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Review

Gut Microbiota in Inflammatory Bowel Disease: Still More Questions Than Answers

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Abstract

Human gut microbiota is a novel concept for pathogenesis in many inflammatory disorders including inflammatory bowel disease. In the last decade, experimental and clinical studies showed that gut microbiota composition is different in ulcerative colitis and Crohn's disease. This review summarizes the gut microbiome association studies and metabolomics of gut microbiota in inflammatory bowel disease. Also, minor components of the gut microbiome (fungi, protozoa, and viruses) and IBD data are discussed. The data about probiotics, prebiotics, and synbiotics are also discussed in inflammatory bowel disease treatment and prevention. Finally, we need more clinical trials on this topic to understand the causative role of microbiota in inflammatory bowel disease.

Keywords: Crohn's disease, gut microbiome, inflammatory bowel disease, mycobiome, ulcerative colitis, probiotics, prebiotics, synbiotics, virome

INTRODUCTION

Microbiota is a general term that defines the whole ecosystem of microorganisms in the human body. This term includes microbiota of the gastrointestinal (GI) system, skin, urogenital, respiratory, conjunctiva, and also oral cavity. Each of these sites harbors their own unique microbiota composition. The term "microbiome" was first mentioned by Lederberg and McCray in 2001, which signifies the genomic repertoire of microorganisms in health and disease states.[1]

The GI system is the main area in the human body that supports colonization of commensal microorganisms with a rich amount of fibers, nutrients, and oxygen levels. The gut microbiome is composed of 100 trillion microorganisms. Beyond bacteria, there are bacteriophages, fungi, archaea, and protozoa in this ecosystem.[2] The genomic material in the gut microbiome is far more diverse than the human genome.[3] The human gut microbiota consisted of *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and in general, *Firmicutes* and *Bacteroidetes* predominate in this environment.

The oral cavity, actually a part of GI tract, confers an ideal environment for these microorganisms since the nutrients, temperature, and mucus layer support the growth. For this reason, microorganisms that constitute the oral microbiome are the second most abundant microbiome in the human body.[4] Approximately 750 species of bacteria can be detected with culture-independent methods.[5]

Crohn's disease (CD) and ulcerative colitis (UC) are inflammatory bowel diseases (IBD). They are chronic inflammatory disorders of the gut.[6,7] The prevalence of IBD is steadily increasing in the United States, North Europe, and China. There are also similar data from countries such as the Middle East, Asia, and South America.[8] Although the exact reason for this rapid increase in incidence is not known, environmental factors rather than host genetics might be suspected. Host-microbiome interaction is controlled by a variety of genes. Genome-wide association studies have detected more than 200 IBD–associated genes, and some of them were involved in the host immune response to gut microbiota (Table 1).[9] Recent data support the role of gut microbiota in the pathogenesis of IBD (Table 2).[10,11] Animal studies have suggested that disturbed microbiota composition (known as "dysbiosis") is IBD; however, extrapolation of these results from basic to clinical trials is still challenging (Table 3).[12,13]

Gut Microbiome as a Potential Biomarker in IBD

Rapid advances in molecular genetic methods enabled differentiation of gut microbiome composition in patients with Irritable bowel syndrome (IBS) from Irritable bowel syndrome or healthy population.[14-16] In patients with CD, *Bacteroides*, *Eubacterium*, *Faecalibacterium*, and *Ruminococcus* are reduced.[17,18] Especially *Akkermansia muciniphila*[19] and *Faecalibacterium prausnitzii* are the most extensively studied bacteria in CD.[20] Lopez-Siles et al[21] investigated *F. prausnitzii* and *Escherichia coli* in 28 healthy controls, 45 patients with CD, 28 patients with UC, and 10 patients with IBS. They found that *F. prausnitzii* is a predictor of "health" in patients with CD (and other gut disorders). *F. prausnitzii* abundance was decreased in CD patients and it was lower than patients with IBS and healthy controls. When *E. coli* was added to *F. prausnitzii* in the diagnostic analysis, it discriminated the ileocolonic vs colonic form of CD patients. The combination of certain bacterial groups might improve the diagnostic value of gut microbiota analysis.[20] Zhou et al found that gut microbiome samples were able to distinguish healthy vs UC and CD patients with diagnostic

