Editorial Commentary

Associations of the gut microbiome and metabolites on biliary tract cancer: insight from a bidirectional two-sample Mendelian randomization study

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Biliary tract cancer (BTC) seriously endangers human health. Recently, with the increasing development of therapeutic strategies, exploration of novel targeted and prognostic biomarkers has become a promising field of study (1). Newly developed biomarker signatures by Zhu *et al.* at the *Biomarker Research* significantly provide the potential prognostic and predictive values of the gut microbiome and metabolites for clinical outcomes in the management of patients with BTC (2). However, causal relationships remain unclear because of confounding factors such as age, environment, dietary patterns, and lifestyle, and it is difficult to effectively control these factors, which limits the causal inference between the gut microbiota and metabolites and BTC.

Randomized controlled trials (RCTs) are considered the best approach for establishing a causal relationship between the gut microbiota and metabolites and BTC. However, it faces many difficulties in implementation for determining the causal associations on BTC. Mendelian randomization (MR) analysis is an epidemiological strategy that utilizes genetic variants to investigate the causal relationships between exposures and BTC. This method has been widely applied to reveal causal inferences in order to avoid confounding and reverse causal biases originating from small sample sizes and cross-sectional designs (3). Herein, we conducted MR analyses based on publicly available genome-wide association study (GWAS) summary data to evaluate the causal associations among the gut microbiome, metabolites, and BTC.

First, comprehensive summary statistics of the gut microbiome in humans were retrieved from the NHGRI-EBI GWAS Catalog, in which 5,959 individuals enrolled in the FINRISK 2002 cohort were included (4). Summary statistics of circulating metabolites were acquired from GWAS analysis of 33 cohorts and 136,016 participants, which quantified 233 metabolites and 13,389,637 single nucleotide polymorphisms (SNPs) (5). GWAS summary data for BTC were retrieved from a large-scale GWAS or the corresponding meta-analyses. For BTC, 339 cases and 195,745 controls were enrolled in the study. All datasets were freely accessed from the IEU Open GWAS Project (https://gwas.mrcieu.ac.uk). As detailed information about the participants was not collected, ethical approval was not required for this study. Detailed dataset information is provided in table available at https://cdn.amegroups.cn/static/public/hbsn-2024-750-1.xlsx.

Second, various methods, including inverse variance weighted (IVW), maximum likelihood (ML), MR-Egger regression, weighted median, weighted model, and MR pleiotropy residual sum and outlier (MR-PRESSO), were

used to examine causal associations. Cochran's *Q* test was used to assess the heterogeneity among the instrumental variables (IVs), and P value <0.05 represented the existence of heterogeneity, and the random-effects IVW method was applied. A sensitivity analysis was performed to assess the robustness of causality. MR-Egger regression and MR-PRESSO were used to assess horizontal pleiotropy (6). The strength of the selected SNPs was evaluated using the F-statistic, where SNPs with an F-statistic <10 were excluded to avoid weak instrument bias in the MR analysis. All statistical analyses in this study were conducted using R software (version 4.3.3).

Finally, using two-sample MR, we identified 13 suggestive associations between gut microbiome and BTC. Significantly protective effects of *Desulfobacterota A* [odds ratio (OR) =0.37], *Desulfovibrionia* (OR =0.46), *Elusimicrobia* (OR =0.08), *ER4 sp002437735* (OR =0.36), *Geobacter C* (OR =0.04), *Prevotella bivia* (OR =0.44) against BTC were detected (all P<0.05). Additionally, *Clostridium E sporosphaeroides* (OR =2.85), *Erysipelatoclostridiaceae* (OR =3.14), *Johnsonella ignava* (OR =69.25), *Mycobacteriaceae* (OR =145.01), *Negativibacillus massiliensis* (OR =5.22), *RUG147 sp900315495* (OR =24.92), *Tepidanaerobacteraceae* (OR =13.66) revealed a causal association with increased risk of BTC (all P<0.05, Figure S1 and Table S1).

The IVW results revealed 37 associations between circulating metabolites and BTC. The protective effects against BTC were identified in the acetate levels (OR =0.25), conjugated linoleic acid (OR =0.34), ratio of conjugated linoleic acid to total fatty acids (OR =0.28), estimated description of fatty acid chain length not actual carbon number (OR =0.42), glycoprotein acetylation (OR =0.52), isoleucine levels (OR =0.40), free cholesterol in large very low-density lipoprotein (VLDL) (OR =0.63), total lipids in large VLDL (OR =0.68), phospholipids in large VLDL (OR =0.63), free cholesterol to total lipids ratio in small high-density lipoprotein (HDL) (OR =0.52), triglycerides to total lipids ratio in small VLDL (OR =0.62), total cholesterol levels in VLDL (OR =0.64), mean diameter of VLDL particles (OR =0.63), cholesteryl esters to total lipids ratio in very large HDL (OR =0.53), free cholesterol in very large VLDL (OR =0.62), total lipids in very large VLDL (OR =0.59), concentration of very large VLDL particles (OR =0.60), phospholipids in very large VLDL (OR =0.62), triglycerides in very large VLDL (OR =0.57), triglycerides to total lipids ratio in very small VLDL (OR =0.64), free cholesterol levels in chylomicrons and extremely large VLDL (OR =0.63), free cholesterol to total lipids ratio in chylomicrons and extremely large VLDL (OR =0.43), total lipid levels in chylomicrons and extremely large VLDL (OR =0.65), concentration of chylomicrons and extremely large VLDL particles (OR =0.63), total cholesterol in very large VLDL (OR =0.59), Phospholipid levels in chylomicrons and extremely large VLDL (OR =0.58) (all P<0.05). Additionally, free cholesterol to total lipids ratio in IDL (OR =1.89), total cholesterol to total lipids ratio in large LDL (OR =1.50), phospholipids to total lipids ratio in medium VLDL (OR =1.92), total cholesterol to total lipids ratio in small HDL (OR =1.77), cholesterol esters in small HDL (OR =1.97), total cholesterol to total lipids ratio in small VLDL (OR =1.90), cholesteryl esters to total lipids ratio in small VLDL (OR =1.68), phospholipids to total lipids ratio in very large HDL (OR =1.73), total cholesterol to total lipids ratio in very large VLDL (OR =2.15), free cholesterol to total lipids ratio in very small VLDL (OR =3.55), phospholipids to total lipids ratio in very small VLDL (OR =1.89) revealed a causal association with increased risk of BTC (all P<0.05, Figure S2 and Table S2). Sensitivity analyses further indicated the absence of heterogeneity and horizontal pleiotropy in these MR analyses (Tables S3,S4).

In conclusion, the research by Zhu and colleagues stands as a substantial contribution to our understanding of immunotherapy-related changes in the gut microbiome and metabolites in patients with BTC. They demonstrated gut microbiomes, and metabolites have potential as prognostic and predictive biomarkers. Our findings support the causal associations of the gut microbiome and circulating metabolites on BTC development. Our findings only serve to improve an already excellent work of research. These indicators provide new insights into the mechanisms underlying BTC and contribute to its prevention, diagnosis, and individualized treatment.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://hbsn.amegroups.com/article/view/10.21037/hbsn-2024-750/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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