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Review Article

Fecal Fungal Microbiota (Mycobiome) Study as a Potential Tool for Precision Medicine in Inflammatory Bowel Disease

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There is growing evidence of the role of fungal microbiota in the pathogenesis of inflammatory bowel disease (IBD). Fungi can exert direct pro-inflammatory effects or modify the bacterial composition via interkingdom interactions. Although several studies have demonstrated alterations in the fecal fungal microbiota composition in IBD, there is a wide variation in the mycobiome in different populations, with no definite pattern that can define the mycobiome in IBD having yet been identified. Recent work has suggested that characterizing the fecal fungal composition may influence therapeutic decisions and help to predict outcomes in a subset of IBD patients. In this study, we review the current literature on the emerging role of the fecal mycobiome as a potential tool for precision medicine in IBD. **(Gut Liver 2023;17:505-515)**

Key Words: Inflammatory bowel diseases; Microbiome; Mycobiome; Crohn disease; Ulcerative colitis

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic systemic condition that encompasses both ulcerative colitis (UC) and Crohn's disease (CD). The etiopathogenesis of IBD is still unknown, but evidence suggests that altered immune responses to gut microbiota perpetuate intestinal inflammation in susceptible individuals.[1-3]

The gut microbiota is an ecosystem consisting of bacteria, virus, fungi, and archaea. Gut microbes interact with the host immune system and help maintaining epithelial integrity and homeostasis. However, alterations in the microbiome may also contribute to chronic intestinal inflammation when alterations of the gut barrier occur, such as in IBD.[4,5] Alterations in the composition of the microbiota (dysbiosis) have been demonstrated both in UC and CD.[6] This has led to an active a search for microbiota-based therapeutic interventions in order to restore balance and to control gut inflammation.[7,8]

Although most of the microbiome studies have focused on the bacterial component, there is increasing evidence of the relevance of viral and fungal dysbiosis in the pathogenesis of IBD.[9-11] Interestingly, gut fungi and bacteria and their metabolites form complex interactions as has been demonstrated by the effect of altering bacterial abundance and diversity on fungal overgrowth.[12,13] Moreover, the presence of disease-specific bacterial-fungal alterations have been repeatedly shown in patients with IBD, supporting a role for fungi in disease pathogenesis and opening the possibility of modulating fungal microbiota as a therapeutic approach.[14,15] In addition, fungal dysbiosis has also been demonstrated in other diseases affecting the gastrointestinal tract, such as in irritable bowel syndrome, *Clostridioides difficile* infection, colorectal cancer, and cirrhosis.[16-19]

Fungi represent only a small fraction of the total human gut microbiome (0.1%) with most of the gut fungal species being unculturable.[20,21] Nevertheless, the implementation of high-throughput sequencing methods in recent years

has allowed for a much more extensive characterization of fungal gut microbiota and the association between fungal dysbiosis and intestinal inflammation.[22,23]

Several studies have shown alterations in the fecal fungal microbiota composition in IBD, with major variations in the *Basidiomycota/Ascomycota* ratio, a decrease in *Saccharomyces*, and an increase in *Candida*.[14,15,24,25] Also, the use of fungal probiotics–such as *Saccharomyces boulardii*–improves the efficacy of conventional treatments and prolongs clinical remission in CD; and certain anti-fungal drugs, like fluconazole, are effective in UC patients with high *Candida* colonization.[26,27]

The mycobiome can also influence the outcomes of therapy in IBD. Recent studies have demonstrated how the abundance of *Candida* may affect the results of fecal microbiota transplantation (FMT) in UC patients; as well as how *Candida* concentrations influence clinical response to infliximab, highlighting the relevance of the fungal microbiota in IBD.[28,29]

Despite the increasing armamentarium to treat IBD in recent years, a third of all patients are primary non-responders to initial treatment, with clinical response declining over time.[30] Also, IBD is a very heterogeneous disease having different phenotypes, often presenting additional extra-intestinal manifestations (arthralgia, skin, ocular manifestations, etc.) complicating therapeutic decisions.[3] Thus, we are in need of better individualization tools to help in the selection of appropriate therapies over time, thereby improving effectiveness and avoiding unnecessary side effects.[31] In this regard, precision medicine can for implementing and integrating the study of different levels of biological information (molecular medicine, genomics, proteomics, immunomics, etc.) in clinical practice.[32,33]

