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Exploring the relationship between gut microbiota and hepatocellular carcinoma through two-sample Mendelian randomization study

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Abstract: Objective To investigate the potential association between 221 types of gut microbiota and hepatocellular carcinoma (HCC) using a two- sample Mendelian randomization approach, providing insights for the prevention and treatment of HCC. **Methods** The HCC data (*n*=197,611) from the IEU OpenGWAS database was used as outcome data, while microbiota and hepatocellular carcinoma (HCC) data were used as outcome data. The HCC data (*n*=197,611) from the IEU OpenGWAS database was used as outcome data, while gut microbiota data (*n*=18,340) obtained from a meta-analysis of a large-scale multi-ethnic genome-wide association study was used as exposure data. The analysis primarily employed the inverse variance weighting method, assessing the results based on the odds ratio (OR) and 95% confidence interval (CI). Quality control was conducted using leave-one-out analysis, heterogeneity tests, and the MR-PRESSO method. **Results** An increased abundance of the genus *Oscillibacter* was associated with a higher probability of developing HCC (*OR*: 1.411, 95%*CI*: 1.049-1.899, *P*=0.025). Leave-one-out analysis indicated that the study results were stable and no instrumental variables had a strong impact on the results. This suggests a positive causal relationship between gut microbiota and HCC, which can eliminate the effects of heterogeneity and horizontal gene pleiotropy on causal-effect estimation. **Conclusion** An increased abundance of the genus *Oscillibacter* may raise the probability of developing HCC.

Keywords: Hepatocellular carcinoma; Gut microbiota; *Oscillibacter*; Two-sample Mendelian randomization; Genome-wide association study

Hepatocellular carcinoma is currently the third leading cause of cancer-related deaths globally, with about 910,000 new cases each year, posing a serious threat to the health of the population[1]. The situation of hepatocellular carcinoma prevention and treatment in China is still rough. The prevalence of hepatocellular carcinoma caused by hepatitis B virus infection and non-alcoholic fatty liver disease accounts for about half of the global prevalence, and the 5-year survival rate is only 12.5%. Therefore, basic and clinical research on hepatocellular carcinoma must be strengthened.

Gut microbes include bacteria, viruses, fungi and other microorganisms, mainly bacteria[2-3], which influence immune regulation, energy metabolism, etc.[4]. The enterohepatic axis enables the host to resist potentially harmful toxins through microbiota, immune regulation, inflammatory metabolism, etc., thus maintaining immune and physiological homeostasis[5]. In recent years, a growing number of studies have identified an association between gut microbiota and hepatocellular carcinoma[6-7]. A prospective cohort study showed that higher levels of *anaerobicum Mycobacterium* had an inhibitory effect on hepatocellular carcinoma. In contrast, *Raoulia* spp. and *Haemophilus* spp. were associated with the development of hepatocellular carcinoma[8]. Although these epidemiologic studies show an association between intestinal flora and hepatocellular carcinoma, it is difficult to establish a causal relationship between intestinal flora and hepatocellular carcinoma through observational epidemiologic studies. Randomized controlled trials are the gold standard for determining causality in epidemiological statistical methods. However, implementation of randomized controlled trials can be difficult due to ethical constraints[9]. Mendelian randomization (MR) analysis is an alternative method that can be used as an alternative to randomized controlled trial studies, which uses the genetic principles of random pairing and genome segregation to simulate the effects of randomized controlled trials in order to assess the causal effects of specific factors on the outcome variable. In addition to lifting ethical constraints, Mendelian randomization allows for using large-scale available genetic data, such as data from genome-wide association studies (GWAS), with genome segregation and pairing, thereby controlling for and reducing the effects of potential confounders[10]. Therefore, this study utilized two-sample MR analysis to investigate the causal relationship between gut microbes and hepatocellular carcinoma using 211 single nucleotide polymorphisms (SNPs) of gut microbes published in *Nature Genetics* in 2021 as the exposure data, and data of hepatocellular carcinoma as the outcome data[11].

1 Materials and methods

1.1 Study design

In this study, the causal relationship between microbial flora and hepatocellular carcinoma was analyzed using the GWAS summary statistics, which included a total of 221 gut microbiota. Subsequently, quality control tests, such as heterogeneity and pleiotropy tests, were performed to ensure the reliability of the causality test results. **[Fig.1]**

