HHS Public Access

Author manuscript

Int J Cancer. Author manuscript; available in PMC 2021 November 15.

Published in final edited form as:

Int J Cancer. 2020 November 15; 147(10): 2743–2753. doi:10.1002/ijc.33051.

Prediagnostic Concentrations of Circulating Bile Acids and Hepatocellular Carcinoma Risk: REVEAL-HBV and HCV Studies

Jessica L. Petrick[1,2], **Andrea A. Florio**[1], **Jill Koshiol**[1], **Ruth M. Pfeiffer**[1], **Baiyu Yang**[1], **Kelly Yu**[1], **Chien-Jen Chen**[3], **Hwai-I Yang**[3], **Mei-Hsuan Lee**[4], **Katherine A. McGlynn**[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA

[2]Slone Epidemiology Center, Boston University, Boston, MA, USA

[3]Genomics Research Center, Academia Sinica, Taipei, Taiwan

[4]Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan

Abstract

Hepatocellular carcinoma (HCC) is the dominant histologic type of liver cancer, accounting for 75% of cases. Growing evidence suggests that the cross-talk between the gut microbiome and metabolome (i.e., gut-liver axis) are related to the development of hepatic inflammation, and ultimately, HCC. Bile acids are metabolites, derived from cholesterol and synthesized in the liver, which may have a critical role in regulation of the gut-liver axis. We investigated whether prediagnostic circulating bile acids were associated with HCC risk, using the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer (REVEAL)-Hepatitis B Virus (HBV) and -Hepatitis C Virus (HCV) cohorts from Taiwan. Fifteen bile acids were quantitated using liquid chromatography, from 185 cases and 161 matched controls in REVEAL-HBV and 96 cases and 96 matched controls in REVEAL-HCV. Odds ratios (ORs) and 95% confidence intervals (CIs) for associations between bile acid levels and HCC were calculated using multivariable-adjusted logistic regression. Higher levels of glycine and taurine conjugated primary bile acids were associated with a 2–8-fold increased risk of HBV- (e.g., glycocholic acid OR_{Q4vsQ1}=3.38,95%CI:1.48–7.71,p_{trend}<0.003) and HCV-related HCC (e.g., OR=8.16,95%CI:2.21–30.18,p_{trend}<0.001). However, higher levels of the secondary bile acid deoxycholic acid were inversely associated with HBV-related HCC risk (OR=0.41,95%CI:0.19–0.88,p_{trend}=0.02). This study provides evidence that higher concentrations of bile acids—specifically, conjugated primary bile acids—are associated with increased HCC risk. However, this study does not support the hypothesis that higher levels of secondary bile acids increase liver cancer risk; indeed, deoxycholic acid may be associated with a decreased HCC risk.

Corresponding author and address: Jessica L. Petrick, PhD, MPH, 72 East Concord Street, L-7, Boston, MA 02118 phone: 617-206-6179 jpetrick@bu.edu.

Disclosure of potential conflicts of interest: BY participated in this research during post-doctoral work at the National Cancer Institute and currently is employed by Roche. Roche had no influence or contribution to this project, nor did it have any financial impact on this project. The remaining authors declare no conflict of interest.

Data Accessibility: The authors confirm that some access restrictions apply to the data underlying the findings. Data are stored at the Genomics Research Center, Academia Sinica, Taipei, Taiwan, and initial requests for data may be directed to Chien-Jen Chen (chencj@gate.sinica.edu.tw), Hwai-I Yang (hiyang@gate.sinica.edu.tw), or Mei-Hsuan Lee (meihlee@ntu.edu.tw).

Keywords

cohort study; mass spectrometry; bile acids; hepatocellular carcinoma; human

Introduction

Primary liver cancer, the major histology of which is hepatocellular carcinoma (HCC), is the second leading cause of cancer death worldwide.[1] Major risk factors for HCC, including hepatitis B virus (HBV), hepatitis C virus (HCV), aflatoxin, excessive alcohol consumption, smoking, obesity and diabetes, all contribute to chronic hepatic inflammation.[2] HCC risk factors such as obesity and alcohol abuse, as well as other insults, can trigger intestinal dysbiosis (i.e., altered microbiota composition and decreased bacterial diversity).[3, 4] As the liver receives approximately 70% of its blood supply from the portal vein,[5] growing evidence suggests that the gut microbiome, and the cross-talk between the microbiome and metabolome (i.e., gut-liver axis), are critically related to the development of hepatic inflammation, liver disease, cirrhosis, and liver cancer.[6–9]

Primary bile acids are derived from cholesterol and synthesized in the liver. They are then conjugated with glycine or taurine, excreted with bile, and stored in the gallbladder. After food ingestion, they are excreted into the intestinal tract to facilitate lipid absorption. Approximately 90–95% of bile acids are reabsorbed in the ileum and enter the enterohepatic circulation, whereby they are transported back to the liver to be recirculated. The remaining 5–10% of bile acids flow into the large intestine, where they are converted to secondary bile acids (e.g., deoxycholic acid [DCA]), by gut microbes. The majority of the secondary bile acids are absorbed by the colonocytes and are returned to the liver for recirculation.[8, 10]

In experimental models, blocking DCA production or reducing the gut microbes that create DCA has been shown to prevent liver cancer development in obese mice.[11] Additionally, ursodeoxycholic acid (UDCA), another secondary bile acid, has been shown to increase elimination of DCA in mice and prevent liver cancer development.[11] UDCA is approved by the US Food and Drug Administration for treating certain liver diseases[12] and has been extensively studied as a potential chemopreventive agent,[13] as it has been shown to inhibit cellular proliferation [14] and suppress DCA-induced apoptosis.[15, 16]

Three recent prospective epidemiologic studies of untargeted metabolomics have reported that glycine and/or taurine conjugates of primary and secondary bile acids were associated with an increased risk of liver cancer.[17] However, no prospective studies have examined a comprehensive targeted panel of primary and secondary bile acids. Thus, to examine circulating bile acids in relationship to risk of liver cancer, we leveraged the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer (REVEAL)-HBV and -HCV cohorts from Taiwan.

Methods

Study Population

The REVEAL-HBV and -HCV cohort studies are based in the same community-based survey, which was conducted to examine the characteristics and risk factors for HCC.[12, 18, 19] Between 1991–1992, 23,820 participants aged 30 to 65 years old from seven townships in Taiwan enrolled in the study, completed questionnaires, and underwent health examinations. Of the study participants, 4,155 were hepatitis B virus surface antigen (HBsAg) seropositive and 1,095 were anti-HCV seropositive. In subsequent years, these two groups were followed as part of either the REVEAL-HBV or REVEAL-HCV cohorts, respectively, which were designed as complementary but discrete cohort studies. Regular examinations consisting of blood collection and abdominal ultrasonography occurred every 6–12 months through December 31, 2008.[12, 20, 21] All participants gave written, informed consent at study enrollment, and the study was approved by the Institutional Review Board of the College of Public Health, National Taiwan University in Taipei.

Case Ascertainment

Diagnosis of HCC was established by ultrasound and α-fetoprotein testing or by data linkage to the Taiwan National Cancer Registry.[12, 18] All cases diagnosed through 2012 were confirmed by medical record verification.[18] Cirrhosis status was ascertained either by ultrasound or data linkage to the National Health Insurance profiles in Taiwan.[12] Ultrasound testing was conducted using high-resolution, real-time ultrasound scanners and was scored according to a previously published and validated algorithm.[22]

In REVEAL-HBV, 185 participants developed HCC during a mean follow-up of 13.0 years. Controls (n=161) were frequency matched to HCC cases on age (5-year categories), sex, and HBV DNA copies at baseline (<10,000, 10,000-<1,000,000, ≤1,000,000 copies/mL).