Several studies have shown variations in microbiota composition and disease features, including activity, phenotypes, response to treatment, and outcomes.[34] However, most of these studies have focused on bacteria. The growing evidence on the role played by fungal dysbiosis in the clinical course of IBD suggests a potential application of mycobiome studies in clinical practice, and eventually a future role in fungal-targeted therapies. In this review, we aim to provide a critical assessment of the evidence of the use of fecal fungal microbiota studies as a tool for precision medicine in IBD and we also suggest further possible strategies for its implementation in clinical practice in the near future.

WHAT IS A NORMAL FECAL MYCOBIOME

Although hindered by the lack of annotations of more than 99% of the existing fungi at the NCBI GenBank database,[35] fungi sequences can account for between 0.01% to 0.1% of the nucleic acid sequences detected by shotgun metagenomics on human microbiome samples.[20,36-38] While the percentage of fungi present in the microbiome is small, their influence in the host might be substantial since their metabolic activities are unique in that they are not likely to be present in procaryotes.[38]

The fecal mycobiome changes with age due to externals factors such as lifestyle,[39,40] nutrition,[41] and the external acquisition of new microorganisms.[42] In addition, the fecal mycobiome shows its highest diversity at the extremes of a human's life existence, at birth and during old age,[42] with these changes highlighting the tight interplay with gut bacteria.[13]

The core mycobiome is dominated by *Ascomycota* and *Basidiomycota*, which are the most abundant phyla in the mucosal samples of healthy human individuals.[43] The classes *Saccharomycetes* and *Tremellomycetes* are the dominant ones in the phyla Ascomycota and Basidiomycota respectively. Moreover, these two classes can be subdivided into ten genera ranked in decreasing abundance: *Candida* (particularly *Candida albicans*), *Saccharomyces* (in particular *Saccharomyces cerevisiae*), *Penicillium*, *Aspergillus*, *Cryptococcus*, *Malassezia* (in particular *Malassezia restricta*), *Cladosporium*, *Galactomyces*, *Debaryomyces,* and *Trichosporon*.[44] In addition to these genera, another 240 fungal genera have been reported but these may be considered environmental or dietary transients.[45-47] In fact, some of these species could be considered symbionts and others transient depending on their stability within the gut environment.[47] Also, the number of fungal species found increase from the ileum to the colon[48] and the presence of each species is associated with the particular architectural niche that they are occupying.[49] For these reasons, it has been proposed that healthy humans might have a "core" mycobiome that could also include a variable and broadly diverse population of less-represented fungi.

METHODS FOR FECAL MYCOBIOME ANALYSIS: CHOICE OF METHODS AFFECTS THE OUTCOME

Our current understanding of fecal microbiota is shaped by methodological strengths and weaknesses. There are many methodological considerations that can affect the results from fecal mycobiome analyses, with there being no consensus as to which methods are the best ones. This is true for everything from sampling strategies through sequencing methodology, to sequence analysis

and interpretation. Thus, special caution should be taken when conducting and interpreting analyses, to avoid overlooking methodological variations potentially skewing the results. For example, studies have shown that as little as a 48-hour storage of fecal samples under different conditions can introduce a considerable amount of variability in the downstream microbial analysis, albeit not always enough to overshadow intra-individual differences.[50] There have also been significant differences in detected fungal microbiome depending on whether the fecal samples were homogenized or if separate aliquots or taken from the same sample.[51] Finally, the choice of DNA isolation method can also affect results significantly, underlining the need for rigorous testing and standardization when used in a clinical setting.[52]

Almost all studies on fecal mycobiome use amplicon sequencing to identify strains, depending on primers targeting the internal transcribed spacer (ITS) within the ribosomal gene region of the fungal genome.[53] ITS amplicon sequencing relies on targeted amplification and sequencing of a small part of the microbial genome, with subsequent alignment to sequences with a known identity. Study protocols typically choose one of several available primer pairs for the amplification, which can be problematic as interpretation is dependent on which primers are used, and both the strain identification and diversity estimates might differ.[54] ITS amplicon sequencing is also dependent on the databases used for alignment and can only be interpreted when the detected sequence is already known.[55]

Alternatively, microbiome shotgun sequencing can be used, allowing for a presumably unbiased characterization of the whole mycobiome independent of amplicons.[56] Shotgun sequencing enables taxonomical characterization, while at the same time allowing identification of the functional genomic clusters and pathways present in the sample independent of species identification. Shotgun metagenomics is slowly becoming the standard in analyses of prokaryotes,[57] but is also gaining use in eukaryote analyses as suitable bioinformatics tools become available.[58,59] As the number of fungal reference genomes increase, and as methods become more sensitive, shotgun sequencing will become the most powerful option for the characterization of fungal microbiota.

