Targeted metabolomics reveals aberrant profiles of serum bile acids in patients with schizophrenia

Ying Qing, Pengkun Wang, Gaoping Cui, Juan Zhang, Kemei Liang, Zhong Xia, Peng Wang, Lin He and Wei Jia

Emerging evidence indicates that bile acids (BAs), which are signaling molecules that regulate metabolism and inflammation, appear to be dysregulated in schizophrenia (SZ). Further investigation is warranted to comprehensively characterize BA profiles in SZ. To address this, we analyzed serum BA profiles in 108 drug-free patients with SZ and in 108 healthy controls (HCs), divided into a discovery set (n = 119) and a validation set (n = 97), using ultraperformance liquid chromatography triple quadrupole mass spectrometry. Forty serum BAs were detected and absolutely quantified using calibration curves. Global BA profiling showed differences in SZ and HC groups in both discovery and validation sets. The concentrations of chenodeoxycholic acid, ursodeoxycholic acid, 3β-cholenoxycholic acid, 7-ketolithocholic acid, 3-dehydrocholic acid, total BAs, and unconjugated BAs were significantly lower in patients with SZ compared with HCs in the two sample sets. The BA deconjugation potentials by gut microbiota and the affinity index of the farnesoid X receptor (FXR) were notably decreased in SZ patients compared to those of HCs. Conjugated BAs and BA deconjugation potentials differed in SZ patients with first versus recurrent episodes, although similar BA profiles were observed in both groups. In addition, a panel of 8 BA variables acted as a potential auxiliary diagnostic biomarker in discriminating SZ patients from HCs, with area under the curve values for receiver operating characteristic curves of 0.758 and 0.732 and for precision-recall curves of 0.750 and 0.714 in the discovery and validation sets, respectively. This study has provided compelling evidence of comprehensive characteristics of circulating BA metabolism in patients with SZ and promoted a deeper understanding of the role of BAs in the pathophysiology of this disease, possibly via the gut microbiota-FXR signaling pathway.

Schizophrenia (2022) 8:65; https://doi.org/10.1038/s41537-022-00273-5

INTRODUCTION

Schizophrenia (SZ) is a severe and complex mental disorder with a global prevalence of around 0.3%1, constituting a substantial health and economic burden on patients and society. Nevertheless, the etiology and pathogenesis of this disease remain elusive. Previous research demonstrates that the pathophysiology of SZ involves disturbed glucose and lipid metabolism2 and chronic low-level inflammation3. Recent evidence shows that bile acids (BAs) play a pivotal regulatory role in inflammation and glucose and lipid metabolism4 and seem to be dysregulated in SZ6, suggesting their potential involvement in SZ by affecting inflammatory response and metabolism. It is therefore important to comprehensively investigate BAs and their role in the development of SZ.

Bile acids are a diverse class of cholesterol-derived amphipathic metabolites that are mainly synthesized in the liver through the classic pathway and alternative pathway and metabolized by gut microbiota5. Notably, BAs can also be synthesized locally in the brain, although the majority of brain BAs are taken up from the systemic circulation8. BAs are critical signaling molecules that modulate metabolic processes by binding to nuclear and membrane receptors such as farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5)9,10, in addition to their well-established function as deterrents that aid in the digestion and absorption of dietary lipids. FXR-mediated BA signaling is capable of modulating BA synthesis11, lipid, glucose and energy metabolism12,13, inflammation14, and also the transcription of several genes, including brain-derived neurotrophic factor (BDNF)15. TGR5-mediated BA signaling in immune cells has been demonstrated to decrease phagocytic activity and pro-inflammatory cytokine production, suggesting an immunomodulatory action of BAs16-20. Elevated levels of pro-inflammatory cytokines21,22 are associated with SZ, so it is possible that changes in BA signaling pathways, such as alterations in BA pool size or composition, could play a role in the pathophysiology of this disease.

Bile acids and the gut microbiome mutually regulate each other in the intestines. Microbiota transform host-derived primary BAs in the gut by carrying out numerous reactions, including deconjugation via bile salt hydrolases (BSH) and 7α-dehydroxylation of the primary BAs glyco-conjugated and tauro-conjugated cholic acid (CA) and chenodeoxycholic acid (CDCA) to form their secondary BAs, deoxycholic acid (DCA) and lithocholic acid (LCA), respectively23. Of the gut microbiota producing BSH24,25, many bacteria, including Bacteroides, Bifidobacterium, Blautia, Clostridium, Enterococcus, Eubacterium, Lactobacillus, and Roseburia, exhibited alterations in patients with SZ25, hinting a potential aberrant deconjugation action in the disease. In turn, BAs regulate the gut microbiome by affecting microbial composition and function and leading to pathological consequences in cases of BA signaling pathway dysfunction26,27.