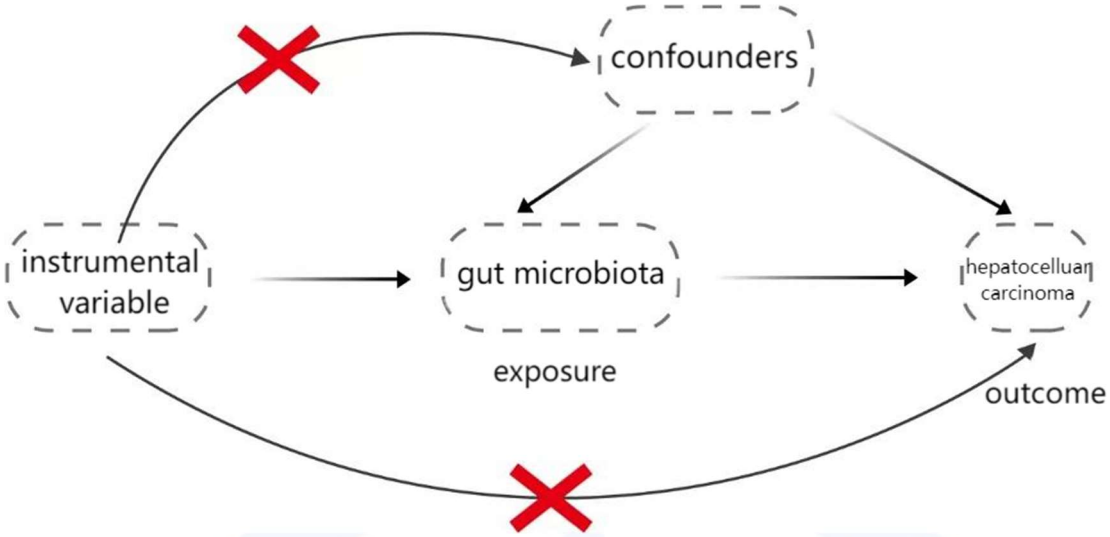

Fig. 1 The Mendelian randomization analysis model used in this study

1.2 Data sources

The GWAS outcome data for hepatocellular carcinoma in this study were obtained from the IEU OpenGWAS database (https://gwas.mrcieu.ac.uk/)[16]. Hepatocellular carcinoma (bbj-a-158) contained 197,611 individuals and 8,885,115 SNPs. In addition, summary statistics on gut microbial classification were obtained from a meta-analysis of a large-scale and multi-ethnic genomic association study covering 18,340 individuals from 24 cohorts [11]. The study characterized microbial composition using three different variant regions of the target-localized 16S rRNA gene. A total of 211 taxonomic orders (131 genera, 35 families, 20 orders, 16 classes and 9 phyla) were included.

1.3 *Instrumental variables*

In order to screen SNPs with significant correlation with hepatocellular carcinoma as instrumental variables, 221 gut microbiota were screened in this study respectively [13]. Considering the generally low abundance of gut microbiota, $P<5\times10^{-5}$ was used as the screening criterion, which is consistent with most of the gut microorganism research. In addition, a linkage disequilibrium coefficient $r^2 < 0.05$ was set in this study, and a window size of 500 kb was used to exclude highly correlated SNPs to ensure that the selected SNPs were independent of each other [16-17]. Finally, SNPs associated with gut microbial abundance were projected to the GWAS summary data for hepatocellular carcinoma, and the corresponding statistical parameters were extracted.

1.4 Statistical analysis

MR analyses were performed using R software (4.2.1) and the "TwoSampleMR" package (0.5.7). In order to assess the causal effect of a single instrumental variable, the Wald ratio method was used; while for assessing the causal effect of multiple instrumental variables, the inverse-variance weighted (IVW), MR-Egger method, Weighted median estimator (WME), Simple mode (SM), and Weighted mode (WM) were used[20-21]. In addition, the risk of hepatocellular carcinoma was assessed using the *OR* and the corresponding 95% *CI*. Regarding the results, the correlation was considered plausible when $P<0.05$ for IVW. The robustness of the results was verified by sensitivity analysis, and heterogeneity was tested using Cochrane's Q test. In addition, genetic pleiotropy was investigated using the MR Egger intercept and MR-PRESSO methods.

2 Results

2.1 Two-sample MR analysis

The initial screening of 221 SNPs of gut microbiota was first performed based on $P<5\times10^{-5}$, and the threshold of linkage disequilibrium analysis. Subsequently, two gut microbiota associated with hepatocellular carcinoma, *Oscillibacter* (id: ebi-a-GCST90017036) and unknown genus 826 (id: ebi-a-GCST90017086), were identified by using IVW, ME, WME, WMO, and SM methods, with IVW method as the main screening criterion, tentatively named genus 826. The results showed that increased abundance of the inflammation-associated genus — *Oscillibacter* (*OR* = 1.411, *95%CI* = 1.049-1.899, *P* = 0.025), as well as the unknown genus 826 (*OR* = 1.627, *95%CI* = 1.163-2.276, *P* = 0.004), increased the risk of hepatocellular carcinoma. The results of the specific IVW, ME, WME, WMO, and SM tests are shown in **Table 1**.

Although there were no statistically significant differences between the four methods except for the IVW method, the scatter plots **[Figure 2]** also demonstrates that the correlations between the two microbiota and hepatocellular carcinoma analyzed by the different methods were all positive, and the trends were essentially similar.