In most studies of IBD fecal microbiota, the main goal has been to identify changes in fungal composition either associated with disease state, progression or treatment response. However, the bioinformatic and statistical tools used for these analyses have differed greatly between studies, and as of yet there is no established consensus concerning the methodology. In previous times, the methods used were typically developed with whole genome gene expression analyses in mind. Metagenome data differ from whole genome gene expression in that the results table is usually sparser, with many species not been detected above threshold within each sample. While this could be solved by filtering raw data, there is currently wide agreement that rarefication of data should be avoided when performing abundance analyses.[60] As studies have shown that the choice of bioinformatic and statistical methods have a great impact on interpretation, future work on the mycobiome should ideally consider results from many different algorithms before attempting any interpretation.[61] Ultimately, it is important that fecal mycobiome studies draw on the experiences gained from bacterial microbiome analyses, and work to reach a best practice consensus when it comes to sampling and analysis.[62] Table 1 summarizes some of the considerations that should be made when conducting and/or interpreting analyses of fecal fungal microbiota.

Table 1. Considerations to Be Made When Planning and Interpreting Studies of Fecal Fungal Microbiota

Step	Consideration
Sample collection	· Are all samples collected similarly? · Is the sample a selection or a whole sample homogenate?
Sample storage	· Is proper and similar sample storage ensured throughout the whole collection process?
Sample processing	· Are all samples processed equally? · Is proper randomization across sample groups ensured?
DNA isolation	· Does the DNA isolation method produce broad and unbiased recovery of good quality DNA from all fungal species represented in the sample? · Are there differences in methods used for subgroups of samples in the study?
Sequencing methodolgy	· Is the same sequencing strategy used for all samples? · If amplicon-based; does the chosen amplicons produce an unbiased capture of all available species? · If shotgun sequencing is used; how are the species identified? · Does the analysis take into consideration biases, strengths and weaknesses of the methods chosen?
Data analysis	· Does the chosen method filter data sets prior to abundance analyses? Are the methods chosen suitable for metagenome data sets?

CHANGES OF THE MYCOBIOME IN IBD AND WITHIN PHENOTYPES

There is increasing evidence supporting a role of the intestinal mycobiome in the pathogenesis of IBD. Fungi can contribute directly to intestinal inflammation via the innate immune receptor, Dectin-1, which recognizes β-1,3-glucans in the fungal cell, thereby activating intracellular signaling through the caspase recruitment domain-containing protein 9 (CARD9) and eliciting a Th1 and Th17 response in the host.[63-65] A recent study has shown that *Debaryomyces hansenii*, a fungus abundant in IBD patients, may impair mucosal healing via the myeloid cell-specific type 1 interferon-CCL5 axis in CD.[66] Interestingly, a polymorphism in the gene for Dectin-1 (CLEC7A) was strongly linked to a severe form of the human UC[25,64] and CARD9 variants are associated with IBD.[67,68]

Elevated levels of anti-*S. cervisae* antibodies (ASCA) is a well-established biomarker for CD and ASCA positivity may predict the development of CD years prior to diagnosis, correlates with disease activity and is found more commonly in healthy relatives of patients with CD.[69-71]

Numerous studies have analyzed the mycobiota in fecal samples or colonic biopsies from CD patients while only few of these have focused on UC.[9] An alteration in the richness and diversity of intestinal mycobiota has been observed in IBD.[14,15,25,72] Most studies show an increase of the *Basidiomycota* phyla and a decrease of the *Ascomycota* phyla.[15,25] In addition, an increase in *Candida* (mainly *C. albicans*) is associated with gut mycobiota dysbiosis in IBD.[44] Table 2 summarizes the main mycobiome studies in patients with IBD.

