Does the Intestinal Microbiota Explain Differences in the Epidemiology of Liver Disease between East and West?

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Key Words

Microbiota · Liver disease · Inflammatory bowel disease · Nonalcoholic fatty liver disease · Nonalcoholic steatohepatitis · East and West

Abstract

Changes in bacterial communities are associated with the pathogenesis of many diseases including inflammatory bowel disease and liver disease. Dysbiosis can induce intestinal inflammation resulting in increased intestinal permeability and bacterial translocation. The majority of chronic liver diseases are associated with bacterial translocation resulting in or enhancing an inflammatory response in the liver. Intestinal inflammation and a dysfunctional intestinal barrier are not sufficient to cause liver disease in the absence of an additional liver insult. In this article, the authors summarize differences in intestinal microbiota composition between Eastern and Western countries. The authors specifically discuss whether differences in microbiota composition could explain the epidemiological differences in liver disease found in Asia and Europe/the USA.

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Introduction

The human body harbors approximately 10^{15} indigenous microbial cells, which colonize various organs and niches of the whole body including the oral cavity, nasal cavity, stomach, intestines, skin, and vagina. The number of microorganisms and the number of species increase in the digestive tract from the stomach to the small and large intestines; there are 10^{12} microbial cells per 1 g feces in the large intestine. The gut microbiome is essential to maintain homeostasis and the health of the host, since it is responsible for vitamin synthesis, energy supply, immune cell maturation, and defense against infectious pathogens [1–5]. On the other hand, it is known that an unbalanced microbiota (dysbiosis) is directly associated with various diseases and involved in the pathogenesis of gastrointestinal diseases such as inflammatory bowel disease (IBD) and liver diseases, but also in the pathogenesis of non-digestive tract diseases including arteriosclerosis, obesity, diabetes, and multiple sclerosis [6–9].

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Fig. 1. Gut-liver axis along with other factors contributing to the onset of chronic liver diseases.

Dysbiosis and Mucosal Inflammation

Intestinal dysbiosis is characterized by a decrease in bacterial diversity in patients with IBD. Butyrate-producing bacteria (*Faecalibacterium prausnitzii*) are lower in IBD patients. Dysbiotic changes in microbiota composition can result in increased mucosal inflammation and have been linked to the onset of IBD [10]. Tumor necrosis factor (TNF)- α is a key cytokine and plays an important role in disease pathogenesis. TNF- α disrupts intestinal tight junctions, induces epithelial cell death, perpetuates inflammation, and contributes to gut barrier dysfunction [11]. IBD is characterized by a mucosal barrier defect, which poses the risk of bacterial translocation from the intestinal lumen to the portal vein and liver. However, only a minority of IBD patients show extra-intestinal liver disease. Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease with inflammation of the biliary epithelium, resulting in destruction and structuring of bile ducts. IBD is a major risk factor for developing PSC; 60–80% of patients with PSC have IBD, while only 4% of patients suffering from ulcerative colitis (one form of IBD affecting the large intestine) have PSC [12]. Similarly, other diseases such as HIV enteropathy are associated with intestinal inflammation and increased intestinal permeability, but only a minority of HIV patients suffers from liver disease [13]. Thus, it appears that intestinal

inflammation and increased intestinal permeability is not sufficient to cause liver disease. As in the case of PSC, other environmental, genetic, or possibly microbiota-related factors must contribute to the onset of chronic liver disease (fig. 1).

Intestinal Inflammation Links Increased Intestinal Permeability and Bacterial Translocation to Chronic Liver Disease

Viral hepatitis, alcoholic liver disease, and nonalcoholic steatohepatitis (NASH) are the leading causes of chronic liver disease. Alcoholic and nonalcoholic liver disease involve changes in dietary patterns: alcohol-dependent patients drink alcohol, while patients with NASH are obese and typically consume a Western diet rich in fat and sugars. The diet strongly modulates the composition of the intestinal microbiota [14]; it is therefore not surprising that patients with alcohol abuse and NASH show intestinal dysbiosis [15–17]. Experimental animal models demonstrate that changes in intestinal bacterial communities are causatively linked to intestinal inflammation, increased intestinal permeability, bacterial translocation, and progression of alcoholic and nonalcoholic liver disease [18–20]. Taken together, dysbiosis-induced intestinal inflammation in combination with a direct liver insult such as alcohol can result in progression of liver disease.

Differences in Microbiota Composition between Eastern and Western Countries

With the significant progress in genome analysis technology, as represented by next-generation sequencing, an association between the gut microbiome and health/disease has been revealed. The International Human Microbiome Consortium, consisting of researchers from the USA, Japan, Europe, and China, was launched in 2007 and has since been promoting the large-scale Human Microbiome Project (HMP) [21]. More than 90% of the adult microbiome is composed of species belonging to four bacterial phyla: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria; however, there are significant differences in composition between individuals. In addition, the composition of the species is characterized by their habitat; Firmicutes are the major species in the intestine, vagina, skin, and oral cavity, while Actinobacteria and Proteobacteria are more dominant in the oral cavity, skin, and nasal cavity. Although the major part of

the gut microbiome in nursing infants is composed of the same four phyla as in adults, the number of species is as small as 1/5 to 1/3 of that in adults, and the species' composition is significantly different as well.