Table 1. Genetic Polymorphisms in IBD Related to Mucosal Immune System-Microbiota Interaction

Gene	Immune Effects	Susceptibility
NOD2/CARD15	Impairment of pathogen recognition	CD
NLRP3	IL-1β synthesis	CD
ATG16L1	Autophagosome formation	CD, UC
IRGM	Process of autophagy	CD, UC
PTPN2	Autophagy in IEC	CD, UC
FUT2	Secretion of blood group ABO antigens and alteration in microbiota	CD
JAK2/STAT3	T-cell activation	CD
ICOSLG	T-cell activation	CD
CCR6	Leukocyte activation and migration	CD

Modified from reference 15.

CD, Crohn's disease; IBD, inflammatory bowel disease; IEC, Intestinal epithelial cells; UC, ulcerative colitis.

accuracy values of 89.5% and 93.2%, respectively.[22] However, fecal samples from Western countries were less accurate in the differentiation of healthy vs IBD patients. We need more studies on IBD to understand the ethnic, geographical, and cultural differences in a microbiome biomarker.

The Composition of gut Microbiome and Metabolic Activity in IBD

The gut bacteria have metabolic activities that regulate the host-microbiota axis.[23] They produce short-chain fatty acids (SCFA) that exert various immunological and metabolic activities. SCFAs have diverse effects on the human body such as regulating histone deacetylase inhibitory activity and inducing some epigenetic and immune responses.[24,25] Also, butyrate is well known for its stimulatory effect on regulatory T cells and modulating macrophages.[26,27] Butyrate levels are lower in feces of IBD patients.[28]

Franzosa et al investigated the stool sample metabolomics of IBD patients.[29] They enrolled 155 discovery cohorts and 65 validation cohorts consisting of IBD and non-IBD controls. The metabolic changes were associated with fecal calprotectin levels. Among more than 8000 metabolites, some of them were expressed in IBD patients, such as increased sphingolipids and bile acids and decreased triglycerides. The metagenomic profiles of IBD patients showed that these bacteria are producing antioxidant metabolites in the inflamed mucosa of IBD patients. Interestingly, 246 enzymes in the IBD population were not synthesized by a certain species of bacteria. This indicates that there is a community-level metabolic shift in IBD patients. In other words, a broad range of bacterial species changed their metabolic functions in the inflammatory gut environment of IBD patients.

They have also identified 122 strong associations between specific bacteria and their metabolites in IBD patients. These specific metabolites are potential therapeutic and diagnostic tools.

Bacteriophages, Protozoa, and Mycobiome

When we talk about the gut microbiome, many scientists think about bacteria. However, the gut microbiota also consisted of fungi, bacteriophages, and protozoa. Of the nonbacterial microorganisms that have been studied in relation to IBD, most of them were accepted as pathogens (such as *Candida albicans*, *Cytomegalovirus*, etc.); however, some of these were actually commensal microorganisms.[30]

Fungi are found in almost every part of our body including skin, urogenital system, mouth, small intestine, large intestine, and so on. Most of these species are *Candida*, *Malassezia*, *and Saccharomyces*, and there are some studies linking them to IBD.[31,32] Anti-*Saccharomyces cerevisiae* antibodies are elevated in CD,[33,34] and the presence of this antibody can increase post-resection recurrence of CD[35] and useful in the differential diagnosis of UC vs CD.[36] *Candida* species especially *C. albicans* are increased in CD and families.[37] In experimental models of CD, *C. albicans* are also increased in the gut microbiome.[38] *Candida* species are opportunistic microorganisms, and their pathogenetic role might be immune stimulation after mucosal barrier dysfunction. Also, in IBD patients, a genetic mutation against fungi (e.g., *DECTIN-1* and *Card9*) may increase fungal colonization and subsequent inflammation.[39]

