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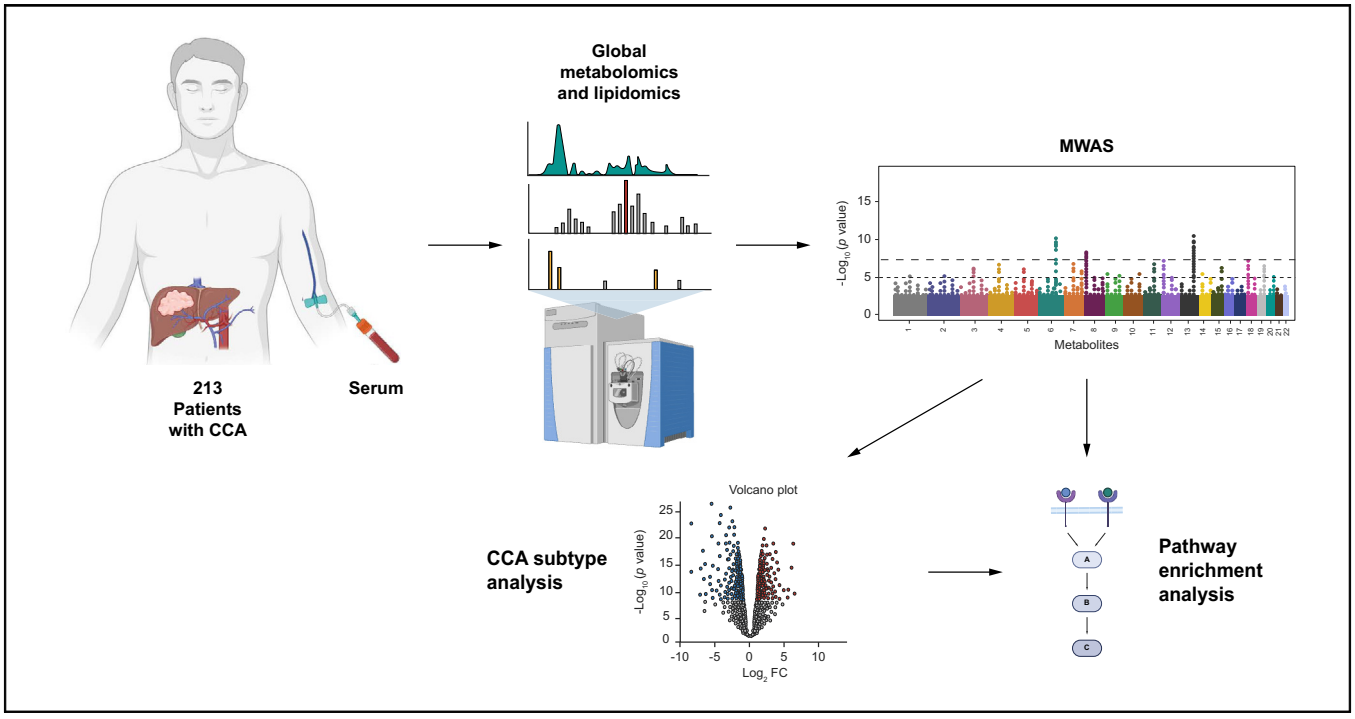
Authors

Linsey E. Jackson, Jennifer L. Tomlinson, Roberto Alva-Ruiz, Lindsey A. Gregory, Seul Kee Byeon, Amro M. Abdelrahman, Dong-Gi Mun, Caroline W. Grant, Zachary C. Fogarty, Chen Wang, Lewis R. Roberts, Rondell P. Graham, Mitesh J. Borad, Sumera I. Ilyas, Gregory J. Gores, Akhilesh Pandey, Arjun P. Athreya, Rory L. Smoot

Correspondence

athreya.arjun@mayo.edu (A.P. Athreya), pandey.akhilesh@mayo.edu (A. Pandey), smoot.rory@mayo.edu (R.L. Smoot).

Graphical abstract



Highlights

- Compilation of serum and clinical data from 213 patients with cholangiocarcinoma.
- Evaluation of all anatomic subtypes of cholangiocarcinoma and surrogates of disease stage.
- Demonstration of the impact of biliary obstruction on serum metabolomic profiles.

Impact and implications

Cholangiocarcinoma (CCA) is a highly lethal hepatobiliary cancer with limited treatment response, highlighting the need for a better understanding of the disease biology. Using a global metabolomics and lipidomics platform, we characterized distinct changes in the serum of 213 patients with CCA compared with healthy controls. The results of this study elucidate novel metabolic pathways in CCA. These findings benefit stakeholders in both the clinical and research realms by providing a foundation for improved disease diagnostics and identifying novel targets for therapeutic design.