2.2 Quality control

Firstly, the effect values of most of the included instrumental variables were shown to be very similar to the total effect values by leave-one-out analysis. **[Figure 3]** Secondly, the results of heterogeneity test showed *Oscillibacter* (Q=3.114, *P*=0.926), unknown genus 826 (*P*=0.887). Gene pleiotropy results showed *Oscillibacter* (*P*=0.703), unknown genus 826 (*P*=0.516). The results of both heterogeneity test and pleiotropy test showed *P*>0.05 and the difference was not statistically significant, suggesting that there is no need to take into account the effect of heterogeneity and pleiotropy on the results.The MR-PRESSO results are shown in **Table 2**.

Tab.1 The five MR methods corresponding to gut microbiota with significant causal relationships in hepatocellular carcinoma

Exposure	Methods	nSNP	Beta	OR	95% *CI*	*P* value
Oscillibacter	IVW	9	0.345	1.411	1.049-1.899	0.025
	WME	9	0.276	1.317	0.890 -1.950	0.167
	ME	9	0.059	1.061	0.251-4.478	0.937
	SM	9	0.238	1.269	0.740-1.269	0.410
	WMO	9	0.243	1.275	0.774-2.100	0.366
Unknown genus 826	IVW	7	0.486	1.627	1.163-2.276	0.004
	WME	7	0.378	1.459	0.936-2.274	0.094
	ME	7	0.090	1.094	0.341-3.507	0.885
	SM	7	0.338	1.402	0.749-2.626	0.330
	WMO	7	0.350	1.420	0.817-2.466	0.259

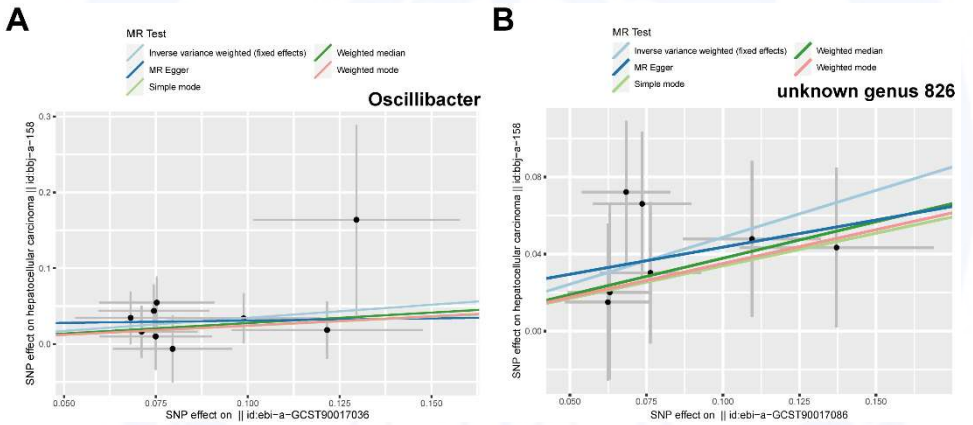

Fig.2 The scatter plots for the five MR models representing gut microbiota with causal relationships to hepatocellular carcinoma

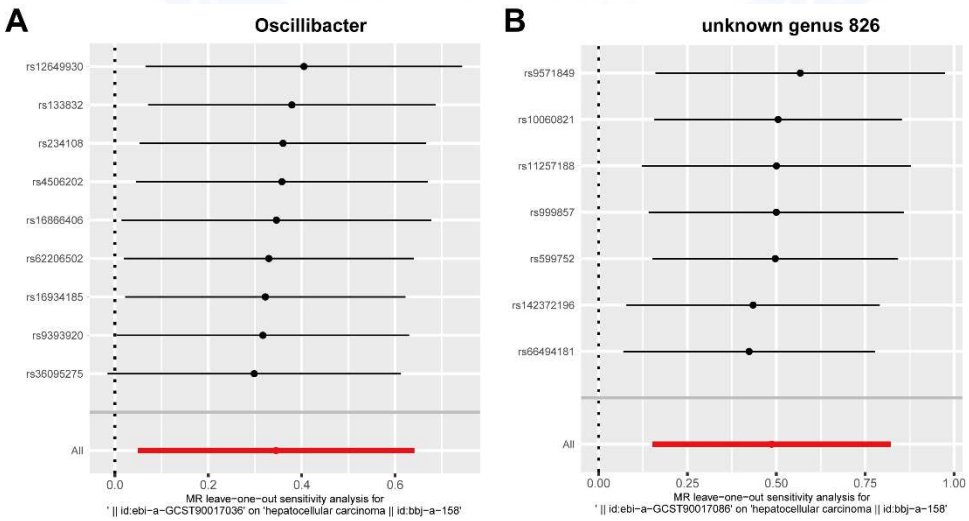

Fig.3 The leave-one-out plot for gut microbiota with causal relationships to hepatocellular carcinoma

