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Causality of blood metabolites on hepatocellular carcinoma and cholangiocarcinoma: a metabolome-wide mendelian randomization study

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Abstract

Background Reportedly, there is an association between body metabolites and the risk of Hepatocellular Carcinoma (HCC) & Cholangiocarcinoma (CCA), possibly due to disrupted metabolic pathways leading to oxidative stress and an imbalance in cell proliferation and apoptosis, thereby increasing the risk of cancer. However, whether metabolites play a role in the onset of HCC or CCA remains inconclusive.

Objective The aim of our study is to explore the potential causal relationship between metabolites and the risk of HCC&CCA.

Methods Our study investigated the causal relationship between 1400 metabolites and HCC&CCA using publicly available genome-wide association study data. Single nucleotide polymorphisms (SNPs) associated with both metabolites and HCC&CCA were chosen as instrumental variables (IVs). The main approaches employed include inverse variance weighted (IVW), MR-Egger regression, and weighted median estimator (WME), with odds ratios (OR) used as the assessment criterion. Heterogeneity testing and sensitivity analyses were conducted to validate the results. We also conducted a reverse MR analysis to further validate the relationship between exposure and disease outcomes.

Results This Mendelian Randomization (MR) study indicates a significant causal relationship between 19 metabolites and the risk of HCC&CCA. Among them, the risk factors include "Bilirubin (E, Z or Z, E) levels,""Bilirubin (Z, Z) to taurocholate ratio,""Dimethylarginine (sdma + adma) levels,""N-methyltaurine levels,""4-vinylguaiacol sulfate levels," "Cholate to adenosine 3',5'-cyclic monophosphate (cAMP) ratio,""Glycohyocholate levels,""Cholesterol levels," and "4-methylguaiacol sulfate levels." The incidence risk of HCC and CCA increases with the elevation of these metabolites. Protective factors include "Ursodeoxycholate levels,""3-hydroxybutyroylglycine levels,""Linoleoylcholine levels," "Nonanoylcarnitine (C9) levels,""Pristanate levels,""Heptenedioate (C7:1-DC) levels,""Mannonate levels,""N-acetyl-L-glutamine levels,""Sphinganine levels," and "N-lactoyl isoleucine levels." The incidence risk of HCC and CCA potentially

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decreases as the levels of these metabolites increase. Heterogeneity tests show that most instrumental variables do not exhibit inter-gene heterogeneity, and the possibility of pleiotropy in the analysis is very low according to the sensitivity analysis. The reverse MR analysis did not yield positive results.

Conclusion Our study has unveiled the intricate causal relationships between metabolites and the risk of HCC&CCA. Through our analysis, we identified nine metabolites, including "Bilirubin (E, Z or Z, E) levels,""Dimethylarginine (sdma + adma) levels,""Cholesterol levels,"ect, as risk factors for HCC&CCA. The incidence risk of HCC and CCA increases with their elevation. On the other hand, ten metabolites, such as "Ursodeoxycholate levels,""Linoleoylcholine levels," "Pristanate levels," ect, were identified as protective factors for HCC&CCA. The risk of developing HCC and CCA decreases with an increase in these metabolites. In conclusion, these findings further explore the physiological metabolic pathways underlying the pathogenesis of HCC and CCA, emphasizing future research directions. They pave the way for researchers to delve into the biological mechanisms of these diseases, facilitating early intervention and treatment strategies for these conditions.

Keywords Hepatocellular carcinoma, Cholangiocarcinoma, Metabolites, Mendelian randomization, Causal relationship

Introduction

Hepatocellular carcinoma (HCC) is the eighth most common cancer globally and the third leading cause of cancer-related deaths [1, 2]. HCC constitutes 80% of all liver cancers. As of 2019, there were approximately 747,000 cases of HCC worldwide [3]. Cholangiocarcinoma (CCA), although relatively rare, is experiencing a gradual increase in incidence and mortality globally. The incidence and mortality rates are particularly high in East Asia. The five-year survival rates for both types of hepatobiliary tumors are below 20% [4]. The intricate interplay of genetic factors, environmental influences, and chronic liver diseases collectively contributes to the development of both HCC and CCA [5].