In REVEAL-HCV, 96 participants developed HCC during a mean follow-up of 14.9 years. Controls (n=96) were individually matched to HCC cases on age (5-year categories), sex, cirrhosis, and HCV-RNA positive rate (undetectable – <25 IU/mL and detectable – ≥25 IU/mL, matched to a control with the closest HCV RNA level).

Laboratory Methods

Prior to shipping, serum samples were stored at −70°C at the Academia Sinica in Taipei, Taiwan. The samples were analyzed at Metabolon, Inc. (Morrisville, NC) for measurement of primary and secondary bile acids, as well as their glycine and taurine conjugates, using liquid chromatography with tandem mass spectrometry (LC-MS/MS; Agilent 1290/Sciex QTrap 6500). The analytes measured were cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), glycodeoxycholic acid (GDCA), glycoursodeoxycholic acid (GUDCA), taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA), taurolithocholic acid (TLCA), tauroursodeoxycholic acid (TUDCA), and glycolithocholic acid (GLCA). Briefly, a solution of labelled internal standards for each of the bile acids was spiked into the

serum samples and then subjected to protein precipitation with acidified methanol. Samples then underwent centrifugation, and a portion of the clear supernatant was evaporated in nitrogen at 40°C. The dried extract was then reconstituted, and an aliquot was injected onto an LC-MS/MS, equipped with a C18 reverse phase HPLC column in negative ion mode. Using internal standards prepared for each run to normalize metabolite concentrations, the assay provided absolute quantitation.

Blinded quality control (QC) samples served as duplicate control samples. One QC sample was included in each batch (n=24 total QC samples), with the matched control sample, to examine within-batch coefficients of variation (CV). Within-batch CVs were less than 19% (range: 4.9–18.8%). The exception was LCA (21.5%), but the CV was similar after batch 21 was excluded (15.4%). Excluding this batch from the analysis did not alter estimates of association (data not shown).

Statistical Analysis

Participant characteristics were examined by calculating frequencies (for categorical variables) or means and standard deviations (for continuous variables). Spearman correlation coefficients were examined for each pairing of bile acids. Missing data for the following covariates were imputed using the PROC MI procedure in SAS (SAS Institute Inc., Cary, NC): body mass index (n=3), alcohol consumption (n=3), and smoking status (n=3). To further examine and adjust for HBV- and HCV-specific markers, imputation was also performed in REVEAL-HBV for hepatitis B e-antigen (HBeAg) serostatus (n=3), which is a marker of active viral replication. For REVEAL-HCV, imputation was also performed for HCV genotype (n=35), as genotype 1 is associated with an increased likelihood of developing HCC.

For analysis, the bile acids were categorized into quartiles. To examine the relative concentrations of secondary to primary bile acids, the ratios of LCA/CDCA and DCA/CA were calculated. Additionally, three bile acid scores were calculated by summing 1) all primary bile acids, 2) secondary bile acids, and 3) total bile acids. In the main analysis, any bile acid value below the lower limit of quantitation (LLOQ) was assigned to the lowest quartile. Unconditional (REVEAL-HBV) and conditional (REVEAL-HCV) logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of the association between bile acids and risk of HCC.[23] Tests of linear trend were performed using quartile-specific log-transformed bile acid concentration medians in the logistic models.

Alcohol consumption (no, yes), education status (illiterate, elementary school, junior high school, high school, college or more), diabetes (yes/no), alanine transaminase (ALT, continuous U/L), and matching factors were included as covariates *a priori*. Additional potential covariates were examined for evidence of confounding by determining whether 1) each covariate was associated with the exposure in the general population (i.e., the controls) and 2) each covariate was associated with liver cancer among the unexposed (i.e., the lowest quartile of bile acid concentration). Finally, covariates that met both criteria were removed one at a time from the fully-adjusted model to determine whether the covariate altered the log(OR) by at least 10%.[24] Fully-adjusted models included age (5-year categories), sex, smoking status (no, yes), baseline cirrhosis status (no, yes), alcohol habit (no, yes), and body

mass index (BMI; continuous kg/m^2). Models of REVEAL-HBV data were further adjusted for number of HBV DNA copies at baseline (<10,000, 10,000–<1,000,000, ≤1,000,000) and HBeAg serostatus (negative, positive), while REVEAL-HCV was further adjusted for HCV genotype (1, non-1). Effect measure modification by sex, smoking status, diabetes, and BMI was assessed. Departures from the null were examined using likelihood ratio tests to compare regression models with and without a multiplicative interaction term.[24] There was no evidence of effect measure modification (Ps≥0.05). All tests for significance were 2-sided. Analyses were conducted using SAS, version 9.4 (SAS Institute Inc., Cary, NC).

Sensitivity Analyses

In both REVEAL-HBV and REVEAL-HCV cohorts, sensitivity analyses were performed, excluding any bile acid value below the LLOQ. Five bile acids had >10% of values below the LLOQ: GLCA, LCA, TDCA, TLCA, and TUDCA. To examine potential reverse causation, analyses were performed excluding any case with an HCC diagnosis within 5 years of baseline date.

Results

As shown in Table 1, HBV- and HCV-related HCC cases had higher BMI, higher ALT levels, and were less educated than controls. HBV-related HCC cases were also more likely to have a diabetes diagnosis, while HCV-related HCC cases were more likely to be smokers. Examining the bile acid pairings, significant correlations were noted between the primary bile acids (i.e., CA and CDCA, ρ=0.800) and their taurine and glycine conjugates (e.g., GCA and GCDCA, ρ=0.869) (Supplemental Table S1). Additionally, CDCA was highly correlated with its epimer secondary bile acid UDCA (ρ=0.806). Circulating concentrations were higher in HCC cases, compared to controls, for almost all bile acids examined (Table 2).

Little to no association was found between unconjugated primary bile acids and risk of HBV-related HCC (OR per one unit change in $\log_2$ CA=1.19, 95% CI: 0.98–1.43 and OR for CDCA=1.17, 95% CI: 0.94–1.45) (Table 3). Conversely, the highest quartiles of CA and CDCA were associated with a 3-fold increased risk of HCV-related HCC (OR=3.40, 95% CI: 1.16–9.97, p_{trend}=0.03 and OR=4.25, 95% CI: 1.54–11.70, p_{trend}=0.02). A doubling of all glycine and taurine conjugates of primary bile acids were associated with 24–91% increased risk of HBV- and HCV-related HCC (e.g., OR per one unit change in $\log_2$ GCDCA for HCV-related HCC=1.91, 95% CI: 1.29–2.83). Similarly, examining the fourth quartile compared to the first, glycine and taurine conjugates of primary bile acids were associated with a 2–8-fold increased risk of HBV- and HCV-related HCC (e.g., OR GCA for HCV-related HCC=8.16, 95% CI: 2.21–30.18, p_{trend}<0.001).