In 2011, the concept of the 'enterotype' was proposed by a group of researchers from Europe and Japan [22]. The human gut microbiome is thereby classified into three enterotypes, and each enterotype has dominant species of any of the following three genera: *Bacteroides*, *Prevotella*, and *Ruminococcus*. Like blood types, enterotypes are not related to race, residential region, or diet, suggesting that they had already existed before the current human species branched off. Furthermore, it has been reported that the human gut microbiome can be classified into four 'community types' according to the composition ratio of the genera *Bacteroides*, *Prevotella*, *Alistipes*, *Faecalibacterium*, and *Ruminococcus* [23]. Since these community types are significantly correlated with the presence or absence of breastfeeding, gender, and educational level, they may become a useful tool for predicting the onset of particular diseases in the future.

With regard to the gut microbiome, even if the enterotypes are not related to race or diet (as mentioned above), there are nonetheless distinctive differences between each race. In the HMP, researchers analyzed various parameters of 242 individuals, including their gut microbiota, the genes involved in each metabolic pathway encoded by the microbiome, and the host characteristics such as age, gender, body mass index, and race (Asian, Black, Mexican, Puerto Rican, and White). As a result, it was reported that race was strongly correlated with specific microbial species and the genes involved in the metabolic pathways [24]. Factors that cause microbial diversity between individuals include diet, drug intake, living environment, health status, and genetic factors. Future studies are required to determine whether the results mentioned above were caused by race-specific characteristics or affected mainly by the individuals' lifestyle including their diet.

Results of a large-scale comparative study of the human microbiome targeted at more than 500 individuals of different races and with varying lifestyles from people in the USA, Amazonian natives in Venezuela, and Malawian natives in Africa were reported in 2012 [25]. Based on a species analysis using 16S rRNA gene sequencing technologies, it was found that there was no significant difference in microbiota between Venezuelan and African natives, while there was a significant difference between Americans and natives in Venezuela/Africa. As one of the differences in microbiota, the Venezuelan and African natives were characterized by a high proportion

of *Prevotella* and a low proportion of *Bacteroides* (which is the major microbiota among Western people consuming a diet rich in meat products). Also, a large difference in genes encoded by the microbiota was observed; while genes that produce glutamate synthase were plentiful in Venezuelan and African natives, glutamine acid degradation enzymes were abundant in Americans. Genes that produce amylase, which is an enzyme degrading starch, were more copious in the microbiota of Venezuelan and African natives than in that of Americans. It is suggested that these results are deeply related to the natives' simple diet, which consists of corn as a staple food and other low-protein foods as compared to the American diet. In addition, according to a report recently released, the gut microbiome of the Amazonian hunter-gatherers called Matsés contained abundant species of the genus *Treponema* as compared to Americans living in big cities [26]. The *Treponema* microbiome observed in the Matsés tribe has a close relationship to the species that help digest carbohydrates and break down dietary fibers in swine, cattle, and termites. The species composition is closely associated with the Matsés' diet, which contains tubers such as potatoes as a staple food.

As an achievement of the Asian Microbiome Project (AMP), an analysis of gut microbiota targeted at children from five Asian countries was reported in 2015 [27]. There were two major patterns of gut microbiome among these Asian children; one was the BB type, with *Bifidobacterium/Bacteroides* as the dominant species, which was common in the children from Japan, China, and Taiwan, and the other was the P type, with *Prevotella* as the dominant species, which was common in Indonesia and Khon Kaen (Thailand). The microbial distribution among the Japanese children was more distinctive, with a high proportion of *Bifidobacterium* and fewer Enterobacteriaceae as compared to other countries or the previous samples from the same generation from Western countries. It is considered that diet and good hygiene may affect the microbiome of the Japanese. However, it is also suggested that their microbiome might be associated with infections, allergic diseases, and autoimmune diseases, which have been continuously increasing in Japan. Studies that directly compared the differences in gut microbial composition between Western and Eastern healthy individuals as well as IBD patients have recently been conducted [28–30]. Although differences in microbiota composition between races were observed in each of the studies, the authors conclude that an influence of diet and the living environment should be considered beside the impact of genetic factors.

Recently, microbial species that directly act on the host immune system have been identified. In 2009, members of the indigenous microbiome called ‘segmented filamentous bacteria’ were reported to be involved in the induction of Th17 cells, which play a key role in host defense against extracellular microbes and the pathogenesis of autoimmune disease [31]. It was also found that *Bacteroides fragilis* and the genus *Clostridium* were the specific microbiota involved in the induction of regulatory T cells [32, 33].