HCC and CCA are characterized by high malignancy and poor prognosis, and they are challenging to diagnose in the early stages [6]. Timely detection and precise diagnosis play a pivotal role in facilitating effective treatment and enhancing outcomes for individuals with liver cancer. A comprehensive understanding of the molecular mechanisms and risk factors linked to HCC and CCA is imperative for the development of targeted therapeutic interventions and the progression of personalized medicine strategies for those affected by these hepatic malignancies.

The liver, a central metabolic organ, plays a crucial role in diverse metabolic processes encompassing lipid, carbohydrate, and protein metabolism [7]. Metabolomics research has brought forth numerous advantages in the diagnosis and treatment of hepatobiliary cancers. By extensively analyzing metabolic products within the body, metabolomics facilitates the identification of biomolecules associated with hepatobiliary cancers. Furthermore, through the analysis of metabolic pathways, it unveils potential therapeutic targets for the diseases. This has profound implications for early diagnosis, disease monitoring, and prognosis assessment of hepatobiliary tumors [8, 9].

Clinical data on metabolomics and Hepatocellular Carcinoma (HCC) and Cholangiocarcinoma (CCA) reveal that researchers have identified specific metabolites within the bodies of liver cancer patients [10]. These metabolites contribute to the early monitoring and assessment of the disease. Studies have also unveiled changes in metabolic pathways involved in the occurrence and development of liver cancer, including alterations in glucose metabolism, lipid metabolism, and amino acid metabolism [11]. Furthermore, research indicates that an increase in alanine levels and abnormalities in its metabolic pathway may be associated with an elevated risk of liver cancer [12]. Metabolic abnormalities related to bilirubin are also linked to liver function and biliary diseases, as the resulting physiological and metabolic changes ultimately increase the risk of hepatobiliary tumors [13]. Certain lipid metabolites, such as phospholipids and cholesterol, are also associated with an increased risk of liver cancer [14]. Cholesterol levels are associated with chronic inflammation and oxidative stress, factors implicated in the occurrence and development of cancer [15]. Studies on the correlation between metabolites and the tumor microenvironment suggest that the interaction between metabolites and the tumor microenvironment influences the biological characteristics and clinical behavior of tumors [16, 17]. In summary, metabolic dysregulation can increase the risk of developing HCC and CCA. Similarly, patients with HCC or CCA exhibit abnormalities in organismal metabolism [18]. Consequently, it is plausible to posit that certain etiological factors contributing to HCC and CCA involve intricate interactions between tumors and metabolites.

Mendelian Randomization (MR) is an epidemiological approach for causal inference analysis based on the principles of Mendelian genetics [19, 20]. Previous

observational studies have identified numerous associations between systemic metabolites and HCC and CCA, suggesting a hypothesis of their correlation. In this study, we conducted a comprehensive MR analysis to establish causal relationships between 1400 systemic metabolites and HCC&CCA.

Materials and methods

Study design

In our study, we utilized publicly accessible, large-sample genome wide association study(GWAS) databases [21]. We considered 1400 metabolites as exposure factors and HCC (hepatocellular carcinoma) & CCA (cholangiocarcinoma) as outcome factors. Employing a two-sample Mendelian randomization (MR) analysis method [19], we iterated the analysis 1400 times and performed False Discovery Rate (FDR) correction on the results [22]. A comprehensive examination was conducted to analyze the causal relationships between the 1400 metabolites and the occurrence of HCC and CCA.

Additionally, Cochran Q test was employed to assess the heterogeneity of instrumental variables [23]. Sensitivity analysis was conducted to validate the reliability of our research findings regarding causal relationships [24]. In the two-sample MR analysis, the association between SNP-exposure and SNP-outcome originated from different research sources and was combined in a ratio format to estimate the impact of exposure on the outcomes [25]. Therefore, MR analysis must adhere to three core assumptions [26]. Our study model is shown in Fig. 1.

