We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,400
Open access books available

174,000
International authors and editors

190M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Chapter

Gut Microbiome and Crohn's Disease: An Enigmatic Crosstalk

Jyoti Sharma, Tuhina Banerjee, Manisha Naithani,
*Navin Kumar, Sudhir Kumar Singh and Somprakas Basu*

Abstract

Crohn's disease (CD) is a chronic, recurrent, immune-mediated inflammatory bowel disease that demonstrates a spectrum of intestinal and extra-intestinal manifestations. The pathogenesis of CD is multifactorial and involves a complex interplay between environmental and microbiological factors in a genetically suscep-tible host. There is robust evidence suggesting the role of gut microbial dysbiosis in the development as well as exacerbation of CD by immune dysregulation and altera-tion in the immune microbiota crosstalk. Patients with CD show reduced commensal microbial diversity, along with increased numbers of pathogenic *Enterobacteriaceae* and *Proteobacteriaceae*. *Faecalibacterium prausnitzii*, an anti-inflammatory molecule-producing bacteria, is also seen in reduced numbers in patients with CD and is associated with an increased risk of recurrence. There has been a paradigm shift in the management of patients of CD, from controlling symptoms to controlling inflam-mation and promoting mucosal healing. Current treatment strategies aim to replace, remove, reset, or redesign the gut microbiota for the therapeutic benefits of patients with CD. These include microbial restoration therapies such as dietary modification, the use of pre-, pro-, and postbiotics, and fecal microbiota transfer (FMT). This chapter focuses on the role of gut microbiota in the pathophysiology of CD and the emerging concepts in microbial therapeutics.

Keywords: microbiome, dysbiosis, gut-immune crosstalk, microbial therapeutics, Crohn's disease

1. Introduction

Crohn's disease (CD) is a chronic relapsing inflammatory disease that can involve any part of the gut from the mouth to the anus [1]. The first documented case of CD dates back to 1761, described by Morgagni [2]. However, it was in 1932, that Crohn et al. elucidated the disease in detail [3]. There has been a rising trend in the incidence of this disease, with more than 6.8 million people affected worldwide [4]. Traditionally, known as the disease of the West, the incidence of CD has also increased in Asian and Southeast Asian countries in the past decade, owing to rapid industrialization and urbanization. The CD is primarily a disease of the young, with a second smaller peak seen in the sixth decade. A female preponderance is seen in
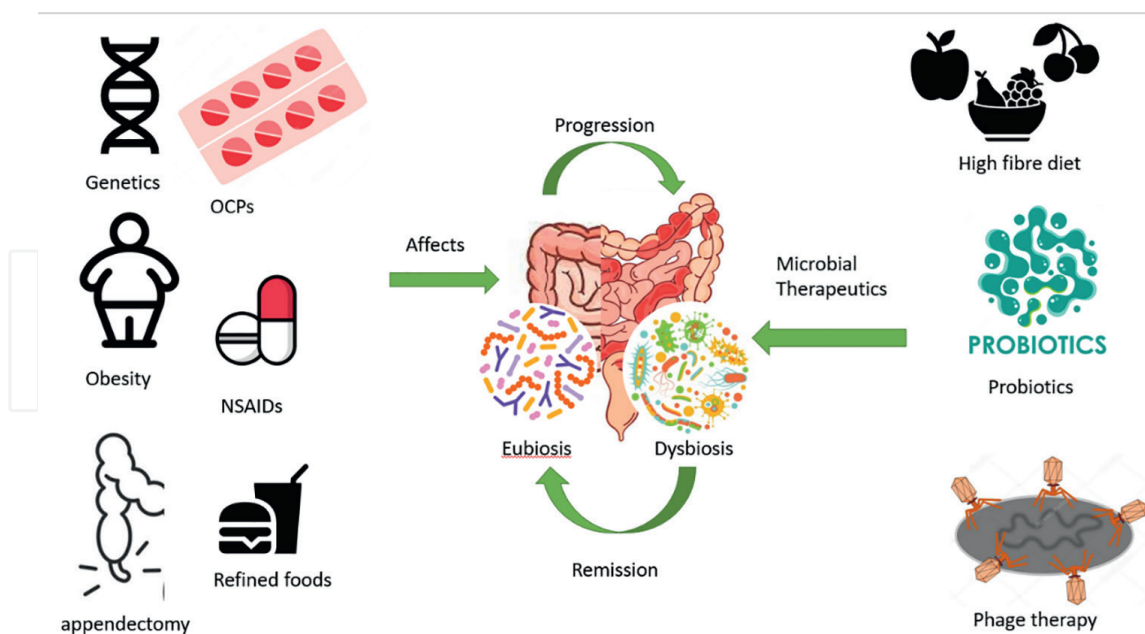
Figure 1.
Interplay of genetic, environmental factors, and microbial dysbiosis in the pathogenesis of CD and the role of microbial therapeutics and diet in the management and remission of CD.

Europe and USA, while the reverse is true in Asia. Younger females are at a lower risk of developing CD as compared to older females [5]. The exact cause of CD is still uncertain. However, the proposed pathophysiology involves an intricate relationship between the genetic, environmental, microbial, and immunological factors [6]. Recent evidence suggesting the role of gut microbial dysbiosis as an important initiator and propagator of CD has found great interest among the researchers (**Figure 1**).

Patients with CD may present with intestinal or extra-intestinal symptoms. Cardinal symptoms include crampy abdominal pain, persistent intermittent diarrhea, bleeding per rectum, weight loss, and fatigue. Severe disease is associated with intestinal strictures, fistulas, intraabdominal-abscesses, or perianal disease in the form of fistula, abscess, etc. These occur due to the transmural intestinal inflammation. Extra-intestinal involvement includes arthropathy, eye and skin manifestations, hepatobiliary and pulmonary involvement, and secondary amyloidosis [6]. The chronic relapsing and remitting course of the disease results in significant morbidity and a decreased quality of life (QOL). Individualized treatment focused on mucosal healing and aimed at remission should be undertaken, thereby improving the patient's QOL and achieving better clinical outcomes. This chapter outlines the pathophysiology, risk factors and the role of gut microbiota in the causation and progression of CD, and the recent advances in the therapeutic strategies of its management.

2. Factors promoting the development of CD

2.1 Host genetics

The onset and progression of CD are influenced by epigenetic changes in a genetically susceptible host. There is evidence suggesting a strong inheritable component of CD which has been obtained through Genome Wide Association Studies (GWAS). A total of 41 chromosomal loci involved in the maintenance of intestinal barrier,

epithelial restitution, regulation of innate and adaptive immunity, autophagy, reactive oxygen species (ROS) production, microbial defense, and cellular hemostasis have been identified [7, 8]. The most extensively studied gene in the pathogenesis of CD is the *nucleotide binding oligomerization domain containing* 2 (NOD2) gene. It is responsible for immunomodulation, and its mutation is associated with the development of CD. Similarly, mutation in autophagy gene autophagy-related 16-like 1 (ATG16L1) is also associated with the development of CD, while *IL23R* gene polymorphisms increases the risk of developing CD. Early onset IBD, as seen mostly in the pediatric patients, is associated with mutations in X-linked inhibitor of apoptosis (XIAP) and interleukin 10 receptor (IL10R) genes. The prevalence of CD in certain specific population groups explains the role of genetic susceptibility to the disease. While variance in NOD-2, ATG16L1, and IL23R predominates in the western population, TNF superfamily member 15 (TNFSF15) mutation is selectively associated with CD in the Asians. Almost half of these genetic alterations are associated with diseases such as psoriasis and ankylosing spondylitis often present as extraintestinal manifestations of CD. However, this genetic variance is seen only in 10–25% of the total cases of CD which suggests the role of epigenetic factors in the causation of CD [9].

