



Gut microbiota dysbiosis in non-alcoholic fatty liver disease

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Introduction

Non-alcoholic fatty liver disease (NAFLD) represents the spectrum of fatty liver that ranges from simple steatosis to steatohepatitis leading to fibrosis and cirrhosis. NAFLD is often considered the hepatic manifestation of metabolic syndrome and it is closely associated with diabetes, insulin resistance and obesity. Prevalence of NAFLD ranges from 10–35% and the differences in prevalence are due to differences in diagnostic methods used to diagnose NAFLD, as well as differences in NAFLD prevalence amongst various ethnic groups. However, the progression of non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH) is seen in 3–5% of the population, and this subgroup is at a high risk of progression to advanced fibrosis including cirrhosis and hepatocellular carcinoma (HCC) (1,2). There is increasing evidence that gut microbiota plays a role in the development of numerous diseases, and thus gut dysbiosis has been implicated in obesity (3,4) cardiovascular disease (5), diabetes (6,7) and metabolic syndromes (8,9) including non-alcoholic fatty liver and NASH (10-12). The study by Boursier *et al.* (13) attempts to link gut dysbiosis with severity of fatty liver disease, finding certain microbial subgroups associated with different stages of NAFLD including NASH and level of fibrosis.

The authors evaluated 57 NAFLD patients, 30 of whom had F0-F1 liver fibrosis at liver biopsy (10 with NASH) and 27 patients with fibrosis stage F >2 (25 with NASH) at liver biopsy. The liver biopsy was assessed by a blinded liver pathologist from NASH Clinical Research Network. The stool samples were collected at the time of liver biopsy and sequenced and analyzed for classification of gut microbiome

using 16S ribosomal RNA gene sequencing. Patients were stratified into three groups: NAFLD without NASH with F0/F1 fibrosis, NASH with F0/F1 fibrosis and NAFLD with (25 patients) or without (2 patients) NASH with F >2 liver fibrosis. There were 12 bacteria phylum, 65 family types and 133 genera in the stool samples; at the phylum level of classification, there were no differences in the gut microbiome between the NAFLD and NASH groups, but at the genus level there was a statistically significant increase in *Bacteroides* and lower abundance of *Prevotella* (*Figure 1*), two species which are in an inverse relationship when patients progressed from NAFL to NASH, even when adjusting for metabolic factors such as metabolic syndrome, BMI, diabetes, lipids and hypertension. Furthermore, Boursier *et al.* found a significant difference between the gut microbial of patients with F0/F1 fibrosis compared to those with F ≥2 fibrosis, with respect to three genera: *Bacteroides*, *Prevotella* and *Ruminococcus*. Those with F ≥2 fibrosis had higher abundances of *Ruminococcus* and *Bacteroides* and lower amounts of *Prevotella*. In addition, *Ruminococcus* counts were independently associated with fibrosis score ≥2. The author's concluded that analysis of gut microbial is predictive of NAFLD severity, and may be involved in the pathogenesis of NAFLD including progression to NASH and advanced fibrosis.

Previous studies based on human microbiota also demonstrated the association between gut microbiota and different NAFLD phenotypes, ranging from simple steatosis to NASH. However, there were several differences in the results, and the level of taxonomy examined (e.g., phylum v. genus) often varied (*Figure 1*). In studies by Mouzaki

Kingdom	Bacteria			
Phylum	Bacteroidetes	Bacteroidetes	Firmicutes	Firmicutes
Class	Bacteroidetes	Bacteroidetes	Clostridia	Clostridia
Order	Bacteroidales	Bacteroidales	Clostridiales	Clostridiales
Family	Bacteroidaceae	Prevotellaceae	Ruminococcaceae	Clostridiaceae
Genus	Bacteroides	Prevotella	Ruminococcus	Clostridium

Figure 1 Gut microbial taxonomical classification.

et al. (14), Zhu *et al.* (15), and Wong *et al.* (16), biopsy proven NASH patients had stool examined for dysbiosis of gut microbiota.

