbiome Project, metagenome-wide association studies on the faecal samples from 290 HC (healthy control), 512 IBD patients and 285 CRC patients were performed. The authors established that the taxonomic and functional composition of the intestinal microbiota in IBD and CRC patients was significantly distinct compared with healthy people. Specifically, IBD patients had low intestinal bacterial diversity while CRC patients had high intestinal bacterial diversity compared to healthy subjects. Consistent with previous studies, the relative abundance of Firmicutes decreased significantly while the relative abundance of Bacteroidetes increased. *Bacteroides* levels in IBD patients were found to be higher compared to both healthy and CRC subjects. The main distinction in the intestinal bacteria between CRC patients and healthy people/IBD subjects was in Fusobacteria, Verrucomicrobia and Proteobacteria. There were significant differences in functional pathways between the three groups and the pathways implicated involved L-homoserine and L-methionine biosynthesis, 5-aminoimidazole ribonucleotide biosynthesis II, L-methionine biosynthesis I, and superpathway of L-lysine, L-threonine, and L-methionine biosynthesis I.
One pilot study also looked at whether the presence of depression as a comorbidity in UC patients correlated with alterations in the gut microbiota. In a cohort of 93 participants (31 healthy controls, 31 UC patients with depression and 31 UC patients without depression), faecal microbiota signatures demonstrated that UC patients with depression had higher disease activity scores and abundance of Gamma-proteobacteria, and lower bacterial richness scores and abundance of Clostridiales species. Whether the microbial changes were linked to increased disease activity or depression was not clarified.

Four studies explored the feasibility of integrating faecal microbiota measures into current practices for disease activity monitoring. Ankerson et al investigated associations between faecal microbiota and disease activity measures in 78 IBD patients tracking disease activity through the use of faecal calprotectin (FC) and the Simple Clinical Colitis Activity Index (SCCAI) over 12 months. Faecal microbiota was analysed using a commercially available DNA test comprising 54 pre-determined markers and 16S rRNA gene sequencing. The authors demonstrated that these methods were able to differentiate between UC and CD, with 16S-seq sequencing able to distinguish less diverse microbiota when comparing CD to UC and left sided/extensive disease to proctitis. Furthermore, Peptostreptococcus anaerobius was found to correlate significantly with FC results, while an additional 24 genera were found to be associated with either FC and/or SCCAI. A further study focused on evaluating the relative abundance of four bacterial groups (Bacteroidetes phylum, class Bacilli, Bifidobacteriaceae family, and Enterobacteriaceae family) in the faecal samples of CD patients and their relation to inflammatory activity. This Brazilian study observed higher levels of Bacteroidetes and a decrease in Bifidobacteriaceae and Bacilli in faecal samples of CD patients compared to healthy controls. However, no association between the phylum Bacteroidetes and clinical activity or disease location was observed. Findings from a European Multicentre Study (IBD-Character) which examined the gut microbiota composition in CD and UC using GA-map Dysbiosis Test revealed that the microbiota composition of new onset IBD differed from healthy individuals and there was a correlation between degrees of inflammation as measured by Montreal classification and dysbiosis, irrespective of disease status. Despite promising findings, these studies were unable to consistently demonstrate the proven diagnostic or predictive capacity of faecal microbiota to enhance clinical decision making. Lin et al demonstrated the superiority of using rectal biopsies compared to faecal samples for differentiating subclinical UC from healthy individuals.

Other studies were interested in investigating whether gut microbiota changes can predict disease prognosis. In a large multicentre IMPACT study involving 370 CD patients and 740 HC, Park et al evaluated differences in microbial abundance in CD patients categorised according to their prognosis. The prognostic categories were: good (CD-G), intermediate (CD-I) and poor (CD-P) based on clinical factors, including use of biologics and intestinal resection. The researchers observed a larger decrease in microbiota alpha diversity in CD-P than CD-G and CD-I and significant differences in gut microbial composition were noted when comparing CD-G to CD-I groups. Within the Firmicutes phylum, Coprococcus and Blautia producta, had decreased abundance in patients with poor prognosis. The main limitation of this study lies in its retrospective, cross-sectional nature. It remains unknown if these specific changes in microbial composition in the three prognostic categories are primary or secondary events. Kwak et al evaluated the potential of using colonic mucosal microbiome signatures as a novel metagenomic biomarker for predicting upper gastrointestinal involvement in CD patients. The authors obtained data from 26 CD (13 with and 13 without UGI involvement) patients from the IBD Multi-omics database. No differences in community richness, phylogenetic diversity and phylogenetic distance were observed between the two groups. DiTaxa analysis demonstrated significant association of Ruminococcus torques with UGI involvement, suggesting that R. torques may potentially be a useful biomarker for UGI involvement and its mechanism of interaction should be further investigated.