This study aims to comprehensively characterize BA profiles in the context of SZ, and to gain understanding of the role of BAs in SZ onset and development. To our knowledge, this is the most...
extensive study of its kind, with large sample size and a high-
coverage targeted metabolomics method, allowing for thorough
scrutiny of BAs. Our study demonstrates that BA pool size and
composition are significantly different in patients with SZ when
compared to HCs, and suggests that BAs may be involved in the
pathophysiology of SZ via the gut microbiota-FXR signaling
pathway.

RESULTS
Demographic characteristics of the study population
This study included 216 participants, of which 108 were drug-free
SZ patients and 108 were healthy controls (HCs). Fifty-nine SZ
patients and 60 HCs were assigned to the discovery set, matched
for age, sex, height, weight, BMI, and smoking habits. The
remaining 49 SZ patients and 48 HCs were assigned to the
validation set. The detailed demographic characteristics of the
participants are shown in Table 1.

Abnormal serum bile acid profiles in patients with SZ
Forty BAs were detected by ultra-performance liquid chromato-
graphy triple quadrupole mass spectrometry (UPLC-TQMS), and
were classified as primary BAs or secondary BAs in conjugated
or unconjugated form (Table S1). To characterize serum BA profiles of
patients with SZ, multivariate and univariate analyses were
performed. Principal component analysis (PCA) performed on all
the samples showed that all nine quality control (QC) samples
were clustered closely in the PCA scores plot (Fig. S1), which
demonstrated the stability of the instrument and the excellent
repeatability of the analysis. Orthogonal partial least squares
discriminant analysis (OPLS-DA) was performed on the discovery
and validation sets to identify differences in global BA profiles
between SZ and HC groups. The score plots of the models showed
a separation between SZ and HC groups in both discovery and
validation sets (Fig. 1A, B). Furthermore, a permutation test with
1,000 iterations was performed to assess statistical significance
and avoid overfitting of the OPLS-DA model, and the result
demonstrated the model’s ability to distinguish between SZ and
HC groups (Fig. 1C, D). The variable importance in projection (VIP)
scores of 40 BAs are listed in Table 2. Following Shapiro-Wilk tests,
the Mann–Whitney U tests were used to compare the concentra-
tions of 40 BAs between patients with SZ and HCs, excluding
outliers less than Q1 - 1.5x IQR or greater than Q3 + 1.5x IQR. Each
BA that had a VIP score > 1 and a false discovery rate (FDR)-
adjusted q-value < 0.05 was defined as significantly different
between SZ and HC groups.

As shown in Table 2 and Fig. 2A, the concentrations of 10 BAs
were significantly different between SZ patients and HCs in the
discovery set. CA and CDCA, two primary bile acids synthesized
from cholesterol, were notably decreased in sera of patients with
SZ when compared to HCs. DCA, the bacterial 7α-dehydroxylation
product of CA, was also significantly reduced in SZ patients
compared to HCs. In addition, patients with SZ had significant lower levels of
ursodeoxycholic acid (UDCA) and 3β-cholenoxycholic acid
(βCDCA), which are two epimers of CDCA, in comparison with
those of HCs. Moreover, SZ patients had notably reduced levels of
7-ketolithocholic acid (7-ketolCA) and 3-dehydrocholic acid (3-
DHCA) and increased levels of apocholic acid (apoCA) and
norcholic acid (NorCA) when compared to those of HCs.

Interestingly, as the decrease in CDCA was more profound than
that observed in CA, a notable increase in the ratio of CA to CDCA
was observed in patients with SZ compared to that of HCs (Fig.
2B), implying that the alternative BA synthetic pathway is impaired
in SZ.

Furthermore, patients with SZ exhibited notable decline in the
concentrations of total BAs (Fig. 2C) and unconjugated BAs (Fig.
2D) compared with HCs. In contrast, the total conjugated BAs were
comparable with no significant differences between the two
groups (Fig. S2A). Likewise, total primary and secondary BAs were
also comparable between SZ patients and HCs (Figs. S2B and S2C).
These findings were suggestive of weakened deconjugation by
gut microbiota in SZ. In the independent validation set, the above
10 individual BAs, total BAs, and unconjugated BAs consistently
exhibited similar changing trends in patients with SZ, among
which CDCA, UDCA, βCDCA, 7-ketolCA, 3-DHCA, total BAs, and
unconjugated BAs reached the threshold of statistical significance
(Table S2 and Fig. S3). The ratio of CA to CDCA was marginally
significant between the two groups (p = 0.073, ratio = 1.56).