Data sources

The HCC&CCA data utilized in our research originates from the IEU Open GWAS database website (https://gwas.mrcieu.ac.uk/), with the GWAS ID: ieu-b-4915. These data were collected from the European population, comprising 372,366 samples and encompassing 7,687,713 single nucleotide polymorphisms (SNPs). The GWAS summary statistics data associated with each metabolite is publicly accessible through the GWAS Catalog, with login IDs ranging from GCST90199621 to GCST90201020. We can access this information on the following website: [https://www.ebi.ac.uk/gwas/homehttps://www.ebi.ac.uk/gwas/home [27]. In our comprehensive analysis, we incorporated data for a total of 1400 distinct metabolites, encompassing 1091 blood metabolites and 309 metabolite ratios [28, 29]. Among the 1,091 plasma metabolites scrutinized, 850 were categorized across eight super pathways, specifically lipid, amino acid, xenobiotics, nucleotide, cofactor and vitamins, carbohydrate, peptide, and energy. The remaining 241 were either labeled as unknown or categorized as "partially" characterized molecules, all while retaining the intended meaning [30]. All datasets used in this study were from the public domain.

Selection of instrumental variables (IVs)

To better capture potential factors related to our research objectives, and considering the specific characteristics of the data as well as insights from preliminary exploratory analysis [31–33], we carefully decided to use a p-value threshold of less than 1×10^{-5} for selecting instrumental variables. This approach ensures that we maintain a valid set of instrumental variables within a reasonable range

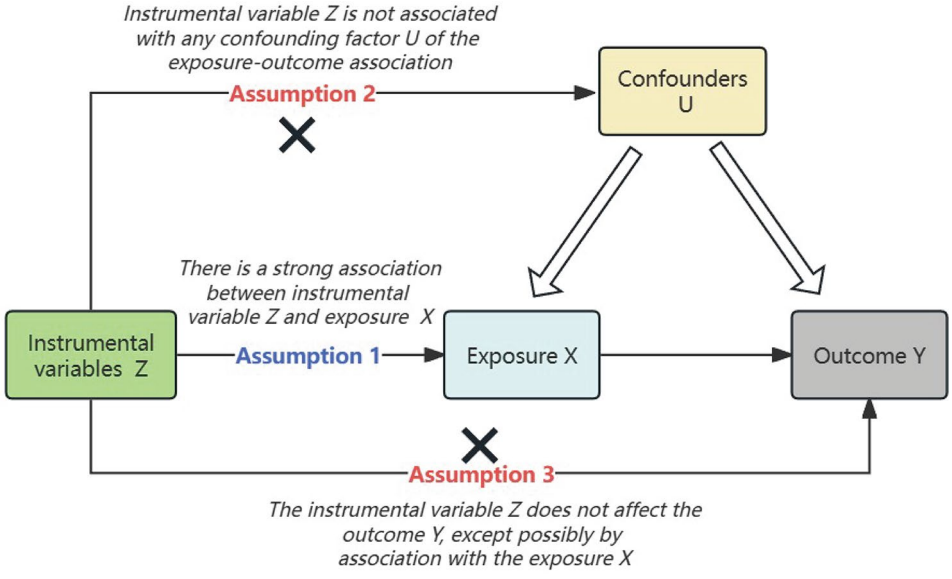

Fig. 1 Model of the two-samples MR analysis

while effectively meeting the needs of our study [34, 35]. Linkage disequilibrium parameters were set to R^2=0.001 and a window size of 10,000 kb [36]. Subsequently, we extracted data on these SNPs from the HCC&CCA composite GWAS database. For HCC&CCA, we applied a more stringent threshold by setting the significance level to 5×10^{-8}. When necessary, we queried SNP information from the PhenoScanner website (http://www.phenoscanner.medschl.cam.ac.uk/) to mitigate confounding f actors. We computed the F-statistic for each instrumental variable to assess its strength, excluding those with low *F*-statistics (<10) to reduce bias from weak instrumental variables. The F-statistic is calculated as follows: $F = \frac{N-k-1}{k} \times \frac{R^2}{1-R^2}$, where N is the sample size of the exposed database, k is the number of SNPs, and R^2 is the proportion of variance explained by SNPs in the exposed database. R^2 is calculated as $R^2 = \frac{2 \times EAF \times (1-EAF) \times \beta^2}{SD^2}$, where EAF is the effect allele frequency, β is the allele effect value, and SD is the standard deviation [23].

