Received: 10 February 2022 | Accepted: 25 April 2022

DOI: 10.1002/hep4.2003

ORIGINAL ARTICLE

A prospective investigation of serum bile acids with risk of liver cancer, fatal liver disease, and biliary tract cancer

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Funding information
National Cancer Institute, Grant/Award Number: Intramural Research Program

Abstract

Bile acids (BAs), major regulators of the gut microbiota, may play an important role in hepatobiliary cancer etiology. However, few epidemiologic studies have comprehensively examined associations between BAs and liver or biliary tract cancer. In the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study, we designed 1:1 matched, nested, case–control studies of primary liver cancer (n = 201 cases), fatal liver disease (n = 261 cases), and primary biliary tract cancer (n = 138 cases). Using baseline serum collected ≤30 years before diagnosis or death, we measured concentrations of 15 BAs with liquid chromatography–tandem mass spectrometry. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) using multivariable conditional logistic regression models, adjusted for age, education, diabetes status, smoking, alcohol intake, and body mass index. We accounted for multiple comparisons using a false discovery rate (FDR) correction. Comparing the highest to the lowest quartile, seven BAs were positively associated with liver cancer risk, including taurocholic acid (TCA) (OR, 5.62; 95% CI, 2.74–11.52; *Q* trend < 0.0001), taurochenodeoxycholic acid (TCDCA) (OR, 4.77; 95% CI, 2.26–10.08; *Q* trend < 0.0001), and glycocholic acid (GCA) OR, 5.30; 95% CI, 2.41–11.66; *Q* trend < 0.0001), and 11 were positively associated with fatal liver disease risk, including TCDCA (OR, 9.65; 95% CI, 4.41–21.14; *Q* trend < 0.0001), TCA (OR, 7.45; 95% CI, 3.70–14.97; *Q* trend < 0.0001), and GCA (OR, 6.98; 95% CI, 3.32–14.68; *Q* trend < 0.0001). For biliary tract cancer, associations were generally >1 but not significant after FDR correction. Conjugated BAs were strongly associated with increased risk of liver cancer and fatal liver disease, suggesting mechanistic links between BA metabolism and liver cancer or death from liver disease.

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INTRODUCTION

In 2020, more than 900,000 people were diagnosed with and more than 830,000 people died from liver cancer worldwide, making it the sixth most diagnosed cancer and the third leading cause of cancer death.[1] Risk factors for liver cancer include chronic liver disease, infection with hepatitis B virus (HBV) or hepatitis C virus (HCV),[2,3] heavy alcohol drinking,[4] obesity,[5,6] and diabetes,[7,8] whereas coffee has been consistently associated with lower liver disease and cancer risk.[9] Biliary tract cancers are less common than liver cancer and are associated with cigarette smoking,[10] alcohol consumption,[10] and obesity.[11]

Mounting evidence also suggests a potential role of the gut microbiota and bile acids (BAs) in the etiology of liver, bile duct, and gallbladder cancer.[12–14] Experimental studies have linked interactions between the gut microbiota and the liver, often called the liver–gut axis, to the pathogenesis of liver diseases, including nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC).[3] BAs have been cited as "the common language of communication along the liver–gut axis,"[15] making them a potential target for prevention of liver disease progression and liver cancer.[15–17] For example, in a NASH-induced HCC mouse model, researchers demonstrated that the secondary BA deoxycholic acid (DCA) promotes liver carcinogenesis.[18]

BAs are a family of steroid-based molecules that are produced from cholesterol and conjugated with glycine or taurine in the liver. Conjugated BAs are stored in the gallbladder and released into the intestinal tract to digest and absorb fat and to regulate intestinal epithelial homeostasis.[19] After synthesis in the liver, primary BAs are secreted into the intestine where approximately 95% are reabsorbed in the terminal ileum in their conjugated form. BAs remaining in the gut can be deconjugated and metabolized by bacteria in the distal small intestine and colon to form secondary BAs,[20] namely DCA and lithocholic acid (LCA),[21] which cause cancer in animal studies.[22] Most BAs are returned to the liver for detoxification and recirculation, with approximately 10% passing through systemic circulation and approximately 5% excreted in feces.[23]

In humans, higher circulating concentrations of primary and secondary BAs have been observed among patients with NAFLD and NASH than controls[17] and among patients with HCC than those with liver cirrhosis.[24,25] Additionally, three untargeted metabolomic studies[26–28] have each identified positive associations between conjugated BAs and liver cancer risk. In an analysis in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study, the BAs glycochenodeoxycholic acid (GCDCA) and glycocholic acid (GCA) were associated with a 4-fold to 7-fold increased risk of liver cancer and fatal liver disease[26]; yet, few epidemiologic studies have comprehensively examined the BA family. Less is known about associations with biliary tract cancers, including cholangiocarcinoma. A review of published studies indicated that these have generally been small and have had discordant results.[29] Here, we used a targeted BA panel to quantify concentrations of 15 BAs, including all major primary and secondary BAs and their conjugates, and estimated associations with incident liver cancer, incident biliary tract cancer, and liver disease mortality.

