The Intestinal Microbiome in Nonalcoholic Fatty Liver Disease

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KEYWORDS

• Nonalcoholic fatty liver disease • Nonalcoholic steatohepatitis • Cirrhosis
• Microbiome • 16S pyrosequencing • Microbiota • Intestinal permeability
• Innate immune system

KEY POINTS

- Nonalcoholic fatty liver disease is a clinicopathologic spectrum with aggressive phenotype nonalcoholic steatohepatitis (NASH) and rapidly rising cause of cirrhosis, liver cancer and liver transplant.
- NAFLD and NASH are commonly associated with obesity and other components of the metabolic syndrome, which are linked to the changes in the intestinal microbiome.
- Although few studies have demonstrated compositional changes intestinal microbiome in both pediatric and adult NAFLD, no consistent signature can be defined.
- Bacteroidetes and Firmicutes are major phyla altered in NAFLD in different studies, and Proteobacteria are among others that are increased in NAFLD.
- Predictive functional changes related to compositional intestinal microbial shifts reflect changes associated with carbohydrate, lipid and amino acid metabolism.

INTRODUCTION

There is growing realization that the human body is a "super" ecosystem where the host that is the body lives in harmony with a large number of microbes. Not only does the body live in harmony but also there is a symbiotic relationship between the

Grant Support: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, RO1 DK 105961) and National Institute on Alcohol Abuse and Alcoholism (NIAAA, K23 AA 021179).
Disclosure: Dr A.J. Sanyal is President of Sanyal Bio and has stock options in Genfit, Exhalenz, Tiziana, Akarna, Hemoshear, and Indalo. He serves on the following advisory boards with remuneration: Lilly, Pfizer, Allergan, Novartis, Ardelyx, Sanofi, and General Electric. He has served on the following advisory boards without remuneration: Merck, Bristol Myers Squibb, Intercept, Syntlogic, Fractyl, Galectin, Gilead, Nitto Denko, Zydus, Jannsen, Nordic BioSiences, and Immuron. He is a consultant to Salix, Cumberland Pharmaceuticals, Nordic Biosciences, Octeta, and Novo Nordisk. Dr P. Puri has nothing to disclose.
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Clin Liver Dis 22 (2018) 121–132
http://dx.doi.org/10.1016/j.cld.2017.08.009 **liver.theclinics.com**

microbes and the body, which is essential for good health. Recently developed tools have allowed the identification of bacteria not previously feasible due to the inability to grow a large number of bacteria in traditional culture media. This has led to new information on changes in the microbiome in various disease states and a growing number of diseases from developmental disorders and behavioral diseases to type 2 diabetes mellitus and autoimmune disorders have been shown to be associated with an altered microflora in the intestine.[1]

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in North America and is growing as a cause of chronic liver disease in many other parts of the world as well. It has 2 principal clinical-pathologic phenotypes: (1) nonalcoholic fatty liver, which is characterized by hepatic steatosis with or without minimal lobular inflammation, and (2) nonalcoholic steatohepatitis (NASH), which is characterized by steatosis, lobular inflammation, and hepatocellular ballooning with varying degrees of fibrosis.[2] The development of both phenotypes is tightly linked to excess body weight and insulin resistance. It thus shares several common clinical and physiologic features with type 2 diabetes mellitus and as with diabetes it too has been associated with specific changes in the intestinal microbiome. This review discusses the emerging tools for the analysis of the microbiome, their limitations, and the existing literature with respect to the intestinal microbiome and their role in NAFLD.

METHODS USED FOR INTERROGATING THE INTESTINAL MICROBIOME

Louis Pasteur and Robert Koch pioneered the original concepts about the microbiome along with Eli Metchnikoff. In the early days of microbiology, the identification of bacteria required their isolation by culture, which was established as the gold standard. This technique was limited, however, by the fact that less than 1% of bacteria can be cultured in traditional aerobic and anaerobic culture conditions.

The ability to clone and sequence polymerase chain reaction (PCR) amplicons of the 16S ribosomal RNA (rRNA) gene from a complex community of bacteria allows generating an abundant profile of different bacterial species in the community. This has been used to further generate community fingerprinting of the microbial diversity using denaturing gradient gel electrophoresis or length heterogeneity fingerprinting to derive the bacterial abundance in terms of operational taxonomic units (OTUs) in a sample. Although this allows profiling of the entire community in 1 run on a sequencer, it does not, however, identify individual species in the OTUs.

Next-generation sequencing technology, for example, the Roche 454 technology, essentially clones 16S rRNA PCR amplicons onto beads producing up to 500,000 reads in a single run. Another innovation was the use of sample bar-coding where multiple samples can be mixed and given unique bar codes and then run on a next-generation sequencer followed by sorting of the sequences in to bins. The current ion torrent technology (Thermo Fisher, MA, USA) and polytomy (Illumina, CA, USA) can produce an even higher number of reads,[3,4] and the ability to generate reads of the microbial sequences is no longer the rate-limiting step in the study of the microbiome, and the main challenges remain in the application of bioinformatics to make sense of the large amount of data generated and ascribe mechanistic roles in various disease states.

Bioinformatics in the Study of the Microbiome

One approach is the identification of raw 16S rRNA sequences and comparing them to bacterial databases to build relative abundance tables. Computational limitations