2.2 Environmental factors

The epidemiologic distribution of CD suggests a possible role of epigenetics along with genetic susceptibility of the individual. With the advent of industrialization, there has been an exponential increase in the incidence of CD, especially in the Asian and Southeast Asian countries, confirming the role of environmental factors [2]. Smoking has been extensively studied as a risk factor in CD and is associated with alterations in autophagy, gut flora, and direct toxicity to the immune and mucus-producing cells [10]. Processed foods are rich in saturated fatty acids but low in fiber, which result in intestinal mucosal inflammation and alteration in the gut microbiota. A diet rich in processed food is associated with an increased risk of CD [11]. High-fiber diet is protective in the development of CD as it is converted into short chain fatty acids (SCFA) that possess anti-inflammatory properties [9, 12]. Sedentary lifestyle and obesity are other risk factors associated with an increased risk of CD [9]. Extensive use of antibiotics in pediatric age group may alter the developing gut

Factors	Paper /year/type of study	Sample size/ no. of studies	Pd of intervention	Role
Smoking	Mahid et al. [15] (2006) Meta-analysis To et al. [10] (2015) Meta-analysis	9	Jan 1980–Jan 2006 1990–July 2015	Twofold increase in CD Early onset CD Higher postoperative disease recurrence
Low fiber diet	Lambert et al. [12] (2021) Meta-analysis	19	Jan 2000–Sept 2020	Higher risk of CD Risk reduction is greatest for fiber derived from fruits
High dietary fat/protein	Ajabnoor et al. [16] (2020) Meta-analysis	13	—	High omega-3 may reduce IBD risk (low quality evidence)

Factors	Paper /year/type of study	Sample size/ no. of studies	Pd of intervention	Role
Lifestyle	Jain et al. [17] (2019) Cohort study Nguyen et al. [18] (2019) Cohort study (nationwide)	4748 patients 42,285 patients	Since 2011 with at least 6-months follow-up Admissions between January to June 2013 and re-admissions until December 2013	Obesity is independently associated with an increased risk of persistent disease activity and relapse Obese patients with IBD had longer hospital stays
Appendectomy	Kaplan et al. [19] (2008) Meta-analysis Fantodji et al. [20] (2022) Cohort study	21 studies 400,520 patients	1966–2007 1970–1974 and followed till 2014	Still debatable
Antibiotics at early age	Ungaro et al. [13] (2014) Meta-analysis	11 studies (7208 participants)	2004–2012	Positive association if used in first year of life
Oral contraceptive use	Ortizo et al. [21] (2017) Meta-analysis	20 studies	1984–2010	Positive association
NSAID use	Moninuola et al. [22] (2018) Meta-analysis	13 studies	1974–March 2017	Positive association
Vitamin D	Pinto et al. [23] (2015) Meta-analysis Li et al. [24] (2019) Meta-analysis	14 studies (1891 participants) 55 studies	Inception—Dec 2014 1982–April 2019	Lower vitamin D levels were associated with high CD risk

Table 1.
Environmental factors and their role in the development of CD.

microbiota and may predispose to CD [13]. "Hygiene hypothesis" or exposure to a "too clean" environment during childhood causes alteration in the evolution of gut microbiota and predisposes the children to CD [14]. Indirect evidence suggesting that most of the environmental factors are associated with an alteration in the gut microbiota reaffirms their possible role in the pathogenesis of CD. However, the role of gut microbiota in either initiation or progression of CD is still uncertain (**Table 1**).

3. Pathophysiology

The pathogenesis of CD is characterized by an impaired intestinal barrier function, dysregulation of the innate and adaptive immune response, and gut microbial dysbiosis [5]. There exists a functional equilibrium between the intestinal epithelium and the luminal contents. This equilibrium is maintained by the intestinal barrier which is composed of the intestinal epithelial cells (IEC), innate immune cells, mucus

layer, and the commensal gut microbiota. It is a dynamic structure that not only acts as a physical barrier but also acts as a chemical and immunological barrier against the pathogenic microbes and helps in maintaining the gut homeostasis [25].

The IECs are divided according to their functions into Goblet cells, entero-absorptive cells, Paneth cells, neuroendocrine cells, and M cells. The Goblet cells produce mucus that acts as a physical barrier and also helps in epithelial cell repair. Paneth cells are associated with maintenance of intestinal stem cell niche and secretion of antimicrobial effectors which are responsible for gut microbial homeostasis [25]. The mucosal innate immune system consists of macrophages, dendritic cells, lymphocytes, and neutrophils that form the first line of defense along with IECs. In a healthy state, the intestinal macrophages exhibit "self-tolerance" where they show attenuated response to the host microbial ligands and cytokines while retaining the bactericidal activity against pathogens. These are a special subset of macrophages that lack CD14. These promote regulatory T cell (Tregs) differentiation by producing anti-inflammatory cytokines. Tregs are a specialized subset of T cells that suppress the immune system and are responsible for maintenance of self-tolerance and homeostasis. These macrophages are also responsible for attenuation of Th1 and Th17 responses. It is observed that patients with CD exhibit another macrophage population that expresses CD14 along with dendritic cell markers, thus producing abundant pro-inflammatory cytokines such as IL-6 and TNF α and resulting in intestinal mucosal inflammation. The dendritic cells form an interface between the innate and adaptive immune system and relay signals to initiate an appropriate adaptive immune response. They perform bacterial sampling by direct dendritic cell to microbe contact which is mediated by CX3CR1-dependent mechanism. Deletion of CX3CR1 results in increased translocation of gut bacteria due to decreased lamina propria macrophages [26, 27].

The microbial products that permeate the intestinal barrier are identified by the antigen-presenting cells (APCs), which initiate a cascade of pro- and anti-inflammatory signals. This activates the local and circulating lymphocytes to migrate to the area of inflammation. The leucocyte migration occurs *via* binding of integrins on the leucocyte surface to the cellular adhesion molecules (CAMs) on the endothelium. The activated endothelium itself produces chemokines to attract leucocytes to the site of inflammation. This disturbed pro- and anti-inflammatory balance with leucocyte migration results in an exaggerated T cell response (Th1 and Th17) that is seen in CD. The APCs and macrophages secrete IL12, IL18, IL23, and TGF-β which cause differentiation of Th1 and Th17 cells. The Th1 and 17 cells secrete IL-17, IFN-ϒ, and TNF-α that in turn stimulate the APCs, macrophages, fibroblasts, and endothelial cells leading to persistent activation of the T cells [28].

Tregs and Th17 cells arise from a common precursor but have opposite actions. In normal state, TGF-β promotes Treg cell differentiation in the lamina propria depending on the local cytokines and microbial signals. But in inflammatory conditions like CD, it leads to Th17 cell differentiation promoted by the presence of other cytokines and microbial signals. This mechanism is responsible for the initiation, persistence, and relapses seen in CD [29].

4. The gut microbiome and dysbiosis

4.1 The gut microbiome

The human gut is niche to a vast variety of commensal, symbiotic, and patho-genic microbial floras that play a pivotal role in various synthetic, metabolic, and

immunologic functions of the human body. Due to its immense functional plasticity, it has often been referred as the "forgotten organ." It co-evolves with the human gut and shares a complex and bi-directional interaction with the host, which helps in maintaining host homeostasis [30]. Gut bacteria form the major biomass, along with archaea, viruses, and eukaryotes. *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* are the four predominant phyla present in the human gut of which *Firmicutes* and *Bacteroidetes* are in maximum abundance, accounting for almost 90% of the total microbiota [29]. *Firmicutes* and *Bacteroidetes*, along with *Bifidobacterium*, synthesize SCFAs, mainly butyrate, which is the principal source of energy for colonic epithelia [31]. *Bifidobacterium* also synthesizes vitamins K and B, which are essential for coagulation [32]. The gut microbiota also plays a crucial role in the development of the host immune system, which, in turn, shapes the gut microbiome [33, 34]. Animal studies have shown that mice deficient in gut microbiota exhibited impaired development of innate immune system [35]. A specific bacterium *Candidatus arthromitis*, also known as segmented filamentous bacteria (SFB), promotes the maturation of mucosal immune system, which is a significant component of the intestinal barrier [36]. *Clostridium* strains IV, XIVa, and XVIII induce Treg cell differentiation and expansion *via* butyrate production [37]. Recent research has demonstrated that *F. prausnitzii*, which belongs to Clostridium cluster IV, has an anti-inflammatory action in the human gut. It produces butyrate and anti-inflammatory bioactive molecules such as shikimic and salicylic acids, inducing the production of IL10 and inhibiting the production of IL12 and interferon-ϒ [38, 39]. In addition, the gut microbiota is also involved in the defense of the host against the intestinal pathogens. The commensal bacteria compete with the pathogenic bacteria thus preventing their colonization. This mechanism is known as "colonization resistance" [40]. They either directly inhibit them by competing for nutrients or, indirectly, by producing inhibitory substances [41]. *Bacteroides thetaiotaomicron*, an abundant commensal bacterium, utilizes the carbohydrates used by *Citrobacter rodentium*, a pathogenic bacteria, and leads to its competitive exclusion [42]. *Bacillus thuringiensis* secretes a bacteriocin that directly targets the spore forming *Clostridia* and *Bacilli* [43]. The microbial products such as lipopolysaccharides and flagellin promote the secretion of IgA from B cells, production of antimicrobial peptide, and the development of Th17 cells [44, 45].

4.2 The gut microbial dysbiosis and CD

"Dysbiosis" or an imbalance in the microbial composition alters the host-microbiota-immune crosstalk and results in disruption of host homeostasis [45]. It may occur due to various environmental factors such as dietary changes, toxins, drugs, and infections [40]. There is a reduction in the beneficial commensal bacteria and a pathological bloom of pathogenic bacteria or "pathobionts," which results in altered synthetic, metabolic, and immunomodulatory functions of the host [46]. Disruption of gut homeostasis results in increased intestinal permeability and translocation of pathogenic bacteria through the intestinal barrier. This activates the gut mucosal immune system, leading to a state of low-grade chronic inflammation [47–49]. This altered host-microbiota-immune crosstalk has been linked to the pathogenesis of various metabolic, cardiovascular, neurological, and neoplastic diseases [46]. However, their association with inflammatory bowel disease (IBD) has been a subject of interest among the researchers since the past few decades.