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Research article

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Metabolome-wide association identifies altered metabolites and metabolic pathways in the serum of patients with cholangiocarcinoma

Linsey E. Jackson,[1,†] **Jennifer L. Tomlinson**,[2,†] Roberto Alva-Ruiz,[2] Lindsey A. Gregory,[2] Seul Kee Byeon,[3] Amro M. Abdelrahman,[2] Dong-Gi Mun,[3] Caroline W. Grant,[4] Zachary C. Fogarty,[5] Chen Wang,[6] Lewis R. Roberts,[7] Rondell P. Graham,[3] Mitesh J. Borad,[8,9,10] Sumera I. Ilyas,[7,11] Gregory J. Gores,[7] Akhilesh Pandey,[3,12,13,*] Arjun P. Athreya,[4,*] Rory L. Smoot[2,14,*]

[1]*Center For Clinical and Translational Science, Mayo Clinic, Rochester, MN, USA;* [2]*Department of Surgery, Mayo Clinic, Rochester, MN, USA;* [3]*Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA;* [4]*Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA;* [5]*Department of Computational Biology, Mayo Clinic, Rochester, MN, USA;* [6]*Department of Quantitative Health Sciences, Mayo Clinic, Rochester, MN, USA;* [7]*Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA;* [8]*Division of Hematology and Medical Oncology, Mayo Clinic, Phoenix, AZ, USA;* [9]*Department of Molecular Medicine, Mayo Clinic, Rochester, MN, USA;* [10]*Mayo Clinic Comprehensive Cancer Center, Mayo Clinic, Phoenix, AZ, USA;* [11]*Department of Immunology, Mayo Clinic, Rochester, MN, USA;* [12]*Center For Individualized Medicine, Mayo Clinic, Rochester, MN, USA;* [13]*Manipal Academy of Higher Education (MAHE), Manipal, India;* [14]*Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA*

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Background & Aims: Metabolomic and lipidomic analyses provide an opportunity for novel biological insights. Cholangiocarcinoma (CCA) remains a highly lethal cancer with limited response to systemic, targeted, and immunotherapeutic approaches. Using a global metabolomics and lipidomics platform, this study aimed to discover and characterize metabolomic variations and associated pathway derangements in patients with CCA.

Methods: Leveraging a biospecimen collection, including samples from patients with digestive diseases and normal controls, global serum metabolomic and lipidomic profiling was performed on 213 patients with CCA and 98 healthy controls. The CCA cohort of patients included representation of intrahepatic, perihilar, and distal CCA tumours. Metabolome-wide association studies utilizing multivariable linear regression were used to perform case–control comparisons, followed by pathway enrichment analysis, CCA subtype analysis, and disease stage analysis. The impact of biliary obstruction was evaluated by repeating analyses in subsets of patients only with normal bilirubin levels.

Results: Of the 420 metabolites that discriminated patients with CCA from controls, decreased abundance of cysteine-glutathione disulfide was most closely associated with CCA. Additional conjugated bile acid species were found in increased abundance even in the absence of clinically relevant biliary obstruction denoted by elevated serum bilirubin levels. Pathway enrichment analysis also revealed alterations in caffeine metabolism and mitochondrial redox-associated pathways in the serum of patients with CCA.

Conclusions: The presented metabolomic and lipidomic profiling demonstrated multiple alterations in the serum of patients with CCA. These exploratory data highlight novel metabolic pathways in CCA and support future work in therapeutic targeting of these pathways and the development of a precision biomarker panel for diagnosis.

Impact and implications: Cholangiocarcinoma (CCA) is a highly lethal hepatobiliary cancer with limited treatment response, highlighting the need for a better understanding of the disease biology. Using a global metabolomics and lipidomics platform, we characterized distinct changes in the serum of 213 patients with CCA compared with healthy controls. The results of this study elucidate novel metabolic pathways in CCA. These findings benefit stakeholders in both the clinical and research realms by providing a foundation for improved disease diagnostics and identifying novel targets for therapeutic design.

Keywords: Metabolome-wide association study; Metabolomic; Lipidomic; Pathway enrichment.

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† These authors contributed equally to this work.

* Corresponding authors. Addresses: Department of Molecular Pharmacology and Experimental Therapeutics, 200 First Street SW, Rochester, MN 55905, USA. Tel.: 507-422-6073 (A.P. Athreya); Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA. Tel: 507-293-9564 (A. Pandey); Department of Surgery and Department Biochemistry and Molecular Biology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA. Tel.: 507-284-1529; Fax: 507 284 5196 (R.L. Smoot).

E-mail addresses: athreya.arjun@mayo.edu (A.P. Athreya), pandey.akhilesh@mayo.edu (A. Pandey), smoot.rory@mayo.edu (R.L. Smoot).

Introduction

Cholangiocarcinoma (CCA) is a group of heterogeneous malignancies that arise from the biliary epithelium, comprising 3% of all gastrointestinal cancers.[1,2] CCAs are classified as intrahepatic (iCCA), perihilar (pCCA), or distal (dCCA), depending on their anatomic site of origin along the biliary tree.[3] Each subtype is characterized by distinct clinicopathologic features, treatment options, and prognosis.[2,3] Despite recent advances in the treatment of CCA, the overall 5-year survival remains at 7–20%, and resistance to systemic therapy, whether primary or acquired, is a common clinical feature.[2,4]

Current therapeutic approaches for this disease include resection, transplantation, or varying combinations of systemic therapy, such as chemotherapy, targeted therapy, and/or immunotherapy.[4–9] Recurrence rates after resection are approximately 70%, and only 20–40% of patients demonstrate an objective response to medical therapies.[10] Thus, new approaches are needed to understand the underlying biology of these tumours and provide new diagnostic and therapeutic targets. It is also imperative that these novel techniques be applied across all anatomic subtypes to understand the molecular heterogeneity of this disease.