Among the secondary bile acids, DCA was inversely associated with HBV-related HCC risk (OR Quartile 4 vs. 1 DCA=0.41, 95% CI: 0.19–0.88) (Table 4). The highest quartile of circulating LCA was associated with a 3-fold increased HCV-related HCC risk, but the CI was wide (OR=3.33, 95% CI: 1.20–9.26, p_{trend}=0.03). Similar associations were observed for TDCA, GLCA, and TUDCA. However, power was limited to examine the majority of secondary bile acids, as >10% of samples were below the limit of quantitation for GLCA,

LCA, TDCA, TLCA, and TUDCA. Circulating GUDCA was associated with an increased risk of HBV-related HCC (OR per one unit change in $\log_2$ GUDCA=1.33, 95% CI: 1.05–1.68), but not HCV-related HCC (OR=1.21, 95% CI: 0.91–1.60).

Examining the ratio of secondary to primary bile acids, higher relative concentrations of DCA/CA were associated with a decreased risk of HBV-related HCC (OR per one unit change in $\log_2$ DCA/CA=0.81, 95% CI: 0.69–0.96), but not HCV-related HCC (Table 4). Summing across bile acids, the primary bile acid score was associated with an increased risk of HBV- (OR=1.34, 95% CI: 1.07–1.69) and HCV-related HCC (OR=1.95, 95% CI: 1.30–2.94; Supplemental Table S2). The secondary and total bile acids scores were only associated with HCV-related HCC (OR=1.55, 95% CI: 1.01–2.38 and OR=2.10, 95% CI: 1.32–3.36, respectively).

Excluding bile acid values below the LLOQ from quartile one resulted in minimally altered estimates (Supplemental Table S3). Similarly, when we conducted lag analyses excluding cases diagnosed in the first five years of follow-up, the results were similar (Supplemental Table S4).

Conclusion

In this study leveraging data from two well-characterized prospective cohort studies, we report that a doubling in the circulating concentrations of glycine and taurine conjugated primary bile acids were associated with a 24–91% increased risk of viral hepatitis-related HCC. Conversely, higher levels of the secondary bile acid DCA were associated only with a decreased risk of HBV-related HCC.

Mechanisms underlying the bile acid-liver cancer association are not fully understood. However, two potential mechanisms include immune homeostasis and metabolic effects mediated by the gut microbiome via the gut-liver axis. The microbiota is the community of microorganisms, including viruses, fungi, and bacteria, that reside within human tissues and biofluids. Dysbiosis (i.e., altered microbiota abundance and composition), observed during HBV[25] and HCV[26, 27] infections, is associated with a dysfunctional intestinal barrier and can lead to bacterial translocation beyond the gut, potentially contributing to hepatocarcinogenesis.[28, 29] Specifically, dysbiosis can lead to the release of cancer-promoting and senescence-promoting metabolites, including the secondary bile acids. In experimental models, blocking DCA production or reducing the gut microbes that create DCA has been shown to prevent liver cancer development in rodents.[11] However, bile acid composition varies substantially between mice and humans.[30–32] In humans, healthy controls have higher levels of autochthonous bacteria (e.g., *Blautia, Ruminococcaceae,* and *Lachnospiraceae*), which are correlated with higher levels of secondary bile acids and higher secondary/primary bile acid ratios.[33] Conversely, patients with liver disease (e.g., HBV, HCV, cirrhosis, and non-alcoholic fatty liver disease) display overgrowth of potentially pathogenic bacteria (e.g., *Enterobacteriaceae*),[25–27, 33, 34] which are correlated with higher levels of primary bile acids.[33] Thus, in liver disease-related dysbiosis, we would expect higher levels of primary bile acids in circulation, which we observed for both HBV- and HCV-related HCC.

Bile acids may also be associated with liver cancer risk via metabolic regulation. The primary function of bile acids is to act as a surfactant that emulsifies dietary fats, allowing for digestion and absorption. However, bile acids also have metabolic effects, regulating glucose, lipids, and energy homeostasis. Specifically, two bile acid receptors modulate the metabolic effects of bile acids – the nuclear farnesoid X receptor (FXR) and the membrane-bound Takeda G protein-coupled receptor (TGR5).[35] Primary bile acids function as the main agonists of FXR, which regulates accumulation of fat and inflammation in the liver and lipid storage in white adipose tissue. The secondary bile acids (i.e., LCA and DCA) function as agonists of TGR5, activation of which increases insulin sensitivity and energy expenditure.[35] Further, bile acids enhance HBV and HCV replication through FXR[36, 37] and high levels of bile acids have been correlated with poor response to interferon-based therapy for HCV.[38–41] Few studies have examined the association between bile acids and direct-acting antiviral (DAA) therapy,[42, 43] likely because new DAA therapies can cure nearly all HCV infections.[44]

Herein, we comprehensively examined 15 circulating bile acids using targeted metabolomics in relation to viral hepatitis-related HCC. Two recent studies conducted in European cohorts, Alpha-Tocopherol, Beta-Carotene Cancer Prevention study (ATBC) and European Prospective Investigation into Cancer and Nutrition (EPIC), have examined the association between untargeted metabolomics and risk of liver cancer.[45, 46] Similar to the current report, both studies reported that GCA and GCDCA were associated with an increased risk of liver cancer. Another cohort study from South Korea (Korean Cancer Prevention Study-II, KCPS-II) utilized an untargeted metabolomic approach and also reported that GCA was upregulated in HCC cases compared to controls. Additionally, the authors' found that GUDCA and TUDCA were upregulated in cases.[17] However, all three of these prospective studies utilized an untargeted platform. Thus, they were unable to comprehensively evaluate all primary and secondary bile acids and their conjugates. While our current study provides the most information to-date on the association between circulating concentrations of bile acids and risk of viral hepatitis-related HCC, we see similar associations across differing etiologies of liver cancer, specifically for conjugated primary bile acids. For ATBC, EPIC, and KCPS-II, 1.7%, 16.8%, and 70.5% of liver cancer cases were HBV-positive, while 5.0%, 21.8% and 0.0% were HCV-positive, respectively.[17, 45, 47] In REVEAL and KCPS-II, unconjugated primary bile acids, specifically GCA, were associated with an increased liver cancer risk, suggesting an etiologic pathway specific to viral hepatitis-related liver cancer. However, the associations with unconjugated primary bile acids were stronger for HCV-related HCC. These associations for CA and TCA were not observed in prior studies, which may suggest that this is an etiologic pathway specific to HCV-related HCC (of which there have been few cases in prior prospective studies). Similarly, several of the secondary bile acids (e.g., LCA) were associated with HCV-related HCC, although power was limited, but there was little to no association with HBV-related HCC.

This study has several strengths, including use of targeted metabolomics for quantification of bile acid levels in serum samples collected prior to the diagnosis of cancer. Prior prospective studies have utilized untargeted metabolomics. Utilizing targeted metabolomics allows for absolute quantification of bile acid levels, through the use of optimized sample preparation and internal standards. Additionally, this allows for a comprehensive

examination of the bile acid metabolome, whereas using untargeted metabolomics would only allow examination of a subset of identified bile acids. All serum samples were collected pre-diagnostically, which ensures that the observed associations are not an artifact of the carcinogenic process. Finally, the REVEAL-HBV and REVEAL-HCV studies are long-standing, population-based cohorts that have been well-characterized for liver disease, including information on the number of HBV DNA copies, HBeAg status, HCV genotype, and cirrhosis.