Tab. 2 The heterogeneity, horizontal pleiotropy test, and MR-PRESSO results for gut microbiota with causal relationships to hepatocellular carcinoma

Exposure	Heterogeneity		Horizontal polytropy	MR-PRESSO	
	Q	*P* value	*P* value	Raw	*P* value
Oscillibacter	3.114	0.926	0.703	1.098 (1.026-1.176)	0.006
Unknown genus 826	2.324	0.887	0.516	1.113 (1.008-1.229)	0.032

3 Discussion

In this study, we investigated the causal relationship between the relative abundance of 221 gut microbiota and hepatocellular carcinoma using a two-sample MR approach utilizing public GWAS data on gut microbiota and GWAS data on hepatocellular carcinoma. The study results showed that the abundance of *Oscillibacter* and unknown genus 826 was positively correlated with the development of hepatocellular carcinoma. The IVW results suggested that the risk of hepatocellular carcinoma in patients with *Oscillibacter* and unknown genus 826 was increased by 1.411-fold and 1.627-fold, respectively, compared with the normal population. Therefore, it is of clinical significance to explore the gut microbiota for the etiology, pre-diagnosis, and comprehensive treatment of hepatocellular carcinoma.

It has been shown that dysregulation of the gut microbiome can be observed in the early stages of chronic liver disease and, if left unchecked, will promote progression of chronic liver disease to hepatocellular carcinoma [18]. Microbe-associated molecular patterns (MAMPs) refer to the gut microbiome and its metabolites as low-level exposures to the liver during disease progression. When the gut microbiome is dysregulated, the levels of MAMPs are elevated, which in turn lead to inflammation and oxidative stress damage in the liver and even hepatocellular carcinoma by binding to lipopolysaccharide and its Toll-like receptor 4 (TLR4), as well as promoting the activation of the NF-kB signaling pathway and the release of pro-inflammatory factors, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1, etc.[19]. In addition, dysregulated gut microbes are also commonly associated with tumor immunomodulation, as shown in a cohort study that included 30 patients with hepatocellular carcinoma, which demonstrated that the response to immunotherapy, represented by programmed cell death protein-1, as well as survival benefit, was correlated with the abundance of the gut microbiota [20]. Therefore, we hypothesized whether some gut microbiomes could contribute directly or indirectly to hepatocellular carcinoma by altering the intestinal barrier and modulating the body's immune response.

In this study, we finally screened 2 gut microbiota that elevate the risk of hepatocellular carcinoma: *Oscillibacter* and unknown genus 826. Although the unknown genus 826 was not identified temporarily, *Oscillibacter* plays an important role in the gut. For example, *Oscillibacter* can produce short-chain fatty acids by fermenting polysaccharides such as glucose and modify dendritic cells with specific caudal lipids from *Oscillibacter* to alter their immunomodulatory functions [21]. It has been suggested that *Oscillibacter* with higher abundance in the healthy group may be a potential probiotic to inhibit the development of colon cancer [22], contrary to the findings of the present study. A prospective study of 75 patients with early-stage hepatocellular carcinoma and 75 healthy controls showed that *Oscillibacter* was significantly more abundant in patients with early-stage hepatocellular carcinoma and could be a marker for early-stage hepatocellular carcinoma [23]. Given the distinct roles played by *Oscillibacter* in colon and hepatocellular carcinomas, the authors hypothesized that gut microbes brought into play by the gut-liver axis cycle may also be affected by the hepatocellular carcinoma microenvironment, thus changing from a probiotic to a microbe that promotes hepatocarcinogenesis. For example, *Lactobacilli*, which were earlier thought to be beneficial to the gut, were found in subsequent studies to reduce the body's cytotoxic T-lymphocyte-mediated adaptive immune response by modulating the cGAS-STING-IFN-I signaling pathway, affecting the efficacy of radiotherapy in hepatocellular carcinoma [24].

However, there are some limitations in this study: (1) unknown genus 826 have not been identified, and their functions cannot be studied and discussed; (2) more cohort studies are needed to confirm the causal relationship between *Oscillibacter* and hepatocellular carcinoma risk; (3) the GWAS data studies mainly focus on European populations, and there are fewer studies on Asian populations. There may be differences in the level of the bacterial flora, which is a specific limitation for the generalization of results; (4) MR analysis initially investigated the causal relationship between *Oscillibacter* and hepatocellular carcinoma, but the specific mechanism remains to be investigated.

In summary, this study utilized hepatocellular carcinoma as an exposure factor, selected significantly associated microbiota—SNPs, as instrumental variables, and sensitivity analyses revealed no pleiotropy or heterogeneity, resulting in the finding that *Oscillibacter* could promote the risk of hepatocellular carcinoma. This approach enabled the identification of a causal relationship, provided candidate genes for gut microbiota in subsequent functional studies, and excluded the possibility of bidirectional causality and selection bias.