Metabolomics is the study of endogenous metabolites in a biological sample, reflecting the cellular functions of the local environment.[11] Lipidomics is the study of molecular lipid species, which are small hydrophobic or amphipathic molecules, on a global scale.[12] Employing metabolomic and lipidomic serum analysis as a diagnostic tool in hepatobiliary disease has been explored, and efforts yielded predictive signatures associated with broad diagnostic categories (normal, hepatocellular carcinoma, CCA, and primary sclerosing cholangitis).[13] However, these algorithms are limited to targeted metabolites and one CCA subtype. Global metabolomics and lipidomics have not been explored. Metabolome-wide association studies (MWASs) have been increasingly used for metabolomic exploration outside of the CCA paradigm and have proven to be powerful tools for the analysis of high-dimensional metabolomic/lipidomic datasets (e.g., sample sizes smaller than the number of measured metabolites) for biomarker discovery.[14–16]

This study hypothesized that CCA induces distinct metabolic differences that can be detected in the sera of patients and the identification of those alterations can be discovered by leveraging multivariate regression analysis. First, MWAS was used to characterize CCA-driven metabolic alterations compared with healthy controls, identifying novel metabolic pathways associated with CCA biology. Next, pathway enrichment was used to delineate metabolic signatures of each CCA subtype and disease stage, as well as a signature correlated with liver dysfunction as denoted by elevated serum bilirubin. To our knowledge, this is the largest study combining global metabolomics and lipidomics providing a novel, detailed landscape of the disease overall and important clinical subsets.

Patients and methods

Study population

Serum samples from patients with *in situ* CCA (n = 329) and healthy controls (n = 98) were collected from January 1, 2010, to December 31, 2017, at the Mayo Clinic in Rochester, MN, USA. Patients included in the study had a confirmed CCA diagnosis by expert consultation using multiple diagnostic modalities (computed tomography scan, magnetic resonance imaging, endoscopic retrograde cholangiopancreatography with biliary brushings, and endoscopic ultrasound) and/or histopathology. Patients were excluded because of the presence of a malignancy other than CCA, additional synchronous malignancies, serum draw status post cancer resection or initiation of cancer treatment, and inadequate serum sample volume for analysis. Ultimately, 213 patients with CCA and 98 controls were included in the final analysis. Given that the samples were collected before the widespread use of next-generation sequencing in CCA, anatomic classification is highlighted over molecular and/or genomic classification. Clinical and laboratory test values were collected from the patients' electronic medical records. Control participants were defined as patients undergoing screening colonoscopy with or without a history of polyps; screening criteria were based on contemporary guidelines provided by the United States Preventive Services Task Force. The research protocol was approved by the Mayo Clinic Institutional Review Board. Informed written consent was obtained from each patient before their samples were used for biomedical research.

Sample preparation and metabolomic and lipidomic analysis

In brief, metabolomic and lipidomic analysis was performed at Metabolon, Inc. (Morrisville, USA), using mass spectrometry to detect metabolites and lipids, as previously described.[17] Further detailed methods are included in the Supplementary Methods.

MWAS and pathway enrichment

MWAS for differential metabolite expression in patients with CCA compared with healthy controls was performed through linear regression, using mass spectrometry-based measurements of metabolites and lipids. In a complete case analysis, age, sex, BMI, cirrhosis, hepatitis B and C, and type 2 diabetes mellitus (T2DM) were used as covariates for the MWAS. The Benjamini–Hochberg false discovery rate (FDR) method was used to correct for multiple comparisons. Features associated with CCA that had a corrected p value less than 0.05 were considered statistically significant. Statistically significant metabolite features were selected for input into MetaboAnalyst version 5.0 for pathway enrichment analysis.[18] The SMPDB library of 99 metabolite sets were used as the reference dataset.

Subset analysis of patients with liver dysfunction

Serum metabolites and lipids from 109 patients with normal bilirubin levels were compared with those from 100 patients with elevated bilirubin levels at the time of serum specimen collection using a Student t test with FDR correction. Of the 213 patients with CCA, four were excluded from analysis owing to missing serum bilirubin measurements. Elevated bilirubin was defined as a serum bilirubin above the upper limit of normal as defined by Mayo Clinic Laboratories (1.2 mg/dL). Fifty features with a constant or single value across samples were found and deleted from the analysis. Metabolites with a fold change greater than 2 and a p value less than 0.05 in patients with normal bilirubin levels compared with patients with elevated bilirubin levels were deemed significantly different and subsequently excluded from the MWAS results. Pathway enrichment analysis was then performed on the metabolites correlated with elevated bilirubin, indicative of underlying liver dysfunction, to characterize a novel metabolic signature.

Results

Characteristics of the patient cohort

A total of 311 participants, including 213 patients with *in situ* CCA and 98 control participants, were included in this study. There were significantly more male patients and patients with cirrhosis in the CCA cohort. Otherwise, the cohorts were well matched. Clinical characteristics are summarized in Table 1, with a full description of clinical parameters presented in Table S1.

Identification of CCA-mediated metabolomic changes in serum with MWAS

Upregulated and downregulated metabolites identified in the serum of patients with CCA through MWAS are depicted in a Manhattan plot (Fig. 1A). Of the 561 metabolites associated with CCA, 397 were upregulated and 164 were downregulated. Fifty-one metabolites had missing values that were imputed. Among the top 20 differentially expressed metabolites, 45% are substrates of primary or secondary bile acid metabolism (Fig. 1A and Table S2). Individual abundances of serum bile acid metabolites from patients with CCA compared to healthy controls are depicted in Fig. 1B. Pathway analysis of the downregulated metabolites identified significant attentuation of caffeine metabolism (p = 0.00004) in patients with CCA (Fig. 2A). Pathway analysis of the upregulated metabolites identified enrichment of amino acid metabolism including methionine metabolism and glycine and serine metabolism (Fig. 2B).