Gut microbial dysbiosis has surfaced as a significant aspect in the pathogenesis of IBD, exhibiting a decrease in the "alpha" or the "within-sample" diversity with

a simultaneous increase in the pathobionts [49]. It is significantly affected by the geographical diversity and epigenetic factors and is more pronounced in patients with CD [50]. The taxonomic shifts in CD are mostly related to dysfunctions of microbial metabolism and bacterial protein signaling. A reduced abundance of bacterial taxa within the phyla *Firmicutes* is the most consistent finding [49]. This leads to significant reduction in SCFAs, mainly butyrate, in the gut that affects the epithelial cell growth as well as Treg cell differentiation and expansion. Other SCFA-producing bacteria such as *Bifidobacterium*, *Lactobacillus*, and *Roseburia intestinalis* are remarkably reduced in patients with CD when compared with healthy individuals. It is seen that the proportions of Clostridium clusters XIVa and IV are significantly lower in CD patients [51, 52]. *F. prausnitzii* belongs to *Clostridium* Cluster IV and possesses anti-inflammatory properties. A significant reduction in its abundance is associated with a decreased resistance of the gut against inflammatory interactions. Thus, decreased abundance of *F. prausnitzii* can be correlated with disease activity and an increased risk of recurrence after surgery [38, 52]. Studies have also shown decreased abundance of *Eubacterium rectale*, *Blautia faecis*, *Roseburia inulinivorans*, *Ruminococcus torques*, and *Clostridium lavalense* along with a decrease in families of *Christensenellaceae*, *Coriobacteriaceae*, and especially *Clostridium leptum* [50, 52–54].

Patients with CD demonstrate abundance of *Proteobacteria* such as *Enterobacteriaceae* and certain species of *Bacteriodetes*. There is a relative abundance of mucosal associated bacteria, mainly *Enteroinvasive E. coli* (EIEC) and *C. rodentium* that have adhesive properties [45]. These bacteria activate the mucosal immune system by adhering to the intestinal epithelium, thereby inducing intestinal inflammation. Decreased abundance of protective bacteria such as *C. arthromitis*, *B. thetaiotaomicron*, and *Bacillus thuringenesis* leads to proliferation of these pathobionts. Certain mucolytic bacteria such as *Ruminococcus gnavas* and *R. torques* are also increased in patients with CD [55]. Increased abundance of *Desulfovibrio*, a sulfate-reducing bacteria, is associated with intestinal epithelial damage due to production of hydrogen sulfate, thereby inducing mucosal inflammation [56]. A predominance of *Clostridium difficile* and *Bacteroides vulgates* is observed in patients with relapse of CD [57]. Abundance of pathobionts such as *Bacteroides fragilis*, strains of *Clostridium hathewayi*, *Clostridium bolteae*, *Actinomycetes* spp., *Veillonella* spp., *Intestinibacter* spp. and a significant increase in *Coprococcus* spp. is also seen in patients with CD when compared to healthy gut flora [58]. Recent studies have also isolated some strains of enterohepatic *Helicobacter* species in these patients suggesting a protective role of these strains in CD [59].

4.3 Fungal dysbiosis and CD

In addition to bacterial dysbiosis, an alteration in the mycobiome (fungal community) is also seen in these patients. Studies have shown significant decrease in the *Saccharomyces cerevisiae* abundance with a significant increase in the *Candida* spp., mainly *Candida albicans* and *tropicalis* [60]. *Malassezia restricta*, a commensal skin fungus, is also found in abundance in CD patients [61].

4.4 Viral dysbiosis and CD

Recent evidence also shows the potential role of gut virome in the pathogenesis of CD [6, 62]. The abundance of *Caudovirales* bacteriophage sequences, including *Myoviridae*, *Siphoviridae*, and *Podoviridae* detected in the intestinal washes and

tissue biopsies of pediatric CD patients, may be utilized as a potential biomarker of early onset CD [63]. An increased abundance of *Synechococcus* phage S CBS1 and Retroviridae family viruses is also observed in these patients [64].

Gut microbial diversity is also affected by the medical treatment protocols of CD. Repeated antibiotic exposure is associated with a significant and consistent reduction in the gut microbial biodiversity with near absence of some specific taxa such as *Acetovibrio*, *Butyricicoccus*, *Collinsella*, *Dorea*, and *Subdoligranulum* [65]. Treatment with 5-aminosalicylic acid showed a significant decrease in *E. coli* with an increase in *Enterococcus* spp., but the results have been conflicting. Anti-TNF therapy demonstrated decreased numbers of *F. prausnitzii* and *E. coli* in some studies [57]. However, the effect of these immunomodulator therapies on the gut microbiome is little known and further research is required.

Postoperative recurrence in CD was characterized by significant abundance in the bacterial counts of *E. coli*, *Bacteroides*, and *Fusobacteria* at the neoterminal ileum. A lesser percentage of *F. prausnitzii* in the resected ileal segment was associated with an early endoscopic recurrence of CD, suggesting a microbial signature that can predict the possibility of recurrence postoperatively [66].

4.5 Genetic variants in CD and their association with microbial dysbiosis

A possible association of the gut microbiome with the genetic loci of CD has long been suspected; however, the results have not been consistent. *NOD2* gene has been extensively studied in the pathogenesis of CD. It is expressed by the Paneth cells and stimulates an immune reaction on recognizing the cell wall peptidoglycan muramyl peptide of gram-positive and gram-negative bacteria. Studies have demonstrated that NOD2 variants of CD show an increased adaptive response to microbial antigens. Risk alleles at NOD2 and ATG16L1 loci were associated with significant taxonomic shifts, especially decreased *Faecalibacterium* and *Roseburia* spp. and increased *Escherichia* spp. strains [67]. Specific genes involved in adhesion, oxidative stress responses, and utilization of mucus favor colonization of *Ruminococcus gnavus* [68]. NOD-like receptor 6 (NLRP 6) has been recognized as the key regulator of a pathobiont *Akkermansia muciniphila* that promotes the development of CD [69]. These associations were associated with a high genetic risk for CD. CLEC7A is a pattern recognition receptor that recognizes glucans with β-1,3 and β-1,6 bonds from fungi. Alteration in C-type lectin domain containing 7A (CLEC7A) is associated with altered macrophage and dendritic cell function and is associated with decreased *Lactobacillus* population [70]. Caspase recruitment domain family member 9 (CARD 9) recognizes fungal motifs and is associated with fungal dysbiosis. It is associated with decreased *Lactobacillus* population and a predominance of *Ascomycota*, *Basidiomycota*, and *Zygomycota* [71]. Alteration in nucleotide-binding oligomerization domain, *Leucine*-rich repeat, and pyrin domain containing protein (NLRP) increases susceptibility to IBD by promoting intestinal inflammation and is associated with an increased abundance of *A. muciniphila* and *Prevotellaceae* family [72]. The common CD-specific genes and their role in pathogenesis of CD and effect on the immune system and intestinal microbiota have been summarized in **Table 2**. However, consistent taxonomic shifts could not be demonstrated in further studies, thus necessitating the need for larger GWAS and higher level of evidence.

It is anticipated that portraying the compositional and functional changes in the microbial diversity will help in developing novel therapeutic options for preventing relapses and inducing remission in CD.

S. no.	Genes	Role	Role in pathogenesis of CD	Effect on the immune system	Effect of genetic variants on the intestinal microbiome
1	NOD 2	Recognizes muramyl dipeptide (MDP) that stimulates autophagy and controls bacterial replication and antigen presentation Regulation of T-cell response via MDP independent pathways	Defective recognition and removal of pathogenic bacteria Defective autophagy Decreased release of defensins	Role in innate and adaptive immunomodulation	Increased *Enterobacteriaceae*, *Erysipelotrichaceae*, *Actinobacteria* group, *Firmicutes* class, and *Bacteroides* spp. Decreased *Faecalibacterium*, *Roseburia*, *Ruminococcaceae*
2	ATG16L1	Autophagy Maintenance of intracellular homeostasis	ATG16L1T300A is associated with increased risk of CD Responsible for increased Th1 and Th17 cells in the lamina propria of ileum and colon without intestinal inflammation	Mutations are associated loss of tolerance to commensal microbiota due to increased production of IgG and IgA against commensal microbiota	Decreased abundance of *Faecalibacterium*, *Roseburia* and *Bacteroidaceae* Increased numbers of *Enterobacteriaceae* such as *Escherichia coli*; Fusobacteriaceae, increase in *Lachnospiraceae*
3	IRGM	Responsible for autophagy Maintenance of intracellular homeostasis	Defective autophagy Decreased production of antimicrobial peptide Abnormal secretory granule development	Plays a role in innate immune response	Decreased abundance of *Roseburia*
4	IL23R [73]	Maintains T-cell dependent immunity by encoding a subunit of IL-23 that is involved in Th-17 cell generation	Role in autoimmunity by expansion of proinflammatory Th17 cells in CD	Responsible for persistent production of pro-inflammatory mediators like *IL6*, *IL12*, *IL17*, *INF-γ*, *TNF-α* and *IL23*	Decreased abundance of *Christensenellaceae*, *Bacteroides caccae* and *Oscillospira*
5	IL-10R [74, 75]	Essential for immune homeostasis in colon	Causes extensive perianal and colonic inflammation Leads to very early onset IBD (VEO-IBD) and extensive perianal disease	Role in immunomodulation, suppresses proliferation and cytokine secretion	Increased numbers of *Enterococcus faecalis*, *E. coli*, and *Helicobacter hepaticus*
6	CLEC7A	Pattern recognition receptor Recognizes various glucan bonds from fungi (β-1,3 and β-1,6 bonds)	Associated with altered macrophage and dendritic cell activity Associated with fungal dysbiosis	Role in innate immunity	Decreased abundance of nonpathogenic *Lactobacillus*, *Saccharomyces* Increased numbers of *Enterobacteriaceae*, *Candida*, and *Trichosporon*