Conflict of interest None

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·论　著·

双样本孟德尔随机化研究探讨肠道微生物与肝癌的关系

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摘要：目的　通过双样本孟德尔随机化方法探讨 221 种肠道微生物与肝癌是否存在关联，为肝癌防治提供思路。**方法**　使用 IEU OpenGWAS 数据库的肝癌数据（n=197 611）作为结局数据，从一项大规模多种族基因组关联研究的元分析中获取肠道微生物数据（n=18 340）作为暴露数据，主要采用逆方差加权法进行分析，根据效应指标优势比（*OR*）和 95%*CI* 评估结果，同时使用留一法和异质性检验以及 MR-PRESSO 方法进行质量控制。**结果**　颤螺菌属（*Oscillibacter*）丰度的提升，可以增加肝癌的患病概率（*OR*=1.411，95%*CI*：1.049～1.899，*P*=0.025）。留一法分析结果表明，该研究的结果稳定，并且未发现对结果产生强烈影响的工具变量。结果显示肠道微生物与肝癌之间存在正向的因果关系，并且可以消除异质性和水平基因多效性对因果效应估计所产生的影响。**结论**　颤螺菌属丰度的提升，可以增加肝癌的患病概率。

关键词： 肝癌；肠道微生物；颤螺菌属；双样本孟德尔随机化；公共基因组关联研究

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Relationship between gut microbiota and hepatocellular carcinoma through two-sample Mendelian randomization study

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Abstract: Objective　To investigate the potential association between 221 types of gut microbiota and hepatocellular carcinoma (HCC) using a two-sample Mendelian randomization approach, providing insights for the prevention and treatment of HCC. **Methods**　The HCC data (n=197 611) from the IEU OpenGWAS database was used as outcome data, while gut microbiota data (n=18 340) obtained from a meta-analysis of a large-scale multi-ethnic genome-wide association study was used as exposure data. The analysis primarily employed the inverse variance weighting method, assessing the results based on the odds ratio (*OR*) and 95% confidence interval (*CI*). Quality control was conducted using leave-one-out analysis, heterogeneity tests, and the MR-PRESSO method. **Results**　An increased abundance of the genus *Oscillibacter* was associated with a higher probability of developing HCC (*OR*=1.411, 95% *CI*: 1.049-1.899, *P*=0.025). Leave-one-out analysis indicated that the study results were stable and no instrumental variables had a strong impact on the results. This showed a positive causal relationship between gut microbiota and HCC, which could eliminate the effects of heterogeneity and horizontal gene pleiotropy on causal effect estimation. **Conclusion**　An increased abundance of the genus *Oscillibacter* may raise the probability of developing HCC.

Keywords: Hepatocellular carcinoma; Gut microbiota; *Oscillibacter*; Two-sample Mendelian randomization; Genome-wide association study

肝癌是目前全球癌症相关死亡的第三大主要原因，每年其相关新增病例数约 91 万，严重威胁居民生命健康[1]。我国肝癌防治的形势仍较严峻，乙肝病毒感染和非酒精性脂肪肝病引起的肝癌，患病率约占全球一半，5 年生存率仅 12.5%。因此，必须加强肝癌的基础与临床研究。

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肠道微生物包括细菌、病毒、真菌和其他微生物，主要为细菌[2-3]，对免疫调节、能量代谢等有重要影响[4]。肠肝轴通过微生物菌群、免疫调节、炎症代谢等使得宿主抵抗潜在有害毒素的侵袭，从而维持免疫以及生理稳态[5]。近年来，越来越多的研究发现肠道微生物和肝癌之间存在关联[6-7]。一项前瞻性队列的研究表明，较高水平的厌氧枝杆菌对肝癌有抑制作用，而拉乌尔菌属和嗜血杆菌则与肝癌发展相关[8]。尽管这些流行病学研究显示肠道菌群与肝癌之间的关系，但很难通过观察性流行病学研究来确定肠道菌群与肝癌之间的因果关系。随机对照试验在流行病统计方法中是判断因果关系的金标准，然而，由于伦理限制，实施随机对照试验可能存在困难[9]。孟德尔随机化（Mendelian randomization，MR）分析是一种可以作为随机对照试验研究的替代方法，它利用遗传学原理中的随机配对和基因组分离来模拟随机对照试验的效果，以评估特定因素对结果变量的因果影响。除了解除了伦理限制，MR 还可以利用大规模的现有遗传数据，例如基因组关联研究数据，并通过基因组分离和配对，从而控制和减少潜在的混杂因素的影响[10]。因此，本研究利用双样本的 MR 分析，使用 2021 年度发表于 *Nature Genetics* 的 211 个肠道微生物的单核苷酸多态性（single nucleotide polymorphism，SNP）作为暴露数据，以肝癌数据作为结局数据，探讨肠道微生物与肝癌的因果关系[11]。