METHODS

Study design

A detailed description of the ATBC study design has been published.[30] From 1985 to 1988, 29,133 Finnish male smokers, aged 50–69 years, with no previous prior malignancy, alcoholism, or major medical conditions, including manifest cirrhosis, were enrolled in ATBC. Following the intervention, participants were passively followed through linkage with the Finnish Cancer Registry, which ascertains nearly 100% of cases,[31] and the Register of Causes of Death. All participants gave written informed consent, and the study was approved by the institutional review boards of the National Cancer Institute and the National Public Health Institute of Finland.

Eligible participants had available unthawed baseline serum, were cancer free at baseline, and were not diagnosed with another rare cancer during study follow-up. Cancer cases and deaths were ascertained through December 31, 2015. Incident primary liver cancer cases (n = 201) were defined as International Classification of Diseases, Ninth Revision (ICD-9): 155.0 and 155.2 or ICD-10: C22. Fatal liver disease cases (n = 261) were defined as ICD-9: 571 or ICD-10: K70–K77; and incident primary biliary tract cancer cases (n = 138) were defined as ICD-9: 156 and intrahepatic bile duct carcinoma as ICD-9: 155.1. Liver cirrhosis deaths were defined such that if an individual had a liver/biliary tract cancer diagnosis before they died of cirrhosis, they were recorded as a liver/biliary tract cancer case only. For each nested study, controls were randomly selected and matched to cases (1:1) on baseline age (±5 year) and date of blood collection (±30 days) and could be shared between cancer (liver or biliary) and fatal liver disease cases but not within cancer sets or within the fatal liver disease set.

At baseline, participants reported information on potential disease risk factors by questionnaires and donated a fasting (overnight) blood sample that was stored at −80°C. HBV and HCV were measured previously for a subset of participants; the methods have been described elsewhere.[32]

Laboratory analysis

Serum samples were analyzed for the following 15 of the most abundant BAs at Metabolon Inc.: chenodeoxycholic acid (CDCA), cholic acid (CA), GCDCA, taurochenodeoxycholic acid (TCDCA), GCA, taurocholic acid (TCA), DCA, LCA, glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TDCA), glycolithocholic acid (GLCA), taurolithocholic acid (TLCA), ursodeoxycholic acid (UDCA), glycoursodeoxycholic acid (GUDCA), and tauroursodeoxycholic acid (TUDCA).[33] Case and matched-control samples were placed next to each other in the same batch but in random order. Blinded quality control (QC) samples (n = 40 study duplicates) were regularly spaced throughout each batch. Intraclass correlation coefficients for the measured BAs all exceeded 0.99, indicating excellent technical reproducibility. Samples were spiked with a solution of corresponding labeled internal standards for each BA and were subjected to protein precipitation with acidified methanol. Samples were centrifuged, and a portion of the clear supernatant was evaporated to dryness in a stream of nitrogen at 40°C. The dried extract was reconstituted, and an aliquot was injected onto an Agilent Infinity II/Sciex QTrap 6500 liquid chromatography–tandem mass spectrometry system equipped with a C18 reverse-phase high-performance liquid chromatography column with acquisition in negative ion mode using electrospray ionization. The peak area of each parent (pseudo-multiple reaction monitoring mode) or product ion was measured against the peak area of the respective internal standard. Quantitation was performed using a weighted linear least squares regression analysis generated from fortified calibration standards prepared immediately before each run.

Statistical analysis

Differences in the distribution of potential risk factors between matched cases and controls were tested using the McNemar test for categorical variables and the Wilcoxon signed-rank test for continuous variables. Data were complete for all covariates except dietary variables that included alcohol intake (6% missing); missing values were assigned to the median value among controls. BA concentrations that fell below the lower limit of quantitation (LLOQ) were assigned to half of the minimum observed concentration for a given metabolite. If more than 50% of participants had levels below LLOQ, we created binary variables, indicating quantifiable or not quantifiable. We examined the association between BAs and hepatobiliary outcomes with three approaches. First, with no prior assumption about the shape of the relations, we defined quartiles (liver cancer and fatal liver disease) or tertiles (biliary tract cancer) of BA concentrations among the controls. Second, we evaluated continuous BA concentrations, which were $\log_2$ transformed to approximate normality because metabolites were generally right skewed; thus, a 1-unit increase was interpreted as a doubling in the concentration. Third, we examined the possibly nonlinear relation between continuous BA measures and risk of each endpoint, using restricted cubic splines fitted to a conditional logistic regression model using the SAS macro lgtphcurv9.[34] To test for nonlinear associations, we used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms.