Depleted bile acid deconjugation potentials and FXR affinity
index in schizophrenic patients
To further evaluate BA deconjugation potentials, we calculated the
pairwise ratios of unconjugated products to their conjugated
precursors. These ratios were CA/(GCA and TCA), CDCA/(GCDCA
and TCDCA), DCA/(GDCCA and TDCA), UDCA/(GUDCA and
TUDCA), LCA/(GLCA and TLCA), and HCA/(GHCA and THCA). Compared to
HCs, patients with SZ had significantly lower ratios for unconju-
gated to conjugated CDCA and its derivatives LCA and UDCA (Fig.
3A), suggesting a hampered CDCA species-specific bacterial
deconjugation manner in SZ. Additionally, FXR affinity indices

---

**Table 1. Demographic characteristics of the study population.**

| Variables          | Discovery Set | Validation Set | p-value |
|--------------------|---------------|----------------|---------|
|                    | SZ (N = 59)   | HC (N = 60)    |         |
| Age, y, mean (SD)  | 37.48 (11.80) | 36.80 (9.74)   | 0.732   |
| Sex (M/F)          | 23/36         | 25/35          | 0.765   |
| Height, cm, mean (SD) | 163.49 (6.95) | 165.00 (6.88) | 0.241   |
| Weight, kg, mean (SD) | 58.62 (7.76)  | 60.78 (8.72)  | 0.162   |
| BMI, kg m⁻², mean (SD) | 21.93 (2.46) | 22.30 (2.75) | 0.448   |
| Smoker, No. (%)    | 15 (25.4)     | 12 (20)        | 0.480   |
| CGI-S, median (IQR) | 6 (0)         | NA             | NA      |

M male, F female, BMI body mass index, CGI-S the clinical global impression-severity of illness scale, SD standard deviation, IQR interquartile range, NA not applicable.

*p-values were calculated by two-tailed Student’s t-test.

The p-values were calculated by the chi-square test.

Smoker represents the number of subjects with a habit of smoking.
were calculated and compared between the two groups, given that induction of FXR target gene expression has been shown to repress the expression of enzymes involved in hepatic BA synthesis. Patients with SZ had lower FXR affinity indices than HCs (Fig. 3B), implying amelioration of FXR-mediated repression of CYP7A1 to produce BAs via the classic pathway.

Altered conjugated bile acids and bile acid deconjugation potentials in patients with first versus recurrent episodes of SZ

Among the 108 patients with SZ included in the study, 62 patients were diagnosed with a first episode (F-SZ) and 46 with recurrent episodes (R-SZ). To evaluate serum BA profiles between these two groups, we compared concentrations of 40 BAs, excluding outliers, and found that 39 BAs had p-values > 0.05 using the Mann–Whitney U test, with only apoCA (p = 0.007; q = 0.298) being screened out after FDR correction with a q-value > 0.05. These results showed that BA profiles were similar between F-SZ and R-SZ groups. Interestingly, R-SZ had significantly higher levels of total conjugated BAs (both glyco-conjugated and taur-conjugated BAs) than F-SZ (Fig. 4A), but showed no remarkable changes in either total BAs or unconjugated BAs. In addition, the ratios of unconjugated to conjugated CA, CDCA, and UDCA species were significantly lower in the R-SZ group than in the F-SZ group (Fig. 4B). These data hinted that recurring SZ states might affect BA conjugation and deconjugation.

Bile acids as candidate auxiliary diagnostic markers for SZ

To explore the diagnostic effectiveness of BAs for SZ prediction, receiver operating characteristic (ROC) curves, precision-recall (P-R) curves, and their area under the curves (AUC) for 40 individually detected BAs and 5 summed BAs, including total BAs, total primary BAs, total secondary BAs, total conjugated BAs, and total unconjugated BAs, were calculated to evaluate the performance of the logistic regression models in the discovery set. Further, different biomarker panels based on the above 45 BA variables were screened to optimize the diagnostic model. According to the posterior probability that each variable is non-zero for Bayesian model averaging, a combination of 8 BA variables, including TCDCA, βCDCA, 6-ketoLCA, apoCA, NorCA, GHCA, THCA, and total

Fig. 1 OPLS-DA based on 40 BAs shows separation between SZ and HC groups. A The scores plot of the discovery set with 95% confidence ellipses drawn. B The scores plot of the validation set with 95% confidence ellipses drawn. C The validation plot of 1000 permutation tests in the discovery set. D The validation plot of 1000 permutation tests in the validation set.
Table 2. Median (IQR) serum concentrations (nM) of 40 BAs in SZ and HC groups in the discovery set.