S. no.	Genes	Role	Role in pathogenesis of CD	Effect on the immune system	Effect of genetic variants on the intestinal microbiome
7	CARD9	Recognizes viral, bacterial, and especially fungal motifs	Associated with fungal dysbiosis	Enhances production of IL-1β and IL-23p19 subunit	Decreased colonies of *Lactobacillus* Dominant *Ascomycota*, *Basidiomycota*, and *Zygomycota*
8	NLRP [72]	Has a molecular domain that helps in self oligomerization and has ATPase activity Can sense endogenous alarmins and microbial ligands	Promotes intestinal inflammation Increases susceptibility to colitis in murine models	Activation of IL-1 family cytokines	Key regulator of *Akkermansia muciniphila*, *Prevotellaceae* family Increased *S. thuringiensis*, *Clostridium*, *Rod bacteria*, and *Proteobacteria*
9	PTPN 2 [76]	Associated with autophagy	Defective autophagosome formation and bacterial elimination Promotes T cell differentiation into Th1 and Th17 types Associated with increased levels of IFN-γ, IL-17, and IL-22 in the serum and intestinal mucosa	High levels of *INF-γ*, *IL17* and *IL22* Role in innate and adaptive immunity	Reduced *Faecalibacterium*, *Bilophila*, *Coprococcus*, *Erysipelotrichaeceae*, *Clostridiales*, and *Ruminococcaceae Bacteroides* were increased in number
10	LRRK-2 [77]	Involved in endocytosis, phagocytosis, and autophagocytosis, lysosomal function Also implicated in intracellular trafficking	Activation of LRRK is associated with increased dendrtitic cell activation, increased expression and release of pro-inflammatory molecules like *IL2* and *TNF-α*	Production of IL-2 and TNF-α and activation of dendritic cells	Increased numbers of *Listeria monocytogenes* and *Salmonella Typhimurium*

Abbreviations: IRGM, immunity related GTPase-M; PTPN-2, protein tyrosine phosphate non-receptor-2; LRRK-2, leucine-rich repeat kinase-2 [70–72].

Table 2.
Genetic variants and their association with intestinal microbiota in CD.

5. Nutrition in CD

Nutrition plays an important role in the management of CD. Dietary changes can influence the gut microbiota and help in restoring the gut homeostasis [78, 79]. In addition, nutritional management is also important in view of CD-associated malnutrition, which results from decreased absorption, intestinal dysbiosis, and CD-related symptoms such as loss of appetite, nausea, and vomiting. Specific dietary strategies have been advised for the management of CD.

5.1 Diets for nutritional optimization in CD

Enteral nutrition (EN) is a liquid dietary regimen that can be given in three formulations, depending on the protein and fat content. These formulations include elemental (easily absorbable low-fat nutrients such as amino acids, mono- or oligosaccharides, and medium-chain triglycerides), semielemental (peptides of different chain length, simple sugars, glucose polymers or starch, and medium-chain triglycerides), and polymeric (whole proteins, complex carbohydrates, and long-chain triglycerides). These formulations are particularly recommended during CD relapses for 6–8 weeks to induce disease remission. These formulations are also advised as a maintenance diet during the remission phase in addition to the usual diet. This type of diet affects the gut microbiota and reduces gut bacterial dysbiosis.

Parenteral nutrition (PN) provides nutrients including macronutrients, micronutrients, and electrolytes through a venous access. Exclusive parenteral nutrition is advised during acute inflammatory phase of CD to provide bowel rest or in conditions such as partial obstruction, high-output fistulae, and bowel ischemia, where the use of enteral nutrition is contraindicated. It is also used as a supplement in patients where enteral nutrition is inadequate to fulfill the energy requirement. Thus, EN often represents the main dietary option, alone, or in association with PN.

5.2 Specific carbohydrate diet

Apart from treatment of celiac disease, this diet is also used in the management of IBD. It includes monosaccharides, dairy products with low lactose content, meat, eggs, oil, and amylose rich vegetables. Products rich in sucrose, maltose, isomaltose, and lactose, along with potatoes, corn, soy, food additives, and preservatives, must be avoided. Studies have shown that this diet improves IBD symptoms and quality of life, and help in maintaining remission.

5.3 Low fermentable, oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) diet

This diet mainly excludes short-chain carbohydrates and limits consumption of honey, apples, watermelon, dates, lentils, and legumes. The drawback of low FODMAP diet is reduced intake of common prebiotics, such as inulin, fructo-oligosaccharides, and fructose. The low FODMAP diet is advisable in patients with quiescent IBD.

5.4 Semivegetarian diet

It is primarily a vegetarian dietary regimen which strongly limits meat and fish, without eliminating them. This diet consists of vegetables, fruits, cereals, eggs, yoghurt, and milk, and excludes processed and refined foods. It is advised as a maintenance treatment in patients with clinical remission.

5.5 Low fat/fiber limited exclusion (LOFFLEX) diet

This is a form of elemental diet which is used to find the potential trigger of CD by reintroducing specific nutrients. It can be customized accordingly by exclusion of nutrients that are commonly considered as triggers of CD, in a well-structured protocol.

Overall, the abovementioned dietary regimens play an essential role in the treatment of IBD, particularly CD. It is apparent that food components have the ability to modulate metabolic pathways, stimulate gene expression, and modify the microbiota composition. Liquid diet is the primary therapy in the management of CD as it reduces inflammation and promotes mucosal healing and helps in reducing the postoperative complications.

6. Therapeutic perspective

Ever since the understanding of natural history of CD became clearer, the therapeutic goals have shifted from controlling symptoms to controlling the inflammation and promoting mucosal healing. The treatment strategies have become more personalized and individual-based, thereby leading to better clinical outcomes. The significant role of gut microbiota in the pathogenesis of CD has influenced the development of novel therapeutic options that selectively target the gut microbiome (**Table 3**). These microbiota-targeted strategies aim at the diagnostic, prognostic, and therapeutic aspects of CD. These treatment strategies aim to replace, remove, reset, or redesign the gut microbiota for therapeutic benefits of patients with CD.

Decreased abundance	Increased abundance
Firmicutes spp.	*Escherichia coli (EHEC O157)*
Bifidobacterium	*Citrobacter rodentium*
Candidatus arthromitis	*Bacteroides fragilis*
Faecalibacterium prausnitzii	*Ruminococcus torques*
Bacteroides thetaiotamicron	*Ruminococcus gnavas*
Bacillus thuringenesis	*Desulfovibrio*
Blautia faecis	*Actinomyces*
Eubacterium rectale	*Veilonella*
Roseburia intestinalis	*Intestinibacter*
Clostridium lavalense	*Closridium hathewayi*
Christensenellaceae	*Clostridium boltae*
Coriobacteriaceae	*Coprococcus*
Clostridium leptum	*Clostridium difficile*
	Virome: *Caudovirales* *Synechococcus* phage S CBS1 Retroviridae family viruses
Mycome: *Saccharomyces cerevisiae*	Mycome: *Candida albicans* *Candida tropicalis* *Malassezia restricta*

Table 3.
Gut microbiota in Crohn's disease.

6.1 Potential biomarkers

Various noninvasive tests such as serum markers, fecal biomarkers, and radiological imaging are available for the diagnosis and monitoring the progression of CD. However, most of these serum and fecal biomarkers are limited to active disease and are surrogate markers; thus, their response to therapy is highly variable. Evaluation of specific microbial biomarkers would help in precise diagnosis and patient stratification in CD. Studies have suggested increased *Faecalibacterium nucleatum* and decreased *F. prausnitzii* counts as a valuable marker for CD [51]. Recent data analysis has identified *Gammaproteobacteria*, *Enterococcus*, and *Enterococcaceae* as potential biomarkers of IBD. Bacterial genera *Collinsella* and *Methanobrevibacter* can be used for differentiation between UC and CD [80, 81]. *F. prausnitzii* and *E. coli* can be used to differentiate between ileal and colonic CD. Ileal CD is characterized by a lower abundance of *F. prausnitzii* with a relative higher abundance of *E. coli* as compared to colonic CD. It has also been noted that AIEC is more abundantly found in the inflamed ileal mucosa of the patients suffering with CD. *Faecalibacterium* and *Papillibacter* can be used as indicators of disease status [82, 83]. They may serve as microbial signatures to diagnose and differentiate between uncertain cases of ulcerative colitis (UC), CD, and irritable bowel syndrome [84]. The microbial shifts may act as biomarkers to predict the outcome of the disease. However, due to high microbial diversity, the predictive value of these biomarkers is considerably less. Thus, they are currently not recommended as a first-line assessment for the diagnosis of CD.

