Metabolism Open 14 (2022) 100178

Contents lists available at ScienceDirect

Metabolism Open

journal homepage: www.sciencedirect.com/journal/metabolism-open

Could gut mycobiome play a role in NAFLD pathogenesis? Insights and therapeutic perspectives

ARTICLE INFO

Keywords
Alcohol related liver disease
Amphotericin B
Candida
Fatty liver disease
Fibrosis
Fungi
Immunity
Liver inflammation
Microbiome
Mycobiome
Non-alcoholic fatty liver disease
Obesity
Steatohepatitis

ABSTRACT

The entire spectrum of nonalcoholic fatty liver disease (NAFLD) ranging from fatty liver to cirrhosis has been considered as the result of specific metabolic pathways and mediators, gut barrier function alterations and inflammatory responses. Previous studies have associated intestinal microbiota with NAFLD pathogenesis, focusing mostly on bacteria. In a recent study by Demir et al. in the *Journal of Hepatology*, researchers characterized the fecal mycobiome of patients with NAFLD and controls. NAFLD severity was linked to a specific fecal mycobiome signature, particularly in patients without obesity, highlighting previously undescribed aspects of the non obese phenotype of NAFLD. There has recently been a growing interest in the pathophysiology and progression of non obese NAFLD, as its actual incidence seems to be higher than previously described. Moreover, the authors demonstrated that in subjects with NAFLD and advanced fibrosis, there was an augmented systemic immune response to *Candida albicans*. Amphotericin B, which has been widely regarded as an antifungal with a good safety profile, low rate of resistance and high efficacy, has already been shown to prevent liver injury and steatosis in mice. Similarly in this study when germ-free mice colonized with feces from patients with NASH were fed with a Western diet, treatment with amphotericin B protected against steatohepatitis and liver fibrosis. In conclusion, this study has provided novel insights into the fecal mycobiome composition in advanced NAFLD especially in the non obese population while suggesting a role for antifungal therapy in the management of NAFLD.

Non-alcoholic fatty liver disease (NAFLD) has become the most common liver disease, affecting approximately 25% of the total adult population in Western countries. It is commonly associated with obesity and metabolic disorders such as dyslipidemia and insulin resistance, and it is considered as the hepatic manifestation of the metabolic syndrome [1]. The spectrum of NAFLD ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis and even hepatocellular carcinoma (HCC). NAFLD has become a significant cause of liver transplantation as approximately 20% of patients with NASH with advanced NAFLD fibrosis Score, F3 fibrosis or compensated cirrhosis will progress to cirrhosis or develop liver decompensation, respectively, over a 2-year period [2].

Although NAFLD was traditionally associated with obesity, there is considerable evidence that NAFLD could be prevalent in up to 10–20% of nonobese Caucasians and Americans, though this entity has been mainly studied in the Asian population [3].

A number of inherited and environmental factors increase the risk of NASH and influence its progression. Metabolic stress, inflammation and fibrosis have been identified as key pathogenic mechanisms. Alterations in the intestinal microbiome have already been linked to hepatic inflammatory signalling that promotes insulin resistance, hepatic steatosis and NASH [4]. Till now, microbiome research has predominantly focused on gut bacteria. However, the gut microbiota also contains, among archaea and viruses, commensal fungi, which are broadly referred to as the mycobiome. A search in Medline identifies over 1400 studies on "NAFLD and microbiome" as opposed to around 10 studies on associations between "NAFLD and mycobiome", indicating the recently emerging role of the mycobiome in the pathogenesis of NAFLD. The fungal community has been considered a minor component of the gut microbiota representing approximately less than 0.1% of the microbial community in the gut [1], while its role has been mainly studied in alcohol related liver disease [5]. Even though bacteria outnumber fungi in the gut, the biomass of fungal cells is important since they are almost 100-fold larger compared to bacteria. Unlike bacteria, fungi are eukaryotes with complex cell structures. They can use more complex biologic processes and produce metabolites that can remain in an organism and cause damage even after they have been eradicated. It is also noteworthy that fungal infections can be resistant to immune responses and difficult to eliminate especially in immunocompromised patients [1].