1　材料与方法

1.1　研究设计　本研究使用公共基因组关联研究（Genome-Wide Association Studies，GWAS）汇总统计数据进行菌群微生物与肝癌之间的因果关系分析，总计包括 221 种肠道微生物。随后，进行异质性检验和基因多效性检验等质量控制验证，以确保所得的因果关系结果的可靠性。如图 1 所示。

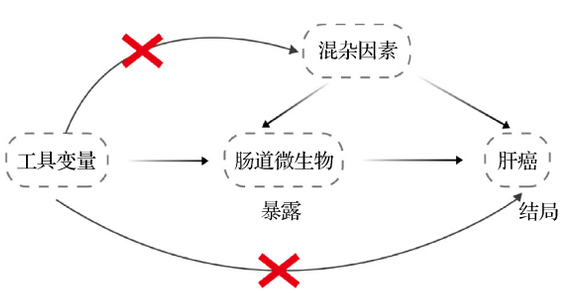


图 1　本研究的 MR 分析模型
Fig. 1　MR analysis model used in this study

1.2　数据来源　本研究的肝癌 GWAS 结局数据来源于 IEU OpenGWAS 数据库（https://gwas.mrcieu.ac.uk/）[12]。肝癌（bbj-a-158）包含了 197 611 例个体和 8 885 115 例 SNP。此外，从一项大规模多种族基因组关联研究的元分析中获取了肠道微生物分类的汇总统计数据，该研究涵盖了来自 24 个队列的 18 340 例个体[11]。该研究使用目标定位 16S rRNA 基因的 3 个不同变异区域，对微生物组成进行描述。总共包括 211 个分类阶元（131 个属，35 个科，20 个目，16 个纲和 9 个门）。

1.3　工具变量　为了筛选与肝癌具有显著相关性的 SNP 作为工具变量，本研究分别筛选了 221 种肠道微生物[13]。考虑到肠道微生物丰度普遍较低，故采用 $P<5\times10^{-5}$ 作为筛选标准，与大多数肠道微生物研究文献一致。此外，本研究还设置了连锁不平衡标准为 $r^2<0.05$，并使用 500 kb 的窗口大小，剔除高度相关的 SNPs，以确保所选的 SNPs 之间相互独立[14-15]。最后，将与肠道微生物丰度相关的 SNPs 投射到肝癌的 GWAS 汇总数据中，并提取相应的统计参数。

1.4　数据分析　利用 R 软件（版本 4.2.1）以及“TwoSampleMR”包（版本 0.5.7）进行 MR 分析。为了评估单一工具变量的因果效应，采用瓦尔德比值（Wald ratio）方法；而对于评估多个工具变量的因果效应，主要使用逆方差加权法（inverse-variance weighted，IVW）、MR-Egger 法、加权中位数法（weighted median，WME）、简单众数法（simple mode，SM），以及加权众数法（weighted mode，WM）[16-17]。此外，使用 *OR* 及相应的 95%*CI* 评估肝癌风险的影响。在结果方面，当 IVW 的 $P<0.05$ 时，认为相关性是可信的。通过敏感性分析验证结果的稳健性，并使用 Cochrane's *Q* 检验测试异质性。此外，还利用 MR Egger 截距和 MR-PRESSO 方法对遗传多效性进行调查。

2　结　果

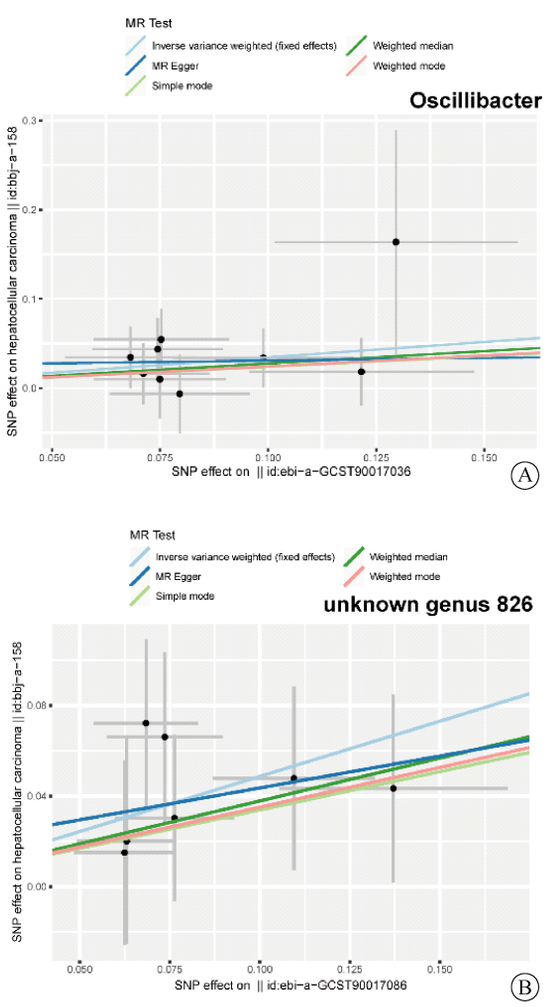
2.1　双样本 MR 分析　首先根据 $P<5\times10^{-5}$，以及连锁不平衡分析的阈值对 221 个肠道微生物的 SNP 进行初步筛选。随后，通过以 IVW、ME、WME、WMO、SM 方法，并以 IVW 方法为主作为筛选标准，鉴定到了 2 个与肝癌相关的肠道微生物，分别为颤螺菌属（*Oscillibacter*，id：ebi-a-GCST90017036）以及暂未命名的微生物（unknown genus 826，id：ebi-a-GCST90017086），暂命名为 826 微生物属。结果显示：与炎症相关的颤螺菌属（*OR*=1.411，95%*CI*=1.049～1.899，*P*=0.025），以及 826 微生物属（*OR*=1.627，95%*CI*=1.163～2.276，*P*=0.004）的丰度增加，均增加了肝癌的患病风险。具体的 IVW、ME、WME、WMO、SM 检验结果