The primary limitation of this study is generalizability of the population, as the REVEAL cohorts are limited to persons who are chronic viral carriers. In addition, the cohorts were started prior to widespread use of nucleos(t)ide analogues as treatment for HBV infection or DAAs as treatment for HCV infection. Thus, it is unclear how these results may translate to populations that are not virally infected or populations where HBV and HCV treatment are more common. However, the results of the current study are similar to the results of two recent untargeted metabolomic studies from Europe. Additionally, our findings are similar to the untargeted metabolomics study from Korea, which was more recently recruited (i.e., 2004–2013 vs. 1991–1992 for REVEAL). Another limitation was that >10% of participants had levels of five bile acids below the LLOQ, which limited our ability to examine them. However, this is not surprising as these bile acids, particularly taurine conjugates of the secondary bile acids, are found in low concentrations in humans.[32]

In conclusion, our study found that conjugated primary bile acids were associated with increased risk of HBV- and HCV-related HCC. However, higher levels of secondary bile acids were not associated with an increased risk of HCC; indeed, DCA may be associated with a decreased risk of HBV-related HCC. Thus, this study does not support the hypothesis that higher levels of secondary bile acids increase liver cancer risk among humans. While intriguing, these findings need to be replicated in other populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial Support: NIH Intramural Research Program, National Cancer Institute, and the Karin Grunebaum Cancer Research Foundation.

Abbreviations

ALT	alanine transaminase
ATBC	Alpha-Tocopherol, Beta-Carotene Cancer Prevention
CA	cholic acid
CDCA	chenodeoxycholic acid
CV	coefficients of variation

Cis	confidence intervals
DCA	deoxycholic acid
DAA	direct-acting antiviral
EPIC	European Prospective Investigation into Cancer and Nutrition
FXR	farnesoid X receptor
GCA	glycocholic acid
GCDCA	glycochenodeoxycholic acid
GDCA	glycodeoxycholic acid
GLCA	glycolithocholic acid
GUDCA	glycoursodeoxycholic acid
HBV	hepatitis B virus
HBeAg	hepatitis B e-antigen
HBsAg	hepatitis B virus surface antigen
HCV	hepatitis C virus
HCC	hepatocellular carcinoma
KCPS-II	Korean Cancer Prevention Study-II
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LCA	lithocholic acid
LLOQ	lower limit of quantitation
ORs	odds ratios
QC	quality control
REVEAL	Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer
TGR5	Takeda G protein-coupled receptor
TCA	taurocholic acid
TCDCA	taurochenodeoxycholic acid
TDCA	taurodeoxycholic acid
TLCA	taurolithocholic acid
TUDCA	tauroursodeoxycholic acid

UDCA	ursodeoxycholic acid

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68: 394–424. [PubMed: 30207593]
2. McGlynn KA, London WT. Epidemiology and natural history of hepatocellular carcinoma. Best practice & research 2005;19: 3–23.
3. Sun L, Ma L, Ma Y, Zhang F, Zhao C, Nie Y. Insights into the role of gut microbiota in obesity: pathogenesis, mechanisms, and therapeutic perspectives. Protein Cell 2018;9: 397–403. [PubMed: 29725936]
4. Engen PA, Green SJ, Voigt RM, Forsyth CB, Keshavarzian A. The Gastrointestinal Microbiome: Alcohol Effects on the Composition of Intestinal Microbiota. Alcohol Res 2015;37: 223–36. [PubMed: 26695747]
5. Son G, Kremer M, Hines IN. Contribution of gut bacteria to liver pathobiology. Gastroenterol Res Pract 2010;2010.
6. Almeida J, Galhenage S, Yu J, Kurtovic J, Riordan SM. Gut flora and bacterial translocation in chronic liver disease. World J Gastroenterol 2006;12: 1493–502. [PubMed: 16570339]
7. Gonzalez FJ, Jiang C, Patterson AD. An Intestinal Microbiota-Farnesoid X Receptor Axis Modulates Metabolic Disease. Gastroenterology 2016;151: 845–59. [PubMed: 27639801]
8. Jia W, Xie G, Jia W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. Nat Rev Gastroenterol Hepatol 2017.
9. Vajro P, Paolella G, Fasano A. Microbiota and gut-liver axis: their influences on obesity and obesity-related liver disease. J Pediatr Gastroenterol Nutr 2013;56: 461–8. [PubMed: 23287807]
10. Nguyen TT, Ung TT, Kim NH, Jung YD. Role of bile acids in colon carcinogenesis. World J Clin Cases 2018;6: 577–88. [PubMed: 30430113]
11. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, Iwakura Y, Oshima K, Morita H, Hattori M, Honda K, Ishikawa Y, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature 2013;499: 97–101. [PubMed: 23803760]
12. Chen C-J, Iloeje UH, Yang H-I. Long-Term Outcomes in Hepatitis B: The REVEAL-HBV Study. Clinics in Liver Disease 2007;11: 797–816. [PubMed: 17981229]
13. Akare S, Jean-Louis S, Chen W, Wood DJ, Powell AA, Martinez JD. Ursodeoxycholic acid modulates histone acetylation and induces differentiation and senescence. Int J Cancer 2006;119: 2958–69. [PubMed: 17019713]
14. Martinez JD, Stratagoules ED, LaRue JM, Powell AA, Gause PR, Craven MT, Payne CM, Powell MB, Gerner EW, Earnest DL. Different bile acids exhibit distinct biological effects: the tumor promoter deoxycholic acid induces apoptosis and the chemopreventive agent ursodeoxycholic acid inhibits cell proliferation. Nutr Cancer 1998;31: 111–8. [PubMed: 9770722]
15. Im E, Martinez JD. Ursodeoxycholic acid (UDCA) can inhibit deoxycholic acid (DCA)-induced apoptosis via modulation of EGFR/Raf-1/ERK signaling in human colon cancer cells. J Nutr 2004;134: 483–6. [PubMed: 14747693]
16. Im E, Akare S, Powell A, Martinez JD. Ursodeoxycholic acid can suppress deoxycholic acid-induced apoptosis by stimulating Akt/PKB-dependent survival signaling. Nutr Cancer 2005;51: 110–6. [PubMed: 15749637]
17. Jee SH, Kim M, Kim M, Yoo HJ, Kim H, Jung KJ, Hong S, Lee JH. Metabolomics Profiles of Hepatocellular Carcinoma in a Korean Prospective Cohort: The Korean Cancer Prevention Study-II. Cancer Prev Res (Phila) 2018;11: 303–12. [PubMed: 29500188]
18. Chen C-J, Yang H-I, Su J, Jen C-L, You S-L, Lu S-N, Huang G-T, Iloeje UH, REVEAL-HBV Study Group ft. Risk of Hepatocellular Carcinoma Across a Biological Gradient of Serum Hepatitis B Virus DNA Level. JAMA 2006;295: 65–73. [PubMed: 16391218]
19. Yang H-I, Yuen M-F, Chan HL-Y, Han K-H, Chen P-J, Kim D-Y, Ahn S-H, Chen C-J, Wong VW-S, Seto W-K. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B):

development and validation of a predictive score. The Lancet Oncology 2011;12: 568–74. [PubMed: 21497551]