Table 2. Human and Animal Studies Providing Evidence for the Role of Microbiome in the Pathogenesis of IBD

Animal Studies	Human Studies
Germ-free environment prevents colitis	IBD disease activity is higher in areas where bacterial populations are high (colon) and where there is relative stasis of fecal material (terminal ileum and rectum).
Fecal transfer from mice with colitis to healthy one induces inflammation	In refractory CD, fecal diversion is beneficial
Naive CD4+ lymphocytes from healthy mice into mice that lack T and B lymphocytes induce colitis	Recurrence of disease occurs after restoration of the fecal stream
CD4+ lymphocyte-induced experimental colitis is dependent on host microbiota composition	Antibiotic therapy might change the course of IBD
	Genetic markers associated with IBD are related to mucosal immunity against gut microbiota
	Specific microorganisms stimulate or suppress gut inflammation.

Modified from reference 14.

CD, Crohn's disease; IBD, inflammatory bowel disease.

Table 3. Gut Microbiome Composition in Various Studies in UC

Decreased	Increased
Bacteroides	*Enterococcus*
Clostridium XIVab	*Escherichia coli*
Lactobacillus	*Actinobacteria*
Akkermansia muciniphila	*Proteobacteria*
Clostridium leptum	*Campylobacter ureolyticus*

Modified from references 23 to 29.

UC, ulcerative colitis.

However, not all fungi are pathogenic or pathobiont. *Saccharomyces boulardii*, a well-documented probiotic, also had a protective effect in mice with carcinogenic colitis.[40,41] The beneficial effect of *S. boulardii* has been shown in CD patients with heterogeneous results.[42] There is evidence that some fungi may be beneficial in IBD, but inconsistent findings indicate that fungi-host interactions are much more complex than we previously thought.

Recent metagenomic studies revealed that gut mycobiome consisted of dozens of fungi, mostly Saccharomycetaceae. In IBD patients, there is a "fungal dysbiosis," the similar term that we used for bacterial dysbiosis.[43-46] Although there are mixed results, 10 studies investigated the composition of mycobiome in IBD patients.[47-52] There is no consensus about which specific fungi dominate in CD and/or UC today, but these findings indicate a potential area of research in combination with bacteria and maybe other microorganisms in the gut microbiome.

Gut protozoa, prevalent in developing countries, are seldomly detected in developed countries. However, the prevalence of IBD is the opposite of this phenomenon. The incidence and prevalence of IBD are higher in developed, westernized countries.[53] Although gut parasites and protozoa are mostly accepted as pathogenic microorganisms, there is evidence that parasites can shift mucosal immune response in IBD.[54-57] *Blastocystis* species and *Dientamoeba fragilis* are found in human feces and they infect humans by the fecal-oral route.[58,59] Although these microorganisms are blamed for endemic gastroenteritis, recent studies did not find any evidence about this causation.[60-64] A recent trial also found that especially *Blastocystis* species are associated with healthy (increased diversity) gut microbiota.[60] Both *Blastocystis* and *D. fragilis* were lower in active UC; however, they are elevated in remission and healthy controls.[65]

The main barrier in studying mycobiome and protozoa species is the low levels of these microorganisms in human gut microbiome. It is difficult to capture sufficient DNA for the detailed analysis. For this reason, the samples are enriched for eukaryotic cells before analysis. Further studies will better delineate and overcome this difficulty. In the future, decreasing costs of these tests, enrichment of reference databases, advanced computational methods, and selecting eukaryotic cells before analysis will improve mycobiome and protozoa analyses in IBD.