见表 1。尽管除了 IVW 法外的 4 种方法均无统计学差异,但散点图 2 还展示了不同方法分析两种微生物和肝癌之间的相关性均为正,且趋势基本一致。

表 1　与肝癌具有显著因果关系的肠道微生物对应的五种 MR 方法

Tab. 1　The five MR methods corresponding to gut microbiota with significant causal relationships in liver cancer

暴露	方法	nSNP	Beta	*OR*	95%*CI*	*P* 值
颤螺菌属	IVW	9	0.345	1.411	1.049~1.899	0.025
	WME	9	0.276	1.317	0.890 ~1.950	0.167
	ME	9	0.059	1.061	0.251~4.478	0.937
	SM	9	0.238	1.269	0.740~1.269	0.410
	WMO	9	0.243	1.275	0.774~2.100	0.366
826 微生物属	IVW	7	0.486	1.627	1.163~2.276	0.004
	WME	7	0.378	1.459	0.936~2.274	0.094
	ME	7	0.090	1.094	0.341~3.507	0.885
	SM	7	0.338	1.402	0.749~2.626	0.330
	WMO	7	0.350	1.420	0.817~2.466	0.259

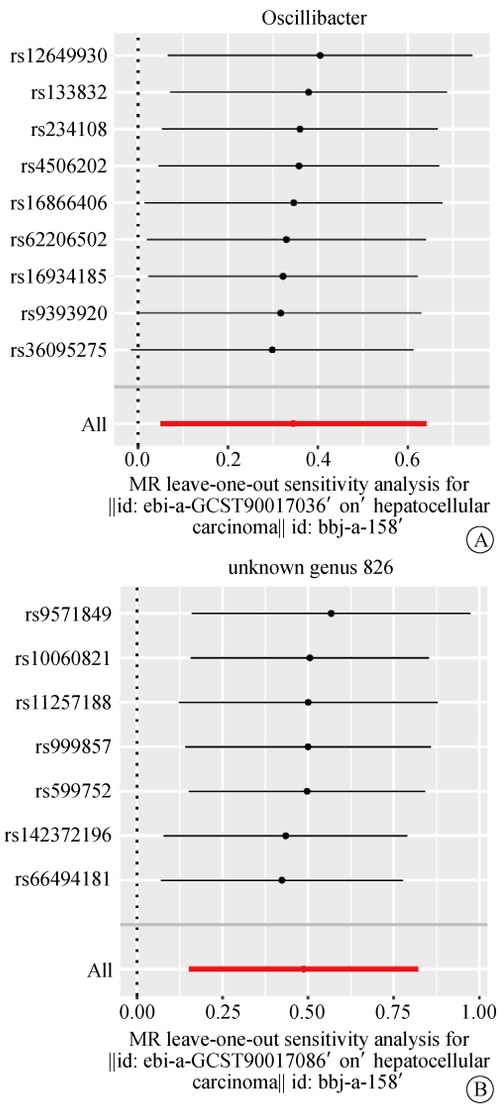


注:A 为颤螺菌属;B 为 826 微生物属。

图 2　与肝癌具有因果关系的肠道微生物的 5 种 MR 模型散点图

Fig. 2　The scatter plots for the five MR models representing gut microbiota with causal relationships to liver cancer

2.2　质量控制　首先通过留一分析法显示纳入的大部分工具变量的效应值与总效应值非常相近。见图 3。其次,异质性检验结果显示颤螺菌属(Q = 3.114, P = 0.926),826 微生物属(P = 0.887)。基因多效性结果显示颤螺菌属(P = 0.703),826 微生物属(P = 0.516)。异质性检验和基因多效性检验的结果均显示 P>0.05,差异无统计学意义,提示无需考虑异质性和基因多效性对结果的影响。MR-PRESSO 结果见表 2。