20. Yang H-I, Tseng T-C, Liu J, Lee M-H, Liu C-J, Su T-H, Batrla-Utermann R, Chan HL-Y, Kao J-H, Chen C-J. Incorporating Serum Level of Hepatitis B Surface Antigen or Omitting Level of Hepatitis B Virus DNA Does not Affect Calculation of Risk for Hepatocellular Carcinoma in Patients Without Cirrhosis. Clinical Gastroenterology and Hepatology 2016;14: 461–8.e2. [PubMed: 26598229]
21. Mei-Hsuan L, Yang H-I, Lu S-N, Jen C-L, Yeh S-H, Liu C-J, Chen P-J, You S-L, Wang L-Y, Chen WJ, Chen C-J. Hepatitis C Virus Seromarkers and Subsequent Risk of Hepatocellular Carcinoma: Long-Term Predictors From a Community-Based Cohort Study. Journal of Clinical Oncology 2010;28: 4587–93. [PubMed: 20855826]
22. Lin DY, Sheen IS, Chiu CT, Lin SM, Kuo YC, Liaw YF. Ultrasonographic changes of early liver cirrhosis in chronic hepatitis B: a longitudinal study. Journal of clinical ultrasound : JCU 1993;21: 303–8. [PubMed: 8514896]
23. Pearce N Analysis of matched case-control studies. BMJ 2016;352: i969. [PubMed: 26916049]
24. Rothman KJ, Greenland S, Lash TL. Modern epidemiology, 3rd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins, 2008 x, 758 p.
25. Wang J, Wang Y, Zhang X, Liu J, Zhang Q, Zhao Y, Peng J, Feng Q, Dai J, Sun S, Zhao Y, Zhao L, et al. Gut Microbial Dysbiosis Is Associated with Altered Hepatic Functions and Serum Metabolites in Chronic Hepatitis B Patients. Front Microbiol 2017;8: 2222. [PubMed: 29180991]
26. Inoue T, Nakayama J, Moriya K, Kawaratani H, Momoda R, Ito K, Iio E, Nojiri S, Fujiwara K, Yoneda M, Yoshiji H, Tanaka Y. Gut Dysbiosis Associated With Hepatitis C Virus Infection. Clin Infect Dis 2018;67: 869–77. [PubMed: 29718124]
27. Preveden T, Scarpellini E, Milic N, Luzza F, Abenavoli L. Gut microbiota changes and chronic hepatitis C virus infection. Expert Rev Gastroenterol Hepatol 2017;11: 813–9. [PubMed: 28621554]
28. Yu LX, Schwabe RF. The gut microbiome and liver cancer: mechanisms and clinical translation. Nat Rev Gastroenterol Hepatol 2017;14: 527–39. Review of the mechanistic links between the microbiome, leaky gut, and liver cancer. [PubMed: 28676707]
29. Cani PD, Jordan BF. Gut microbiota-mediated inflammation in obesity: a link with gastrointestinal cancer. Nat Rev Gastroenterol Hepatol 2018;15: 671–82. Review suggesting that leakage of microbial components across the intestinal barrier could be the mechanism underlying obesity and liver cancer risk. [PubMed: 29844585]
30. Wahlstrom A, Kovatcheva-Datchary P, Stahlman M, Khan MT, Backhed F, Marschall HU. Induction of farnesoid X receptor signaling in germ-free mice colonized with a human microbiota. J Lipid Res 2017;58: 412–9. [PubMed: 27956475]
31. Sayin SI, Wahlstrom A, Felin J, Jantti S, Marschall HU, Bamberg K, Angelin B, Hyotylainen T, Oresic M, Backhed F. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell metabolism 2013;17: 225–35. [PubMed: 23395169]
32. Garcia-Canaveras JC, Donato MT, Castell JV, Lahoz A. Targeted profiling of circulating and hepatic bile acids in human, mouse, and rat using a UPLC-MRM-MS-validated method. J Lipid Res 2012;53: 2231–41. [PubMed: 22822028]
33. Kakiyama G, Pandak WM, Gillevet PM, Hylemon PB, Heuman DM, Daita K, Takei H, Muto A, Nittono H, Ridlon JM, White MB, Noble NA, et al. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. J Hepatol 2013;58: 949–55. [PubMed: 23333527]
34. Shen F, Zheng RD, Sun XQ, Ding WJ, Wang XY, Fan JG. Gut microbiota dysbiosis in patients with non-alcoholic fatty liver disease. Hepatobiliary Pancreat Dis Int 2017;16: 375–81. [PubMed: 28823367]
35. Molinaro A, Wahlstrom A, Marschall HU. Role of Bile Acids in Metabolic Control. Trends Endocrinol Metab 2018;29: 31–41. [PubMed: 29195686]
36. Schupp AK, Graf D. Bile acid-induced modulation of virus replication. Eur J Med Res 2014;19.

37. Scholtes C, Diaz O, Icard V, Kaul A, Bartenschlager R, Lotteau V, Andre P. Enhancement of genotype 1 hepatitis C virus replication by bile acids through FXR. J Hepatol 2008;48: 192–9. [PubMed: 18096266]

38. Chhatwal P, Bankwitz D, Gentzsch J, Frentzen A, Schult P, Lohmann V, Pietschmann T. Bile acids specifically increase hepatitis C virus RNA-replication. PLoS One 2012;7: e36029. [PubMed: 22558311]

39. Iwata R, Stieger B, Mertens JC, Muller T, Baur K, Frei P, Braun J, Vergopoulos A, Martin IV, Schmitt J, Goetze O, Bibert S, et al. The role of bile acid retention and a common polymorphism in the ABCB11 gene as host factors affecting antiviral treatment response in chronic hepatitis C. J Viral Hepatitis 2011;18: 768–78.

40. Jorquera F, Monte MJ, Guerra J, Sanchez-Campos S, Merayo JA, Olcoz JL, Gonzalez-Gallego J, Marin JJG. Usefulness of combined measurement of serum bile acids and ferritin as additional prognostic markers to predict failure to reach sustained response to antiviral treatment in chronic hepatitis C. J Gastroen Hepatol 2005;20: 547–54.

41. Iwata R, Stieger B, Mertens JC, Muller T, Baur K, Frei P, Braun J, Vergopoulos A, Martin IV, Schmitt J, Goetze O, Bibert S, et al. The role of bile acid retention and a common polymorphism in the ABCB11 gene as host factors affecting antiviral treatment response in chronic hepatitis C. J Viral Hepat 2011;18: 768–78. [PubMed: 20723035]

42. Stauber RE, Fauler G, Rainer F, Leber B, Posch A, Streit A, Spindelboeck W, Stadlbauer V, Kessler HH, Mangge H. Anti-HCV treatment with ombitasvir/paritaprevir/ritonavir +/− dasabuvir is associated with increased bile acid levels and pruritus. Wien Klin Wochenschr 2017;129: 848–51. [PubMed: 28980064]

43. Zha J, Badri PS, Ding B, Uchiyama N, Alves K, Rodrigues L Jr., Redman R, Dutta S, Menon RM. Drug Interactions Between Hepatoprotective Agents Ursodeoxycholic Acid or Glycyrrhizin and Ombitasvir/Paritaprevir/Ritonavir in Healthy Japanese Subjects. Clin Ther 2015;37: 2560–71. [PubMed: 26505529]

44. Sulkowski MS, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinestrosa F, Thuluvath PJ, Schwartz H, Nelson DR, et al. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. N Engl J Med 2014;370: 211–21. [PubMed: 24428467]

45. Loftfield E, Rothwell JA, Sinha R, Keski-Rahkonen P, Robinot N, Albanes D, Weinstein SJ, Derkach A, Sampson J, Scalbert A, Freedman ND. Prospective investigation of serum metabolites, coffee drinking, liver cancer incidence, and liver disease mortality. J Natl Cancer Inst 2019.