Candida is the main genera identified in several studies in fecal or mucosal biopsy samples from patients with IBD. *C. albicans* and *Candida parapsilosis* have been uniformly found to be increased in fecal samples from CD patients, while changes in *Candida tropicalis* vary among different studies.[9,29] Recently, an increased abundance of *Candida* was observed in both CD and UC patients who did not respond to infliximab treatment.[29] Interestingly, a higher percentage of *C. albicans*-reactive T cells was observed in blood samples from CD patients compared to healthy controls.[73] This implies that intestinal *C. albicans* might promote inflammation by inducing the development of Th17-reactive cells.

An increase in *Malassezia* sequences has been observed in CD patients.[68] In addition, Limon *et al.*[68] showed that the presence of *M. restricta* was associated with the S12N mutation in CARD9, a genetic alteration linked to the development of both CD and UC. In contrast, Nelson *et al.*[74] did

Table 2. Main Studies of the Fecal Mycobiome in IBD

Author (year)	IBD type	Type of samples	Main alterations
Ott *et al.* (2008)[72]	CD/UC	Mucosa samples	More fungal richness/diversity in CD. A non-significant difference was observed in UC patients.
Li *et al.* (2014)[24]	CD	Mucosa and fecal samples	Increased fecal fungal diversity in CD and increased richness and diversity in inflamed mucosa vs non-inflamed mucosa.
Mukhopadhya *et al.* (2015)[80]	Pediatric IBD	Mucosa samples	*Basidio* predominance in pediatric IBD patients.
Chehoud *et al.* (2015)[25]	Pediatric IBD	Fecal samples	*Candida* was significantly more abundant in IBD patients compared to controls.
Liguori *et al.* (2016)[43]	CD	Surgical mucosa samples	*Candida glabrata* was overrepresented in CD, and in particular in flare.
Sokol *et al.* (2017)[15]	CD	Fecal samples	Reduced diversity in IBD and increased *Candida* spp. abundance in CD patients compared to healthy controls.
Lewis *et al.* (2017)[76]	Pediatric CD	Fecal samples	Five yeasts were positively associated with CD, in particular in the context of greater bacterial dysbiosis.
Imai *et al.* (2019)[77]	CD/UC	Fecal samples	Composition of the fungal microbiome of a Japanese population was considerably different from that of a Western population. *Candida* was significantly higher in CD patients than in healthy controls and UC patients.
El Mouzan *et al.* (2018)[81]	Pediatric IBD	Mucosa and fecal samples	Better prediction obtained for the diagnosis of pediatric CD using stool samples.
Lemoinne *et al.* (2020)[75]	PSC/IBD patients	Fecal samples	PSC/IBD patients presented a higher fungal diversity compared to patients with only IBD diagnosis. PSC was associated with an alteration of the bacterial-fungi interaction.
Qiu *et al.* (2020)[78]	CD	Fecal samples	*Candida* and *Aspergillus* were the two most abundant genera identified in the gut. Correlation between major bacteria and fungi.
Nelson *et al.* (2021)[74]	CD	Fecal samples	No influence of NOD2 variants on mycobiota in CD patients on remission.
Zeng *et al.* (2022)[79]	CD	Fecal samples	Phenotype and activity in CD patients. *Candida* was significantly higher in abundance in the non-B1 group.

IBD, Inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; PSC, primary sclerosing cholangitis.

not confirm an increase in *Malassezia* in CD patients. In this study, the influence of NOD2 variants was evaluated in CD adult patients in remission. No significant differences in relation to abundance of Ascomycota or Basidiomycota or genera was observed.[74] The Basidiomycota/Ascomycota abundance ratio was not found to be significantly different according to NOD2 variants.[74]

There are variations in the proportion of *Saccharomyces* species reported in different studies. *S. cerevisiae* was observed to be decreased in the stool of IBD patients, especially in those with an active inflammation.[15,43]

There is little information available about how the mycobiome correlates with the presence of extraintestinal manifestations. Lemoinne *et al.*[75] found that patients with primary sclerosing cholangitis (PSC) displayed a significantly higher fungal diversity and fungal dysbiosis distinct from that of patients with IBD. Also, PSC was associated with increases in *Exophiala* genus and *Sordariomycetes* class and a decrease in the *Saccharomycetales* order, *Saccharomycetes* class, *Saccharomycetaceae* family and *S. cerevisiae* species. However, the authors failed to observe a strong association between the IBD activity and fungi taxa, probably due to the absence of patients with active IBD in this study.[75]