Correlations between BAs were estimated using Spearman correlation coefficients. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) for BA variables using multivariable conditional logistic regression models. Models were conditioned on matching factors and adjusted for potential confounders, including age, education, diabetes status, smoking intensity, smoking duration, alcohol intake, and body mass index. Models were further adjusted for dietary variables previously associated with BAs,[35] including coffee, fat, fiber, and energy intake, using the nutrient density method of energy adjustment. To test for a linear trend across categories, we treated quartiles or tertiles as an ordinal variable in the model, with statistical significance determined by the Wald test. For spline models, covariate adjustments were the same as those used in the conditional logistic regression models. The spline models generally did not significantly improve the model fit ($p > 0.05$) for BAs, except for associations of GCA, TCA, DCA, and GDCA with fatal liver disease (Figures S1–S3). Therefore, we reported the associations for quantiles and $\log_2$-transformed BA models.

In secondary analyses, we created total BA variables by summing together the BAs overall or by group (e.g., total primary BAs). We examined the relative concentrations of secondary to primary BAs by using ratios of LCA/CDCA and DCA/CA. We also evaluated potential reverse causation by estimating ORs among cases that occurred within the first 10 years versus those occurring more than 10 years after baseline, and in separate analyses, by excluding cases that occurred within 1 year of baseline. Finally, we estimated ORs among the subset with HBV and HCV data, excluding those with positive HBV or HCV tests. Among those evaluated for hepatitis B and C infections, n = 266 matched liver cancer case–control sets, n = 432 fatal liver disease case–control sets, and n = 187 biliary tract cancer case–control sets had data on HBV and HCV. In total for this analysis, we excluded those who were seropositive for HBV or HCV (n = 30 for liver cancer set; n = 30 for liver disease set; n = 17 for biliary tract cancer set). The final study sample for the conditional models included n = 206 (103 cases and 103 matched controls) for liver cancer, n = 374 (187 cases and 187 matched controls) for fatal liver disease, and n = 134 (67 cases

and 67 matched controls) for biliary tract cancer. We also ran sensitivity analyses excluding participants who had a self-reported history of gallstones (n = 66) or gallstone operation (n = 47) or cirrhosis (n = 6).

All statistical tests were two sided. We calculated false discovery rate (FDR)-corrected *Q* values by the Benjamini-Hochberg procedure for each study outcome.[36] Differences were considered statistically significant at $Q < 0.05$ (main analyses) or $p < 0.05$ (secondary analyses). Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc, Cary, NC, USA) or with R version 4.0.2 (R Core Team, Vienna, Austria; https://www.R-project.org/).

RESULTS

Baseline characteristics of primary liver cancer, fatal liver disease, and primary biliary tract cancer cases with their matched controls are compared in Table 1. Overall, cases tended to be less well educated and to smoke more cigarettes per day than their matched controls. Liver cancer and biliary tract cancer cases were slightly older than matched controls, despite matching (±5 years), whereas liver cancer and fatal liver disease cases reported higher alcohol but lower coffee intake. On average, liver cancer cases had higher body mass index than their matched controls and were more likely to report a diabetes diagnosis and to test positive for past HBV infection. Among controls, BAs within a given classification (e.g., conjugated primary) were strongly positively correlated (Figure S4), and median BA concentrations tended to be higher among cases than controls (Tables S1–S3).

In multivariable-adjusted models, we observed positive associations ($Q < 0.05$) between seven BAs and liver cancer risk (Figure 1; Table S4) and between 11 BAs and fatal liver disease risk (Figure 2; Table S5) across BA quartiles. The strongest associations were observed for primary conjugated BAs. For liver cancer, participants in the highest quartile of GCDCA, TCDCA, GCA, or TCA had a 4.1-fold to 5.6-fold higher odds of liver cancer compared to those in the lowest quartile (Figure 1; Table S4). Associations for conjugated primary BAs with fatal liver disease were of greater magnitude, with those in the highest quartile of GCDCA, TCDCA, GCA, or TCA having a 4.7-fold to 9.7-fold higher odds of fatal liver disease compared to those in the lowest quartile (Figure 2; Table S5). While we observed positive associations for conjugated primary BAs with biliary tract cancer in the highest tertile for GCDCA and TCDCA compared to the lowest tertile, trends across BA tertiles were not statistically significant following correction for multiple comparisons (Figure 3; Table S6).