Interactions between intestinal fungal community and liver are anatomically and functionally bidirectional. Antigens derived from the gut commensal fungi can cross the gastrointestinal barrier and be transported via the portal vein to the liver, thus having an impact on its function. The immune cells in the liver can contribute to the host homeostasis through immune responses to the intestinal fungi [1]. Thus, it has become apparent that gut mycobiome has a profound influence in modulating local as well as peripheral immune responses. However, like bacteria, fungi can be beneficial to host immunity but they may also have deleterious effects in pathologic conditions [6].

https://doi.org/10.1016/j.metop.2022.100178
Received 11 March 2022; Accepted 13 March 2022
Available online 14 March 2022

Based on previous studies showing that associations between the gut bacterial microbiota composition and more severe liver disease are particularly observed in non-obese subjects [7], Demir et al. in their cross-sectional study analysed compositional changes in faecal mycobiome using Internal Transcribed Spacer (ITS)2 sequencing in subjects with NAFLD further dividing them according to obesity. Beta diversity (an estimate of dissimilarity between specimens) analysis captured more differences among non-obese subjects than obese subjects. In the non-obese group, patients with NAFLD at advanced stages (NASH or F2–F4 fibrosis) had different fecal mycobiome compositions than patients with NAFLD at earlier stages (NAFLD or F0–F1 fibrosis). When assessing alpha-diversity (estimate of similarity or dissimilarity within a sample), apart from a lower fungal richness in non-obese patients with NAFLD and more severe liver disease, the authors did not observe significant differences among groups [8]. Pathophysiological mechanisms leading to the lean phenotype of NAFLD are not yet understood but may include apart from a different pattern of gut microbiota, a dysfunctional adipose tissue, altered body composition, genetic differences and epigenetic changes [3].

In line with the observation of Lee et al. that altered fecal bacterial microbiome represents a risk factor for disease progression in non obese NAFLD patients [7], the authors hypothesize that compositional changes in the fungal microbiome could similarly represent a prominent pathogenetic factor of advanced NAFLD in this group of patients [8]. In order to investigate specific compositional changes between groups, Demir et al. performed differential multinomial regression analyses using the novel tool Songbird. Specific fungi including *Candida albicans* and *Mucor* spp. were associated with the presence of NASH whereas different taxa were associated with NAFLD. When determining log ratios for specific fungal taxa, they detected significantly higher log ratios for *Candida albicans/Saccharomyces cerevisiae*, unknown *Pleosporales/S. serevisae* and *Pichia barkeri/Saccharomyces cerevisiae* in non obese patients with NASH as compared τo non obese patients with NAFLD.

For non obese NAFLD patients with more severe fibrosis (F2–F4 stages), *Mucor* spp and *Candida albicans* were among others associated positively with F2–F4 fibrosis [8]. *Mucor* sp., which has been classified as an opportunistic pathogen in immunocompomised patients, particularly in hematologic patients under chemotherapy, and subjects with uncontrolled diabetes, was associated with high blood glucose levels in the study. Furthermore, *Candida albicans* has been extensively investigated while belonging to *Candida*, one of the most common genera in the gastrointestinal tract (together with *Saccharomyces* and *Cladosporium*).

In previous studies, *Candida albicans* has shown increased abundance in high-fat-diet-fed mice [9], being implicated in the exacerbation of experimental colitis in mice [6].

Recent data have highlighted that some commensal fungal species, especially *Candida albicans* and *Malassezia* spp., are potent inducers of antigen-specific T-helper cell responses in humans, being altered in inflammatory diseases [10,11]. This induction seems to occur during a homeostatic interaction with the host even in the absence of obvious inflammation. *Candida albicans* specific T-cell responses also broadly modulate human anti-fungal Th17 immunity via increasing Th17 cells cross-reactivity to other fungal species, such as *Aspergillus fumigatus*, that rather provokes a regulatory T-cell response in healthy humans [11]. Apart from the homeostatic interaction, *Candida* overgrowth is linked to several diseases, including inflammatory bowel disease (IBD), alcohol related liver disease, obesity, and liver cirrhosis [6]. Th-17 cell mediated responses provide protection against fungi via the production of interleukin (IL)-17 and IL-22. The IL-17 receptor is expressed in almost all types of liver cells, implying that the antifungal immune response could lead to liver damage [1]. Interestingly, patients with an impairment in Th17 immunity mainly suffer from mucocutaneous candidiasis [12].