The fecal mycobiome has also been studied in pediatric populations. Chehoud *et al.*[25] reported a higher prevalence of *Candida* in IBD while *Cladosporium* was more frequently found in healthy controls. In a another prospective study, Lewis *et al.*[76] found that *S. cerevisiae*, *Clavispora lusitaniae*, *Cyberlindnera jadinii*, *C. albicans*, and *Kluyveromyces marxianus* were positively associated with CD, particularly in the setting of greater bacterial dysbiosis.

The correlation between fungal and bacterial microbiota composition has been analyzed in some studies. Imai *et al.*[77] described that *Candida* was negatively correlated with the genus *Citrobacter* in UC patients, but was positively correlated with the genera *Lactococcus* and *Veillonella* in CD patients. Also, in CD patients, the genus *Faecalibacterium* was negatively correlated with the order *Eurotiales*, whereas the genus *Enterococcus* was positively correlated with the genus *Malassezia*. Additionally, CD patients presented a positive correlation between the genus *Ruminococcus* and *Sarocladium* and *Ustilago*.[77]

A recent study including 25 CD patients from China observed a negative correlation between the abundance of *Bifidobacterium* and *Candida* and a positive correlation between *Roseburia* and *Ruminococcus* as well as *Fusicatenibacter*.[78] Interestingly, in a previous study fungi-to-bacteria diversity ratio was observed to be significantly higher in patients with PSC and IBD than in those with only IBD.[75]

There is few information available on IBD phenotype and mycobiota. Qiu *et al.*[78] evaluated recently the association of mycobiota and CD phenotype. In terms of disease behavior (B Montreal classification), no significant differences in alfa or beta diversity or at phylum level were observed between inflammatory (B1) with non-B1. However, although *Candida* was significantly enriched in non-B1-type CD compared to B1-type CD no significant difference was observed at species level (*C. albicans*, *Candida glabrata* or *C. tropicalis*) between groups.[79] Interestingly, patients with strictures (B2) showed a marked increase in *C. albicans* compared with patients with inflammatory phenotype (B1) in Norwegian IBD patients.[82]

A recent study in Norwegian IBD patients found relevant differences in the mycobiome associated with the location of CD (L).[82] Interestingly, patients with pure ileal forms (L1) showed a depletion in *C. tropicalis* and a significant increase in *D. hansenii* compared with colonic and ileocecal forms (L1/L3).[82] A recent study demonstrated that *D. hansenii* is enriched in inflamed ileal biopsies of patients with CD and impairs mucosal healing through the myeloid cell–specific type 1 interferon–CCL5 axis.[66] Interestingly, Sokol *et al.*[15] reported a significant decrease diversity in CD patients without ileal involvement suggesting the possibility that key ileal functions like producing antimicrobial peptides and absorbing bile acids could affect fungal diversity.

Perianal disease is associated with a decrease in quality of life and bad outcomes in CD patients. Zeng *et al.*[79] compared the mycobiota of CD patients regarding the presence or absence of perianal lesions. There were no significant differences at the phylum level between the two groups and the proportion of *Candida* was also similar. Interestingly, several fungi genres were increase in patients with perianal disease (*Clonostachys*, *Humicola*, *Lophiostoma*, *Fusarium*, *Lecanicillium*, and *Gibberella)* while *Saccharomyces* and *Dipodascus* were enriched in CD patients without perianal lesions.[79] The same study showed that *Candida* was significantly enriched in type non-B1 CD, suggesting that *Candida* may be potentially associated with stenosis and penetrating lesions in CD.[79]

There is insufficient information about the variations of the mycobiota in relation to the extension of disease in UC. Catalàn-Serra *et al.*[82] found a much higher abundance of *Penicillium* in patients with proctitis (Montreal E1), compared to left-side colitis and extensive colitis (Montreal E2 and E3). Furthermore, *Penicillium* genus was almost depleted in patients with total colitis (E3) and a significant decrease in *Candida sake* and *Debaryomyces microsporus* was observed in left-side colitis compared to extensive colitis.[82]

More studies are still needed in order to assess the differential composition of the mycobiota according to the disease extension in patients with UC or the behavior in patients with CD (inflammatory vs stenosing-perforating). In addition, more information is needed on perianal involvement, one of the most complex therapeutic challenges in patients with CD.