Virome is another frontier for gut microbiome research. Some authors claim that gut virome is the "dark matter of IBD pathogenesis".[66,67] There are two kinds of viruses in the gut: viruses infecting eukaryotic cells (e.g., human cells) and viruses infecting bacteria (bacteriophages). Most of the human gut virome is composed of bacteriophages.[68] Human gut bacteriophage trials in IBD showed consistent results. Although there is a paucity of trials, most of them indicated an increased abundance of *Caudovirales* and a decreased virome diversity in CD and UC.[69-70] There are many limitations of virome analysis. Similar to fungal genetic material, viral DNA is a small proportion of the total genome in a sample. Viruses have tiny genetic materials and are highly susceptible to mutation. For these obstacles, standardization of virome research is needed. Bacteriophages are also potential therapeutic tools, thus modulating bacteriomes in the gut environment. Bacteriophages can modify gut bacteria into two proposed mechanisms: first, phage particles can induce immunological response (direct action). Second, phages can transfer genes to bacteria (horizontal gene transfer—indirect action).[71]

Although there is no clinical therapeutical trial with bacteriophages in humans, further studies are needed to fill this gap of knowledge in IBD therapy.

Probiotics, Prebiotics, and Synbiotics in IBD

Probiotics can be defined as live microorganisms, which when administered in adequate amounts, confer health benefits on the host.[72] The term probiotics usually refers to bacteria predominantly found in fermented products such as yogurt, kefir, pickles. Prebiotics are mainly fibers that are selectively fermented by commensal bacteria providing a health benefit. Prebiotics are classified as polyols (sugar alcohols), oligosaccharides, and soluble fibers.[73] Synbiotics are a combination of probiotics and prebiotics.

Recent meta-analysis showed that probiotics have a beneficial clinical effect on IBD course, especially UC.[74] The consumption of these products (literally food supplements) is theoretically supposed to increase the number of *Bifidobacteria* and *Lactobacilli* in the gut. The mechanism of action of pro-, pre-, or synbiotics in IBD is not completely understood. The proposed mechanisms are the metabolic activity of beneficial bacteria (synthesis of SCFAs), anti-inflammatory action, modulating the microbial balance, mucosal immunity, and improving regulatory T cells and barrier function.[75] The effects of pro-, pre-, and synbiotics on the gut microbiota are also controversial. Some trials showed an increase in the number of *Bifidobacteria* and *F. prausnitzii*.[76,77] Prebiotics are also studied in IBD. Benjamin et al showed that fructo-oligosaccharide supplementation in active CD showed null effects on gut microbiota composition.[78] VSL3 is a multispecies probiotic combination. In UC, there are sufficient evidence for inducing remission and prevention of relapse as an adjuvant therapy with mesalazin.[79]

As a result, pro-, pre-, and synbiotics could have a positive impact on the clinical course of UC by probably increasing the number of beneficial bacteria (especially *Bifidobacteria*). However, there is a significant bias in trials and further multicenter studies are needed to reach a firm conclusion.

CONCLUSION

IBD is a heterogeneous, multifactorial autoinflammatory condition. Although genetic factors are well-established environmental factors, epigenetic and microbiome-related effects are still under investigation. Gut microbiome and related parameters (metabolome and proteome, etc.) enable a therapeutic potential that we can modify (unlike genetics). Current data indicate a correlation between gut microbiota composition-function and IBD etiology and natural disease course. There are still unanswered questions about IBD and gut microbiota connection. What is the longitudinal effect of microbiota from birth to adulthood? What is the effect of gut microbiome modulation on the prevention of IBD, disease severity, phenotype, and response to therapy. What is the

effect of the oral microbiome on the gut microbiome and IBD course? We still have more questions than answers on this topic, and standardized methods and high-quality studies investigating the causal effect of gut microbiota on IBD are needed.