46. Stepiena M, Keski-Rahkonen P, Kiss A, Gunter M, Scalbert A, Jenaba M. Untargeted serum metabolomics of hepatocellular carcinoma in a prospective cohort of European populations. European Congress of Epidemiology / Revue d'Épidémiologie et de Santé Publique 2018;55: S298.

47. Fedirko V, Tran HQ, Gewirtz AT, Stepien M, Trichopoulou A, Aleksandrova K, Olsen A, Tjonneland A, Overvad K, Carbonnel F, Boutron-Ruault MC, Severi G, et al. Exposure to bacterial products lipopolysaccharide and flagellin and hepatocellular carcinoma: a nested case-control study. BMC Med 2017;15: 72. [PubMed: 28372583]

NOVELTY AND IMPACT

Evidence suggests that the gut microbiome, including gut-derived circulating secondary bile acids, is related to the development of liver cancer. In this study, higher concentrations of glycine and taurine conjugated primary bile acids were associated with a 2–8-fold increased risk of HBV- and HCV-related HCC. However, this study does not support the hypothesis that higher levels of secondary bile acids increase liver cancer risk; indeed, deoxycholic acid may be associated with a decreased HCC risk.

Table 1.

Baseline characteristics from included participants from the REVEAL-HBV and REVEAL-HCV cohorts.

	REVEAL-HBV					REVEAL-HCV				
Covariate	**Controls (N=161)**		**HCC Cases (N=185)**		**P-value**	**Controls (N=96)**		**HCC Cases (N=96)**		**P-value**
Mean age (SD), years	50.7	(9.4)	51.3	(9.0)	0.6	54.5	(7.3)	54.4	(6.8)	0.8
Age, No. (%)										
30 – <40	24	(14.9)	24	(13.0)		4	(4.2)	4	(4.2)	
40 – <50	53	(32.9)	58	(31.4)		14	(14.6)	14	(14.6)	
50 – <60	51	(31.7)	68	(36.8)		54	(56.2)	54	(56.2)	
60+	33	(20.5)	35	(18.9)	0.8	24	(25.0)	24	(25.0)	1.0
Sex, No. (%)										
Male	130	(80.8)	150	(81.1)		56	(58.3)	56	(58.3)	
Female	31	(19.3)	35	(18.9)	0.9	40	(41.7)	40	(41.7)	1.0
Body mass index, No. (%)										
<18.5, kg/m^2	5	(3.1)	7	(3.8)		0	(0.0)	1	(1.0)	
18.5 – <23, kg/m^2	68	(42.2)	52	(28.1)		45	(46.9)	28	(29.2)	
23 – <25	42	(26.1)	45	(24.3)		19	(19.8)	19	(19.8)	
≥25, kg/m^2	46	(28.6)	81	(43.8)	0.01	32	(33.3)	48	(50.0)	0.04
Alcohol consumption, No. (%)										
No	136	(84.5)	149	(80.5)		86	(89.6)	87	(90.6)	
Yes	25	(15.5)	36	(19.5)	0.4	10	(10.4)	9	(9.4)	0.8
Smoking status, No. (%)										
No	104	(64.6)	113	(61.1)		67	(69.8)	55	(57.3)	
Yes	57	(35.4)	72	(38.9)	0.5	29	(30.2)	41	(42.7)	0.07
Education, No. (%)										
Illiterate	20	(12.4)	24	(13.0)		35	(36.4)	39	(40.6)	
Elementary school	69	(42.9)	98	(53.0)		45	(46.9)	37	(38.6)	
Junior high school	19	(11.8)	25	(13.5)		4	(4.2)	13	(13.5)	
High school	32	(19.9)	25	(13.5)		7	(7.3)	5	(5.2)	
College or more	21	(13.0)	13	(7.0)	0.1	5	(5.2)	2	(2.1)	0.1
Mean ALT (SD), u/L	18.3	(18.4)	32.5	(44.8)	<0.001	31.5	(30.7)	43.9	(42.5)	0.006
Diabetes at Baseline, No. (%)										
No	160	(99.4)	175	(94.6)		91	(94.8)	92	(95.8)	
Yes	1	(0.6)	10	(5.4)	0.01	5	(5.2)	4	(4.2)	0.7
Liver Cirrhosis at Baseline, No. (%)										
No	161	(100.0)	158	(85.4)		93	(96.9)	93	(96.9)	
Yes	0	(0.0)	27	(14.6)	<0.001	3	(3.1)	3	(3.1)	1.0
HBeAg										
Negative	142	(88.2)	107	(57.8)						
Positive	19	(11.8)	78	(42.2)	<0.001					

	REVEAL-HBV			REVEAL-HCV				
Covariate	**Controls (N=161)**	**HCC Cases (N=185)**	**P-value**	**Controls (N=96)**		**HCC Cases (N=96)**		**P-value**
HCV Genotype								
Genotype 1				45	(46.9)	66	(68.8)	
Genotype non-1				51	(53.1)	30	(31.2)	0.002

Abbreviations: HBV=hepatitis B virus, HCV=hepatitis C virus, HCC=hepatocellular carcinoma, SD=standard deviation, kg=kilogram, m=meter.

[1] P-values calculated using the Chi-square test (categorical variables) or the Wilcoxon test (continuous variables).

Table 2.

Mean (SD) levels of baseline biomarker concentrations[1] among included cases and controls from the REVEAL-HBV and REVEAL-HCV cohorts.

	REVEAL-HBV (N=346)			REVEAL-HCV (N=192)		
Bile Acids	**Controls**	**HCC Cases**	**P-value[2]**	**Controls**	**HCC Cases**	**P-value[2]**
Cholic Acid (CA)	150.84 (300.0)	214.71 (476.7)	0.1	202.01 (445.3)	221.28 (510.9)	0.8
Glycocholic Acid (GCA)	429.73 (974.0)	866.53 (1976.7)	0.01	232.18 (550.7)	800.21 (3609.3)	0.1
Taurocholic Acid (TCA)	111.08 (344.1)	271.44 (904.4)	0.04	89.40 (317.8)	357.58 (1960.0)	0.2
Chenodeoxycholic Acid (CDCA)	319.62 (528.9)	372.02 (620.0)	0.4	346.06 (584.2)	347.43 (436.3)	1.0
Glycochenodeoxycholic Acid (GCDCA)	1052.47 (1665.2)	1804.57 (3155.2)	0.01	727.37 (1189.4)	1396.41 (2488.6)	0.02
Taurochenodeoxycholic Acid (TCDCA)	239.40 (489.0)	617.91 (1612.1)	0.005	191.06 (419.2)	458.40 (939.3)	0.01
Deoxycholic Acid (DCA)	195.43 (209.5)	163.80 (209.3)	0.2	178.53 (273.3)	179.49 (161.6)	1.0
Glycodeoxycholic Acid (GDCA)	313.82 (673.4)	364.77 (551.4)	0.5	173.39 (202.9)	292.83 (444.5)	0.02
Taurodeoxycholic Acid (TDCA)	76.09 (191.6)	96.37 (139.3)	0.3	54.26 (114.0)	82.58 (131.8)	0.2
Lithocholic Acid (LCA)	8.92 (8.9)	9.12 (11.5)	0.9	9.93 (9.9)	12.27 (10.3)	0.3
Glycolithocholic Acid (GLCA)	13.17 (18.6)	10.73 (9.6)	0.2	10.30 (9.3)	14.96 (18.7)	0.1
Taurolithocholic Acid (TLCA)	7.00 (4.0)	7.20 (7.5)	0.9	7.21 (9.2)	7.46 (6.2)	0.9
Ursodexoycholic Acid (UDCA)	68.73 (131.6)	72.65 (109.1)	0.8	86.35 (168.01)	69.90 (105.2)	0.4
Glycoursodeoxycholic Acid (GUDCA)	144.40 (249.5)	205.02 (419.8)	0.1	138.94 (449.7)	152.53 (366.3)	0.8
Tauroursodeoxycholic Acid (TUDCA)	14.13 (23.2)	25.20 (69.4)	0.1	16.47 (47.0)	20.39 (60.2)	0.7
Secondary to Primary Bile Acid Ratios						
LCA/CDCA	0.40 (0.6)	0.22 (0.3)	0.01	0.50 (0.9)	0.38 (0.7)	0.4
DCA/CA	5.27 (6.7)	3.07 (4.2)	0.0004	4.55 (8.8)	3.79 (5.4)	0.5
Bile Acid Scores						
Primary	2278.56 (3389.69)	4139.88 (7397.81)	0.004	1768.98 (2764.30)	3559.02 (9136.91)	0.1
Secondary	770.26 (1040.06)	882.46 (974.82)	0.3	586.16 (741.76)	756.93 (846.96)	0.1
All	3048.82 (4266.01)	5022.34 (8122.12)	0.01	2355.15 (3201.39)	4315.96 (9695.47)	0.1