CHANGES OF THE MYCOBIOME WITH THE ACTIVITY OF THE DISEASE

A few published studies have addressed the association between fecal mycobiome composition and disease activity in order to try to understand the potential pathogenic role of fungal dysbiosis in IBD.

Sokol *et al.*[15] found a decreased fungal diversity and a higher *Basidiomycota*/*Ascomycota* ratio in flares. Such a difference was not detected between controls and inactive IBD patients.[15,77] A decrease in *Penicillium* was observed more marked in active IBD, especially in CD patients.[15,78] However, other studies found no differences in *Penicillium* abundance between IBD patients and controls.[74] Some studies have also reported an increase in the global fungal load in active CD.[43]

Candida abundance has been associated with active disease in several studies. Qiu *et al.*[78] observed that *Candida* was overrepresented in active CD patients compared with controls, whereas no difference was found between CD patients in remission and healthy controls.

Active IBD patients have also shown an increase in three different *Candida* species (*C. dubliniensis*, *C. lusitaniae* and *C. sake*) and *Galactomyces candidus*; while *Saccharomyces pastorianus* and *Saccharomyces bayanus* were depleted.[82] Furthermore, CD active patients have an increased abundance of *C. sake* and a very marked depletion of *S. pastorianus* compared with CD in remission.[82]

A potential protective anti-inflammatory effect of *Saccharomyces* was also suggested in previous studies where fecal *S. cerevisiae* was decreased in IBD patients and patients in flare, and increased in IBD remission,[15] moreover, Zeng *et al.*[79] have recently reported in a study of mucosal biopsies that *C. albicans* and *C. tropicalis* were more abundant in biopsies from inflamed regions whereas, in contrast, *S. cerevisiae* and *S. castelli* were less abundant in the inflamed mucosa.

A recent publication compared fecal fungi between CD patients in flare and in remission.[79] The phylum Ascomycota dominated fungi in both groups and no significant differences were found in *Candida*. Of note, *Exophiala* and *Saccharomyces* were enriched in the CD-flare group, and Aspergillus and Clonostachys were enriched in the CD-remission group. Specifically, *Exophiala dermatitidis* was enriched in CD patients in flare and it highly associated with clinical activity and laboratory inflammatory markers of CD suggesting a pathogenic role.[79]

The correlation between intestinal fungi with clinical and laboratory markers of activity has been evaluated in IBD. Li *et al.*[24] found a significantly positive correlation between the diversity indices and serum C-reactive protein concentrations in active CD patients. Also, the species richness showed positive correlation with Crohn's Disease Activity Index (CDAI) in CD patients and the diversity indices were also correlated positively with CDAI.[24]

A recent study found that a positive correlation between *Nigrospora* and erythrocyte sedimentation rate.[79] On the other hand, *Verticillium* correlated negatively with erythrocyte sedimentation rate and C-reactive protein. At the species level, *C. albicans*, *Verticillium dahliae*, *Wallemia canadensis*, *Aspergillus penicillioides* and *Nigrospora oryzae* correlated with laboratory inflammation markers.[79] *E. dermatitidis* correlated positively with CDAI and laboratory activity index, demonstrating that it may be closely related to CD activity.[79]

Less is known about the mycobiome and disease activity in UC. Catalàn-Serra *et al.*[80] found a significant increase in *Penicillium kluyveri* and *G. candidus* in active UC patients compared with patients in remission. On the other hand, *C. dubliniensis*, *S. pastorianus,* and *Penicillium sclerotigenum* were less frequent in activity.

In pediatric CD population, fungal dysbiosis after diet-based therapy was analyzed in a longitudinal study at baseline and 1 week into therapy. *Candida*, *Clavispora*, and *Cyberlindnera* were all reduced in abundance after 1 week of therapy. Similarly, abundance of *Clavispora, Cyberlindnera*, and *Kluyveromyces* were significantly reduced from baseline to week 8 only in the exclusive enteral nutrition-treated group but not in anti-tumor necrosis factor-treated patients.[76]

CAN FUNGAL SIGNATURES PREDICT OUTCOMES IN IBD?