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REFERENCES

1. Lederberg J, McCray AT. Ome sweet omics—a genealogical treasury of words. *Scientist*. 2001;15(7):8.
2. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clin J Gastroenterol*. 2018;11(1):1-10. [CrossRef]
3. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. *World J Gastroenterol*. 2015;21(29):8787-8803. [CrossRef]
4. Dewhirst FE, Chen T, Izard J, et al. The human oral microbiome. *J Bacteriol*. 2010;192(19):5002-5017. [CrossRef]
5. Paster BJ, Olsen I, Aas JA, Dewhirst FE. The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Periodontol 2000*. 2006;42(1):80-87. [CrossRef]
6. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. *Annu Rev Immunol*. 2010;28:573-621. [CrossRef]
7. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature*. 2011;474(7351):307-317. [CrossRef]
8. Kaplan GG, Ng SC. Understanding and preventing the global increase of inflammatory bowel disease. *Gastroenterology*. 2017;152(2):313-321.e2. [CrossRef]
9. Liu JZ, van Sommeren S, Huang H, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat Genet*. 2015;47(9):979-986. [CrossRef]
10. Goldsmith JR, Sartor RB. The role of diet on intestinal microbiota metabolism: downstream impacts on host immune function and health, and therapeutic implications. *J Gastroenterol*. 2014;49(5):785-798. [CrossRef]
11. Sheehan D, Moran C, Shanahan F. The microbiota in inflammatory bowel disease. *J Gastroenterol*. 2015;50(5):495-507. [CrossRef]
12. Lupp C, Robertson ML, Wickham ME, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. *Cell Host Microbe*. 2007;2(2):119-129. [CrossRef]
13. Marsilio S, Pilla R, Sarawichitr B, et al. Characterization of the fecal microbiome in cats with inflammatory bowel disease or alimentary small cell lymphoma. *Sci Rep*. 2019:9(1):19208.
14. Verma R, Verma AK, Ahuja V, Paul J. Real-time analysis of mucosal flora in patients with inflammatory bowel disease in India. *J Clin Microbiol*. 2010;48(11):4279-4282. [CrossRef]
15. de Meij TGJ, de Groot EFJ, Peeters CFW, et al. Variability of core microbiota in newly diagnosed treatment- naive paediatric inflammatory bowel disease patients. *PLoS ONE*. 2018;13(8):e0197649. [CrossRef]
16. Zhou Y, Xu ZZ, He Y, et al. Gut microbiota offers universal biomarkers across ethnicity in inflammatory bowel disease diagnosis and infliximab response prediction. *mSystems*. 2018;3(1):e00188-17. 16.
17. Wills ES, Jonkers DM, 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):e90981. [CrossRef]
18. Takahashi K, Nishida A, Fujimoto T, et al. Reduced abundance of butyrate-producing bacteria species in the fecal microbial community in Crohn's disease. *Digestion*. 2016;93(1):59-65. [CrossRef]
19. Yu Y, Yang W, Li Y, Cong Y. Enteroendocrine cells: sensing gut microbiota and regulating inflammatory bowel diseases. *Inflamm Bowel Dis*. 2020;26(1):11-20. [CrossRef]
20. Fukuda K, Fujita Y. Determination of the discriminant score of intestinal microbiota as a biomarker of disease activity in patients with ulcerative colitis. *BMC Gastroenterol*. 2014;14:49. [CrossRef]
21. Lopez-Siles M, Martinez-Medina M, Busquets D, et al. Mucosa-associated *Faecalibacterium prausnitzii* and *Escherichia coli* co-abundance can distinguish irritable bowel syndrome and inflammatory bowel disease phenotypes. *Int J Med Microbiol*. 2014;304(3-4):464-475.
22. Zhou Y, Xu ZZ, He Y, et al. Gut microbiota offers universal biomarkers across ethnicity in inflammatory bowel disease diagnosis and infliximab response prediction. *mSystems*. 2018;3(1):e00188. [CrossRef]
23. Dorrestein PC, Mazmanian SK, Knight R. Finding the missing links among metabolites, microbes, and the host. *Immunity*. 2014;40(6):824-832. [CrossRef]