In this regard, Demir et al. examined the *anti-C. albicans* IgG antibodies in plasma samples of NAFLD and controls, and found significantly higher IgG levels in NAFLD patients with advanced liver fibrosis. The authors assume that this probably indicates increased immune response to *Candida albicans* either due to the increased abundance of intestinal *C. albicans* or the relatively more frequent systemic exposure to *C. Albicans* [8].

With regards to obesity, previous studies have demonstrated a significant difference in the fungal composition between obese subjects and controls. Nonetheless, *Candida, Nakaseomyces*, and *Penicillium* were the most predominant genera identified in obese subjects, whereas *Mucor racemosus* and *M. fuscus* were the most represented in nonobese patients. Additionally, *Mucor* genus was relatively increased in obese subjects upon weight loss [13]. Unfortunately, it is uncertain yet what a healthy or normal gut mycobiome comprises, and whether these mycobiota changes are a cause or an effect of obesity. Since Demir's et al. study of the mycobiome has displayed different mycobiota compositions in obese and non obese NAFLD patients, the feature of obesity should probably be included in any type of analysis when interpreting compositional changes of mycobiome in subjects with NAFLD.

Inflammation is known to play a major role in the progression of NAFLD and NASH. Liver immune cells when exposed to fungal antigens and fungi derived metabolites elicit anti-inflammatory cytokines and chemokines, some of which can lead to liver damage [1]. The fungal cell wall polysaccharide β-glucan can induce chronic liver inflammation by continuously activating the cellular inflammasome pathway leading to hepatocyte damage [5]. Interestingly, Demir et al. demonstrated that alterations in the fecal fungal microbiota are more associated with inflammatory features than with steatosis or ballooning in the liver. Specifically, both *Mucor* sp./*S. Cerevisiae* and *Candida albicans/S. cerevisiae* log ratios were independently associated with higher inflammatory activity [8]. Liver inflammation is bidirectionally interrelated with hepatocyte injury whose healing process may lead to liver regeneration and fibrosis. However, on the other hand, there are studies showing that insufficiency in inflammatory signals may lead to a loss of the microbiota ability to maintain intestinal barrier functions [4].

Fungi and bacteria interact in many ways regarding growth, nutrition, reproduction and pathogenicity [14]. Alterations in the complex interconnections between fungi and bacteria (linkage patterns) have been observed in cirrhotics [15], while reductions in intestinal bacteria facilitate colonization with *Saccharomyces cerevisiae* or *Candida albicans* [16].

Most studies have been focusing on the interactions between *Candida* species and bacterial species demonstrating the existence of an interaction between *Candida* and *Enterococcus faecalis* or *Clostridium difficile* not necessarily with pathogenic implications. Both fungi and bacteria can benefit from their interaction via the formation of a mixed species biofilm that strengthens their virulence [6]. Demir et al. went one step further by using bacterial and fungal features to discriminate mild from advanced liver disease. Moreover, fungal features had an overall higher importance in non obese patients compared to the obese ones [8]. Hence, the mechanisms by which bacteria and fungi regulate each other in commensal communities are diverse and may offer targets for manipulating the microbiome.

Chronic alcohol consumption is a well-known factor of increased intestinal permeability, and changes in the intestinal microbiota composition may contribute to the development of alcohol related liver disease. Alcoholic liver disease has already been linked to a decrease in fungal diversity along with *Candida* overgrowth independent of stages of ALD [5]. In line with this study, Demir et al. have shown that NAFLD patients with advanced liver disease were mainly characterized by increased *Mucor* spp., whereas patients with advanced ALD fibrosis were characterized by increased *Blumeria, Candida* and *Debaryomyces* spp., indicating that specific changes in fecal mycobiome could be attributed to different liver disease etiologies [8]. Yang et al. have already suggested that the main pathogenic mechanism for mycobiota associated progression of liver disease may be an increase in intestinal fungal populations, suggesting that manipulation of the intestinal mycobiome could attenuate alcohol related liver disease [5].