As exemplified in the studies above, the application of gut microbiota signatures in regular clinical practice is significantly limited by interindividual variations and the complexity of the required analyses. Chen et al hypothesized that these challenges could be overcome by the use of large data exploratory pattern analysis. The study was conducted with a large data set (n = 173,221) of nonselective patient stool sample test results. The data set included assays for detection of 24 selected commensal microorganisms and several biomarkers.
Patients were classified based on levels of inflammatory markers including FC, eosinophil protein X and IgA, with an index score for intestinal inflammation-associated dysbiosis (IAD) developed. A microbial profile strongly associated with faecal inflammation biomarkers and the IAD score, which was distinct between patients with IBD than those with IBS or celiac disease was identified. The development of an index to predict gut dysbiosis in different gastrointestinal conditions using real world data may provide a simpler alternative to interpreting gut microbial status and, hence improving clinical assessment. Another study explored the potential of developing diagnostic tools using machine learning algorithms. Xu et al.55 created LightCUD, an IBD diagnostic program based on whole-genome sequencing and 16S data from 349 human gut microbiota samples (IBD, healthy controls). This program was shown to discriminate IBD patients from healthy controls as well as further distinguishing between specific types of IBD with high accuracy. In a blind validation cohort, LightCUD maintained good performance (AUC = 0.809, AP = 0.971) in discriminating healthy controls from CD patients. Additionally, LightCUD showed 76.9% accuracy when discriminating CD from UC. The model performed poorer when distinguishing UC from CD compared to IBD from healthy controls and this could be attributable to the fewer number of training sets in the database. Nevertheless, the use of machine learning algorithms to interpret large microbiota dataset is promising.

CLOSTRIDIODES DIFFICILE INFECTION

Patients with IBD, particularly those with UC are at an increased risk of Clostridiodes difficile infections (CDI). Two independent studies investigating CDI and gut microbiota signatures were published during this reporting period. Lee et al.57 hypothesized that specific faecal microbial changes were associated with UC flares and recurrent CDI (rCDI). The longitudinal study identified distinct temporal changes in the gut microbiota that were associated with rCDI. They observed that patients with larger intraindividual community structure differences 14 days after completion of antibiotics compared to their baseline samples were at the highest risk for rCDI. Notably, increases in Ruminococaceae and Enterobacteriaceae were associated with increased risk of rCDI while increases in community richness and Faecalibacterium were protective. Lee et al.57 highlighted the potential for the use of microbial features at baseline and after therapy to predict rCDI risk in patients with and without UC. Hellmann et al.58 analysed the relationship between CDI and bowel resection surgery in a cohort of 75 paediatric CD patients. They reported that the rate of bowel resection surgery increased from 21% in those without CDI to 67% in those with CDI. Specifically, a positive CDI test during the first-year post-diagnosis was associated with shortened time to first bowel resection surgery. Reduction of beneficial bacterial species (Alistipes and Ruminococcus) and methionine biosynthesis were observed in patients with Clostridiodes difficile carriage, which could contribute to increased risk for surgery.