[1] All bile acid concentrations are absolute quantitation.

[2] Calculated using the analysis of variance test.

Table 3.

Multivariate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between primary bile acid concentrations and hepatocellular carcinoma risk among participants of the REVEAL-HBV and REVEAL-HCV cohorts.

		REVEAL-HBV (N=346)				REVEAL-HCV (N=192)[1]			
		Controls	Cases	OR[2]	95% CI	Controls	Cases	OR[3]	95% CI
Primary Bile Acids		n=161	n=185			n=96	n=96		
Cholic Acid (CA)									
	Quartile 1	42	38	1.00		24	15	1.00	
	Quartile 2	40	38	1.22	(0.55, 2.73)	24	23	1.84	(0.61, 5.56)
	Quartile 3	39	47	1.02	(0.45, 2.31)	24	20	1.48	(0.50, 4.34)
	Quartile 4	40	62	1.87	(0.88, 3.96)	24	38	3.40	(1.16, 9.97)
	P-value for trend[4]				0.08				0.03
	Continuous (log_2)			1.19	(0.98, 1.43)			1.20	(0.93, 1.54)
Glycocholic Acid (GCA)									
	Quartile 1	42	16	1.00		24	9	1.00	
	Quartile 2	40	39	1.68	(0.71, 4.00)	24	14	2.77	(0.67, 11.43)
	Quartile 3	41	47	1.51	(0.64, 3.53)	24	18	1.78	(0.45, 7.09)
	Quartile 4	38	83	3.38	(1.48, 7.71)	24	55	8.16	(2.21, 30.18)
	P-value for trend[4]				0.003				<0.001
	Continuous (log_2)			1.26	(1.05, 1.52)			1.68	(1.23, 2.30)
Taurocholic Acid (TCA)									
	Quartile 1	51	25	1.00		34	15	1.00	
	Quartile 2	38	35	1.79	(0.80, 4.01)	21	8	0.92	(0.27, 3.14)
	Quartile 3	38	37	1.60	(0.73, 3.48)	21	28	2.29	(0.76, 6.93)
	Quartile 4	34	88	2.97	(1.38, 6.38)	20	45	5.30	(1.93, 14.56)
	P-value for trend[4]				0.01				<0.001
	Continuous (log_2)			1.24	(1.05, 1.45)			1.52	(1.19, 1.94)
Chenodeoxycholic Acid (CDCA)									
	Quartile 1	41	36	1.00		26	14	1.00	

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	REVEAL-HBV (N=346)				REVEAL-HCV (N=192)[1]			
	Controls	Cases	OR[2]	95% CI	Controls	Cases	OR[3]	95% CI
Primary Bile Acids	**n=161**	**n=185**			**n=96**	**n=96**		
Quartile 2	40	42	0.86	(0.38, 1.96)	23	27	2.61	(0.91, 7.44)
Quartile 3	41	51	1.62	(0.74, 3.54)	24	15	1.69	(0.54, 5.28)
Quartile 4	39	56	1.53	(0.70, 3.32)	23	40	4.25	(1.54, 11.70)
P-value for trend[4]				0.2				0.02
Continuous (log_2)			1.17	(0.94, 1.45)			1.35	(1.02, 1.79)
Glycochenodeoxycholic Acid (GCDCA)								
Quartile 1	42	24	1.00		24	8	1.00	
Quartile 2	41	45	1.32	(0.58, 2.97)	24	14	2.38	(0.50, 11.25)
Quartile 3	41	41	1.28	(0.56, 2.93)	24	36	11.94	(2.55, 55.99)
Quartile 4	37	75	2.20	(0.99, 4.88)	24	38	7.61	(1.73, 33.44)
P-value for trend[4]				0.04				0.01
Continuous (log_2)			1.30	(1.05, 1.61)			1.91	(1.29, 2.83)
Taurochenodeoxycholic Acid (TCDCA)								
Quartile 1	43	21	1.00		24	6	1.00	
Quartile 2	40	40	1.73	(0.76, 3.95)	24	11	1.80	(0.45, 7.23)
Quartile 3	42	37	1.14	(0.49, 2.67)	24	30	4.67	(1.26, 17.29)
Quartile 4	36	87	2.85	(1.28, 6.34)	24	49	7.27	(1.99, 26.62)
P-value for trend[4]				0.01				0.001
Continuous (log_2)			1.30	(1.07, 1.58)			1.70	(1.26, 2.29)

[1] Models with categories of <5 cases are suppressed.

[2] Unconditional logistic regression, adjusted for age (5-year categories), sex, baseline cirrhosis status, and HBV DNA copies at baseline (<10,000, 10,000–<1,000,000, ≤1,000,000), smoking status, HbeAg (negative, positive), alcohol habit (no, yes), BMI (continuous), education level, ALT (continuous), and diabetes status at baseline.

[3] Conditional logistic regression, adjusted for matching factors (age (5-year categories), sex, baseline cirrhosis status, and HCV-RNA-positive rate), smoking status, alcohol habit (no, yes), BMI (continuous), education status, HCV genotype, ALT (continuous), and diabetes status at baseline.

[4] P-value for trend calculated using the Wald test.

Table 4.

Multivariate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between secondary bile acid concentrations and secondary/primary bile acid ratios and hepatocellular carcinoma risk among participants of the REVEAL-HBV and REVEAL-HCV cohorts.