Dysregulated bile acid metabolism and identification of metabolomic signature

CCA is commonly associated with either segmental or central biliary obstruction, which can lead to secondary alterations in liver function.[1] Given the marked number of differentially expressed bile acid metabolites observed on the MWAS and the desire to eliminate global underlying liver dysfunction as a confounder in our metabolic analysis, serum from patients with CCA and elevated bilirubin at the time of collection was compared with that of patients with CCA and normal bilirubin levels. Serum from patients with CCA and elevated serum bilirubin showed upregulation of 195 metabolites and downregulation of 71 metabolites. Pathway enrichment of metabolites with increased abundance showed upregulation of tryptophan metabolism, malate–aspartate shuttle, and carnitine synthesis in patients with CCA and liver dysfunction (Fig. S1A). The valine, leucine, and isoleucine degradation pathway (p = 0.0001) was found to be significantly altered following pathway analysis of the metabolites with decreased abundance in patients with CCA and liver dysfunction (Fig. S1B).

Serum metabolomic profiling of CCA following removal of liver dysfunction-associated metabolites

Metabolites associated with elevated serum bilirubin and liver dysfunction were removed from the MWAS results. Of the 597 significantly altered metabolites in the MWAS, 177 were associated with elevated serum bilirubin, leaving 420 remaining. Notably, even after removing liver dysfunction-associated metabolites, several bile acid metabolites remained within the top 20 hits (Table 2). This finding suggests that CCA biology is associated with specific alterations in bile acid metabolism. Glutamate metabolism was also highly represented in the top metabolites (Table 2). With liver dysfunction-related metabolites removed, pathway analysis of upregulated and downregulated metabolites was repeated. Caffeine metabolism remained the only significantly downregulated pathway ($p = 1.4 \times 10^{-5}$) (Fig. 2C). Again, enrichment of pathways related to amino acid metabolism was observed among the metabolites with increased abundance (Fig. 2D).

Metabolomic analysis of CCA subtypes

Differences in the metabolic profiles of iCCA, pCCA, and dCCA were measured in comparison with healthy controls. The metabolites associated with each CCA subtype were then used for pathway enrichment (Fig. S2A and B). Separate analyses per CCA subtype were conducted for patients with normal serum bilirubin to identify signatures independent of liver dysfunction. Patients with dCCA had seven upregulated and one downregulated metabolites when compared with healthy controls, which did not provide enough data for pathway analysis (Fig. S3A). Pathway analysis of metabolites with increased abundance in iCCA and pCCA identified significant enrichment of the malate–aspartate shuttle pathway (Fig. 3A). In addition, enrichment of amino acid metabolism pathways was observed in serum from patients with pCCA, with both the glucose–alanine and methionine metabolism being significantly upregulated (Fig. 3A). Serum from patients with pCCA also demonstrated

Table 1. Demographics of patient cohorts and baseline characteristics of controls and patients with cancer.

Variables	Controls (n = 98)	Cases (n = 213)	*p* value
Sex, n (%)			
Male	45 (45.9)	133 (62.4)	**0.006***
Female	53 (54.1)	80 (37.6)	
BMI (kg/m^2)			
Mean ± SD	29.4 ± 5.8	28.3 ± 6	0.13†
Median (IQR)	28.4 (25.5–32.7)	27.8 (23.7–31.9)	
Range	19.8–49.6	18.1–55.6	
Age (years)			
Mean ± SD	62.9 ± 9.9	60.2 ± 14.3	0.09†
Median (IQR)	63 (58–70)	61 (49–70)	
Range	32–85	23–94	
Comorbidities, n (%)			
Cirrhosis	0	34 (16.1)	**<0.01***
Hepatitis B	0	0	
Hepatitis C	1 (1)	3 (1.4)	1*
Type 2 diabetes mellitus	15 (15.3)	45 (20.9)	0.28*

Differences in clinical variables were compared using the *Chi-square test or Fisher's exact test for categorical variables and †two-sample t tests for continuous variables; p <0.05 indicates statistical significance; p-values that meet significance are bolded.

