Review Article

Gut microbiota and non-alcoholic fatty liver disease

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BACKGROUND: Non-alcoholic fatty liver disease (NAFLD) is a common disorder with poorly understood pathogenesis. Beyond environmental and genetic factors, cumulative data support the causative role of gut microbiota in disease development and progression.

DATA SOURCE: We performed a PubMed literature search with the following key words: "non-alcoholic fatty liver disease", "non-alcoholic steatohepatitis", "fatty liver", "gut microbiota" and "microbiome", to review the data implicating gut microbiota in NAFLD development and progression.

RESULTS: Recent metagenomic studies revealed differences in the phylum and genus levels between patients with fatty liver and healthy controls. While bacteroidetes and firmicutes remain the dominant phyla among NAFLD patients, their proportional abundance and genera detection vary among different studies. New techniques indicate a correlation between the *methanogenic archaeon* (*methanobrevibacter smithii*) and obesity, while the bacterium *akkermanshia municiphila* protects against metabolic syndrome. Among NAFLD patients, small intestinal bacterial overgrowth detected by breath tests might induce gut microbiota and host interactions, facilitating disease development.

CONCLUSIONS: There is evidence that gut microbiota participates in NAFLD development through, among others, obesity induction, endogenous ethanol production, inflammatory response triggering and alterations in choline metabolism. Further studies with emerging techniques are needed to further elucidate the microbiome and host crosstalk in NAFLD pathogenesis.

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KEY WORDS: non-alcoholic fatty liver disease; non-alcoholic steatohepatitis; gut microbiota; 16S rRNA sequencing; archaea

Introduction

Non-alcoholic fatty liver disease (NAFLD), the liver manifestation of metabolic syndrome, is the main cause of liver enzymes abnormalities in Western countries.[1, 2] NAFLD definition requires lack of ongoing or recent excessive alcohol consumption (>20 g/d for men and >10 g/d for women, respectively) and exclusion of other causes of liver steatosis.[3] Histologic evidence of steatosis or in the absence of histology, γ-GT and/or aminotransferases elevations as well as compatible sonographic findings suffice for diagnosis. The spectrum of the disease encompasses liver steatosis, non-alcoholic steatohepatitis (NASH), NASH-related cirrhosis and hepatocellular carcinoma.[4] Moreover, NAFLD patients are at greater risk to develop cardiovascular diseases[5] and their overall mortality is higher than that of a matched general population.[6]

Obesity, diabetes mellitus and hypertriglyceridemia are the main risk factors for the development and progression of NAFLD.[7] Given the mounting prevalence of overweighed and obese individuals (65% and 30%, respectively in the USA),[8] it is apparent that NAFLD and its consequences represent major public health issues. NAFLD prevalence ranges from 3% to 30%,[7, 9] depending on the used diagnostic methods (biochemical markers, radiology, histology). NAFLD and NASH prevalence among an urban USA population is estimated to 20%[10] and 4%,[2] respectively. However, epidemiological data vary in special populations: Hispanics show higher prevalence than non-Hispanics, whereas non-Hispanics black individuals[11] as well as populations from Alaska[12] and American-Indians,[13] exhibit significantly lower prevalence (0.6%-2%) of NAFLD.

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A "two hits" hypothesis about NAFLD pathogenesis has been proposed.[14] The first hit is increased triglycerides accumulation in the liver, whereas a second one (e.g. oxidative stress) induces liver parenchyma inflammation leading to NASH. Recently, investigators have proposed the "multiple parallel" hits hypothesis:[15, 16] inflammation may either precede or follow simple steatosis with multiple factors, namely lipotoxicity, increased oxidative stress, mitochondrial dysfunction and iron overload acting in parallel to promote NASH.[16] Moreover, a genetic predisposition to the disease is possible since a mutation in the patatin-like phospholipase domain-containing 3 gene has been recognized to strongly predict liver fat accumulation and disease progression.[17]

The aim of this review is to highlight key issues on gut microbiota-host crosstalk regarding the pathogenesis of NAFLD and NASH. We present herein the yield of a PubMed search from 1995 to 2014 using the key words "non-alcoholic fatty liver disease", "non-alcoholic steatohepatitis", "fatty liver", "gut microbiota" and "microbiome".

