The gut microbiome and nicotine metabolism in NAFLD

Lei Miao[a], Giovanni Targher[b], Christopher D. Byrne[c], Ming-Hua Zheng[d,e*]

[a]Department of Gastroenterology, The Second Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China

[b]Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University of Verona, Verona 37129, Italy

[c]Southampton National Institute for Health and Care Research Biomedical Research Centre, University Hospital Southampton, Southampton General Hospital, Southampton SO166YD, UK

[d]MAFLD Research Center, Department of Hepatology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China

[e]Key Laboratory of Diagnosis and Treatment for The Development of Chronic Liver Disease in Zhejiang Province, Wenzhou 325000, China

***Corresponding author.**

E-mail address: zhengmh@wmu.edu.cn (M.-H. Zheng)

A history of cigarette smoking is present in approximately 40% of patients with chronic liver diseases and there is considerable interest in better understanding if and how cigarette smoking may affect chronic liver diseases [1]. Non-alcoholic fatty liver disease (NAFLD) is currently the most common cause of chronic liver disease, affecting up to 30% of the world's adults [2]. Genetic and environmental factors contribute to the development and progression of NAFLD, and increasing evidence shows that smoking is associated with more severe liver disease in NAFLD [3,4]. Despite convincing evidence of this association between smoking and liver disease, the underlying mechanism(s) linking smoking with NAFLD is still poorly understood.

NAFLD and smoking are both associated with metabolic syndrome that is characterized by insulin resistance and visceral ectopic fat accumulation [5, 6] and now the relationship between smoking and NAFLD is becoming better understood. Tobacco smoking exposure may increase the permeability of the intestinal mucosa and elevate the intestinal pH, which enables some harmful bacteria to thrive, causing intestinal dysbiosis. Exposure to tobacco smoking can also alter lipid and bile acid metabolism due to intestinal dysbiosis, which may increase hepatic fat accumulation and exacerbate systemic insulin resistance [7]. Moreover, higher serum and fecal bile acid levels have also been shown to be associated with advanced fibrosis in NAFLD [8]. Thus, it is plausible to hypothesize that lung-gut crosstalk occurs with smoking exposure. It is known that cigarette smoking has a differential role in inflammatory

bowel disease worsening Crohn's disease and alleviating ulcerative colitis and it is plausible that intestinal dysbiosis may be a mediator of liver injury, thereby leading to hepatic steatosis and inflammation in NAFLD. To date, observational research shows that tobacco smoke could affect gut microbiota composition by increasing the phylum of Firmicutes, Proteobacteria, and decreasing the phylum of Bacteroidetes, Lactobacillus, and Bifidobacterium. These gut microbiota alterations may produce several metabolites such as ceramides, ethanol, lipopolysaccharide (LPS), short-chain fatty acids (SCFAs), and pathogen-associated molecular patterns (PAMPs). Thus, it is plausible that microbial metabolite alterations may play a crucial role in the progression of NAFLD, possibly via effects on the lung-gut-liver axis (**Fig. 1**) [9-11].

A cross-sectional population-based study from China (involving 8580 subjects) showed that former and current heavy smoking was associated with an increased risk of suffering NAFLD, even after adjusting for potential confounding factors (odds ratio (OR) = 1.45, 95% confidence interval (CI) 1.05–2.00 and OR = 2.29, 95% CI 1.30–4.03), compared with never smoking. These data also showed that passive smoking and heavy smoking both had a synergistic adverse effect on the risk of NAFLD [12]. Additionally, in a prospective cohort of 1315 individuals, the authors examined the association between childhood and adulthood passive smoking with NAFLD in midlife and found that passive smoking in both children and adults was associated with an increased risk of adult NAFLD. Either passive smoking in childhood or adulthood was associated with a higher risk of NAFLD, even after

adjusting for age, sex, adulthood physical activity, childhood socioeconomic status, and alcohol consumption (relative risk =1.41, 95% CI 1.01–1.97 for children and relative risk = 1.35, 95% CI 1.01–1.82 for adults) [13]. In order to better elucidate the possible impact of smoking on the risk of NAFLD development, a prospective cohort study involving nearly 200,000 Korean adults without NAFLD (median follow-up time, 4.1 years) was studied. After adjusting for diet, alcohol intake, physical activity, and socioeconomic factors, the adjusted hazard ratios of developing NAFLD for the man having 10–19.9 smoking pack-years and ≥20 pack-years were 1.25 (95% CI 1.21–1.29) and 1.36 (1.30–1.42) respectively, comparing to 0 pack-years. What's more, smoking pack-years was also associated with an increased risk for NAFLD with a high liver fibrosis score [14].