注:A 为颤螺菌属;B 为 826 微生物属。

图 3　与肝癌具有因果关系的肠道微生物的留一法图

Fig. 3　The leave-one-out plot for gut microbiota with causal relationships to liver cancer

表 2　与肝癌存在因果关系的肠道微生物的异质性、水平多效性测试和 MR-PRESSO 结果

Tab. 2　The heterogeneity, horizontal pleiotropy test, and MR-PRESSO results for gut microbiota with causal relationships to liver cancer

暴露	异质性		水平多效性	MR-PRESSO	
	Q 值	*P* 值	*P* 值	Raw	*P* 值
颤螺菌属	3.114	0.926	0.703	1.098（1.026~1.176）	0.006
826 微生物属	2.324	0.887	0.516	1.113（1.008~1.229）	0.032

3 讨　论

本研究利用公共的肠道微生物 GWAS 数据和肝癌 GWAS 数据,采用双样本 MR 方法,研究了 221 种肠道微生物相对丰度与肝癌之间的因果关系。研究结果显示,颤螺菌属和 826 微生物属的丰度与肝癌的发病呈正相关。IVW 结果提示,颤螺菌属和 826 微生物属的患者患病风险分别是正常人群肝癌的 1.411 倍和 1.627 倍。所以,对于肝癌患者,探讨肠道菌群对其病因、前期诊断、综合治疗具有一定的临床意义。

有研究表明,肠道微生物组的失调在慢性肝病的早期阶段即可观察到,如果不加以控制,将促进慢性肝病进展为肝癌[18]。微生物相关的分子模式(microbe-associated molecular patterns, MAMPs)是指在疾病进展过程中,肠道微生物组及其代谢产物是对肝脏的低水平暴露。当肠道微生物组失调时,MAMPs 水平会升高,通过结合脂多糖及其 Toll 样受体 4,以及促进 NF-κB 信号通路激活、释放促炎因子,如 TNF-α、IL-6、IL-1 等,进而导致肝脏发生炎症和氧化应激损害,甚至导致肝癌发生[19]。此外,失调的肠道微生物还通常与肿瘤免疫调节有关,一项队列研究纳入了 30 例肝癌患者,其研究结果显示以程序性细胞死亡蛋白-1 为代表的免疫治疗应答以及生存获益,与肠道微生物群的丰度相关[20]。因此,笔者推测一些肠道微生物组是否可以通过改变肠道屏障和调节机体免疫反应来直接或间接促进肝癌的发生。

本研究最终筛选出 2 个升高肝癌风险的肠道微生物,分别为颤螺菌属、826 微生物属。尽管 826 微生物属暂时未鉴定,但颤螺菌属在肠道中发挥着重要作用。例如颤螺菌属可以通过发酵葡萄糖等多糖类物质产生短链脂肪酸,并通过来自颤螺菌属的特定尾氨酸类脂质修饰树突状细胞,从而改变其免疫调节功能[21]。有研究认为健康组丰度较高的颤螺菌属可能是抑制结肠癌发生发展的潜在益生菌[22],本研究结论与之相反。一项包含 75 例早期肝癌和 75 例健康对照人群的前瞻性研究显示,颤螺菌属在早期肝癌患者中的丰度显著上升,并可作为早期肝癌的标志物[23]。鉴于颤螺菌属在结肠癌和肝癌发挥的截然不同的作用,笔者推测肠肝轴循环带来的肠道微生物可能还会受到肝癌微环境的影响,从而从一种益生菌变成促进肝癌发生的微生物。例如早期被认为属于对肠道有益的乳酸菌,在后续的研究中却发现乳酸菌能通过调控 cGAS-STING-IFN-I 信号通路来降低机体细胞毒性 T 淋巴细胞介导的适应性免疫应答,影响肝癌放疗疗效[24]。

但本研究也存在一些局限性:(1) 826 微生物还未鉴定,其功能还无法进行研究和讨论;(2) 后续还需要更多的队列研究来证实颤螺菌属和肝癌风险的因果关系;(3) GWAS 数据研究主要以欧洲人群为主,亚洲人群的研究较少,在菌群层面可能会有差异,对于结果的推广具有一定的局限性;(4) MR 分析初步探讨了颤螺菌属与肝癌的因果关系,具体的作用机制还有待探究。

综上所述,本研究利用肝癌作为暴露因素,选择显著相关的微生物菌群 SNPs 作为工具变量,且敏感性分析发现无多效性、异质性,结果发现颤螺菌属可以促进肝癌的发生风险。通过这种方法能确定两者的因果关系,为后续的功能研究提供候选肠道微生物,并排除了双向因果关系和选择性偏倚的可能性。

利益冲突　无

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