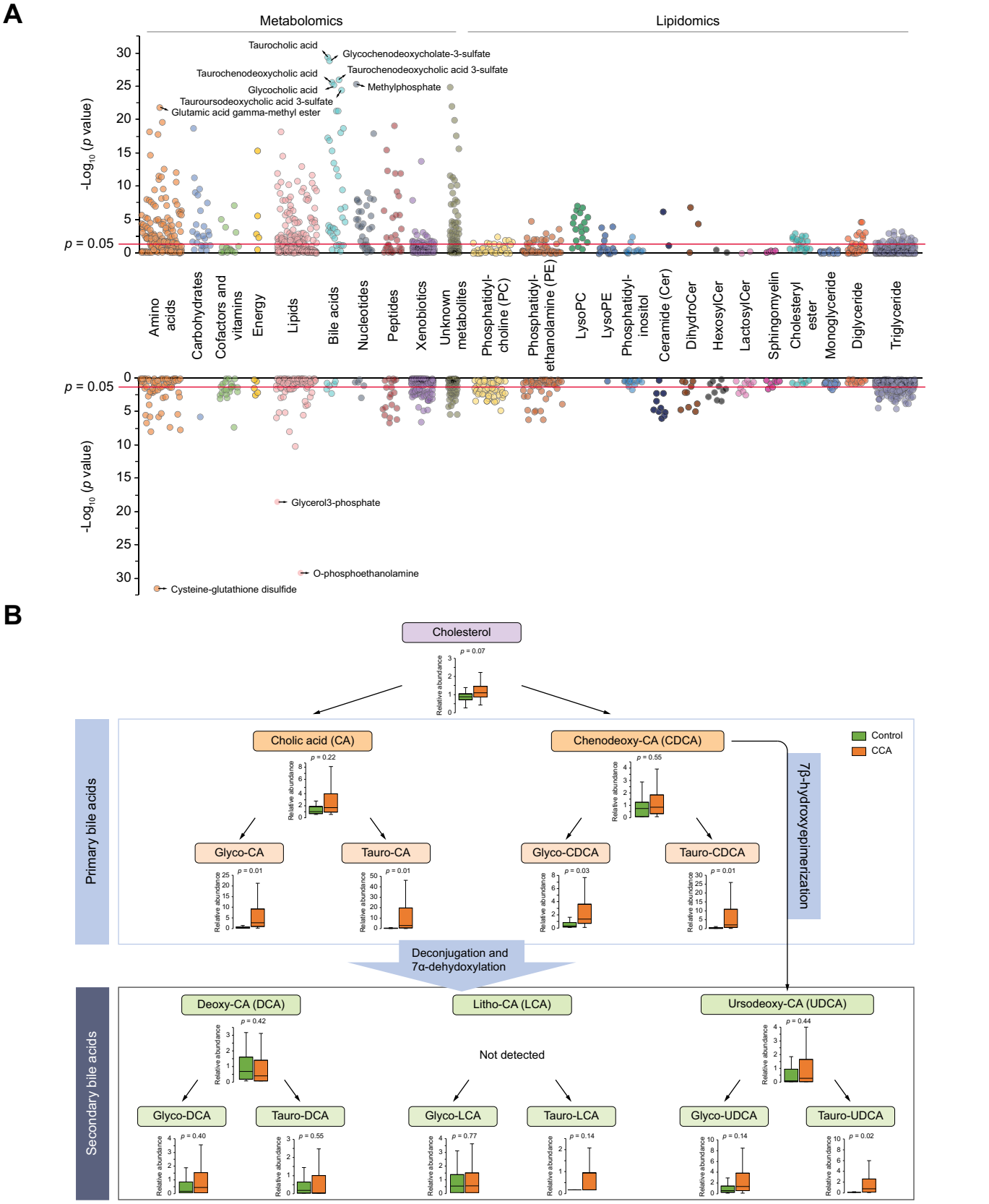

Fig. 1. Global MWAS of serum from patients with CCA compared with controls. (A) Manhattan plot of MWAS results. Metabolites with increased (upper panel) and decreased (lower panel) abundance in serum of patients with CCA compared with controls are demonstrated. Differential analysis was performed using a linear regression model, p-value < 0.05 indicating statistically significant. (B) Bile acid metabolic pathway overlayed with box plots of individual bile acid metabolite abundances in patients with CCA compared with controls. Comparison of metabolite abundance was conducted using Student's *t* test, with FDR-adjusted p <0.05 indicating statistical significance. CA, cholic acid; CCA, cholangiocarcinoma; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; FDR, false discovery rate; LCA, lithocholic acid; MWAS, metabolome-wide association study; UDCA, ursodeoxycholic acid.

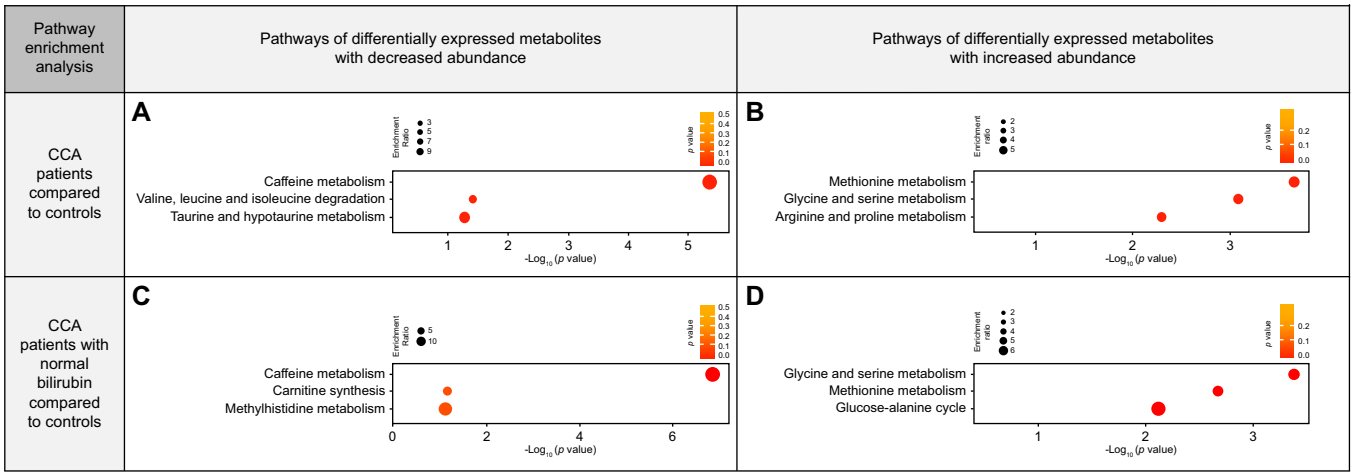

Fig. 2. Pathway enrichment of differentially expressed metabolites in patients with CCA compared with healthy controls. Top three enriched metabolic pathways of metabolites with (A) decreased and (B) increased differential expression in patients with CCA compared with controls. Top three enriched metabolic pathways of metabolites with (C) decreased and (D) increased differential expression in patients with CCA compared with controls following the removal of metabolites correlated with liver dysfunction. Pathways were identified using Fisher's test, with FDR-adjusted p <0.05 indicating statistical significance. CCA, cholangiocarcinoma; FDR, false discovery rate.

global downregulation of lipid/fatty acid metabolism, the most significant being phosphatidylcholine biosynthesis (Fig. 3B).