In categorical analyses, DCA and LCA were not significantly associated with risk of liver cancer. However, the *Q* trends for GDCA, were statistically significant, with those in the highest quartile having 3.3-fold higher odds of both liver cancer and fatal liver disease compared to those in the first quartile (Figures 1 and 2; Tables S4 and S5). For BAs in which >50% individuals had levels below LLOQ, presence versus absence of TCDCA, GLCA, or TLCA was associated with a 2.2-fold to 3.9-fold higher odds of fatal liver disease (Figure 2; Table S5). For biliary tract cancer, associations were generally positive but not statistically significant after FDR correction (Figure 3; Table S6). Two tertiary conjugated BAs, GUDCA and TUDCA, were associated with a 3–4 higher odds of liver cancer and liver disease mortality when examining the fourth quartile compared to the first (Figures 1 and 2; Tables S4 and S5).

For total BA concentrations, those in the highest compared to the lowest quartile had over a 4-fold higher odds of liver cancer (Table S7) and a 3.8-fold higher odds of fatal liver disease (Table S8), with the total primary BA load largely driving the association for both liver cancer and fatal liver disease risk. We found no associations between total BA concentrations and biliary tract cancer (Table S9).

Adjusting for dietary variables marginally attenuated ORs; however, ORs remained statistically significant, and few estimates were changed by >10% (Tables S4–S6). Associations between BAs and liver cancer were generally similar for cases occurring within 10 years of baseline and those occurring more than 10 years after baseline (Tables S10–S12); we did, however, observe stronger ORs for conjugated primary BAs and fatal liver disease during the first 10 years of follow-up. Excluding participants who tested positive for HBV or HCV infection minimally altered the ORs for liver cancer, fatal liver disease, and biliary tract cancer (Tables S13–S15). Excluding cases diagnosed in the first year of follow-up also minimally altered ORs (Tables S13–S15). Lastly, excluding individuals with a self-reported history of gallstones or gallstone operation or cirrhosis did not meaningfully change the results (Tables S16–S18).

DISCUSSION

We measured concentrations of 15 BAs, including GCDCA and GCA,[26] and examined associations with incident liver cancer, incident biliary tract cancer, and chronic liver disease mortality over 30 years of follow-up. After adjusting for multiple comparisons, we found that conjugated primary BAs were strongly associated with liver cancer and fatal liver disease risk, with ORs ranging from 4.1 to 9.7 when comparing those in the highest to the lowest quartile of a given BA. Additionally, we discovered that higher concentrations of conjugated secondary and tertiary BAs were associated with both liver cancer and fatal liver disease risk. While we observed positive associations in the highest tertile for

TABLE 1 Baseline characteristics of the ATBC study population for primary liver cancer, fatal liver disease, and primary biliary tract cancer

Baseline characteristics	Primary liver cancer			Fatal liver disease			Primary biliary tract cancer		
	Controls (n = 201)	Cases (n = 201)	*p* value[a]	Controls (n = 261)	Cases (n = 261)	*p* value[a]	Controls (n = 138)	Cases (n = 138)	*p* value[a]
Age (years), mean (SD)	56.9 (4.5)	57.5 (4.8)	0.01	55.2 (4.3)	55.2 (4.0)	0.75	57.2 (4.8)	57.8 (5.0)	0.01
Education (≤elementary), n (%)	39 (19.4)	56 (27.9)	0.05	59 (22.6)	74 (28.4)	0.15	26 (18.8)	32 (23.2)	0.44
Leisure PA (3+times/week), n (%)	38 (18.9)	40 (19.9)	0.90	39 (14.9)	53 (20.3)	0.15	25 (18.1)	30 (21.7)	0.54
Self-reported diabetes (yes), n (%)	5 (2.5)	23 (11.4)	0.0009	9 (3.5%)	9 (3.5)	1.00	1 (0.72)	6 (4.4)	0.13
Alcohol (g ethanol/day), mean (SD)	14.4 (14.3)	25.3 (29.4)	<0.0001	21.0 (23.0)	38.2 (36.2)	<0.0001	18.3 (24.9)	15.6 (17.5)	0.81
Smoking intensity (cigarettes/day), mean (SD)	18.8 (8.6)	21.0 (9.5)	0.02	20.7 (8.1)	22.1 (9.3)	0.05	18.8 (8.4)	21.0 (8.9)	0.03
Smoking duration (years), mean (SD)	34.2 (8.9)	36.4 (8.3)	0.01	33.7 (7.9)	33.6 (8.8)	0.78	36.9 (6.9)	36.7 (8.5)	0.61
Coffee intake (cups/day), mean (SD)	2.8 (1.0)	2.4 (1.1)	0.01	2.6 (1.1)	2.1 (1.1)	<0.0001	2.7 (1.1)	2.8 (1.1)	0.50
BMI (kg/m^2), mean (SD)	26.2 (3.2)	27.7 (4.6)	0.0002	26.7 (3.8)	26.8 (4.0)	0.96	26.1 (3.4)	26.4 (3.7)	0.43
HBV (anti-HBc, yes[b]), n (%)	7 (5.3)	19 (14.3)	0.03	13 (6.0)	14 (6.5)	1.00	4 (4.8)	12 (14.5)	0.08
HBV (HBsAg, yes[b]), n (%)	2 (1.5)	2 (1.5)	1.00	2 (0.9)	1 (0.5)	1.00	1 (1.2)	0 (0)	NA[c]
HCV (anti-HCV, yes[b]), n (%)	2 (1.5)	8 (6.0)	0.11	2 (0.9)	6 (2.8)	0.29	0 (0)	1 (1.2)	NA[c]