Dysbiosis can alter gut homeostasis leading to an impairment in intestinal barrier integrity and a pro-inflammatory immune response.[6] Although the use of microbial signatures to predict outcomes has not yet reached the clinical practice yet, several lines of evidence support an association of certain bacteria with the prognosis of IBD patients. For example, a deficiency in *Faecalibacterium prausnitzii* in CD patients undergoing surgery has been

associated with a higher risk of recurrence,[83] whereas the presence of adherent and invasive *Escherichia coli* is predictive of postoperative recurrence.[84]

It is still not known whether fungal microbiota signatures can be used as a predictive tool in clinical practice remains unknown. Few studies have follow-up cohorts to study the association of mycobiome composition and IBD outcomes, and there is a lack of well-designed longitudinal prospective studies.

A recent study that analyzed the fecal mycobiome of Norwegian IBD patients and controls showed a clear association between some fungal species and complicated disease or risk of surgery.[82] Of the total of 89 IBD patients included in this study, 40 of these were followed clinically for a period of 6 years after the sample collection. Patients with complicated diseases (defined as the need for intensified medical treatment) had significantly more *Clavispora* and less *Phaeococcomyces* and *Penicillium* than those with an uncomplicated course. At species level, a complicated course was also associated with more abundant *C. sake* and *Galactomyces pseudocandidus* but with a reduction in several *Penicillium* species.

Moreover, *Candida carnescens* was significantly overrepresented in CD patients who needed surgery during follow up while *C. tropicalis*, *Debaryomyces nepalensis,* and *D. hansenii* were depleted. Studies aiming to replicate these findings in an independent prospective cohort of IBD patients are currently underway.

Interestingly, the fungal composition was also shown to influence the response to treatment in IBD. Leonardi *et al.*[28] demonstrated that increased abundance of *Candida* was associated with the clinical response and an increased bacterial diversity in UC treated with FMT. In turn, a subsequent decrease in *Candida* post-FMT was indicative of ameliorated disease activity, pointing to the relevance of fungi as a predictor of response when considering FMT treatment in UC.[28]

A recent Finnish study showed that a high abundance of *Candida* was associated with a poor response to infliximab in a 1-year longitudinal study highlighting the potential role of the mycobiome as biomarker.[29]

CONCLUSIONS AND FUTURE DIRECTIONS

Fungi are an indispensable component of the human gut microbiome and perform a key role in modulating the immune response and in the development of chronic intestinal inflammation. Fungal microbiome dysbiosis is a common feature in IBD and several lines of research have highlighted the potential role of the mycobiome in the pathogenesis of the disease.

The introduction of fecal sequencing-based analysis in recent years has increased our understanding of the normal and pathological fungal composition, which has allowed its association with the different phenotypes, disease activity and prognosis in IBD to be investigated, thereby identifying the use of the mycobiome as a potential precision medicine tool.

Growing evidence has shown that fecal mycobiome dysbiosis occurring in IBD, as well as variations being found in the different phenotypes and in the fungal composition with disease activity. In addition, some studies have revealed an association between fungi composition and disease outcomes, as well as deleterious effect on intestinal inflammation been provoked by certain fungal species. Nevertheless, the majority of the studies only demonstrate an association–rather than causation–and very few of these have been validated externally or in prospective studies. Thus, this review aims to summarize the current evidence and the deficiencies in mycobiome research which need to be resolved before it can be implemented in clinical practice.

Indeed, many questions remain unsolved. One of the most urgent aspects is to be able to characterize better what a normal gut mycobiome is. Although several species seem to be predominant, there are a great variation concerning this aspect in the literature. Specifically, two aspects need to be further addressed: (1) which fungi are resident, and which are transient and (2) what variations exist in the mycobiome composition regarding geographical and ethnical differences. In addition, it is important to obtain a better characterization of the effect of local or specific diets on the mycobiome, and related to this, also to have a better understanding of the use of diet as a potential therapeutic tool (exclusive enteral nutrition, CD exclusion diet, parenteral nutrition, etc.) in the fungal microbiome. Some microbiome-targeted interventions, such as specific diets with a focus on fungi (like the mycobiome diet),[85] micronutrient supplementation with zinc[86] or the use of some probiotics containing *S. boulardii*[87] have shown positive effects on the mycobiome, specifically by decreasing *Candida* spp. Nevertheless, more research is still necessary to understand therapeutic interventions and, in particular, mechanistic studies should be carried out on the effects of certain species/genera, including *Candida*, *Malassezia* or *Debaryomyces*. Several suggestions for further study on the role of the mycobiome in intestinal inflammation and IBD are summarized in Fig. 1.