Analysis of tumour stage-correlated metabolites

Using t tests, we identified 39 metabolites associated with tumour stage (Fig. S4 and Table S3), 43% of which are involved in fatty acid metabolism. Linear regression, adjusting for age, sex, BMI, cirrhosis, hepatitis B and C, and T2DM, was performed to validate these findings and identified 51 metabolites (Table S4). Of the 12 named metabolites to reach statistical significance in both tests, 50% were fatty acid metabolites. Metabolites exhibited the same pattern of downregulation in early-stage disease and upregulation in late-stage disease in both analyses. Pathway enrichment revealed alterations in both acetyl group transfer into the mitochondria and glycine/serine metabolism in early-stage disease (Fig. S5A and B).

Discussion

This is a comprehensive characterization of global serum metabolic changes in patients with CCA. The use of a large disease cohort that included all anatomic CCA subtypes provided novel insights into the serum metabolic perturbations of CCA and liver dysfunction, and defined signatures associated with both

Table 2. Top 20 differentially abundant metabolites in patients with CCA compared with controls following removal of metabolites correlated with liver dysfunction.

Pathway	Chemical name	p value	Association
Primary bile acid metabolism	Glycochenodeoxycholate-3-sulfate	1.65E-29	Positive
Secondary bile acid metabolism	Taurochenodeoxycholic acid 3-sulfate	1.05E-26	Positive
Secondary bile acid metabolism	Tauroursodeoxycholic acid	3.74E-25	Positive
Glutamate metabolism	Glutamic acid gamma-methyl ester	1.72E-22	Positive
Primary bile acid metabolism	Glycochenodeoxycholic acid 3-glucuronide	4.83E-22	Positive
Glutamate metabolism	Glutamate	2.90E-20	Positive
Gamma-glutamyl amino acid	Gamma-glutamylphenylalanine	9.10E-20	Positive
Secondary bile acid metabolism	Glycohyocholic acid	1.83E-19	Positive
Amino sugar metabolism	*N*-Acetylglucosamine/*N*-acetylgalactosamine	1.94E-19	Positive
Endocannabinoid	Stearoyl ethanolamide	5.98E-19	Positive
Primary bile acid metabolism	Glycocholic acid 3-sulfate	9.37E-19	Positive
Unknown	X-25433	1.11E-18	Positive
Fibrinogen cleavage peptide	Fibrinopeptide B	3.79E-16	Positive
Secondary bile acid metabolism	Glycoursodeoxycholic acid 3-sulfate	4.69E-16	Positive
Unknown	X-21470	8.43E-16	Positive
Endocannabinoid	Palmitoylethanolamide	3.23E-15	Positive
Food component/plant	Mannonate	1.70E-14	Positive
Secondary bile acid metabolism	Tauroursodeoxycholic acid	3.19E-14	Positive
Partially characterized molecules	GlcNAc sulfate conjugate of $C_{21}H_{34}O_2$ steroid	4.63E-14	Positive
Fatty acid, dicarboxylate	Octadecadienoate (C18:2-DC)	1.21E-13	Positive

Pathway, chemical name, p value, and association trend of metabolites with differential abundance in patients with CCA. Differential analysis performed using a linear regression model. FDR-adjusted p value <0.05 indicates statistical significance.

