ONLINE-ONLY SUPPLEMENTARY MATERIAL

Supplementary Table 1. Identified bile acids based on targeted metabolomics analysis (using UPLC-MS/MS)

	Name	MetaClass	HMDB ID	KEGG ID
CA	cholic acid	Primary unconjugated	HMDB0000619	C00695
HCA	hyocholic acid	Primary unconjugated	HMDB0000760	C17649
CDCA	chenodeoxycholic acid	Primary unconjugated	HMDB0000518	C02528
TαMCA	tauro α-muricholic acid	Primary Tau-conjugated	NA	NA
THCA	taurohyocholic acid	Primary Tau-conjugated	HMDB0011637	C15516
TCA	taurocholic acid	Primary Tau-conjugated	HMDB0000036	C05122
TCDCA	taurochenodeoxycholic acid	Primary Tau-conjugated	HMDB0000951	C05465
GHCA	glycohyocholic acid	Primary Gly-conjugated	HMDB0240607	NA
GCA	glycocholic acid	Primary Gly-conjugated	HMDB0000138	C01921
GCDCA	glycochenodeoxycholic acid	Primary Gly-conjugated	HMDB0000637	C05466
βUCA	β-ursocholic acid	Secondary unconjugated	NA	NA
UCA	ursocholic acid	Secondary unconjugated	HMDB0000917	C17644
6-ketoLCA	6-ketolithocholic acid	Secondary unconjugated	NA	NA
βUDCA	3β-ursodeoxycholic acid	Secondary unconjugated	HMDB0000686	C17662
UDCA	ursodeoxycholic acid	Secondary unconjugated	HMDB0000946	C07880
HDCA	α-hyodeoxycholic acid	Secondary unconjugated	HMDB0000733	C15517
7-DHCA	7-ketodeoxycholic acid	Secondary unconjugated	HMDB0000391	NA
βCA	3β-cholic acid	Secondary unconjugated	HMDB0000419	NA
dehydroLCA	dehydrolithocholic acid	Secondary unconjugated	NA	NA
alloLCA	allolithocholic acid	Secondary unconjugated	HMDB0000381	NA
isoLCA	isolithocholic acid	Secondary unconjugated	HMDB0000717	C17658
LCA	lithocholic acid	Secondary unconjugated	HMDB0000761	C03990
7-ketoLCA	7-ketolithocholic acid	Secondary unconjugated	HMDB0000467	NA
12-ketoLCA	12-ketolithocholic acid	Secondary unconjugated	HMDB0000328	NA
βDCA	3β-deoxycholic acid	Secondary unconjugated	HMDB0000438	NA
DCA	deoxycholic acid	Secondary unconjugated	HMDB0000626	C04483
βCDCA	3β-chenodeoxycholic acid	Secondary unconjugated	HMDB0000361	C17660
TUDCA	tauroursodeoxycholic acid	Secondary Tau-conjugated	HMDB0000874	C16868

TLCA	taurolithocholic acid	Secondary Tau-conjugated	HMDB0000722	C02592
TDCA	taurodeoxycholic acid	Secondary Tau-conjugated	HMDB0000896	C05463
GUDCA	glycoursodeoxycholic acid	Secondary Gly-conjugated	HMDB0000708	NA
GDCA	glycodeoxycholic acid	Secondary Gly-conjugated	HMDB0000631	C05464
GLCA	glycolithocholic acid	Secondary Gly-conjugated	HMDB0000698	C15557
GLCA-3S	glycolithocholic acid-3-sulfate	Sulfated BAs	HMDB0002639	C11301
LCA-3S	lithocholic acid-3-sulfate	Sulfated BAs	HMDB0000907	NA
CDCA-3Glu	chenodeoxycholic acid-3-β-d-glucuronide	Glucuronidation	HMDB0002430	NA
NorCA	norcholic acid	Others	NA	NA
NorDCA	23-nordeoxycholic acid	Others	NA	NA

Note: NA indicates not available in HMDB or KEGG database.

Supplementary Table 2. Previously published cross-sectional studies examining circulating BAs in NAFLD.