Statistical analysis, heterogeneity, and pleiotropy testing for mendelian randomization

In our study, we utilized tools such as the R program (version 4.2.1) and the Two-Sample MR package (version 0.5.6) to integrate and assess data. To evaluate the potential causal relationship between metabolites and the risk of HCC and CCA, we employed robust Mendelian randomization (MR) analysis methods based on different assumptions to estimate the causal relationship between exposure and outcome. In this research, the primary analytical approach was the Inverse Variance Weighted (IVW) method [37]. Additionally, we employed MR-Egger regression [38], Weighted Median Estimator (WME) [39], simple mode, and weighted mode [40] as supplementary methods for MR analysis. We collectively applied these analytical methods to assess the causal relationship between 1400 metabolites and HCC&CCA. Furthermore, we conducted comprehensive sensitivity analyses to eliminate scenarios that could potentially violate the core assumptions of MR.

Next, we performed an analysis to assess heterogeneity. This assessment was designed to identify variations among IVs [41]. Cochran's Q statistic and its associated P-value were utilized to ascertain the existence of heterogeneity [42]. The primary purpose of the pleiotropy test was to identify horizontal pleiotropy among multiple instrumental variables (IVs). In our investigation, we employed the P-value derived from the pleiotropy test to evaluate the presence of pleiotropy in our analysis [43]. For a more in-depth sensitivity assessment, we conducted leave-one-out sensitivity tests, systematically excluding individual IVs one at a time and recalculating MR results. Furthermore, in this study, we utilized the MR-pleiotropy residual sum outlier (MR-PRESSO) to detect and manage potential pleiotropy in our analysis [44].

Finally, to investigate the causal relationship between HCC and CCA and metabolites, we employed IVs generated from the summary statistics of HCC&CCA Genome-Wide Association Studies (GWAS), conducting reverse MR analysis.

Results

Causal relationship between metabolites and HCC&CCA

To investigate the causal associations between metabolites and the occurrence of Hepatocellular Carcinoma (HCC) and Cholangiocarcinoma (CCA), we conducted a two-sample MR analysis, with the Inverse Variance Weighted (IVW) method as the primary analytical approach. Ultimately, utilizing the computational methods mentioned earlier, we identified a total of 19 metabolites or metabolite ratios from among 1400 metabolites that exhibit a causal relationship with HCC&CCA.

The odds ratios (ORs) calculated through the IVW method enabled us to determine that metabolites such as "Bilirubin (E, Z or Z, E) levels", "Bilirubin (Z, Z) to taurocholate ratio", "Dimethylarginine (sdma + adma) levels", "N-methyltaurine levels", "4-vinylguaiacol sulfate levels", "Cholate to adenosine 3',5'-cyclic monophosphate (cAMP) ratio", "Glycohyocholate levels", "Cholesterol levels", and "4-methylguaiacol sulfate levels" act as risk factors for HCC and CCA. The incidence risk of HCC and CCA increases with the elevation of these metabolites. Conversely, "Ursodeoxycholate levels", "3-hydroxybutyroylglycine levels", "Linoleoylcholine levels", "Nonanoylcarnitine (C9) levels", "Pristanate levels", "Heptenedioate (C7:1-DC) levels", "Mannonate levels", "N-acetyl-L-glutamine levels", "Sphinganine levels", and "N-lactoyl isoleucine levels", were identified as protective factors for HCC and CCA. The incidence risk of HCC and CCA potentially decreases as the levels of these metabolites increase.