24. Lane ER, Zisman TL, Suskind DL. The microbiota in inflammatory bowel disease: current and therapeutic insights. *J Inflamm Res*. 2017;10:63-73. [CrossRef]
25. Kim S, Kim JH, Park BO, Kwak YS. Perspectives on the therapeutic potential of short-chain fatty acid receptors. *BMB Rep*. 2014;47(3):173-178. [CrossRef]
26. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*. 2013;341(6145):569-573. [CrossRef]
27. Fernando MR, Saxena A, Reyes J-L, McKay DM. Butyrate enhances antibacterial effects while suppressing other features of alternative activation in IL-4-induced macrophages. *Am J Physiol Gastrointest Liver Physiol*. 2016;310(10):G822-831.27.
28. Marchesi JR, Holmes E, Khan F, et al. Rapid and noninvasive metabonomic characterization of inflammatory bowel disease. *J Proteome Res*. 2007;6(2):546-551. [CrossRef]
29. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, et al. Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat Microbiol*. 2019;4(2):293-305. [CrossRef]
30. Guzzo GL, Andrews JM, Weyrich LS. The neglected gut microbiome: fungi, protozoa, and bacteriophages in inflammatory bowel disease. *Inflamm Bowel Dis*. 2022:izab343, [ePub].
31. Sartor RB, Wu GD. Roles for intestinal bacteria, viruses, and fungi in pathogenesis of inflammatory bowel diseases and therapeutic approaches. *Gastroenterology*. 2017;152(2):327-339.e4.
32. Miyoshi J, Sofia MA, Pierre JF. The evidence for fungus in Crohn's disease pathogenesis. *Clin J Gastroenterol*. 2018;11(6):449-456. [CrossRef]
33. Darroch CJ, Barnes RM, Dawson J. Circulating antibodies to *Saccharomyces cerevisiae* (bakers'/brewers' yeast) in gastrointestinal disease. *J Clin Pathol*. 1999;52(1):47-53.
34. Quinton JF, Sendid B, Reumaux D, et al. Anti-*Saccharomyces cerevisiae* mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut*. 1998;42(6):788-791. [CrossRef]
35. Forcione DG, Rosen MJ, Kisiel JB, Sands BE. Anti-*Saccharomyces cerevisiae* antibody (ASCA) positivity is associated with increased risk for early surgery in Crohn's disease. *Gut*. 2004;53(8):1117-1122. [CrossRef]
36. Reese GE, Constantinides VA, Simillis C, et al. Diagnostic precision of anti-*Saccharomyces cerevisiae* antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *Am J Gastroenterol*. 2006;101(10):2410-2422. [CrossRef]
37. Standaert-Vitse A, Sendid B, Joossens M, et al. *Candida albicans* colonization and ASCA in familial Crohn's disease. *Am J Gastroenterol*. 2009;104(7):1745-1753. [CrossRef]
38. Zwolinska-Wcislo M, Brzozowski T, Budak A, et al. Effect of *Candida* colonization on human ulcerative colitis and the healing of inflammatory changes of the colon in the experimental model of colitis ulcerosa. *J Physiol Pharmacol*. 2009;60(1):107-118.
39. Richard ML, Lamas B, Liguori G, Hoffmann TW, Sokol H. Gut fungal microbiota: the Yin and Yang of inflammatory bowel disease. *Inflamm Bowel Dis*. 2015;21(3):656-665. [CrossRef]
40. Garcia Vilela E, De Lourdes De Abreu Ferrari M, Oswaldo Da Gama Torres H, et al. Influence of *Saccharomyces boulardii* on the intestinal permeability of patients with Crohn's disease in remission. *Scand J Gastroenterol*. 2008;43(7):842-848. [CrossRef]
41. Wang C, Li W, Wang H, et al. *Saccharomyces boulardii* alleviates ulcerative colitis carcinogenesis in mice by reducing TNF-α and IL-6 levels and functions and by rebalancing intestinal microbiota. *BMC Microbiol*. 2019;19(1):246. [CrossRef]
42. Bourreille A, Cadiot G, Le Dreau G, et al. *Saccharomyces boulardii* does not prevent relapse of Crohn's disease. *Clin Gastroenterol Hepatol*. 2013;11(8):982-987. [CrossRef]
43. Hallen-Adams HE, Suhr MJ. Fungi in the healthy human gastrointestinal tract. *Virulence*. 2017;8(3):352-358. [CrossRef]
44. Limon JJ, Skalski JH, Underhill DM. Commensal fungi in health and disease. *Cell Host Microbe*. 2017;22(2):156-165. [CrossRef]