Ref.	Year	Cohort	Main results
29	2020	171 Asians with biopsy-proven NAFLD and 31 non-NAFLD controls	Levels of unconjugated and conjugated BAs were increased in stools of NAFLD subjects with fibrosis, and the total stool BAs were elevated in **non-obese patients** with significant fibrosis. **Primary bile acids (CA, CDCA, GCDCA) and UDCA** increased significantly with worsening fibrosis severity. Stool **LCA** level was elevated in **obese subjects** with significant fibrosis.
41	2019	Twin and Family cohort (n=156): 36 NAFLD patients; Biopsy-proven NAFLD cohort (n=156)	Twin and Family cohort: **Higher chenodeoxycholyl-conjugates and lower glycohycholate** was observed in NAFLD compared to non-NAFLD-controls. Biopsy-proven-NAFLD cohort: No differences in total BA were observed between NAFL versus NASH. The total unconjugated BA significantly decreased across NASH categories. The **total serum BA increas**ed with increasing fibrosis stage. The **primary-conjugated-BA proportion increased**, whereas **unconjugated BA, unconjugated-cholyl and chenodeoxycholyl-conjugates significantly decreased** with increasing fibrosis stage.
42	2021	NAFLD patients (n=102) and controls (n=50)	Several glycine conjugated forms of BAs showed significant associations with higher severity of liver inflammation and fibrosis. Plasma **7-Keto-DCA** levels showed the strongest association with advanced stages of fibrosis [odds ratio(95% confidence interval)] 4.2(1.2-16.4), NASH 24.5(4.1-473), and ballooning 18.7(4.8-91.9). Plasma 7-Keto-LCA was associated with NASH 9.4(1.5-185) and ballooning 5.9(1.4-28.8).
43	2018	NAFLD patients (25 NAFL, and 37 NASH) and 24 controls	**Increased total primary BAs and decreased secondary BAs** characterized patients with NASH. **Total conjugated primary BAs** were higher in NASH versus NAFL and versus controls. NASH patients had **higher conjugated to unconjugated CDCA, CA, and total primary BAs.** The **total CA/CDCA** ratio was higher in NAFLD, regardless of presence or absence of diabetes. **Conjugated CA and TCA** directly and **secondary to primary BA ratio** inversely correlated to histological NAFLD activity score. A higher ratio of **total secondary to primary BA decreased and higher conjugated CA** increased the likelihood of having significant fibrosis.
44	2020	Healthy controls (n=20), NASH without (n=23) or with cirrhosis (n=11), and NASH-HCC without (n=14) or with cirrhosis (n=19)	**Circulating levels of total and individual BAs** were higher in NASH patients compared with healthy controls. Levels of the **GCA**, TCA, GCDCA, and TCDCA, were significantly increased in cirrhotic vs noncirrhotic NASH patients, independent of HCC occurrence.
45	2019	Training cohort (n=87): 15 healthy controls, 24 non-NASH	The **ratio of circulating conjugated CDCA to MCA** increased from healthy controls to non-NASH individuals and NASH individuals in a stepwise manner in the

		and 48 NASH patients; Testing cohort (n=47): 25 non-NASH and 22 NASH patients	training cohort and was associated with greater histological severity of NASH: steatosis, lobular inflammation, ballooning, and fibrosis stage. This ratio was also increased in the validation cohort of NASH patients, and it was able to predict NASH (AUROC: 75%) and significant fibrosis (AUROC: 71%) in these two cohorts.
46	2021	Discovery set of early chronic liver disease study (n=122): 87 NAFLD and NASH patients, and 35 healthy controls; testing set (n=33): 12 NAFL and 21 NASH patients	Total BAs and conjugated BAs increased gradually. **GCA/TCA and GDCA/TDCA** were altered with the severity of disease. Prediction model based on **GCA/TCA and GDCA/TDCA** obtained AUROC values of 0.91 and 0.94 in the discovery set and testing set, respectively.
50	2020	390 subjects were screened with liver elastography, detecting significant liver fibrosis in 58 subjects and steatosis in 186 subjects.	Total BAs were significantly higher in subjects with fibrosis, with CDCA displaying the greatest increase among individual BAs. The primary conju- gated BAs, GCA and GCDCA, displayed the strongest association with fibrosis by logistic regression. High LCA levels were strongly associated with advanced fibrosis. In contrast, DCA and total unconjugated secondary BAs were positively associated with steatosis, whereas relative GUDCA was negatively associated.

Supplementary Figure 1. Diagnostic performance of selected individual biomarkers for identifying mild or significant fibrosis in different subgroups of NAFLD patients.