6.2 Live biotherapeutic products (LBP)

Probiotics are selected viable microorganisms that modulate the intestinal microbiota and exert a beneficial effect on the host by modulating the intestinal microbiota and alleviating intestinal dysbiosis [85]. Theoretically, probiotics produce metabolites that inhibit the growth of the pathobionts and promote the growth of commensal bacteria, thus restoring the normal gut microbiome. They also induce an anti-inflammatory effect and improve and restore gut barrier function [86]. Various bacterial strains have been tested in human clinical trials, including *Bifidobacterium* spp., *E. coli* Nissle 1917, *Saccharomyces boulardii*, and *Lactobacillus* spp. and found to have beneficial effect on gut health [87]. However, their efficacy in the management of Crohn's disease has been controversial. Clinical trials have suggested a positive clinical effect of VSL#3, a probiotic containing four *Lactobacilli* (three *Bifidobacterium* spp. and *Streptococcus salivarius* subsp. thermophilus) in patients with active UC. However, it failed to prove its efficacy in patients with CD. These incongruences can in part be explained by the variety of probiotics used. It is imperative to note that the human gut-derived microbiota will have the best colonization and the most compatible therapeutic effect in patients with CD. Traditionally, the probiotics have been isolated from various dairy and nondairy products. These next-generation probiotics are derived from human feces or saliva and have a higher resistance to gastric enzymes and bile salts. In addition, they are also beneficial in patients with lactose intolerance [88, 89]. However, the feces-derived probiotics are not easily accepted by the patients due to the general perception of it being unhygienic. Recently, the concept of "synbiotics" has surfaced, which means adding a prebiotic to the probiotic [90]. A prebiotic is a substance selectively utilized by the probiotic, such as insulin and fructo-oligosaccharides [85, 86], and its use significantly improves remission rates, clinical activity, and histological scores in active CD [91]. Postbiotics are metabolites

produced by live microbes and are essential in maintaining the gut homeostasis. They include organic acids such as short-chain fatty acids (SCFA), tryptophan, and some bacteriocins. They exert an anti-inflammatory and anti-oxidant effect in the human gut and inhibit the growth of pathobionts. Administration of SCFAs and tryptophan have shown remission of inflammation in animal models; however, its efficacy in humans is controversial and under trial [92].

6.3 Live bacterial consortia (gut 103 and 108)

Gut 103 and 108 are used to supplement deficient microbiota and correct dysbiosis in patients with CD. Gut 103 consists of 17 bacterial strains, while Gut 108 is a purified version of Gut 103 and utilizes 11 human bacteria associated with the 17 strains. These bacterial formulations have shown to decrease pathobionts, expand the resident flora, decrease mucosal inflammation, and re-establish gut homeostasis. Moreover, these formulations allow the bacteria to stay longer in the colon as compared to other probiotics thereby increasing their efficacy [93].

6.4 Antibiotic therapy

Antibiotic therapy has shown benefits in some patient groups with active CD. They aim at controlling the pathogenic bacterial blooms, thereby reducing the gut microbial dysbiosis. This helps in reducing the gut mucosal inflammation, thereby decreasing the disease activity and inducing remission. Anti-mycobacterial drugs, fluoroquinolones, and rifaximin have shown positive results in active CD remission in certain population groups [94]. A small randomized controlled trial compared the effect of Ciprofoxacin and Mesalazine in patients with mild to moderate CD and observed complete remission with Ciprofloxacin [95]. Another randomized trial showed early benefits of antibiotics in 213 patients receiving either Clarithromycin, Rifabutin, or Clofazimine, with no significant difference in the relapse rates were noted in follow-up [96]. Antibiotic therapy is also used to prevent postoperative recurrence of CD and in treatment of complications of CD like perianal abscess and fistula. The current limitation of antibiotic therapy is the collateral damage to the healthy gut microbiome due to its nonspecific effect and development of antibiotic resistance. Further research is required to establish a definitive role of antibiotics in the management of CD.

6.5 Phage therapy

Phage therapy consists of using highly specific lytic bacteriophages to target strains within one bacterial species. This therapy is more advantageous than antibiotic therapy as it targets a specific strain of pathogenic bacteria with a limited impact on the normal gut microbiota [97]. Enteroinvasive *E. coli* (EIEC) are abundantly present in the ileum of patients with CD and have been linked to gut mucosal inflammation. Specific bacteriophages against EIEC have been isolated, and it has been observed that administration of "phage cocktail" (2×10^9 PFU/mL) could significantly reduce EIEC colonization [98]. A recent study on transgenic mice model of dextran sulfate sodium (DSS)-induced colitis showed that a single-day treatment with oral phage cocktail significantly reduced the colonization of EIEC and reduced intestinal symptoms

over a period of 2 weeks. Another crossover trial suggested that administration of phage cocktail over 28 days selectively reduced fecal EIEC without disrupting the commensal gut microbiota [99]. Federici et al. developed an orally administered lytic five-phage combination that targets the antibiotic resistant *Klebsiella pneumoniae* clade and demonstrated its feasibility in the management of IBD [100]. Although promising, the major concern of phage therapy is safety and the dosing schedule which remains as future challenges.

6.6 Bacterial vectors

The role of genetically engineered bacteria as a vector for therapeutic agents has been an area of interest. *Lactococcus lactis* is an innocuous vector as it is noninfective and noninvasive for the human body and hence has been widely studied. Oral formulations of genetically engineered *L. lactis* secreting IL-10, AG011 are undergoing various clinical trials and have been reported to reduce adverse drug reactions [101]. Other substances recombined into *L. lactis* are murine TNF neutralizing antibodies and IL-1 antagonists, which have shown promising results [102, 103].

6.7 Fecal microbiota transplantation (FMT)

FMT aims to restore the gut microbiota in CD patients by transferring these from a healthy donor to the affected recipient. The prevailing concept is that FMT might correct the gut microbial dysbiosis and lead to restoration of normal gut microbiota [104]. FMT has shown high efficacy in patients with recurrent *C. difficile* infection and has raised a possibility of its benefit in other diseases associated with gut dysbiosis like CD [105]. The inoculum can be given as fresh or frozen sample via various enteral routes. A recent systematic review published in 2021 concluded a 79% clinical response rate and a 62% clinical remission rate in CD patients. Moreover, it was noted that the rate of clinical remission was higher in patients treated with fresh stools as compared to frozen stools [48]. FMT is generally well tolerated and safe in CD with rare serious adverse effects. However, there is meager evidence on the long-term immunological effects of FMT. There are also certain limitations to this therapy such as heterogeneity in the technique, frequency of administration, and the ideal time to perform FMT. These factors affect the clinical outcome of treatment. Moreover, the multifactorial pathogenesis of CD and the dubious role of dysbiosis as a cause or consequence of the disease limit the effectiveness of this therapy. Thus, larger and well-designed studies and clinical trials are necessary to evaluate the effectiveness and optimal technique of FMT.

6.8 Role of *F. prausnitzii*

F. prausnitzii belongs to *Clostridium* cluster IV and is one of the main butyrate producers of the human gut. It exhibits anti-inflammatory properties by producing butyrate and inducing a tolerogenic cytokine profile. This includes decreased secretion of IL-12 and IFN-ϒ and increased secretion of IL-10 [38]. *F. prausnitzii* along with *E. coli* (F-E index) can help differentiate CD from irritable bowel syndrome (IBS) and UC. The F-E index can also be used to distinguish between ileal and colonic CD. *F. prausnitzii* levels can be used as a biomarker to assess disease progression and clinical response [39]. High fecal *F. prausnitzii* counts are associated with a lower CD

activity. *F. prausnitzii* has shown promising results as a good microbial biomarker; however, larger well-designed studies are essential to achieve a consensus.

7. Future direction

Understanding dysbiosis and specific microbial pathways in the causation of CD has led to adaptation of more targeted treatment strategies. Microbiome-targeted therapies aim at diagnosis, treatment, stratification, and assessment of high-risk population groups.

Profiling the gut microbiota may provide essential information related to the pathogenesis and treatment efficacy in patients with CD. Microbiome multiomics provide information on the interaction of specific microbiota with its environment and may help in understanding the functional aspect of dysbiosis in CD. They help in identifying and isolating the microbiota. Various methods for isolation of the gut microbiome have emerged lately. Organoids in 2D culture and "Gut on chip" are novel techniques developed to isolate the gut microbes and monitor host-microbial interactions [58]. Microbial multiomics, combined with precision medicine provides a more specific, "personalized" treatment to an individual and predicts a better treatment response and clinical outcome.