Few studies have examined a potential fungal therapeutical

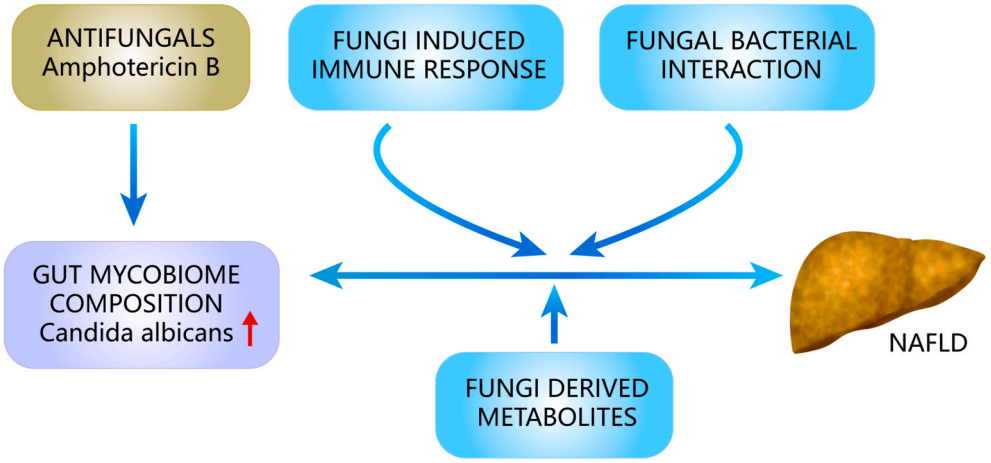

Fig. 1. A synoptic presentation of the mycobiome-liver interaction and its parameters that contribute to the development of NAFLD. Abbreviations: NAFLD, Non-Alcoholic Fatty Liver Disease.

approach for liver diseases. Given that oral amphotericin B does not present considerable systemic side effects, is well tolerated by patients and has the broadest spectrum of activity including *Candida albicans* and the lowest rate of resistance of all known antifungals, it remains a preferred choice for antifungal therapy [17]. Amphotericin has already been shown to prevent ethanol induced liver damage in mice [5], as mice receiving amphotericin B while on an ethanol diet did not develop intestinal fungal overgrowth and had lower levels of liver injury and steatosis. Demir et al. were the first to show that amphotericin B can prevent Western diet-induced steatohepatitis in fecal microbiome humanized mice. Importantly, the antifungal treatment did not induce any changes in the intestinal bacterial microbiota [8]. Although the authors have suggested strong therapeutic implications of amphotericin in NAFLD, it would rather be most important to define the specific subgroups of patients that would mostly benefit from antifungal therapy given the diversity of mycobiome in the heterogeneous human population.

In summary, Demir et al. provide novel insights into unknown aspects of gut mycobiome composition in NAFLD. Currently, the rising incidence of NAFLD with its different phenotypes has rendered imperative its better understanding and effective treatment [18–26]. There is agreement that a balanced gut mycobiome contributes to the maintenance of host immune homeostasis [27,28]. More large-scale, longer-term, longitudinal studies are required to determine whether changes that occur in the bacteriome and mycobiome are causal or consequent of NAFLD progression. There is also need to examine the dysfunctional adipose tissue and the role of adipocytokines and hepatokines in NAFLD etiopathogenesis [29–38]. Furthermore, specific metabolic pathways and mediators, an intact gut barrier and bacterial-fungal interactions may play an important role in this process, as summarized in Fig. 1 [27,28]. The majority of studies has focused on descriptive findings of the gut microbiome; however, joint analyses on gut mycobiome with other advanced tools, such as metagenomics and metabolomics, are needed to provide new perspectives into the pathophysiology of NAFLD [1].

Conflict of interest

None.

Funding information

None.

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Dimitrios Fotis*
Gastroenterology Department, General Hospital of Trikala, 56 Karditsis, 42100, Trikala, Greece

Junli Liu
Shanghai Jiao Tong University School of Medicine, Shanghai Jiao Tong University Affiliated 6th People's Hospital, Shanghai Diabetes Institute, Shanghai, China
E-mail address: liujunli@sjtu.edu.cn.

Maria Dalamaga**
Department of Biological Chemistry, Medical School, National and Kapodistrian University of Athens, 75 Mikras Asias, Goudi, 11527, Athens, Greece

* Corresponding author.

** Corresponding author. National and Kapodistrian University of Athens, Medical School, Greece.
E-mail address: dimitriosfotis@gmail.com (D. Fotis).
E-mail address: madalamaga@med.uoa.gr (M. Dalamaga).