Detailed results of the IVW method are presented in Fig. 2, while the analysis outcomes of the other four methods are available in the supplementary materials following the main text.

Results of heterogeneity and pleiotropy tests

The Cochran's Q test for the IVW and MR-Egger methods indicated that the majority of SNPs did not exhibit intergenic heterogeneity ($P > 0.05$). However, occasional instances of heterogeneity were unavoidable due to the large sample size. Consequently, a small number of instrumental variables for certain metabolites showed evidence of intergenic heterogeneity. The pleiotropy tests suggested a low likelihood of pleiotropy in causal analysis ($P > 0.05$). The results of MR-PRESSO showed that no horizontal pleiotropy was detected ($P > 0.05$). For more detailed results, please refer to Table 1.

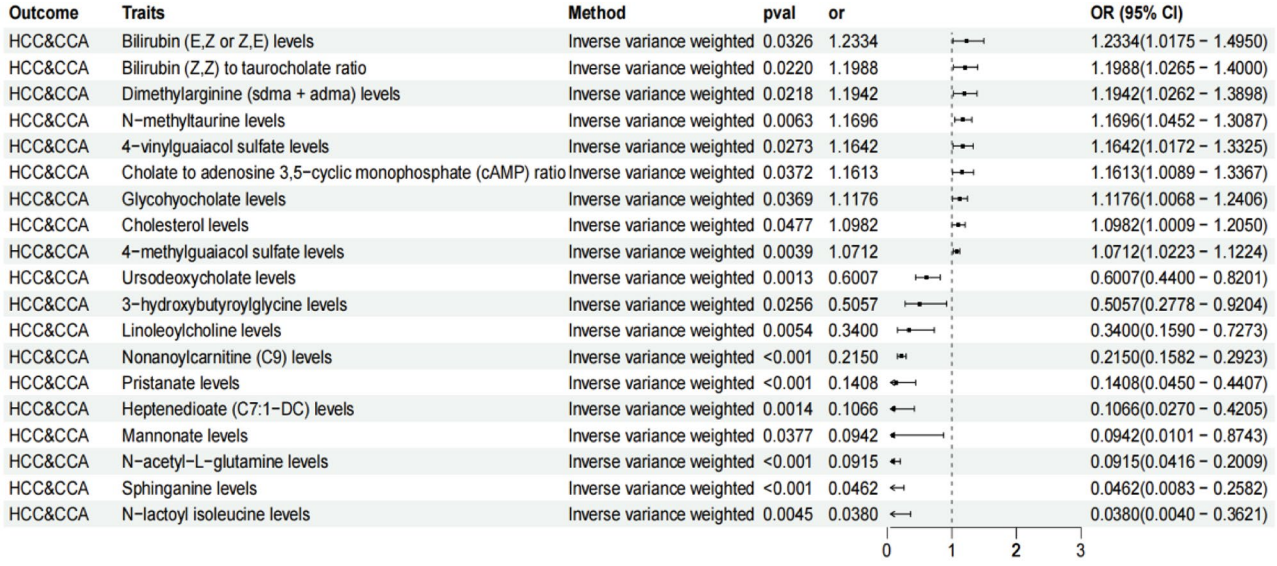

Fig. 2 Forest plot illustrating the causal associations between 19 metabolites and HCC&CCA. Abbreviations: OR, Odds ratio; CI, confidence interval