Abbreviations: ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention; BMI, body mass index; HBc, hepatitis B core antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; n, number; PA, physical activity.

[a]*p* values calculated using the McNemar test (categorical variables) or the Wilcoxon signed-rank test (continuous variables).

[b]Data on hepatitis B and C virus status were available for 133 liver cancer cases and matched controls, 216 chronic liver disease deaths and matched controls, and 83 biliary tract cancer cases and matched controls.

[c]No statistics are computed because there are fewer than two levels that are not missing.

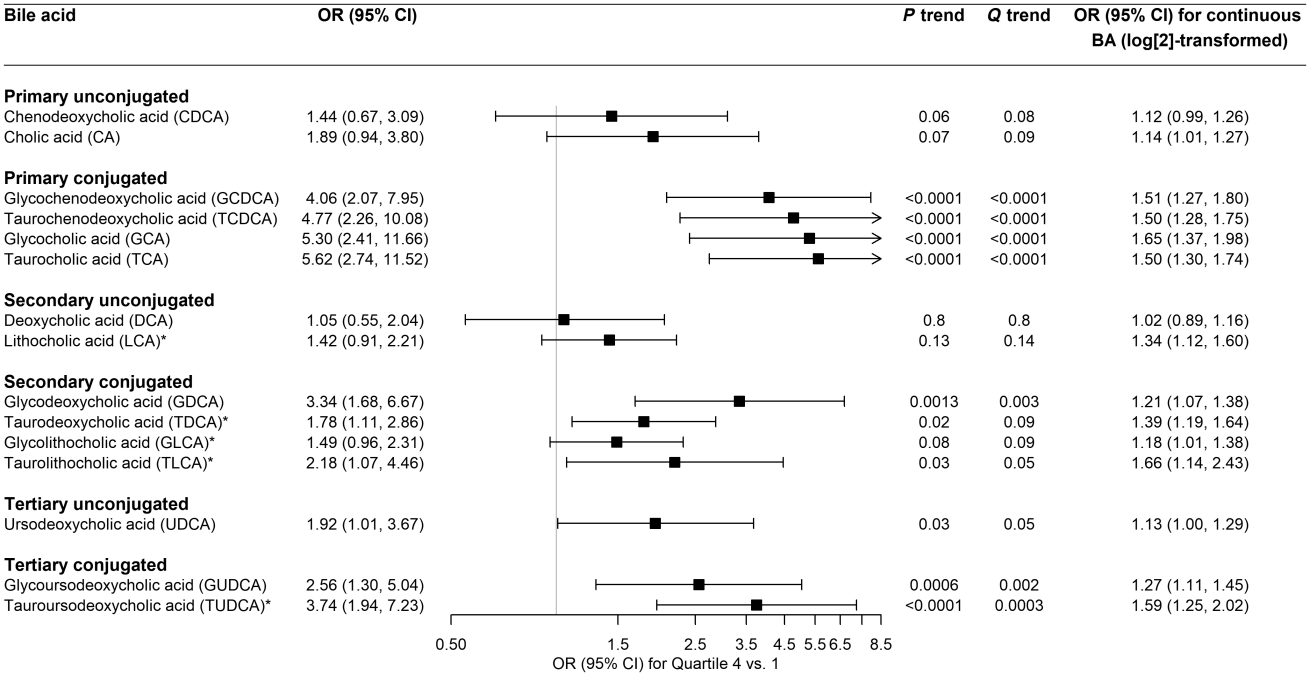

FIGURE 1 Associations of individual BAs with risk of primary liver cancer estimated in a nested case–control study of 201 liver cancer cases and 1:1 matched controls in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) cohort. ORs and 95% CIs are for those in the highest quartile of BA concentrations (ng/mL) compared to those in the lowest quartile based on the BA distribution in controls. Asterisks indicate BAs having more than 50% of concentrations below the lower limit of quantitation with ORs comparing those with quantifiable concentrations to those with concentrations below the limit of quantification. *p* trends were corrected for multiple comparisons, and the false discovery rate-corrected *Q* trend is reported. Analyses were adjusted for age at baseline (years), education (≤ to or > elementary education), number of cigarettes/day, smoking duration (years), body mass index (kg/m^2), history of diabetes (yes/no), and alcohol (none, <10.8 g/day, ≥10.8 g/day). BA, bile acid; CI, confidence interval; OR, odds ratio.

conjugated primary and secondary BAs compared to the lowest tertile with biliary tract cancer, these associations were no longer statistically significant after FDR correction. Overall, our findings suggest that higher circulating concentrations of BAs, particularly conjugated BAs, are associated with higher risk of liver cancer and fatal liver disease, suggesting that a higher BA load may play an important role in disease etiology. Our observation that conjugated rather than unconjugated BAs are more strongly associated with liver cancer and liver disease mortality may also reflect the accumulation of conjugated BAs resulting from subclinical hepatic damage, which leads to significantly increased concentrations of conjugated primary and secondary BAs in circulation.[37,38]