Mouzaki *et al.* (14) compared the gut microbiota of 50 patients with NAFL (simple steatosis, n=11), NASH (n=22) and healthy controls (n=17) established through liver biopsy. Differences in age, gender and BMI were present in the groups at baseline, and degree of liver fibrosis was not stated. This study found higher fecal *Clostridium cocoides* levels in patients with NASH compared to simple steatosis, though this difference disappeared when controlled for BMI and recent fat intake. Percentage of the class Bacteroidetes (containing both *Bacteroides* and *Prevotella*) was significantly lower in patients with NASH compared to both simple steatosis and health controls, even when controlling for BMI and fat intake. This was one of the first studies to link gut dysbiosis and NAFLD, and evaluated gut microbial at the level of class, but not genus as was the case in the Boursier *et al.* article, which limits comparisons between these two studies. This study also did not control for other metabolic factors as was done in the Boursier study, which may have influenced the outcomes as well. Additionally, this study was also limited by small sample size, large differences in the comparison groups at baseline, and lack of racial diversity of the study participants.

Zhu *et al.* (15) evaluated and compared gut microbial composition in 63 children and adolescents (NASH *vs.* obese *vs.* normal control) defined as having NASH on liver biopsy (n=22), obese without NASH (BMI >95th percentile with normal liver enzymes, n=25), or healthy controls (BMI <85th percentile, n=16). Authors reported that the obese and NASH subjects had more similar microbiomes to each other than the healthy control group. Contrary to the Mouzaki *et al.* study, Zhu *et al.* found a higher number of *Bacteroides* and decrease in Firmicutes in the obese and NASH groups, compared to the healthy control group. *Prevotella* was markedly more abundant in the NASH and obese groups compared to the healthy group (Figure 1).

This is a contrast to the Boursier study which found *Prevotella* in lower numbers in the group which progressed to NASH or fibrosis.

Wong *et al.* (16) evaluated 16 biopsy proven NASH, and 22 healthy controls with normal liver function tests who had no history of liver disease, aged 24–69. The primary goal of this study was to evaluate the effect of probiotics on gut microbiota. However, baseline data were analyzed for the gut microbiota of participants and found that at the phylum level, Bacteroidetes was found to be most abundant type of microbe in both NASH and healthy controls, but Firmicutes abundance was increased in healthy patients compared to NASH at baseline, similar to results from Mouzaki *et al.* The order Aeromonadales, families *Succinivibrio* and *Porphyromonadaceae* and genera *Parabacteroides* and *Allisonella* were more abundant in NASH patients than healthy controls, and the order Clostridiales and genera *Faecalibacterium* and *Anaerospore* were less abundant in NASH patients. The limitations of this study are that no liver biopsy was done on “healthy controls”, they were assumed to be free of NAFLD because liver enzymes were normal. This study analyzed bacteria at many levels of the taxonomical classification (i.e., Phylum, class, order and genus) and this makes comparison of results with other studies difficult.

Conclusions

Overall, Boursier *et al.* did an excellent study correlating gut microbial composition with NAFLD spectrum severity but there are several limitations of this study. While the authors found the only limitation of this study to be sample size, this study is also limited by the composition of the sample which is predominately male, and age above 50. This study was also conducted amongst people of European descent, limiting applicability to persons of other racial origin. Furthermore, this study not only lacks healthy control patients without NAFLD, but also without age, sex, race

and co-morbid condition matches, which would present a more robust study. It also did not attempt to control for diet or medications [including probiotics, proton pump inhibitors (PPI), antibiotics] of the participants, which may influence gut microbiota composition. This study also does not consider the differences in gut microbiota amongst subjects of different ages, which has previously been characterized (17) or consider in more detail the different levels of advanced fibrosis above $F \geq 2$, as persons with cirrhosis has been seen to have a significantly different microbial profile than healthy controls (18,19). Though all of the studies cited found differences in gut microbiota based on NAFLD spectrum severity, the correlation of which bacteria is associated with NASH is contradictory or difficult to compare, as not all studies compare data at the same bacterial classification, and sequencing of gut microbiota was not standardized (20). The Boursier *et al.* study does not address these differences in results, and standardization of the approaches for characterizing the gut microbiota may be useful for application of study results in the future. Boursier *et al.*'s decision to characterize the differences in gut microbiota at the genus level should be commended, as more specific levels of categorization of gut microbiota are more useful than more general categorization (e.g., at the level of phylum) for understanding the role of gut microbiota in the pathogenesis of disease and studying interventions (i.e., pre and probiotics) for intervention in disease progression.

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Footnote

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to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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