Table 1 Results of heterogeneity and pleiotropy tests

Trait	GWAS ID	*P* of Cochran Q		*P* of pleiotropy	*P* of MR-PRESSO
		MR Egger	IVW		
Bilirubin (E, Z or Z, E) levels	GCST90200687	0.4337	0.5590	0.7338	0.0627
Bilirubin (Z, Z) to taurocholate ratio	GCST90200829	0.8497	0.8758	0.5341	0.1379
Dimethylarginine (sdma + adma) levels	GCST90199832	< 0.05	0.1175	0.7392	0.2126
N-methyltaurine levels	GCST90199924	0.5374	0.5850	0.5149	0.0733
4-vinylguaiacol sulfate levels	GCST90199982	0.9524	0.9675	0.7611	0.1986
Cholate to adenosine 3',5'-cyclic monophosphate (cAMP) ratio	GCST90200830	0.4982	0.5058	0.3648	0.3742
Glycohyocholate levels	GCST90199935	0.3513	0.3444	0.3214	0.2587
Cholesterol levels	GCST90200368	0.8251	0.8811	0.9847	0.0553
4-methylguaiacol sulfate levels	GCST90199989	0.9272	0.9512	0.9426	0.0873
Ursodeoxycholate levels	GCST90200297	0.9776	0.9930	0.8521	0.0926
3-hydroxybutyroylglycine levels	GCST90200174	0.9301	0.7832	0.6193	0.4591
Linoleoylcholine levels	GCST90200130	0.9369	0.9440	0.8691	0.0592
Nonanoylcarnitine (C9) levels	GCST90200031	0.3828	< 0.05	0.2002	0.0782
Pristanate levels	GCST90200409	0.7851	0.1342	0.1306	0.6967
Heptenedioate (C7:1-DC) levels	GCST90200163	0.1540	< 0.05	0.2612	0.0683
Mannonate levels	GCST90199932	0.9519	0.9040	0.5646	0.0864
N-acetyl-L-glutamine levels	GCST90200676	0.8133	0.8735	0.6467	0.0762
Sphinganine levels	GCST90200374	< 0.05	< 0.05	0.2916	0.0978
N-lactoyl isoleucine levels	GCST90200197	0.6065	< 0.05	0.4090	0.2713

Abbreviations: IVW: inverse variance weighted

To enhance the robustness of the results and visualize the outcomes, we additionally created "Leave-one-out" sensitivity analysis plots, scatter plots, and funnel plots. The above content further confirmed the stability of the research findings. The aforementioned parts are detailed in the Supplementary materials.

Reverse mendelian randomization findings

To further investigate the causal relationship between HCC & CCA and metabolites, we treated HCC & CCA as the exposure factor and conducted a comprehensive reverse Mendelian Randomization (MR) analysis, considering each of the 1400 metabolites as outcome factors. Unfortunately, the reverse MR analysis did not yield statistically significant results as we had hoped. This issue is expected to be further explored in future research.

Discussion

Leveraging a substantial dataset of publicly available genetic information, we investigated the causal relationships between 1,091 blood metabolites, 309 metabolite ratios, and hepatocellular carcinoma (HCC) &

cholangiocarcinoma (CCA). This study may be the first to employ Mendelian Randomization (MR) analysis to explore the causal links between such a wide array of metabolites and hepatobiliary tumors. Overall, we identified 19 metabolites or metabolite ratios with causal associations to HCC & CCA. Of these, 9 were identified as risk factors and 10 as protective factors.

In our tests for pleiotropy, heterogeneity, and MR-PRESSO, we found no significant evidence of horizontal pleiotropy. However, we did observe some instances of heterogeneity. In large-scale studies, heterogeneity is common and often unavoidable due to differences in the effects of instrumental variables, natural genetic variation, environmental influences, and multiple underlying mechanisms. Despite this, as long as there is no undesirable horizontal pleiotropy (where SNPs directly affect the outcome rather than through the exposure), Mendelian randomization analysis can still provide valid causal inferences. By employing appropriate statistical methods and sensitivity analyses, we can obtain robust and reliable results even in the presence of heterogeneity. In the following sections, I will focus on discussing several common metabolites or those with controversial relationships with hepatobiliary tumors.

Our study reveals that bilirubin levels serve as a risk factor for HCC&CCA. Current literature also supports this viewpoint. Conditions such as chronic liver disease or bile duct obstruction can lead to elevated bilirubin levels. The oxidative damage and inflammatory responses resulting from this elevation may trigger abnormal apoptosis and proliferation of liver cells, laying the foundation for tumor formation and increasing the risk of developing hepatobiliary tumors [45, 46]. However, some scholars have pointed out that elevated bilirubin levels may serve as markers for liver disease but might not necessarily have a direct causal relationship with hepatobiliary tumors. The association between the two could be influenced by various factors, and a more in-depth study is still required for a comprehensive understanding [13].