POUCHITIS, PSC, PERSISTENT DIARRHOEA, ANKYLOSING SPONDYLITIS

Restorative proctocolectomy with ileal-anal pouch anastomosis (IAPA) is performed in patients with UC refractory to medical therapy. Pouchitis is a common complication affecting more than 40% of patients within a year post-surgery. Gut dysbiosis, including low species richness, is implicated in UC patients with an IAPA. Peterson et al.59 assessed differences in microbiota and E. coli phylogroups in active and inactive pouchitis. Twenty UC patients with IAPA were recruited, of which half had active pouchitis. Disease activity was determined using the modified pouch disease activity index and by faecal calprotectin, while microbiota diversity and E. coli phylogroup were assessed by 16S rRNA gene sequencing and triplex PCR, respectively. IAPA patients had a greater abundance of Proteobacteria when compared to healthy controls and IBD patients. In particular, high levels of Fusobacteria were noted in IAPA patients with marked pouch inflammation; indicated by high faecal calprotectin levels. The established link between Fusobacteria and inflammation may be valuable to guide diagnostics and treatment for pouchitis.
Primary sclerosing cholangitis (PSC), a chronic progressive biliary system disease, is highly comorbid with IBD in which approximately 75% of patients with PSC have IBD. Gut microbial dysbiosis is also a recognised risk factor in PSC-IBD. An exploratory study by Quraishi et al. aimed to investigate the pathophysiological differences between PSC-IBD and UC by using a comparative and integrative approach to analyse colonic gene expression, gut microbiota profiles, and immune changes in both conditions. The study demonstrated, for the first time, that colonic inflammation in PSC-IBD and UC is immune mediated, with upregulation of immune responses involving predominant Th17 and IL17-producing CD4 cell response compared to healthy controls. Further investigation revealed that PSC-IBD is transcriptionally and microbiobially distinct from UC, with dysregulation of genes implicated in colonic bile acid homeostasis. Further work to establish the role of mucosal bile acid toxicity in driving inflammation in PSC-IBD and its relationship with the gut microbiota is required.

In patients with IBD, residual symptoms, including persistent diarrhoea, may persist despite mucosal healing. Boland et al. analysed biopsy specimens from 215 IBD patients and 48 healthy controls to determine if microbiota composition was associated with the symptoms in patients with endoscopic healing. The authors observed that alpha-diversity measures of the tissue-associated intestinal microbiome remained lower in CD patients compared to controls despite achieving endoscopic remission. In particular, there was a reduction in Chao1 diversity and greater dysbiosis in intestinal microbiota of patients with residual symptoms of IBD. Thus, highlighting that microbiota composition could be associated with persistent diarrhoea.

Cardoneanu et al. characterised gut dysbiosis in patients with chronic immune mediated IBD and ankylosing spondylitis (AS) since both diseases share a similar aetiopathogenesis. The prospective case-control study analysed faecal samples from 124 subjects (20 CD, 27 UC, 28 AS, 17 IBD with AS, 32 controls). They found an increased percentage of Bacteroides and E. coli and a decrease in Clostridium cocoides, Clostridium leptum and F. prausnitzii in all disease groups compared to healthy controls. In particular, patients with AS (axial form) were found to have significantly increased Bifidobacterium levels compared to peripheral disease. In patients with both IBD and AS, Crohn Disease Activity Index scores were inversely correlated with the total bacterial group and directly correlated with Lactobacillus. This study demonstrates that intestinal dysbiosis is implicated in both IBD and AS. However, further work is needed to elucidate how dysbiosis leads to intestinal and articular inflammation.

EXTRA-INTESTINAL MANIFESTATIONS

“Creeping fat (CF)”, an extra-intestinal manifestation in IBD, involves hypertrophy of mesenteric adipose tissue (MAT) adjacent to inflamed regions of the gut. This is a proactive response where MAT migrates to sites of gut barrier dysfunction to prevent systemic dissemination of pathogenic bacteria that have translocated from the gut lumen. This phenomenon is closely related to disease activity. Two independent studies evaluated the link between CF development and the microbiota. In a gnotobiotic mouse experiment, Ha et al. demonstrated that bacterial translocation from the gut to MAT occurred to a larger extent in CD than in healthy states. Clostridium innocuum was found to translocate and remains viable in the lipid-rich MAF environment. The presence of activated immune cells responding to C. innocuum may cause the stimulation of MAT restructuring and formation of CF. In contrast, Serena et al. hypothesized that CF itself is a reservoir of bacteria. The authors conducted 16S rRNA gene sequencing of MAT from patients with active and inactive CD as well as controls. The data revealed that Proteobacteria was the most abundant phylum in both CF and MAT. Disease activity was found to be associated with microbial composition with a reduction in the abundance of pathogenic microbes observed in patients with inactive disease.