45. Nash AK, Auchtung TA, Wong MC, et al. The gut mycobiome of the Human Microbiome Project healthy cohort. *Microbiome*. 2017;5(1):153. [CrossRef]
46. Sokol H, Leducq V, Aschard H, et al. Fungal microbiota dysbiosis in IBD. *Gut*. 2017;66(6):1039-1048. [CrossRef]
47. Ott SJ, Kühbacher T, Musfeldt M, et al. Fungi and inflammatory bowel diseases: alterations of composition and diversity. *Scand J Gastroenterol*. 2008;43(7):831-841. [CrossRef]
48. Qiu X, Ma J, Jiao C, et al. Alterations in the mucosa-associated fungal microbiota in patients with ulcerative colitis. *Oncotarget*. 2017;8(64):107577-107588.
49. Liguori G, Lamas B, Richard ML, et al. Fungal dysbiosis in mucosa-associated microbiota of Crohn's disease patients. *J Crohns Colitis*. 2016;10(3):296-305. [CrossRef]
50. Hoarau G, Mukherjee PK, Gower-Rousseau C, et al. Bacteriome and mycobiome interactions underscore microbial dysbiosis in familial Crohn's disease. *mBio*. 2016;7(5):e01250-16. [CrossRef]
51. Jain U, Ver Heul AM, Xiong S, et al. *Debaryomyces* is enriched in Crohn's disease intestinal tissue and impairs healing in mice. *Science*. 2021;371(6534):1154-1159. [CrossRef]
52. Chehoud C, Albenberg LG, Judge C, et al. Fungal signature in the gut microbiota of pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis*. 2015;21(8):1948-1956. [CrossRef]
53. GBD. Inflammatory bowel disease collaborators. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol*. 2017;5(1):17-30.
54. Croese J, O'neil J, Masson J, et al. A proof of concept study establishing *Necator americanus* in Crohn's patients and reservoir donors. *Gut*. 2006;55(1):136-137. [CrossRef]
55. Fortun P, Shepherd V, Moroz V, Pritchard D, Hawkey CJ. OC-004 effect of hookworm treatment on active Crohn's disease. *Gut*. 2010;59(Suppl 1):A2.1-A2A2. [CrossRef]
56. Sandborn WJ, Elliott DE, Weinstock J, et al. Randomised clinical trial: the safety and tolerability of Trichuris suis ova in patients with Crohn's disease. *Aliment Pharmacol Ther*. 2013;38(3):255-263. [CrossRef]
57. Summers RW, Elliott DE, Qadir K, Urban JF, Thompson R, Weinstock JV. *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. *Am J Gastroenterol*. 2003;98(9):2034-2041. [CrossRef]
58. Garcia LS. *Dientamoeba fragilis*, one of the neglected intestinal protozoa. *J Clin Microbiol*. 2016;54(9):2243-2250. [CrossRef]
59. Wawrzyniak I, Poirier P, Viscogliosi E, et al. Blastocystis, an unrecognized parasite: an overview of pathogenesis and diagnosis. *Ther Adv Infect Dis*. 2013;1(5):167-178. [CrossRef]
60. Brands MR, Van de Vijver E, Haisma SM, Heida A, van Rheenen PF. No association between abdominal pain and *Dientamoeba* in Dutch and Belgian children. *Arch Dis Child*. 2019;104(7):686-689. [CrossRef]
61. Holtman GA, Kranenberg JJ, Blanker MH, Ott A, Lisman-van Leeuwen Y, Berger MY. *Dientamoeba fragilis* colonization is not associated with gastrointes- tinal symptoms in children at primary care level. *Fam Pract*. 2017;34(1):25-29. [CrossRef]
62. Jokelainen P, Hebbelstrup Jensen B, Andreassen BU, et al. *Dientamoeba fragilis*, a commensal in children in Danish day care centers. *J Clin Microbiol*. 2017;55(6):1707-1713. [CrossRef]
63. Dullaert-de Boer M, Schuurs TA, Vermeer M, et al. Distribution and relevance of *Dientamoeba fragilis* and *Blastocystis* species in gastroenteritis: results from a case-control study. *Eur J Clin Microbiol Infect Dis*. 2019;39(1):197-203.