Other aspects, for example, the variations in the gut mycobiome over time in the disease course and the effect of common medications (steroids, azathioprine, anti-tumor

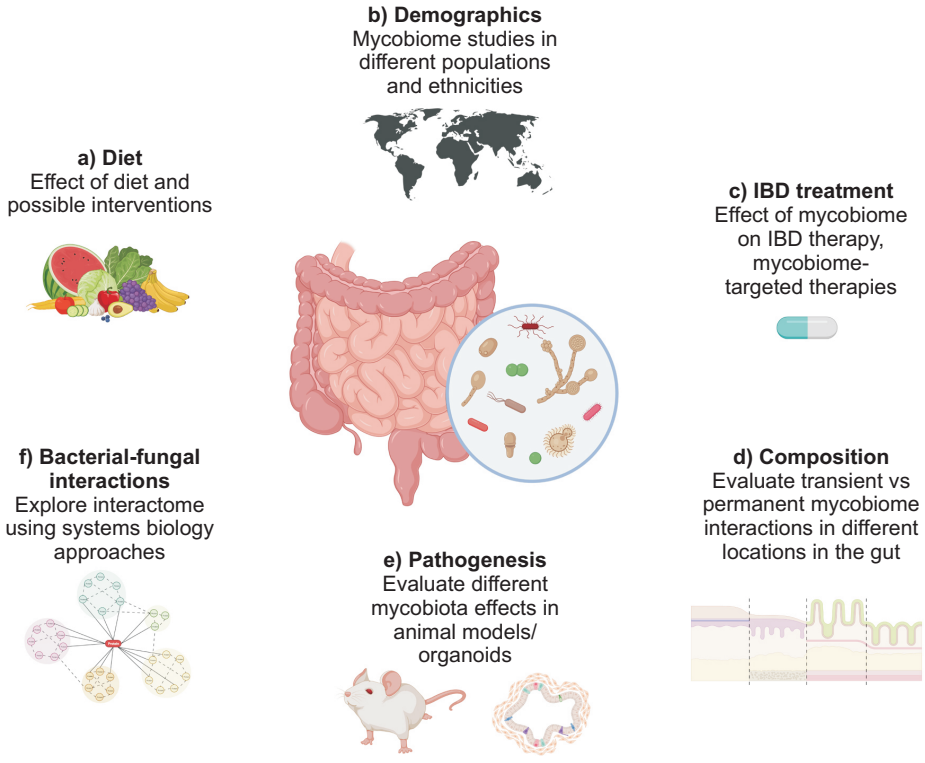

Fig. 1. Future studies on fungal microbiota to promote implementation in clinical practice.
IBD, inflammatory bowel disease.

necrosis factor) on its composition need to be established. Furthermore, there is increasing interest in exploring how new biologic therapies and small molecules impact the mycobiome, and if the mycobiome composition could be used as a biomarker for therapeutic response.

Recent studies linking the fungal composition (*Candida*) with response to treatment with FMT or infliximab should encourage a more dedicated effort to focus on fungi.[28,29] Additionally, prospective longitudinal cohorts including newly diagnosed patients naïve to therapy are currently underway for this purpose.[88]

Lastly, we need to obtain a better understanding of the interaction between gut fungi and bacteria better. Although the effects of antimicrobials such as antibiotics on fungal composition is well established, less is known about the effect of altering the mycobiome (with antifungals or specific diets/probiotics for example) on bacteria. An integrative analysis using systems biology approaches should enable a better understanding of the trans-kingdom network (bacteria, fungi, virus, and archaea) and how it influences the immune response in the human gut. Future coordinated efforts should now address these issues to permit these findings on the role of fungi in IBD to be incorporated into clinical practice.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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