GENETICS

Genome-wide association studies (GWAS) have identified over 240 IBD-risk loci through comparison of unrelated IBD cases and controls. Risk loci in genes associated with microbial recognition and clearance, including NOD2, ATG16L1 and IL-23 were all identified through seminal
GWAS. However, genetic heritability estimation of common GWAS variants has not shown to be a significant risk. Family history of IBD has been identified as a strong risk factor for IBD, with first-degree relatives shown to have up to a 15-fold higher life-time risk of IBD compared to the general population. To further identify the host genetic and gut microbial signatures in familial IBD, Park et al. undertook genome-wide single nucleotide polymorphism genotyping and whole exome sequencing to calculate weighted genetic risk scores from known IBD-associated common variants and to identify rare deleterious protein-altering variants specific to patients with familial IBD in 8 Korean families that each included more than 2 affected first-degree relatives (FDRs) and their unaffected FDR(s)\(^6\). Stool microbial community analysis by 16S rRNA sequencing was undertaken in parallel. The findings indicated that common disease-risk variants were not enriched in familial IBD cases. However, they found a distinct change in the microbiota profiles in IBD patients compared to their unaffected family relatives. Lower alpha diversity and changes in several previously known taxa were shown, although additional familial-specific taxa were identified, which need further investigation. These include a reduction in *Bacteroides uniformis* and *Bacteroides* unclassified and an increase in several Gammaproteobacteria genera. The power of combining multiple independent cohorts for genomic association analyses was demonstrated in the manuscript of Ruhlemann et al.\(^6\). Undertaking GWAS on almost 9,000 subjects identified 38 genetic loci which were associated with single bacteria and overall microbiome composition. Further analyses confirmed the identified associations of ABO histo-blood groups and FUT2 secretor status with *Bacteroides* and *Faecalibacterium* spp.

To evaluate the impact of host genetics on the gut microbiota of patients with IBD, a large study from The Netherlands used whole exome sequencing of the host genome and whole genome shotgun sequencing of 1464 faecal samples from 525 IBD patients and 939 population-based controls\(^6\). Novel associations between common genomic variants located in IBD implicated genes (MYRF, IL17REL, SEC16A and WDR78) or immune-related genes (CABIN1) to the gut microbial features have been identified in both IBD and the general population cohort. The cohort analysis confirmed associations determined in previous studies (NOD2 variants and CD; HLA loci SNPs associated with IBD). In addition, similar microbiome signatures of IBD were identified (reduced Firmicute and increased proteobacteria), resulting in predicted pathway analysis of a decreased abundance of genes involved in SCFA metabolism. Further interrogation of the impact of impaired NOD2 expression on intestinal microbial community stability and dynamics was also assessed during the reporting period\(^6\). Using WT and Nod2 knockout mice, treated with broad-spectrum antibiotics, demonstrated Nod2 impairment was associated with reduced weight gain and delayed recovery of the intestinal microbiota. Subsequent transfer of the Nod2 knockout faecal microbiota, to germ-free recipient mice results in increased intestinal inflammation compared to conventional/wild type mice faecal microbiota.

A further study looking at the relationship between the gut microbiota and specific IBD risk genotypes in healthy first-degree relatives of IBD patients - specifically CD, was also published during the reporting period\(^6\). Using a cohort of 1546 asymptomatic first-degree relatives of CD patients, associations between three individual NOD2 variants and the significance in clustering patterns of the microbiome composition were undertaken. No association was seen when the presence of any single polymorphism was correlated with microbiome composition. Similar findings were seen when the presence of any single polymorphism was compared to individuals with no variant. Further analysis looked at associations between NOD2 polymorphisms and specific microbial taxa. The findings showed that the NOD2 risk allele (rs2066845) was associated with an increased relative abundance of Erysipelotrichaceae family. This study is one of the largest studies of healthy subjects, specifically first-degree IBD relatives, examining the specific association of the NOD2 genotype with the healthy human gut microbiota.

Zhang et al.\(^7\) evaluated the causal relationship between gut microbiota and IBD, as well as identify key pathogenic bacteria using the Mendelian Randomization analysis. The study used mendelian randomization analysis, evaluated the causal relationship between gut microbes and IBD; identifying *Verrucomicrobiaceae* and two genera *Akkermansia* and *Dorea* as implicated in IBD pathogenesis. The analysis provides a new approach to using publicly available large-scale GWAS data in order to mine existing data.
SPECIFIC MICROBES