64. Petersen AM, Stensvold CR, Mirsepasi H, et al. Active ulcerative colitis associated with low prevalence of *Blastocystis* and *Dientamoeba fragilis* infection. *Scand J Gastroenterol*. 2013;48(5):638-639. [CrossRef]
65. Tito RY, Chaffron S, Caenepeel C, et al. Population-level analysis of *Blastocystis* subtype prevalence and variation in the human gut microbiota. *Gut*. 2019;68(7):1180-1189. [CrossRef]
66. Clooney AG, Sutton TDS, Shkoporov AN, et al. Whole-virome analysis sheds light on viral dark matter in inflammatory bowel disease. *Cell Host Microbe*. 2019;26(6):764-778.e5.
67. Carding SR, Davis N, Hoyles L. Review article: the human intestinal virome in health and disease. *Aliment Pharmacol Ther*. 2017;46(9):800-815. [CrossRef]
68. Lewis JD, Chen EZ, Baldassano RN, et al. Inflammation, antibiotics, and diet as environmental stressors of the gut microbiome in pediatric Crohn's disease. *Cell Host Microbe*. 2017;22(2):247. [CrossRef]
69. Pérez-Brocal V, García-López R, Vázquez-Castellanos JF, et al. Study of the viral and microbial communities associated with Crohn's disease: a metagenomic approach. *Clin Transl Gastroenterol*. 2013;4(6):e36. [CrossRef]
70. Wagner J, Maksimovic J, Farries G, et al. Bacteriophages in gut samples from pediatric Crohn's disease patients: metagenomic analysis using 454 pyrosequencing. *Inflamm Bowel Dis*. 2013;19(8):1598- 1608. [CrossRef]
71. Norman JM, Handley SA, Baldridge MT, et al. Disease-specific alterations in the enteric virome in inflammatory bowel disease. *Cell*. 2015;160(3):447-460. [CrossRef]
72. Pérez-Brocal V, García-López R, Nos P, Beltrán B, Moret I, Moya A. Metagenomic analysis of Crohn's disease patients identifies changes in the virome and microbiome related to disease status and therapy, and detects potential interactions and biomarkers. *Inflamm Bowel Dis*. 2015;21(11):2515-2532. [CrossRef]
73. Duerkop BA, Kleiner M, Paez-Espino D, et al. Murine colitis reveals a disease-associated bacteriophage community. *Nat Microbiol*. 2018;3(9):1023-1031. [CrossRef]
74. Maronek M, Link R, Ambro L, Gardlik R. Phages and their role in gastrointestinal disease: focus on inflammatory bowel disease. *Cells*. 2020;9(4):1013.
75. Swanson KS, Gibson GR, Hutkins R, et al. The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nat Rev Gastroenterol Hepatol*. 2020;17(11):687-701.
76. Gibson GR, Hutkins R, Sanders ME, et al. Expert consensus document: the international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol*. 2017;14(8):491-502.
77. Zhang XF, Guan XX, Tang YJ, et al. Correction to: clinical effects and gut microbiota changes of using probiotics, prebiotics or synbiotics in inflammatory bowel disease: a systematic review and meta-analysis. *Eur J Nutr*. 2021;60(5):2877. [CrossRef]
78. Fan H, Du J, Liu X, et al. Effects of Pentasa-combined probiotics on the microflora structure and prognosis of patients with inflammatory bowel dis- ease. *Turk J Gastroenterol*. 2019;30(8):680-685. [CrossRef]
79. Su H, Kang Q, Wang H, et al. Effects of glucocorticoids combined with probiotics in treating Crohn's disease on inflammatory factors and intestinal micro-flora. *Exp Ther Med*. 2018;16(4):2999-3003.