Gut microbiome

Gut microbiota is a group of commensal microorganisms that live synergistically with the host. They process complex, otherwise indigestible, polysaccharides to short-chain fatty acids, thus providing extra energy for the host. They also participate in the synthesis of vitamins (e.g. vitamin K) and in the development and maintenance of the immunity at the intestinal lumen level.[18, 19]

The adult type gut microbiota is made up from bacteria, viruses, protozoa, archaea, eukaryotes, yeasts and parasites. It counts more than 10^{14} cells,[20] more than 100 different bacterial species;[18] their genome counts up to 300 000 genes,[21] 100 times the number of the human genome. Among them, bacteria predominate with the Gram-positive short-chain fatty acids-producing firmicutes and the Gram-negative hydrogen-producing bacteroidetes being the main phyla, followed by proteobacteria, actinobacteria, bifidobacteria, etc.[22, 23] Based on the abundant genera, two basic enterotypes are recognized:[24] Enterotype 1, where *bacteroides spp.* dominate and enterotype 2, with abundance of *prevotella spp.*. The existence of a third enterotype--enterotype H[25]--with abundance of both *bacteroides spp.* and *prevotella spp.* has also been proposed.

Evaluating gut microbiota in NAFLD

In order to reveal microbiota composition, culture-dependent and culture-independent techniques have been implicated.[26] Traditional culture allows detection and semi-quantification of many bacterial groups. Nevertheless, since a large amount of gut bacteria requires special conditions (e.g. anaerobic environment) to grow, a loss of 80% of the detectable bacteria is anticipated.[22] Trying to overcome this problem, culture-independent techniques have been developed. Apart from the widely used quantitative real-time polymerase chain reaction (qRT-PCR), additional techniques based on the diversity in the sequence of the bacterial 16S ribosomal RNA (16S rRNA) gene are now utilized: either the entire or conserved regions of the 16S rRNA gene are amplified, the results are compared with the sequence-containing libraries, thus accurate bacterial species identification or the partial 16S rRNA sequencing (pyrosequencing) provides information about the number, nature and abundance of the diverse bacterial species[27] without need of an *ex vivo* bacterial culture or DNA cloning.

Furthermore, the "-omics" studies are reliable methods to specify the functional species that interact with the host. Accordingly, the entire bacterial genome (metagenomics), the expressed mRNA (metatranscriptomics), the obtained proteins from the investigated microenvironment (metaproteomics), and the produced metabolites (metabolomics) are used to distinguish different phenotypes as well as, potential host-microbiome interactions.

Table 1 summarizes data from human studies investigating stool microbiota in NAFLD. Zhu et al[25] studied a pediatric population of 22 biopsy-proven NASH subjects, 25 obese children and 16 healthy controls using 16S rRNA sequencing in stool samples. Sequencing revealed a significant increase in bacteroidetes with decreased levels of firmicutes among obese and NASH individuals. Actinobacteria were significantly decreased in NASH patients, whereas proteobacteria counts showed a gradual increase from healthy to obese and NASH patients. Authors also provided evidence of increased ethanol levels in NASH children, postulating a possible role of abundant ethanol-producing bacteria (such as *Escherichia*) in NASH progression.

The increased bacteroidetes/firmicutes ratio detected by Zhu et al[25] was not confirmed by Raman et al,[28] who studied 30 obese adult patients with clinical, biochemical and radiological suspicion of NAFLD and 30 healthy controls using multi-tag pyrosequencing in a single stool sample per patient. Although differences in family and genera level were detected, the authors could not demonstrate a significant difference in the phylum level between the two groups. Similarly, using qRT-PCR in stool samples of 33 patients with biopsy-proven NAFLD (22 with NASH) and 17 healthy controls, Mouzaki et al[29]revealed significantly lower counts of Bacteroides in NASH patients than patients with simple steatosis and healthy controls. Firmicutes, proteobacteria and actino-