	REVEAL-HBV (N=346)				REVEAL-HCV (N=192)[1]			
	Controls	Cases	OR[2]	95% CI	Controls	Cases	OR[3]	95% CI
Secondary Bile Acids	**n=161**	**n=185**			**n=96**	**n=96**		
Deoxycholic Acid (DCA)								
Quartile 1	51	67	1.00		32	27	1.00	
Quartile 2	36	47	0.92	(0.45, 1.88)	21	15	0.53	(0.18, 1.57)
Quartile 3	36	39	0.68	(0.32, 1.44)	22	26	1.14	(0.46, 2.80)
Quartile 4	38	32	0.41	(0.19, 0.88)	21	28	2.05	(0.78, 5.41)
P-value for trend[4]				0.02				0.08
Continuous (log_2)			0.88	(0.72, 1.07)			1.11	(0.88, 1.39)
Glycodeoxycholic Acid (GDCA)								
Quartile 1	48	44	1.00		30	23	1.00	
Quartile 2	38	40	1.04	(0.49, 2.21)	22	13	0.76	(0.29, 2.00)
Quartile 3	38	33	0.62	(0.28, 1.37)	22	22	1.22	(0.44, 3.35)
Quartile 4	37	68	1.40	(0.68, 2.86)	22	38	2.18	(0.89, 5.32)
P-value for trend[4]				0.3				0.06
Continuous (log_2)			1.01	(0.86, 1.19)			1.18	(0.96, 1.46)
Taurodeoxycholic Acid (TDCA)								
Quartile 1	69	58	1.00		51	31	1.00	
Quartile 2	31	30	1.39	(0.65, 2.97)	15	16	1.35	(0.49, 3.75)
Quartile 3	32	36	1.00	(0.48, 2.10)	15	21	1.85	(0.67, 5.08)
Quartile 4	29	61	1.42	(0.69, 2.91)	15	28	3.54	(1.21, 10.30)
P-value for trend[4]				0.4				0.02
Continuous (log_2)			1.04	(0.87, 1.24)			1.42	(1.10, 1.82)
Lithocholic Acid (LCA)								
Quartile 1	101	108	1.00		63	46	1.00	

	REVEAL-HBV (N=346)				REVEAL-HCV (N=192)[1]			
	Controls	Cases	OR[2]	95% CI	Controls	Cases	OR[3]	95% CI
Secondary Bile Acids	**n=161**	**n=185**			**n=96**	**n=96**		
Quartile 2	19	26	1.64	(0.74, 3.66)	11	16	2.32	(0.82, 6.63)
Quartile 3	20	24	0.53	(0.22, 1.25)	11	12	1.57	(0.51, 4.88)
Quartile 4	21	27	0.52	(0.22, 1.23)	11	22	3.33	(1.20, 9.26)
P-value for trend[4]				0.06				0.03
Continuous (log_2)			0.87	(0.65, 1.16)			1.5	(1.07, 2.11)
Glycolithocholic Acid (GLCA)								
Quartile 1	88	85	1.00		66	46	1.00	
Quartile 2	24	35	1.36	(0.62, 2.99)	10	18	2.61	(0.89, 7.71)
Quartile 3	25	33	1.17	(0.54, 2.53)	10	15	2.16	(0.68, 6.90)
Quartile 4	24	32	1.07	(0.51, 2.27)	10	17	4.31	(1.28, 14.53)
P-value for trend[4]				0.9				0.02
Continuous (log_2)			0.97	(0.76, 1.25)			1.7	(1.18, 2.45)
Taurolithocholic Acid (TLCA)								
Quartile 1	137	145	1.00		87	67		
Quartile 2	9	10	0.63	(0.20, 2.00)	3	12		
Quartile 3	8	17	1.22	(0.39, 3.77)	3	5		
Quartile 4	7	13	0.77	(0.22, 2.64)	3	12		
P-value for trend[4]				0.8				
Continuous (log_2)			1.05	(0.72, 1.54)			2.88	(1.55, 5.38)
Ursodexoycholic Acid (UDCA)								
Quartile 1	51	52	1.00		32	17	1.00	
Quartile 2	35	46	1.56	(0.75, 3.27)	21	21	1.99	(0.68, 5.82)
Quartile 3	38	35	1.21	(0.56, 2.61)	22	48	4.11	(1.55, 10.91)
Quartile 4	37	52	1.74	(0.85, 3.57)	21	10	1.55	(0.48, 5.02)
P-value for trend[4]				0.2				0.9
Continuous (log_2)			1.15	(0.93, 1.43)			1.1	(0.85, 1.42)
Glycoursodeoxycholic Acid (GUDCA)								

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	REVEAL-HBV (N=346)				REVEAL-HCV (N=192)[1]			
	Controls	Cases	OR[2]	95% CI	Controls	Cases	OR[3]	95% CI
Secondary Bile Acids	**n=161**	**n=185**			**n=96**	**n=96**		
Quartile 1	43	26	1.00		27	16	1.00	
Quartile 2	40	47	1.98	(0.87, 4.53)	23	23	1.37	(0.52, 3.63)
Quartile 3	41	44	2.08	(0.91, 4.73)	23	33	2.02	(0.84, 4.85)
Quartile 4	37	68	2.99	(1.33, 6.72)	23	24	2.04	(0.67, 6.21)
P-value for trend[4]				0.02				0.2
Continuous (log_2)			1.33	(1.05, 1.68)			1.21	(0.91, 1.60)
Tauroursodeoxycholic Acid (TUDCA)								
Quartile 1	91	70	1.00		60	45	1.00	
Quartile 2	24	15	0.65	(0.26, 1.60)	12	12	0.92	(0.32, 2.64)
Quartile 3	23	53	3.03	(1.48, 6.21)	12	16	1.37	(0.47, 3.95)
Quartile 4	23	47	1.80	(0.87, 3.72)	12	23	3.77	(1.28, 11.08)
P-value for trend[4]				0.03				0.02
Continuous (log_2)			1.32	(1.06, 1.66)			1.44	(1.04, 1.98)
Secondary/Primary Ratios								
LCA/CDCA								
Quartile 1	100	115	1.00		63	51	1.00	
Quartile 2	19	28	0.78	(0.35, 1.75)	11	20	1.51	(0.50, 4.51)
Quartile 3	20	27	0.96	(0.42, 2.19)	11	9	1.73	(0.55, 5.43)
Quartile 4	22	15	0.34	(0.13, 0.92)	11	16	1.50	(0.57, 3.93)
P-value for trend[4]				0.05				0.4
Continuous (log_2)			0.86	(0.72, 1.03)				
DCA/CA								
Quartile 1	50	77	1.00		31	35	1.00	
Quartile 2	36	41	0.71	(0.35, 1.47)	22	20	0.52	(0.20, 1.34)
Quartile 3	37	47	0.68	(0.34, 1.35)	21	21	1.39	(0.54, 3.55)
Quartile 4	38	20	0.27	(0.11, 0.65)	22	20	0.65	(0.26, 1.67)

	REVEAL-HBV (N=346)				REVEAL-HCV (N=192)[1]			
	Controls	**Cases**	**OR[2]**	**95% CI**	**Controls**	**Cases**	**OR[3]**	**95% CI**
Secondary Bile Acids	**n=161**	**n=185**			**n=96**	**n=96**		
P-value for trend[4]				0.005				0.6
Continuous (log_2)			0.81	(0.69, 0.96)			0.96	(0.81, 1.15)

[1] Models with categories of <5 cases are suppressed.

[2] Unconditional logistic regression, adjusted for age (5-year categories), sex, baseline cirrhosis status, and HBV DNA copies at baseline (<10,000, 10,000–<1,000,000, ≤1,000,000), smoking status, HbeAg (negative, positive), alcohol habit (no, yes), BMI (continuous), education level, ALT (continuous), and diabetes status at baseline.

[3] Conditional logistic regression, adjusted for matching factors (age (5-year categories), sex, baseline cirrhosis status, and HCV-RNA-positive rate), smoking status, alcohol habit (no, yes), BMI (continuous), education status, HCV genotype, ALT (continuous), and diabetes status at baseline.

[4] P-value for trend calculated using the Wald test.