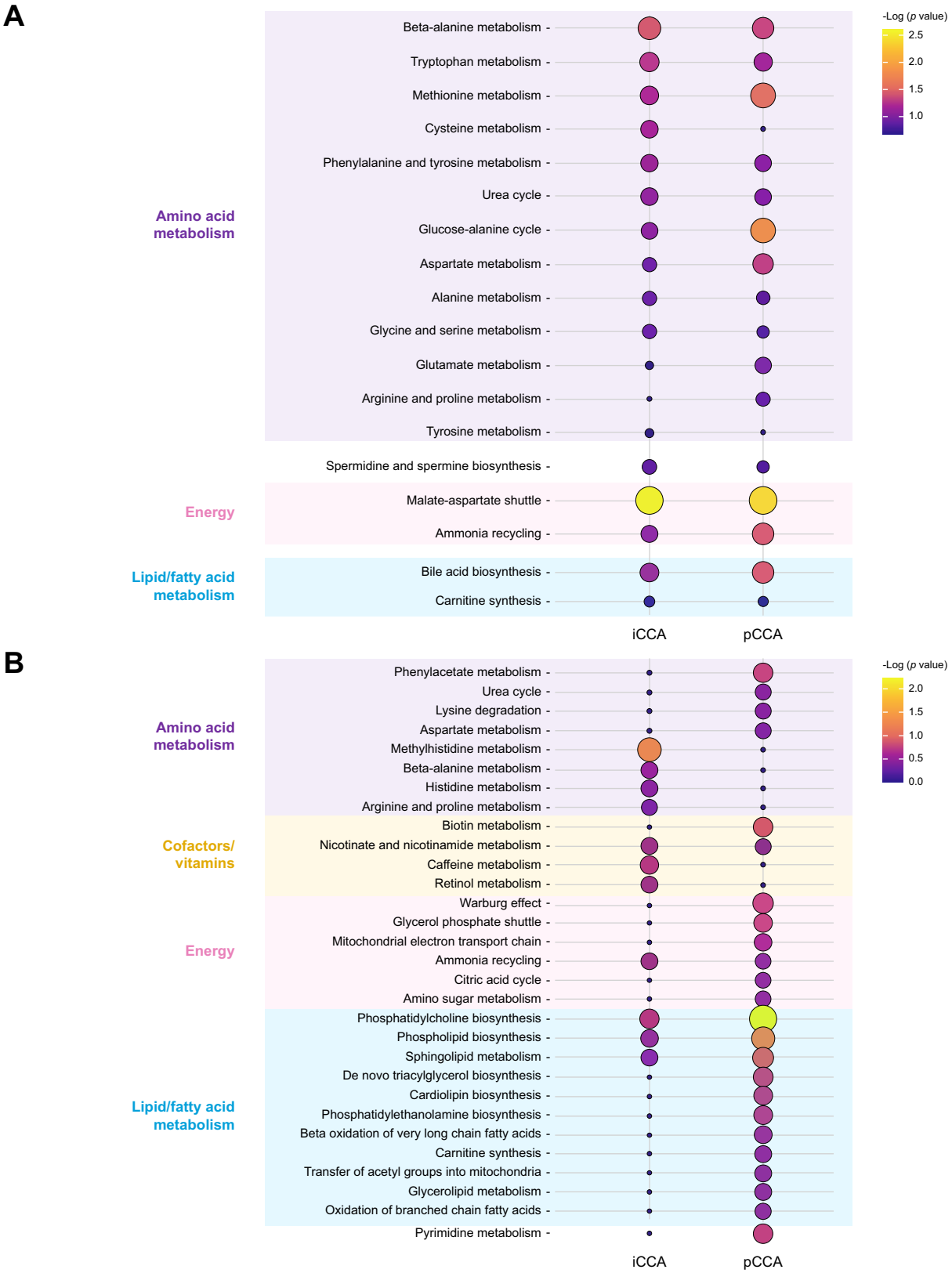

Fig. 3. Comparison of metabolite profile by CCA subtype following removal of metabolites correlated with liver dysfunction. Enriched pathways following pathway analysis of metabolites with (A) increased and (B) decreased differential expression in patients with iCCA or pCCA compared with controls. Colour and size of points in the plot correlates with -log(p). Pathways were identified using Fisher's test, with FDR-adjusted p <0.05 indicating statistical significance. CCA, cholangiocarcinoma; FDR, false discovery rate; iCCA, intrahepatic cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma.

conditions. The biological implications of the metabolomic changes observed in this study require additional research efforts. However, several notable observations were made including perturbation of bile acid metabolite abundance even in the absence of elevated serum bilirubin levels, changes in caffeine metabolism, and alterations in multiple mitochondrial redox-associated pathways/metabolites such as the malate–aspartate shuttle and glutathione (GSH). These findings are discussed below in detail.

Multiple metabolites identified by MWAS were substrates in primary or secondary bile acid metabolism. This trend remained following the identification and removal of liver dysfunction-associated metabolites. All substrates were conjugated bile acids to either taurine or glycine. Similar to our findings, a previous study concluded that serum from patients with CCA was observed to have a higher ratio of conjugated bile acids to unconjugated bile acids when compared to patients with benign biliary disease and/or healthy controls.[19] The role of conjugated bile acids in CCA cancer biology was explored by Dai *et al.*,[20] who reported that conjugated bile acids induced the growth of human CCA cells via enhanced activation of NF-κB. In addition, conjugated bile acids have been shown to decrease the expression of farnesoid x receptor (FXR), a liver tumour suppressor.[21] Conjugated bile acids also have been observed to promote extracellular signal-regulated kinase 1/2 and protein kinase B signaling predominantly through activation of sphingosine 1-phosphate receptor 2 (S1PR2), leading to cell growth and invasion in preclinical models of CCA.[22,23] Previously, a bile acid biomarker panel consisting of chenodeoxycholic acid (CDCA) and taurochenodeoxycholic acid (TCDCA) demonstrated improved AUC, sensitivity, and specificity than carbohydrate antigen 19-9 (CA19-9) in differentiating patients with CCA from those with benign biliary disease and healthy controls.[19] Similarly, glycocholic acid (GCA) and TCDCA were recognized as phenotype-specific biomarkers for CCA.[24]

Cysteine-glutathione disulfide (CSSG) was highly downregulated and emerged as the most differentially abundant metabolite between patients with CCA and healthy controls. CSSG is a mixed disulfide formed upon oxidation of GSH.[25] Given the instability of GSH in plasma, most GSH is converted rapidly to CSSG.[26] Thus, CSSG can be interpreted as a surrogate of GSH levels in our study. Our data revealed that CSSG was significantly decreased in patients with CCA compared with normal controls. Oxidative stress is an important mechanism of carcinogenesis and tumour progression through signal alteration and by inflicting DNA damage. GSH is one of the most abundant reducing agents found in living organisms and is responsible for maintaining intracellular redox homeostasis. Accordingly, GSH is rapidly depleted in times of oxidative stress.[27] Both increased and decreased levels of GSH have been implicated in carcinogenesis.[28] GSH deficiency, as observed in our data, is suggested to result in increased cell susceptibility to oxidative stress, inflammation, and tumour progression.