There is ongoing research on the safety and routes of administration of FMT. Oral FMT capsules have emerged as a novel noninvasive method for FMT. A recent meta-analysis examining the safety and efficacy of oral FMT capsules concluded that this method is easy with an overall efficacy of 82.1% [106]. However, safety of FMT is a big concern, as the donor feces may contain unknown pathogenic microbiota. Due to these concerns, a Canadian group has mass cultivated probiotics from processed feces which has shown positive results in *C. difficile* colitis [106]. However, these probiotics are still under speculation and need further research to determine its safety.

8. Conclusion

There is compelling evidence demonstrating the association of gut microbial dysbiosis in the pathogenesis of CD; however, its causal relationship is still uncertain. Microbial dysbiosis has been observed in asymptomatic patients with genetic susceptibility and patients with an inactive disease, suggesting that the microbial changes are present long before inflammation. This indicates the potential role of microbial dysbiosis in the causation of CD. Moreover, postoperative recurrence at neo-terminal ileum again suggests the causal role of dysbiosis in CD. The advent of bacteriotherapy has led to more targeted treatment strategies in patients with CD. However, the biggest challenge that still exists is the inconsistency and heterogeneity of data on the dysbiotic microbial composition that limits effective microbial therapies. In addition, their role in predicting the response to therapy is still unanswered. It is anticipated that better designed studies and advanced genetic sequencing technology will lead to a more defined role of gut microbiome in the pathogenesis and treatment of CD.

Conflict of interest

The authors report no conflict of interest.

Author details

Jyoti Sharma[1], Tuhina Banerjee[2], Manisha Naithani[3], Navin Kumar[3],
Sudhir Kumar Singh[3] and Somprakas Basu[3]*

1 All India Institute of Medical Sciences Delhi, Delhi, India

2 Banaras Hindu University, Varanasi, India

3 All India Institute of Medical Sciences Rishikesh, India

*Address all correspondence to: somprakas.surg@aiimsrishikesh.edu.in

IntechOpen

References

[1] Fichera A, Krane MK, editors. Crohn's disease: Basic principles. 1st ed. Switzerland: Springer; 2015. p. 306. DOI: 10.1007/978-3-319-14181-7

[2] Jairath V, Feagan BG. Global burden of inflammatory bowel disease. The Lancet Gastroenterology & Hepatology [Internet]. 2020;**5**(1):2-3. DOI: 10.1016/S2468-1253(19)30358-9

[3] Park SH. Update on the epidemiology of inflammatory bowel disease in Asia: Where are we now? Intestinal Research. 2022;**20**(2):159-164

[4] Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology [Internet]. 2012;**142**(1):46-54.e42. DOI: 10.1053/j.gastro.2011.10.001

[5] Greuter T, Manser C, Pittet V, Vavricka SR, Biedermann L. Gender differences in inflammatory bowel disease. Digestion. 2020;**101**(Suppl. 1):98-104

[6] Roda G, Chien Ng S, Kotze PG, et al. Crohn's disease. Nature Reviews Disease Primers [Internet]. April 2020;**6**(1):26. DOI: 10.1038/s41572-020-0156-2

[7] Liu JZ, Van Sommeren S, Huang H, Ng SC, Alberts R, Takahashi A, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nature Genetics. 2015;**47**(9):979-986

[8] Huang H, Fang M, Jostins L, Umićević Mirkov M, Boucher G, Anderson CA, et al. Fine-mapping inflammatory bowel disease loci to single-variant resolution. Nature [Internet]. 2017;**547**(7662):173-178. DOI: 10.1038/nature22969

[9] Gajendran M, Loganathan P, Catinella AP, Hashash JG. A comprehensive review and update on Crohn's disease. Disease-a-Month [Internet]. 2018;**64**(2):20-57. DOI: 10.1016/j.disamonth.2017.07.001

[10] To N, Gracie DJ, Ford AC. Systematic review with meta-analysis: The adverse effects of tobacco smoking on the natural history of Crohn's disease. Alimentary Pharmacology & Therapeutics. 2016;**43**(5):549-561

[11] Tomasello G, Mazzola M, Leone A, Sinagra E, Zummo G, Farina F, et al. Nutrition, oxidative stress and intestinal dysbiosis: Influence of diet on gut microbiota in inflammatory bowel diseases. Biomedical Papers. 2016;**160**(4):461-466

[12] Lambert K, Pappas D, Miglioretto C, Javadpour A, Reveley H, Frank L, et al. Systematic review with meta-analysis: Dietary intake in adults with inflammatory bowel disease. Alimentary Pharmacology & Therapeutics. 2021;**54**(6):742-754

[13] Ungaro R, Bernstein CN, Gearry R, Hviid A, Kolho KL, Kronman MP, et al. Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: A meta-analysis. The American Journal of Gastroenterology. 2014;**109**(11):1728-1738

[14] Cholapranee A, Ananthakrishnan AN. Environmental hygiene and risk of inflammatory bowel diseases.

Inflammatory Bowel Diseases. 2016;**22**(9):2191-2199

[15] Mahid SS, Minor KS, Soto RE, Hornung CA, Galandiuk S. Smoking and inflammatory bowel disease: A meta-analysis. Mayo Clinic Proceedings. 2006;**81**(11):1462-1471

[16] Ajabnoor SM, Thorpe G, Abdelhamid A, Hooper L. Long-term effects of increasing omega-3, omega-6 and total polyunsaturated fats on inflammatory bowel disease and markers of inflammation: A systematic review and meta-analysis of randomized controlled trials. European Journal of Nutrition [Internet]. 2021;**60**(5):2293-2316. DOI: 10.1007/s00394-020-02413-y

[17] Jain A, Nguyen NH, Proudfoot JA, et al. Impact of Obesity on Disease Activity and Patient-Reported Outcomes Measurement Information System (PROMIS) in Inflammatory Bowel Diseases. American Journal of Gastroenterol. 2019;**114**(4):630-639

[18] Nguyen NH, Ohno-Machado L, Sandborn WJ, Singh S. Obesity is independently associated with higher annual burden and costs of hospitalization in patients with inflammatory bowel diseases. Clinical Gastroenterology and Hepatology [Internet]. 2019;**17**(4):709-718.e7. DOI: 10.1016/j.cgh.2018.07.004

[19] Kaplan GG, Jackson T, Sands BE, Frisch M, Andersson RE, Korzenik J. The risk of developing Crohn's disease after an appendectomy: A meta-analysis. The American Journal of Gastroenterology. 2008;**103**(11):2925-2931

[20] Fantodji C, Jantchou P, Parent ME, Rousseau MC. Appendectomy and risk for inflammatory bowel disease: Effect of age and time post appendectomy—A cohort study. BMJ Open Gastroenterology. 2022;**9**(1):1-8

[21] Ortizo R, Lee SY, Nguyen ET, Jamal MM, Bechtold MM, Nguyen DL. Exposure to oral contraceptives increases the risk for development of inflammatory bowel disease: A meta-analysis of case-controlled and cohort studies. European Journal of Gastroenterology & Hepatology. 2017;**29**(9):1064-1070

[22] Moninuola OO, Milligan W, Lochhead P, Khalili H. Systematic review with meta-analysis: Association between acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs) and risk of Crohn's disease and ulcerative colitis exacerbation. Alimentary Pharmacology & Therapeutics. 2018;**47**(11):1428-1439

[23] Del Pinto R, Pietropaoli D, Chandar AK, Ferri C, Cominelli F. Association between inflammatory bowel disease and vitamin D deficiency. Inflammatory Bowel Diseases. 2015;**21**(11):2708-2717

[24] Li XX, Liu Y, Luo J, Huang ZD, Zhang C, Fu Y. Vitamin D deficiency associated with Crohn's disease and ulcerative colitis: A meta-analysis of 55 observational studies. Journal of Translational Medicine. 2019;**17**(1):1-19

[25] Vancamelbeke M, Vermeire S. The intestinal barrier: A fundamental role in health and disease. Expert Review of Gastroenterology & Hepatology. 2017;**11**(9):821-834

[26] Peterson LW, Artis D. Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. Nature Reviews Immunology [Internet]. 2014;**14**(3):141-153. DOI: 10.1038/nri3608

[27] Jakobsson HE, Rodríguez-Piñeiro AM, Schütte A, Ermund A, Boysen P, Bemark M, et al. The composition of the gut microbiota shapes the colon mucus barrier. EMBO Reports. 2015;**16**(2):164-177

[28] DeFilippis EM, Longman R, Harbus M, Dannenberg K, Scherl EJ. Crohn's disease: Evolution, epigenetics, and the emerging role of microbiome-targeted therapies. Current Gastroenterology Reports. 2016;**18**(3):1-9

[29] de Souza Heitor SP, Claudio F. Immunopathogenesis of IBD: Current state of the art. Nature Reviews. Gastroenterology & Hepatology. 2017;**14**(1):3

[30] Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. Role of the normal gut microbiota. World Journal of Gastroenterology. 2015;**21**(29):8836-8847