Our results build on prior untargeted metabolomic studies in ATBC,[26] the European Prospective Investigation into Cancer and Nutrition cohort,[28] and the Korean Cancer Prevention Study II cohort[27] that found higher BA levels of GCA, GCDCA, and TUDCA were associated with liver cancer risk. A recent study in the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer (REVEAL)-HBV and REVEAL-HCV cohorts from Taiwan used a targeted platform to estimate associations between BAs and HCC in individuals with hepatitis[39] and found that glycine- and taurine-conjugated primary BAs were associated with a 2-fold to 8-fold increased risk of HBV- and HCV-related HCC. Unlike the REVEAL studies, chronic hepatitis infection is not the primary risk factor for liver cancer in our study population. In fact, we found that serum BAs were associated with liver cancer and fatal liver disease risk among those who tested negative for HBV and HCV infection at baseline.

The complex interactions between the gut microbiome, BAs, and liver are not well understood. However, dysbiosis, which is characterized by a decrease in gut microbial diversity and an increase in proinflammatory species, has been shown to accompany chronic liver disease.[16,40,41] Experimental studies have demonstrated that BAs can promote liver cancer through a variety of diet and obesity-related mechanisms. For example, the administration of a high-fat diet to mice was shown to increase the abundance of bacteria that can convert primary to secondary BAs, thereby increasing hepatic levels of DCA and resulting in the secretion of inflammatory and tumor-promoting factors and HCC development in mice.[18] Another study in mice demonstrated that secondary BAs promote obesity-associated liver cancer through prostaglandin E_2-mediated suppression of antitumor immunity.[42]

Another line of evidence linking BAs to liver cancer and liver disease concerns BA signaling and the nuclear farnesoid X receptor (FXR). BA reabsorption