A larger-scale study including age- and gender-matched individuals with a wide range of molecular information is needed to clarify whether or not the differences in the gut microbiome between individuals from Eastern and Western countries could contribute to the onset of particular diseases.

Differences in the Prevalence of Chronic Liver Diseases between East and West

Among chronic liver diseases, PSC shows a difference in prevalence between Eastern and Western countries. The prevalence of PSC ranges from 6 to 16 cases per 100,000 inhabitants, while its incidence is approximately 1 per 100,000 persons in North America and Europe [12]. The prevalence and incidence rates are lower in Southern Europe and in Asia [34]. Singapore has a reported prevalence rate of 1.3 per 100,000 inhabitants [35]. The rate of developing PSC after a diagnosis of ulcerative colitis was lower in Korea when compared to a population in Northern Europe [36, 37]. The prevalence of IBD among patients with PSC is reportedly also lower in Japan than in Western countries [38].

Differences in incidence and prevalence rates have been attributed to differences in genetic susceptibility. However, incidence rates are increasing over time [34], which cannot be explained by differences in genetic background. It is therefore tempting to speculate that changes in dietary patterns enhance intestinal dysbiosis, which in turn contributes to the onset of PSC. Whether this hypothesis is true or not has to be determined with temporal clinical studies that include monitoring of the microbiome, intestinal inflammation, and the systemic inflammatory response. Although it is an intriguing hypothesis, there are several points that deserve additional attention and might argue against it. Although incidence and prevalence rates are lower in Asian countries, there are no studies reporting differences in disease progression.

Even if the microbiota is in fact a driver of intestinal inflammation, bacterial translocation, and progression of liver disease, liver diseases caused via different etiologies such as alcohol abuse or viral hepatitis might result in differences in prevalence, incidence, or disease progression as well. Studies reporting on such differences are lacking.

The prevalence of nonalcoholic fatty liver disease (NAFLD) overall is lower in Asia than in Western countries. Urban areas in India and other parts of Asia that have adapted a ‘Western’ diet report prevalence rates for NAFLD and NASH of 10–24 and 3–4%, respectively, which is similar to their prevalence rates in the West. In addition, the prevalence of NAFLD in an obese population was similarly high in Asia and Western countries [39]. Thus, differences in NAFLD etiology and prevalence are more closely related to dietary patterns than geographic differences.

Bile Acids as Modulators of Liver Disease

Bile acids are important molecules that mediate communication between the liver and the intestine. Bile acids are synthesized in the liver from cholesterol, conjugated to glycine or taurine in hepatocytes, and excreted into the duodenum via bile ducts. Conjugated bile acids are part of micelles, which aid in the digestion and absorption of dietary fat and fat-soluble particles. Once they reach the terminal ileum, they are transported via the apical sodium-dependent bile acid transporter (SLC10A2) into enterocytes. Intracellular bile acids bind to the nuclear receptor FXR and induce the expression of fibroblast growth factor 15 (FGF15, termed FGF19 in humans). The enterokine FGF15 is secreted into the portal circulation. After binding to its receptor on hepatocytes, an intracellular cascade causes a downregulation of bile acid synthesis via repression of Cyp7a1, the rate-limiting enzyme in the de novo synthesis of bile acids from cholesterol. This closes the loop of a negative feedback regulation of bile acid synthesis by bile acids within the enterohepatic circulation. Intestinal bacteria (mostly) in the large intestine generate secondary bile acids by deconjugation and dihydroxylation. Secondary bile acids are passively absorbed in the colon and return to the liver via the portal circulation [40].

Changes in the intestinal microbiota have been linked to differences in bile acid homeostasis and disease. For example, the absence of microbiota in germ-free rodents resulted in dramatic changes of bile acid profiles [41]. Absence of the intestinal microbiota exacerbated hepatobiliary disease in a model of primary sclerosing cholangitis

using Mdr2-deficient mice [42]. The results from this study and a similar study from our laboratory [43] suggest that the commensal microbiota and/or its metabolites protect against biliary injury and liver fibrosis.

On the other hand, the microbiota modifies bile acids and can change the preference of bile acids for binding to their receptors FXR and the G protein-coupled receptor TGR5. FXR-deficient mice are protected from cholestatic liver fibrosis following bile duct ligation [44]. Activation of TGR5 by bile acids in brown adipose tissue and muscle increased energy expenditure and attenuated diet-induced obesity in mice [45]. It is therefore feasible that the modulation of bile acid profiles by a different intestinal microbiota composition affects chronic liver disease progression.

Conclusion

Differences in microbiota composition exist between Eastern and Western countries. These are most likely explained by differences in diet. Although there is a divergence in the prevalence of chronic liver disease, future studies need to assess whether variations in microbiota

and the prevalence of liver disease are causatively linked. Such studies require the integration of microbiomics and metabolomics with clinical studies.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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