[31] Silva JPB, Navegantes-Lima KC, Oliveira ALB, Rodrigues DVS, Gaspar SLF, Monteiro VVS, et al. Protective mechanisms of butyrate on inflammatory bowel disease. Current Pharmaceutical Design. 2018;**24**(35):4154-4166

[32] Ramakrishna BS. Role of the gut microbiota in human nutrition and metabolism. Journal of Gastroenterology and Hepatology. 2013;**28**(S4):9-17

[33] Wu HJ, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes. 2012;**3**(1):4-14

[34] Honda K, Littman DR. The microbiota in adaptive immune homeostasis and disease. Nature. 2016;**535**(7610):75-84

[35] Fomby P, Cherlin AJ. Role of microbiota in immunity and inflammation. National Institutes of Health. 2011;**72**(2):181-204

[36] Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell [Internet]. 2009;**139**(3):485-498. DOI: 10.1016/j.cell.2009.09.033

[37] Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature. 2013;**500**(7461):232-236

[38] Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(43):16731-16736

[39] Parsaei M, Sarafraz N, Moaddab SY, Ebrahimzadeh LH. The importance of Faecalibacterium prausnitzii in human health and diseases. New Microbes and New Infections [Internet]. 2021;**43**:100928. DOI: 10.1016/j.nmni.2021.100928

[40] Pickard JM, Zeng MY, Caruso R, Núñez G. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. Immunological Reviews. 2017;**279**(1):70-89

[41] Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nature Reviews Immunology [Internet]. 2013;**13**(11):790-801. DOI: 10.1038/nri3535

[42] Kamada N, Kim YG, Sham HP, Vallance BA, Puente JL, Martens EC, et al. Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. Science (80–). 2012;**336**(6086):1325-1329

[43] Huang T, Zhang X, Pan J, Su X, Jin X, Guan X. Purification and characterization of a novel cold shock protein-like bacteriocin synthesized

by Bacillus thuringiensis. Scientific Reports [Internet]. 2016;**6**(April):1-14. DOI: 10.1038/srep35560

[44] Pilmis B, Le Monnier A, Zahar JR. Gut microbiota, antibiotic therapy and antimicrobial resistance: A narrative review. Microorganisms. 2020;**8**(2):1-17

[45] Melissa K, Carles U, Al E. Bacterial flagellin stimulates TLR5-dependent defense against vancomycin-resistant enterococcus infection. Bone. 2006;**23**(1):1-7

[46] Sharma J, Huda F, Naithani M, Singh SK, Kumar N, Basu S. Role of gut microbiome and enteric bacteria in gallbladder cancer. In: Rodrigo L, editor. Immunology of the GI Tract— Recent Advances [Internet]. London: IntechOpen; 2022. Available from: https://www.intechopen.com/ chapters/81344. DOI: 10.5772/ intechopen.104404

[47] Llewellyn SR, Britton GJ, Contijoch EJ, Vennaro OH, Mortha A, Colombel JF, et al. Interactions between diet and the intestinal microbiota alter intestinal permeability and colitis severity in mice. Gastroenterology [Internet]. 2018;**154**(4):1037-1046.e2. DOI: 10.1053/j.gastro.2017.11.030

[48] Cheng F, Huang Z, Wei W, Li Z. Fecal microbiota transplantation for Crohn's disease: a systematic review and meta-analysis. Techniques in Coloproctology. 2021;**25**:495-504

[49] Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: Current status and the future ahead. Gastroenterology [Internet]. 2014;**146**(6):1489-1499. DOI: 10.1053/j.gastro.2014.02.009

[50] Qiu P, Ishimoto T, Fu L, Zhang J, Zhang Z, Liu Y. The gut microbiota in inflammatory bowel disease. Frontiers in Cellular and Infection Microbiology. 2022;**12**(February):1-14

[51] Takahashi K, Nishida A, Fujimoto T, Fujii M, Shioya M, Imaeda H, et al. Reduced abundance of butyrate-producing bacteria species in the fecal microbial community in Crohn's disease. Digestion. 2016;**93**(1):59-65

[52] Varela E, Manichanh C, Gallart M, Torrejón A, Borruel N, Casellas F, et al. Colonisation by Faecalibacterium prausnitzii and maintenance of clinical remission in patients with ulcerative colitis. Alimentary Pharmacology & Therapeutics. 2013;**38**(2):151-161

[53] Pittayanon R, Lau JT, Leontiadis GI, Tse F, Yuan Y, Surette M, et al. Differences in gut microbiota in patients with vs without inflammatory bowel diseases: A systematic review. Gastroenterology [Internet]. 2020;**158**(4):930-946.e1. DOI: 10.1053/j. gastro.2019.11.294

[54] Rolhion N, Darfeuille-Michaud A. Adherent-invasive Escherichia coli in inflammatory bowel disease. Inflammatory Bowel Diseases. 2007;**13**(10):1277-1283

[55] Liu S, Zhao W, Lan P, Mou X. The microbiome in inflammatory bowel diseases: From pathogenesis to therapy. Protein & Cell [Internet]. 2021;**12**(5):331-345. DOI: 10.1007/ s13238-020-00745-3

[56] Loubinoux J, Bronowicki JP, Pereira IAC, Mougenel JL, Le Faou AE. Sulfate-reducing bacteria in human feces and their association with inflammatory bowel diseases. FEMS Microbiology Ecology. 2002;**40**(2):107-112

[57] Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut

microbiota in the pathogenesis of inflammatory bowel disease. Clinical Journal of Gastroenterology [Internet]. 2018;**11**(1):1-10. DOI: 10.1007/s12328-017-0813-5

[58] Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature. 2019;**569**(7758):655-662

[59] Rokkas T, Gisbert JP, Niv Y, O'Morain C. The association between helicobacter pylori infection and inflammatory bowel disease based on meta-analysis. United European Gastroenterology Journal. 2015;**3**(6):539-550

[60] Sokol H, Leducq V, Aschard H, Pham HP, Jegou S, Landman C, et al. Fungal microbiota dysbiosis in IBD. Gut. 2017;**66**(6):1039-1048

[61] Limon JJ, Tang J, Li D, Wolf AJ, Michelsen KS, Funari V, et al. Malassezia is associated with Crohn's disease and exacerbates colitis in mouse models. Cell Host & Microbe [Internet]. 2019;**25**(3):377-388.e6. DOI: 10.1016/j.chom.2019.01.007

[62] Zuo T, Ng SC. The gut microbiota in the pathogenesis and therapeutics of inflammatory bowel disease. Frontiers in Microbiology. 2018;**9**(Sep):1-13

[63] Norman JM, Handley SA, Baldridge MT, Droit L, Liu CY, Keller BC, et al. Disease-specific alterations in the enteric virome in inflammatory bowel disease. Cell [Internet]. 2015;**160**(3):447-460. DOI: 10.1016/j.cell.2015.01.002

[64] Pérez-Brocal V, García-López R, Vázquez-Castellanos JF, Nos P, Beltrán B, Latorre A, et al. Study of the viral and microbial communities associated with Crohn's disease: A metagenomic approach. Clinical and Translational Gastroenterology. 2013;**4**(6):e36. Published 13 Jun 2013. DOI:10.1038/ctg.2013.9

[65] Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biology. 2012;**13**(9):R79. Published 16 Apr 2012. DOI: 10.1186/gb-2012-13-9-r79

[66] Zhuang X, Tian Z, Li N, Mao R, Li X, Zhao M, et al. Gut microbiota profiles and microbial-based therapies in post-operative Crohn's disease: A systematic review. Frontiers in Medicine. Lausanne. 2021;7:615858. Published 28 Jan 2021. DOI: 10.3389/fmed.2020.615858

[67] Imhann F, Vich Vila A, Bonder MJ, Fu J, Di G, Visschedijk MC, et al. Interplay of host genetics and gut microbiota underlying the onset and clinical presentation of inflammatory bowel disease. Gut. 2018;**67**(1):108-119

[68] Hall AB, Yassour M, Sauk J, Garner A, Jiang X, Arthur T, et al. A novel Ruminococcus gnavus clade enriched in inflammatory bowel disease patients. Genome Medicine. 2017;**9**(1):1-12

[69] Seregin SS, Golovchenko N, Schaf B, Chen J, Pudlo NA, Mitchell J, et al. Erratum: NLRP6 protects il10−/− mice from colitis by limiting colonization of (Cell Reports (2017) 19(4) (733-745) (S2211124717304576) (10.1016/j.celrep.2017.03.080)). Cell Reports [Internet]. 2017;**19**(10):2174. DOI: 10.1016/j.celrep.2017.05.074

[70] Jarmakiewicz-Czaja S, Zielińska M, Sokal A, Filip R. Genetic and epigenetic etiology of inflammatory bowel disease: An update. Genes (Basel).