As high throughput sequencing studies identify associations between specific microbes and IBD, greater focus on mechanistic interrogation of individual microbes is being published. These studies are largely split into defining beneficial factors and understanding potential pathogenic traits/influences. *F. prausnitzii* is considered a key microbe in IBD as well as being a general health biomarker, although mechanistic insight into anti-inflammatory properties is still lacking. Transcriptomic analysis of *F. prausnitzii* stimulated human epithelial cells (HT-29) identified Dact3, linked to the Wnt/JNK pathway, as the most upregulated gene; an effect that was lost following gene silencing and also validated in murine models of colonic inflammation. The study provides new insight into host molecular mechanisms involved in the anti-inflammatory effects of the beneficial commensal bacterium *F. prausnitzii*. Further demonstration of the anti-inflammatory effect of *F. prausnitzii* was shown by Zhou et al. Using a TNBS-induced colitis mouse model, treated with *F. prausnitzii* and its supernatant demonstrated significantly improved colitis-related symptoms, increased microbial diversity and mitigated gut dysbiosis with an increase in SCFA-producing bacteria and a decrease in serum TNF-α.

Three studies investigated the role of specific microbes as IBD triggers, including both bacterial *Helicobacter saguini* and *E. coli* and the fungal species *Debaryomyces hansenii*. In the *H. saguini* study, the potential of the *Helicobacter* infection to naturally transmit and infect subsequent generations of germfree IL-10/ mice whilst causing significant intestinal inflammation was determined. The study confirmed that horizontal transmission via the faecal-oral route was the likely mechanism for transmitting *H. saguini* infection from the dams to offspring. The study also used Whole-genome comparative analyses, identifying host- and generation-dependent variant genes from *H. saguini* isolates, which suggests that IBD-associated microbes may adapt for colonization and survival in chronic inflammatory environments. The study provides further support for the need to assess pathogenic potential of such microorganisms in the context of IBD pathogenesis. *Debaryomyces hansenii* commonly found in various dairy products, was shown to be abundant within inflamed mucosal tissues of both pre-clinical and CD patient samples. In studies designed to fulfil Koch’s postulates, *D. hansenii* cultures isolated from inflamed lesions impaired colonic healing when introduced into injured conventionally raised or gnotobiotic mice. Mechanistically, *D. hansenii* was shown to impair mucosal healing through the myeloid cell-specific type 1 interferon-CCL5 axis. The ability of a UC *E. coli* pathobiont strain p19A to induce colitis in a genetically susceptible murine model, was also investigated in the assessment period. UC pathobiont p19A was able to colonize the intestines of genetically susceptible mice following antibiotic treatment, causing modest inflammation but worsened DSS-induced colitis. The researchers also undertook a mechanistic assessment of the strain and determined that strain p19A lacks many of the virulence factors usually expressed by pathogenic strains, such as capsular polysaccharides and type III secretion systems. Lack of these pathogenic factors potentially allows strain p19A to colonise the host without excessive recognition. Interestingly, strain p19A potentiated inflammation in the DSS-induced colitis model as well as dramatically increasing in numbers in the perturbed environment. The ability of p19A to potentiate colitis was shown to be dependent on α-hemolysin, a pore forming toxin that can cause cell lysis and induce tissue damage as well as the adhesin FimH. Both studies highlight the need to embrace mechanistic studies in the context of microbial involvement in disease pathogenesis.

TECHNICAL STUDIES

There are numerous factors contributing to variability in microbiome signatures from clinical/environmental samples. These include sample collection protocols, DNA extraction and sequencing techniques, as well as bioinformatics analysis pipelines; understanding the impact of these factors is essential. To evaluate the extent of technical variability, Szamosi et al. analysed human intestinal biopsy samples resected from individuals with CD (n = 12), UC (n = 10), and non-IBD controls (n = 10) in 3 independent laboratories. Independent protocols for
DNA extraction, library preparation, 16S rRNA gene sequencing, and bioinformatics were employed. The data indicated that DNA extraction methods and sequencing protocols did not significantly affect microbial community characterisation. Bioinformatic processing, however, led to significant inconsistencies in taxonomic assignment and abundance estimates.

**Conflict of Interest**

E.B., G.H., C.M. declare no conflict of interest. H.S. received consultancy, or lecture fees, from Carenity, Abbvie, Astellas, Danone, Ferring, Mayoly Spindler, MSD, Novartis, Roche, Tillots, Enterome, Maat, BionX, Biose, Novartis, and Takeda and is also a co-founder of Exeliom Bioscience. N.B. received lecture fees from Tillots.

**REFERENCES**