Increased abundance of glutamate and other metabolites involved in the glutamate metabolism pathway was identified by MWAS and disease stage analysis. Metabolic reprogramming is one of the hallmarks of cancer in order to meet the increased energy requirements of cancer cells; CCA is no exception.[29] Glutamine and its major downstream product glutamate are critical bioenergy substrates for cell growth.[30] Glutamate not only supports oxidative phosphorylation and the TCA cycle, but also functions as a substrate for the synthesis of fatty acids, amino acids, antioxidants, and nucleotides.[31,32] These findings suggest a reliance of tumour cells on glutamine for anabolic metabolism and altered signaling, thus making glutamine metabolism an attractive target for cancer therapy.[32,33]

Downregulation of caffeine metabolism was the only pathway alteration that reached significance when evaluating all patients with CCA compared to healthy controls in the MWAS, and the trend remained once metabolites associated with liver dysfunction were removed. Caffeine undergoes demethylation and/or hydroxylation to form four distinct metabolite byproducts: paraxanthine, theobromine, theophylline, and 1,3,7-trimethyluric acid.[34] Of these byproducts, all except theobromine were significantly decreased in abundance in patients with CCA. Although patient caffeine intake data were outside the scope of this study, theobromine levels were comparable between healthy controls and patients with CCA, suggesting that the differences noted in the other caffeine metabolite levels were intrinsic to caffeine metabolism rather than to environmental effects. Caffeine metabolism occurs in the liver and is predominantly catalyzed by cytochrome P450 (CYP) 1A2.[35] These intriguing data suggest that CYP1A2 function may be reduced in patients with CCA. The impact of this finding on the biology and natural history of patients with CCA is an area for future research. However, alterations in CYP family enzymes have previously been linked to worse overall and recurrence-free survival in several cancers, including hepatocellular carcinoma.[36,37]

Pathway analysis also identified upregulation of the malate–aspartate shuttle in patients with iCCA and pCCA compared with controls. The malate–aspartate shuttle is used in various tissue types, such as the heart and liver, to facilitate mitochondrial ATP production downstream of glycolysis by transporting electrons across the inner mitochondrial membrane.[38] However, the role of the malate–aspartate shuttle in cancer cells is unknown, as aerobic glycolysis and fermentation can occur at rates much faster than mitochondrial respiration. Recent exploration in glioma cells has suggested that the malate–aspartate shuttle functions to maintain decreased NADH to NAD+ ratios in the cytosol, allowing for the elevated rates of glycolysis by the cancer cells.[39] In these glioma cells, inhibition of the malate–aspartate shuttle using a selective inhibitor decreased the intracellular ATP levels and increased apoptosis, effects not observed in primary cell cultures. Similarly, colon cancer cells were found to use the shuttle to maintain NADH/NAD+ ratios, facilitating mitochondrial oxidative phosphorylation.[40] These data suggest that similar to glioma and colon cancer cells, CCA may use the malate–aspartate shuttle to support high rates of glycolysis. Targeted inhibition of this shuttle and/or the mitochondrial redox machinery could represent a novel therapeutic approach, if off-target toxicity can be limited.

Limitations of this study include retrospective data collection from a single institution. The inclusion of larger and more diverse cohorts of patients in this dataset is necessary to validate the characterized associations between CCA and unique serum changes representative of the disease process, including the addition of disease controls. Future studies using preclinical models of CCA are needed to determine the mechanistic

significance of the altered metabolites and lipids. In addition, investigation is needed to establish specific clinical applications for the metabolic biomarkers suggested in this study, whether as part of diagnosis, prognosis, and/or disease monitoring.

Conclusion

In conclusion, this study characterizes the global serum metabolomic and lipidomic profile of patients with CCA and provides novel insights into serum metabolic pathway perturbations of CCA.

Abbreviations

CA19-9, carbohydrate antigen 19-9; CCA, cholangiocarcinoma; CDCA, chenodeoxycholic acid; CSSG, cysteine-glutathione disulfide; CYP, cytochrome P450; dCCA, distal cholangiocarcinoma; FDR, false discovery rate; FXR, farnesoid X receptor; GCA, glycocholic acid; GSH, glutathione; iCCA, intrahepatic cholangiocarcinoma; MWAS, metabolome-wide association study; pCCA, perihilar cholangiocarcinoma; S1PR2, sphingosine-1-phosphate receptor 2; T2DM, type 2 diabetes mellitus; TCDCA, taurochenodeoxycholic acid.

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Conflicts of interest

The authors have no relevant conflicts of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Conceptualization: LRR, GJG, SII, RPG, MJB, AP, APA, RLS. Resources: LRR, AP, APA, RLS. Data curation: LEJ, JLT, RAR, LG, ZCF. Formal analysis: LEJ, JLT, AMA, ZCF, APA, RLS. Methodology: LEJ, ZCF, CW, APA. Software: LEJ, DGM, CWG, ZCF, APA. Validation: LEJ, JLT, APA. Visualization: LEJ, JLT, AMA. Investigation: JLT, RAR, LG, SKB, DGM. Project administration: AP, APA, RLS. Supervision: APA, RLS. Funding acquisition: RLS. Writing – original draft: LEJ, JLT. Writing – review and editing: LEJ, JLT, RAR, LG, SKB, AMA, DGM, CWG, ZCF, CW, LRR, GJG, SII, RPG, MJB, AP, APA, RLS.

Data availability statement

In the absence of publicly available datasets, the data underlying this article will be shared on reasonable request to the corresponding author(s).

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2024.101068.

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Author names in bold designate shared co-first authorship

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