2022;**13**(12):2388. Published 16 Dec 2022. DOI: 10.3390/genes13122388

[71] Liu TC, Stappenbeck TS. Genetics and pathogenesis of inflammatory bowel disease. Annual Review of Pathology: Mechanisms of Disease. 2016;**11**(7351):127-148

[72] Zhen Y, Zhang H. NLRP3 inflammasome and inflammatory bowel disease. Frontiers in Immunology. 2019;**10**(Feb):1-10

[73] Zakrzewski M, Simms LA, Brown A, Appleyard M, Irwin J, Waddell N, et al. IL23R-protective coding variant promotes beneficial bacteria and diversity in the ileal microbiome in healthy individuals without inflammatory bowel disease. Journal of Crohn's & Colitis. 2019;**13**(4):451-461

[74] Krawiec P, Pawłowska-Kamieniak A, Pac-Kożuchowska E. Interleukin 10 and interleukin 10 receptor in paediatric inflammatory bowel disease: From bench to bedside lesson. Journal of Inflammation (United Kingdom). 2021;**18**(1):1-5

[75] Shouval D, Ouahed J, Biswas A, Al E. Interleukin 10 receptor signaling: Master regulator of intestinal mucosal homeostasis in mice and humans. Advances in Immunology. 2014;**122**(3):177-210

[76] Yilmaz B, Spalinger MR, Biedermann L, Franc Y, Fournier N, Rossel JB, et al. The presence of genetic risk variants within ptpn2 and ptpn22 is associated with intestinal microbiota alterations in Swiss IBD cohort patients. PLoS One. 2018;**13**(7):1-19

[77] Herbst S, Gutierrez MG. LRRK2 in infection: Friend or foe? ACS Infectious Diseases. 2019;**5**(6):809-815

[78] Caio G, Lungaro L, Caputo F, Zoli E, Giancola F, Chiarioni G, et al. Nutritional treatment in Crohn' s disease. Nutrients. 2021;**13**(5):1628

[79] Núñez-Sánchez MA, Melgar S, O'Donoghue K, Martínez-Sánchez MA, Fernández-Ruiz VE, Ferrer-Gómez M, et al. Crohn's disease, host–microbiota interactions, and immunonutrition: Dietary strategies targeting gut microbiome as novel therapeutic approaches. International Journal of Molecular Sciences. 2022;**23**(15)

[80] Guo X, Huang C, Xu J, Xu H, Liu L, Zhao H, et al. Gut microbiota is a potential biomarker in inflammatory bowel disease. Frontiers in Nutrition. 2022;**8**(January):1-11

[81] Ananthakrishnan AN. Microbiome-based biomarkers for ibd. Inflammatory Bowel Diseases. 2020;**26**(10):1463-1469

[82] Wills ES, Jonkers DMAE, Savelkoul PH, Masclee AA, Pierik MJ, Penders J. Fecal microbial composition of ulcerative colitis and Crohn's disease patients in remission and subsequent exacerbation. PLoS One. 2014;**9**(3):1-10

[83] Rehman A, Rausch P, Wang J, Skieceviciene J, Kiudelis G, Bhagalia K, et al. Geographical patterns of the standing and active human gut microbiome in health and IBD. Gut. 2016;**65**(2):238-248

[84] Lopez-Siles M, Martinez-Medina M, Busquets D, Sabat-Mir M, Duncan SH, Flint HJ, et al. Mucosa-associated Faecalibacterium prausnitzii and Escherichia coli co-abundance can distinguish irritable bowel syndrome and inflammatory bowel disease phenotypes. International Journal of Medical Microbiology [Internet]. 2014;**304**(3-4):464-475. DOI: 10.1016/j.ijmm.2014.02.009

[85] Ad B, Ag T, Ak A, Butterworth AD, Thomas AG, Akobeng AK. Probiotics for induction of remission in Crohn ' s disease (review) probiotics for induction of remission in Crohn' s disease. Library (London). 2008;**3**:3-5

[86] Abraham BP, Quigley EMM. Probiotics in inflammatory bowel disease. Gastroenterology Clinics of North America [Internet]. 2017;**46**(4):769-782. DOI: 10.1016/j. gtc.2017.08.003

[87] Fedorak RN, Feagan BG, Hotte N, Leddin D, Dieleman LA, Petrunia DM, et al. The probiotic vsl#3 has anti-inflammatory effects and could reduce endoscopic recurrence after surgery for Crohn's disease. Clinical Gastroenterology and Hepatology [Internet]. 2015;**13**(5):928-935.e2. DOI: 10.1016/j.cgh.2014.10.031

[88] Sornplang P, Piyadeatsoontorn S. Probiotic isolates from unconventional sources: A review. Journal of Animal Science and Technology [Internet]. 2016;**58**:1-11. DOI: 10.1186/ s40781-016-0108-2

[89] Bazireh H, Shariati P, Azimzadeh Jamalkandi S, Ahmadi A, Boroumand MA. Isolation of novel probiotic lactobacillus and enterococcus strains from human salivary and fecal sources. Frontiers in Microbiology. 2020;**11**(December):1-12

[90] Schrezenmeir J, de Vrese M. Probiotics, prebiotics, and synbiotics— Approaching a definition. The American Journal of Clinical Nutrition. 2001; **73**(2 Suppl):361S-364S. DOI: 10.1093/ ajcn/73.2.361s

[91] Oka A, Sartor B. Terapias basadas en microbios y dirigidas a microbios para enfermedades inflamatorias del intestino. Physiology & Behavior. 2020;**176**:139-148

[92] Belizário JE, Faintuch J. Microbiome and gut dysbiosis. Experientia. Supplementum. 2018;**109**:459-476

[93] van der Lelie D, Oka A, Taghavi S, Umeno J, Fan TJ, Merrell KE, et al. Rationally designed bacterial consortia to treat chronic immune-mediated colitis and restore intestinal homeostasis. Nature Communications. 2021;**12**(1):1-17

[94] Bernstein CN. Treatment of IBD: Where we are and where we are going. The American Journal of Gastroenterology [Internet]. 2015;**110**(1):114-126. DOI: 10.1038/ ajg.2014.357

[95] Wu XW, Ji HZ, Wang FY. Meta-analysis of ciprofloxacin in treatment of Crohn's disease. Biomedical Reports. 2015;**3**(1):70-74

[96] Wang SL, Wang ZR, Yang CQ. Meta-analysis of broad-spectrum antibiotic therapy in patients with active inflammatory bowel disease. Experimental and Therapeutic Medicine. 2012;**4**(6):1051-1056

[97] Qv L, Mao S, Li Y, Zhang J, Li L. Roles of gut bacteriophages in the pathogenesis and treatment of inflammatory bowel disease. Frontiers in Cellular and Infection Microbiology. 2021;**11**(November):1-11

[98] Zhilu X, Xiangqian D, Keli Y, Caroline C, Jingwan Z, Yu L, et al. Association of adherent-invasive Escherichia coli with severe gut mucosal dysbiosis in Hong Kong Chinese population with Crohn's disease. Gut Microbes [Internet]. 2021;**13**(1):1-13. DOI: 10.1080/19490976.2021.1994833

[99] Galtier M, De Sordi L, Sivignon A, de Vallée A, Maura D, Neut C, et al. Bacteriophages targeting adherent

invasive Escherichia coli strains as a promising new treatment for Crohn's disease. Journal of Crohn's & Colitis. 2017;**11**(7):840-847

[100] Federici S, Kredo-Russo S, Valdés-Mas R, et al. Targeted suppression of human IBD-associated gut microbiota commensals by phage consortia for treatment of intestinal inflammation. Cell. 2022;**185**(16):2879-2898.e24. DOI: 10.1016/j.cell.2022.07.003

[101] Martín R, Chain F, Miquel S, Natividad JM, Sokol H, Verdu EF, et al. Effects in the use of a genetically engineered strain of Lactococcus lactis delivering in situ IL-10 as a therapy to treat low-grade colon inflammation. Human Vaccines & Immunotherapeutics. 2014;**10**(6):1611-1621

[102] Chiabai MJ, Almeida JF, De Azevedo MGD, Fernandes SS, Pereira VB, De Castro RJA, et al. Mucosal delivery of Lactococcus lactis carrying an anti-TNF scFv expression vector ameliorates experimental colitis in mice. BMC Biotechnology. 2019;**19**(1):1-12

[103] Namai F, Shigemori S, Ogita T, Sato T, Shimosato T. Microbial therapeutics for acute colitis based on genetically modified Lactococcus lactis hypersecreting IL-1Ra in mice. Experimental & Molecular Medicine [Internet]. 2020;**52**(9):1627-1636. DOI: 10.1038/s12276-020-00507-5

[104] Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host & Microbe. 2014;**15**(3):382-392

[105] Saha S, Mara K, Pardi DS, Khanna S. Long-term safety of fecal microbiota transplantation for recurrent *Clostridioides difficile* infection.

Gastroenterology [Internet]. 2021;**160**(6):1961-1969.e3. DOI: 10.1053/j.gastro.2021.01.010

[106] Du C, Luo Y, Walsh S, Grinspan A. Oral Fecal microbiota transplant capsules are safe and effective for recurrent *Clostridioides difficil*e infection: A systematic review and meta-analysis. Journal of Clinical Gastroenterology. 2021;**55**(4):300-308. DOI: 10.1097/MCG.0000000000001495