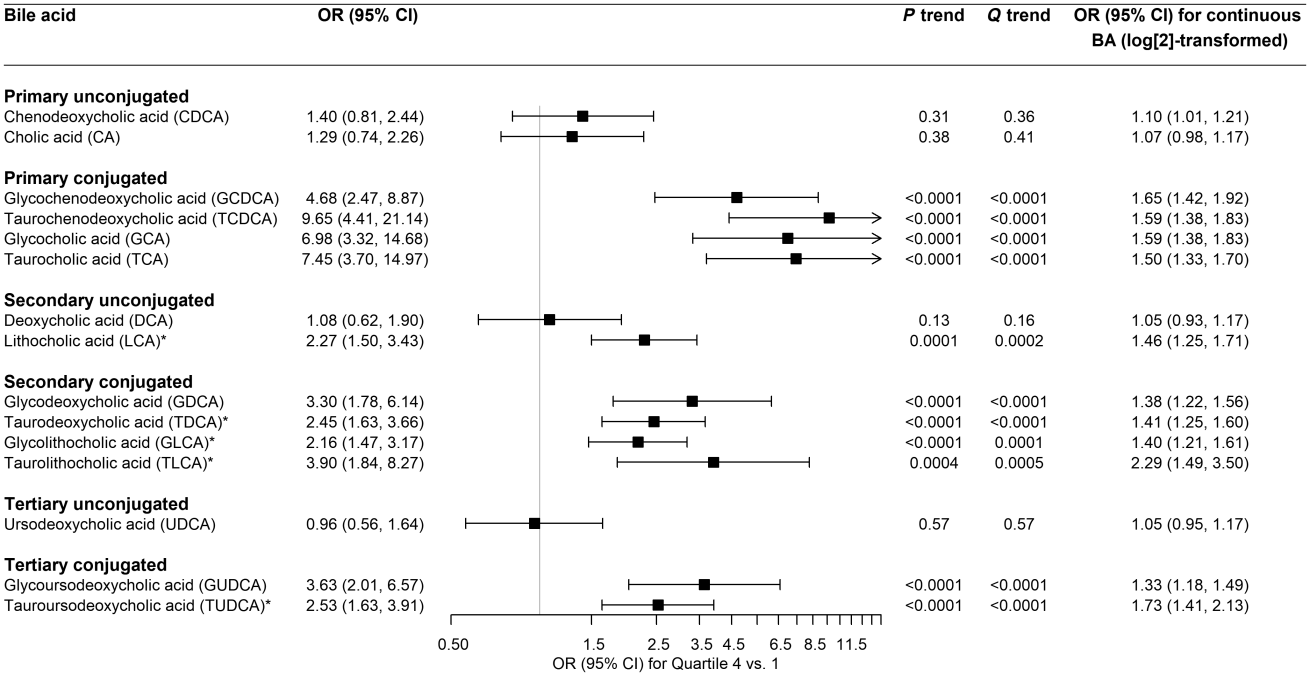

FIGURE 2 Associations of individual BAs with risk of fatal liver disease estimated in a nested case–control study of 261 fatal liver disease cases and 1:1 matched controls in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) cohort. ORs and 95% CIs are for those in the highest quartile of BA concentrations (ng/mL) compared to those in the lowest quartile or tertile, based on the BA distribution in controls. Asterisks indicate BAs having more than 50% of concentrations below the lower limit of quantitation with ORs comparing those with quantifiable concentrations to those with concentrations below the limit of quantification. *p* trends were corrected for multiple comparisons, and the false discovery rate-corrected *Q* trend is reported. Analyses were adjusted for age at baseline (years), education (≤ to or > than elementary education), number of cigarettes/day, smoking duration (years), body mass index (kg/m^2), history of diabetes (yes/no), and alcohol (none, <10.8 g/day, ≥10.8 g/day). BA, bile acid; CI, confidence interval; OR, odds ratio.

from the small intestine is facilitated by nuclear FXR,[43] which is known to regulate BA homeostasis and production by repressing the rate-limiting enzyme of the synthetic pathway. Mice lacking nuclear FXR have increased hepatic BAs and a high incidence of liver tumors.[44] Additionally, altered BA/FXR signaling has been associated with gastrointestinal and hepatic diseases, including inflammatory bowel disease, diarrhea-predominant irritable bowel syndrome, cirrhosis, and nonalcoholic liver disease in human and experimental studies.[45,46]

Strengths of our study include its prospective study design with baseline blood collected up to 30 years before liver/biliary tract cancer diagnosis or liver disease death, our use of a targeted panel for quantifying circulating BA concentrations, and extensive information on hepatobiliary cancer and liver disease risk factors, including HBV and HCV positivity, which was very low in our study population. Furthermore, our study was strengthened by availability of data on HBV and HCV; results were similar when we excluded participants with positive HBV or HCV tests, suggesting that a potential BA mechanism is relevant in the absence of hepatitis infection.

Limitations of our study include that it is limited to Finnish male smokers; thus, our findings should be replicated in diverse populations, including women, non-Europeans, and nonsmokers. We lacked information on asymptomatic liver disease at baseline; however, we found similar results for cases occurring in the first 10 years or 10–30 years of follow-up, which provides some reassurance to this concern. While we cannot rule out potential confounding by unmeasured (e.g., primary sclerosing cholangitis) or unknown risk factors,[46] our results were not meaningfully altered by adjustment for measured risk factors (i.e., diabetes and alcohol intake) or in sensitivity analyses excluding individuals with a self-reported history of gallstones or cirrhosis. Our study lacked serial blood collections, and serum BA concentrations may vary considerably within individuals over time[33]; thus, longitudinal studies documenting changes in BA concentrations over time would be informative. Finally, although associations between BAs and biliary tract cancer were no longer significant after adjusting for multiple comparisons, to our knowledge, this is the first epidemiologic study of serum BAs and biliary tract cancer. Future studies with larger sample sizes or pooling efforts, which are facilitated by our targeted approach, are needed.