Regarding cholesterol levels, our study indicates that it is a risk factor for HCC & CCA, consistent with some current clinical research findings. Some studies suggest that elevated cholesterol levels may be associated with an increased risk of liver cancer. This association could be attributed to the link between high cholesterol levels and liver diseases such as non-alcoholic fatty liver disease (NAFLD) and cirrhosis, which are precursors to liver cancer. Additionally, high cholesterol levels may be related to chronic inflammation and oxidative stress, both of which are implicated in the occurrence and development of cancer. On the other hand, there are studies proposing that low cholesterol levels may be associated with the severity of chronic liver disease, malnutrition, and a reduced capacity of the liver to synthesize bile acids, potentially contributing to an increased risk of liver cancer [15]. Animal experiments have also confirmed that elevated cholesterol levels can enhance the anti-tumor function of natural killer cells, reducing the growth of liver tumors [47]. It is evident that the relationship between cholesterol and hepatobiliary tumors is not consistent across studies and depends on various factors, including the specific numerical values of cholesterol levels, individual health conditions, lifestyle factors, etc. Further in-depth research is needed to gain a comprehensive understanding of this complex relationship.

Several other risk factors identified in our study in relation to hepatobiliary tumors currently lack substantial clinical and foundational research. The variations in "Bilirubin (Z, Z) to taurocholate ratio" may reflect the metabolism and functional status of the hepatobiliary system [48], but their precise association with hepatobiliary tumors has yet to be clearly studied. Similarly, "Dimethylarginine (sdma + adma) levels" is associated with various physiological processes, including vascular function, inflammation, and metabolic disorders [49], but evidence for its causal relationship with hepatobiliary tumors is currently lacking. "N-methyltaurine levels", as a metabolite of bile acids, lacks clear research results regarding its causal relationship with hepatobiliary tumors, and further investigation is needed to understand its specific physiological role in the body. Concerning "4-vinylguaiacol sulfate levels", "Cholate to adenosine 3',5'-cyclic monophosphate (cAMP) ratio", and "Glycohyocholate levels", each involves different metabolites or cellular signals, but their specific relationships with hepatobiliary tumors have yet to be conclusively studied. As for "4-methylguaiacol sulfate levels", there is currently no research on its relationship with hepatobiliary tumors, and thus, the specific impact of "4-methylguaiacol sulfate levels" remains unknown. Overall, a deeper understanding of the precise roles and associations of these metabolites in the development of hepatobiliary tumors requires further clinical and experimental research.

Regarding the protective factors identified in our study, there is considerable clinical and foundational research on the levels of ursodeoxycholic acid (UDCA), and the following studies support the findings we obtained. Research suggests that UDCA, as a bile acid, may have protective effects on certain liver diseases, particularly primary biliary cholangitis (PBC) and non-alcoholic fatty liver disease (NAFLD), contributing to improved liver function and reduced inflammation [50, 51]. Early studies have also indicated a potential preventive role of UDCA in liver cancer, especially among patients with cirrhosis [52, 53]. However, the exact impact of UDCA on hepatobiliary tumors is complex and requires further in-depth research for a comprehensive understanding.

Regarding the levels of pristanate, there is partial support for our findings from existing research. Some studies suggest that pristanate may play a regulatory role in preventing liver diseases such as fatty liver, potentially reducing the risk of liver cancer [54]. However, contrasting views have been proposed; for instance, some research indicates that an increase in pristanate levels may be correlated with an elevated risk of liver cancer. This association could be attributed to the relationship between pristanate metabolism and liver function [55], where abnormalities in certain metabolic pathways may be linked to the occurrence of hepatobiliary tumors [56, 57].The relationship between pristanate and hepatobiliary tumors is currently inconclusive, and existing evidence is insufficient to precisely define the role of pristanate in the development of hepatobiliary tumors.