A prospective cohort study based on histological analysis also supports an association between smoking and the severity and progression of liver disease in NAFLD. It indicated that advanced liver fibrosis was independently associated with age, type 2 diabetes, and smoking pack-years. It was shown that a history of smoking ≥10 pack-years was more common among NAFLD patients with advanced fibrosis (P <0.0001). Moreover, multivariate analysis showed a correlation between a smoking history of ≥10 pack-years and advanced fibrosis (OR=1.63, 95% CI 1.19–2.24). What's more, among the subjects without type 2 diabetes, a history of ≥10 pack-years was strongly associated with advanced fibrosis (OR=2.48, 95% CI 1.68–3.67) [15]. These data suggest that smoking may aggravate the progression of NAFLD, mostly by promoting

insulin resistance and metabolic dysfunction. All of these studies provide robust evidence suggesting that smoking contributes to the development and progression of NAFLD.

However, the potential mechanisms of the lung-gut crosstalk are still unknown and to better elucidate the mechanisms underlying the complex link between gut microbiota and smoking-related progression of NAFLD, pre-clinical studies in mice have been undertaken [16, 17]. Recently, it has been reported that nicotine accumulated in the gut during smoking and promoted the progression of NAFLD, and also identified the gut bacterium *Bacteroides xylanisolvens* as an effective nicotine degrader in nicotine-exposed mice [17]. The study also sampled 83 patients with NAFLD, and divided them into smokers (n = 41) and non-smokers (n = 42). It was interesting to note that levels of *B. xylanisolvens* were negatively correlated with NAFLD severity, but the correlation was not found in non-smokers. What's more, the fecal nicotine levels were positively associated with the progression of NAFLD, while the nicotine degradation product 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) was negatively correlated with NAFLD progression. Smoking-induced changes in gut microbiota could activate the intestinal AMPKα-SMPD3, which will promote NAFLD progression by increasing intestinal ceramide production. This study provides a new potential mechanistic insight into the interaction between nicotine, gut microbiome and NAFLD. It proved that intestinal nicotine accumulation could accelerate the progression of NAFLD and identified *B. xylanisolvens* as an effective nicotine degrader. It is interesting to note

that SMPD3 inhibition might be a novel therapeutic strategy that requires further investigation in the quest to find novel pharmacotherapies for non-alcoholic steato hepatitis (NASH) [17].

In conclusion, it is becoming clear that the influence of nicotine on intestinal microbiota is important in the pathogenesis of NAFLD. Smoking can cause intestinal dysbiosis, exacerbating hepatic steatosis through AMPK inactivation, regulation of SMPD3 and increase in ceramides, catecholamine and glucagon that can contribute to the progression of NAFLD. We propose that further prospective cohort studies are needed that also examine the changing status of smoking behavior on the progression and resolution of NAFLD. Further pre-clinical studies are also urgently needed to elucidate the mechanistic pathways underlying smoking-induced changes in the microbiome and intestinal function in NAFLD.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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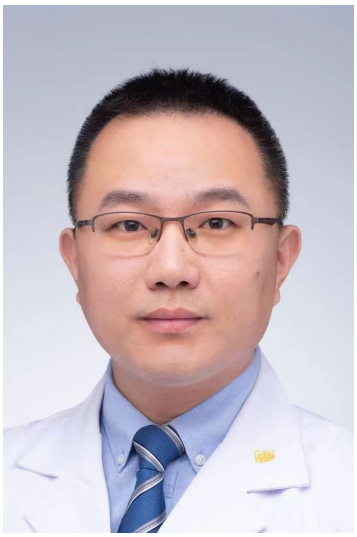

Ming-Hua Zheng is a Professor at MAFLD Research Center, Department of Hepatology, the First Affiliated Hospital of Wenzhou Medical University, the core member of the Asian Pacific Association for the study of the liver (APASL) Metabolic fAtty lIver DiseasE coNsortium (MAIDEN), and the leader of CHESS-MAFLD consortium. His research interest focuses on clinical, basic, and translational research on fatty liver disease.

Figure Legend

Fig. 1. Smoking influence in lung-gut-liver axis.

The gut microbiome is essential for human life by profoundly impacting human nutrition and physiology. It plays a key role in the progression of NAFLD: (1) smoking toxic substances will damage the lung and produce a variety of proinflammatory cytokines such as interleukin (IL)-6, IL-13, transforming growth

factor (TGF)-β, tumor necrosis factor (TNF)-α, which induce the production of reactive oxygen species (ROS) in the liver leading to NASH. (2) What's more, smoking toxic substances will cause gut dysbiosis too (decrease in microbial diversity and pathogen multiplication, such as increase in the phylum of Firmicutes, Proteobacteria, and decrease in the phylum of Bacteroidetes, Lactobacillus, and Bifidobacterium). Microbial metabolites alterations (including ceramides, ethanol, short-chain fatty acids (SCFAs), lipopolysaccharide (LPS), pathogen-associated molecular patterns (PAMPs), cell wall components and flagellin) will exacerbate the progression of NAFLD. (3) Intestinal dysbiosis will be influenced by smoking toxic substances, however, the gut bacteria and its metabolites may contribute to changes in lung immunity, which may directly or indirectly cause illness and disorders, including lung diseases.