In summary, higher concentrations of conjugated primary, secondary, and tertiary BAs, measured in serum up to 30 years before cancer diagnosis or death, were associated with increased risk of liver cancer and fatal liver disease, indicating that the accumulation of BAs

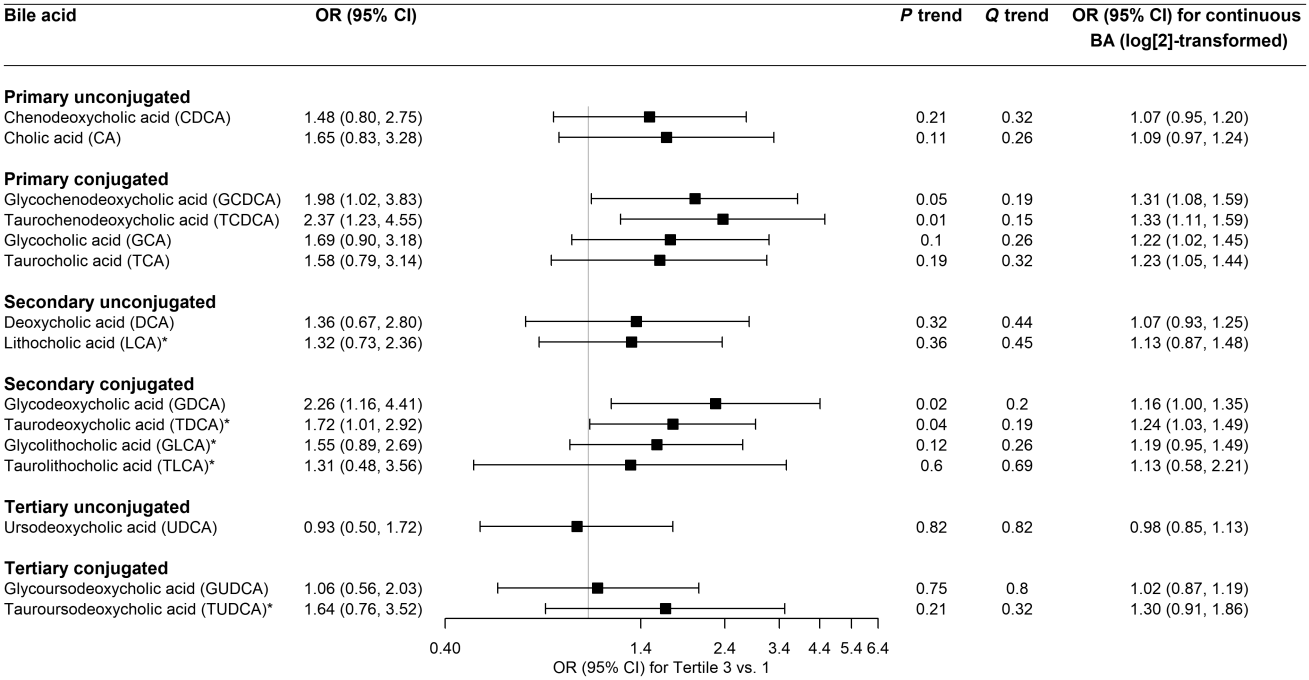

FIGURE 3 Associations of individual BAs with risk of primary biliary cancer estimated in a nested case–control study of 138 biliary tract cancer cases and 1:1 matched controls in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) cohort. ORs and 95% CIs are for those in the highest tertile of BA concentrations (ng/mL) compared to those in the lowest tertile, based on the BA distribution in controls. Asterisks indicate BAs having more than 50% of concentrations below the lower limit of quantitation with ORs comparing those with quantifiable concentrations to those with concentrations below the limit of quantification. *p* trends were corrected for multiple comparisons, and the false discovery rate-corrected *Q* trend is reported. Analyses were adjusted for age at baseline (years), education (≤ to or > than elementary education), number of cigarettes/day, smoking duration (years), body mass index (kg/m^2), history of diabetes (yes/no), and alcohol (none, <10.8 g/day, ≥10.8 g/day). BA, bile acid; CI, confidence interval; OR, odds ratio.

may play an important role in liver disease progression and cancer development. Future prospective studies in diverse populations with serial blood collections are needed to better understand how BA concentrations vary over time and in response to changing disease risk factors, including infections, alcohol consumption, obesity, and diabetes.

CONFLICT OF INTEREST

Nothing to report.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Farhat Z, Freedman ND, Sampson JN, Falk RT, Koshiol J, Weinstein SJ, et al. A prospective investigation of serum bile acids with risk of liver cancer, fatal liver disease, and biliary tract cancer. Hepatol Commun. 2022;6:2391–2399. https://doi.org/10.1002/hep4.2003