We consider "Nonanoylcarnitine (C9) levels" to be a protective factor for HCC&CCA. It is a metabolite of fatty acid metabolism and represents a form of fatty acyl carnitine that aids in the β-oxidation of fatty acids within the mitochondria. Its levels may be influenced by the fatty acid metabolic pathways [58], and in certain conditions, abnormalities in fatty acid metabolism may be associated with the occurrence and development of hepatobiliary tumors. However, there is currently a lack of definitive research results on this matter.

Our research has identified additional protective factors for HCC&CCA. "3-hydroxybutyroylglycine levels" are associated with ketone body metabolism and may serve as an indicator of metabolic status in the context of metabolomics and related studies. "Linoleoylcholine levels" represent a fatty acid glycine, potentially influencing liver lipid metabolism, inflammatory responses, and other physiological processes. "Heptenedioate (C7:1-DC) levels" are a product of fatty acid metabolism [59]. "N-acetyl-L-glutamine levels" are a derivative of glutamate, participating in various biological processes [60]. "Sphinganine levels", a crucial lipid molecule in the body, are involved in regulating cell signaling, survival, and other biological processes [61]. While research on the levels of "Mannonate", associated with multiple biological processes and metabolic pathways, lacks clarity regarding its relationship with liver diseases, including hepatobiliary tumors. Similarly, research on "N-lactoyl isoleucine levels", this particular metabolite, is relatively scarce. In summary, specialized studies are required to validate and confirm the precise roles and associations of these metabolites in the development of hepatobiliary tumors.

In summary, the relationship between metabolites and hepatobiliary tumors is a complex and multifaceted research area. Some metabolites are considered risk factors for hepatobiliary tumors, including certain fatty acids, bile acids, and amino acids. Conversely, an increase in the levels of certain metabolites may be associated with a reduced risk of hepatobiliary tumors, such as specific bile acids and antioxidants. Studying the relationship between metabolites and hepatobiliary tumors contributes to a deeper understanding of the pathogenesis of hepatobiliary tumors, identification of potential biomarkers, and the exploration of new directions for the prevention and treatment of hepatobiliary tumors. However, there are still many mysteries in this field, and further clinical and basic research is needed to unravel the exact relationship between metabolites and hepatobiliary tumors.

This study employs the Mendelian Randomization (MR) analysis method to investigate the causal relationship between metabolites and HCC&CCA. The dataset used is large, publicly available, and adopts the MR approach for robust analysis. In this study, Instrumental Variables (IVs) selection is detailed, comprehensive, and reliable, with careful consideration and exclusion of SNPs that could potentially impact the outcomes. However, the study has limitations, particularly in elucidating the biological mechanisms of the causal effects between exposure and outcome. In conclusion, through a thorough MR analysis, we have identified several causal relationships between metabolites and HCC&CCA. This may open new avenues for researchers to explore the biological mechanisms of HCC and CCA, contributing to early intervention and treatment strategies for these diseases.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-13690-3.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Acknowledgements

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Author contributions

Study conception and design: L N, ZH G, JG S; Analyses: L N, ZH G, D C, J H, GY X; Draft: L N, ZH G; Supervision: JG S; Interpretation of results, critical editing, and manuscript approval: all authors. L N and ZH G have equal contributions to this manuscript and are co-first authors.

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Data availability

All data is from the public domain, The datasets [Exposure/Outcome] for this study can be found in the [IEU Open GWAS] [https://gwas.mrcieu.ac.uk/] and [GWAS Catalog] [https://www.ebi.ac.uk/gwas/home].

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

STROBE-MR checklist
I ensure that my manuscript complies with the STROBE-MR guidelines for reporting Mendelian randomization studies (https://www.strobe-mr.org/), and I have included the complete STROBE-MR checklist in the supplementary files.

Competing interests
The authors declare no competing interests.

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