| BAs          | BAs in discovery set (nM) | ratio | p-value | q-value | VIP |
|--------------|---------------------------|-------|---------|---------|-----|
| CA           | 12.6 (25.38)              | 0.54  | 0.009   | 0.04    | 1.68|
| GCA          | 31.14 (32.63)             | 0.69  | 0.031   | 0.09    | 1.18|
| TCA          | 3.13 (3.56)               | 0.97  | 0.565   | 0.752   | 0.72|
| CDCA         | 31.89 (89.3)              | 0.30  | 0.001   | 0.01    | 1.64|
| GCDCA        | 269.41 (372.09)           | 0.97  | 0.635   | 0.752   | 0.04|
| TCDCDA       | 18.87 (28.86)             | 0.92  | 0.656   | 0.752   | 0.16|
| DCA          | 47.22 (48.23)             | 0.60  | 0.001   | 0.01    | 1.62|
| GDCA         | 46.15 (64.18)             | 0.75  | 0.087   | 0.204   | 0.64|
| TDCA         | 4.19 (6.85)               | 0.90  | 0.539   | 0.752   | 0.03|
| UDCA         | 9.35 (23.51)              | 0.39  | 0.001   | 0.01    | 1.61|
| GUDCA        | 36.49 (55.14)             | 0.91  | 1       | 1       | 0.73|
| TUDCA        | 1.01 (1.62)               | 0.95  | 0.956   | 1       | 0.39|
| LCA          | 1.69 (3.18)               | 0.63  | 0.267   | 0.428   | 0.04|
| GLCA         | 2.67 (6.47)               | 0.87  | 0.658   | 0.752   | 0.03|
| TLCA         | 0.16 (0.56)               | 1.00  | 0.634   | 0.752   | 0.4 |
| HCA          | 2.48 (4.64)               | 0.70  | 0.213   | 0.398   | 0.29|
| GHCA         | 3.01 (2.84)               | 0.78  | 0.031   | 0.09    | 1.04|
| THCA         | 1.66 (1.34)               | 0.83  | 0.006   | 0.04    | 0.75|
| jUUDCA       | 41.72 (56.49)             | 0.82  | 0.08    | 0.2     | 0.03|
| βCDCA        | 19.73 (24.7)              | 0.64  | 0.001   | 0.01    | 1.97|
| βDCA         | 10.33 (16.91)             | 0.57  | 0.007   | 0.04    | 1.31|
| jUCA         | 0.23 (0.57)               | 0.88  | 0.683   | 0.759   | 0.07|
| jICA         | 1.02 (0.81)               | 0.82  | 0.205   | 0.398   | 0.75|
| jIMCA        | 0.26 (0.75)               | 0.90  | 0.613   | 0.752   | 0.3 |
| UCA          | 0.21 (1.23)               | 0.81  | 0.354   | 0.544   | 1.29|
| TuMCA        | 0.93 (0.51)               | 0.84  | 0.018   | 0.06    | 1.33|
| alloLCA      | 0.28 (0.39)               | 1.33  | 0.231   | 0.398   | 1.11|
| isoLCA       | 2.19 (4.73)               | 0.64  | 0.073   | 0.194   | 0.79|
| NorDCA       | 0.57 (0.99)               | 1.08  | 0.232   | 0.398   | 0.84|
| 6-ketoLCA    | 0.72 (0.47)               | 0.94  | 0.165   | 0.348   | 0.79|
| 7-ketoLCA    | 2.02 (3.23)               | 0.52  | 0.016   | 0.047   | 1.27|
| 12-ketoLCA   | 1.66 (2.74)               | 0.76  | 0.239   | 0.398   | 0.17|
| apoCA        | 1.44 (2.41)               | 1.60  | 0.012   | 0.047   | 1.94|
| LCA-3S       | 1.82 (5.84)               | 1.05  | 0.978   | 1       | 0.25|
| GLCA-3S      | 47.13 (75.5)              | 1.14  | 0.656   | 0.752   | 0.09|
| HDCA         | 0.85 (0.56)               | 0.91  | 0.123   | 0.273   | 0.52|
| NorCA        | 2.28 (2.34)               | 1.55  | 0.003   | 0.027   | 1.79|
| 3-DHCA       | 0.2 (0.31)                | 0.63  | 0.009   | 0.04    | 1.28|
| 7-ketoDCA    | 0.71 (1.18)               | 0.89  | 0.719   | 0.778   | 0.78|
| CDCA-3Gln    | 5.43 (5.6)                | 1.03  | 0.561   | 0.752   | 0.51|

BAs bile acids, VIP variable importance in the projection, CA cholic acid, GCA glycocholic acid, TCA taurocholic acid, CDCA chenodeoxycholic acid, GCDA glycochenodeoxycholic acid, TCDCA taurochenodeoxycholic acid, DCA deoxycholic acid, GDCA glycodeoxycholic acid, TDCDCA taurodeoxycholic acid, UDCA ursodeoxycholic acid, GUDCA glycodeoxycholic acid, TUDCA taurodeoxycholic acid, LCA lithocholic acid, GLCA glycolithocholic acid, TLA taurolithocholic acid, HCA hyocholic acid, GHCA glychocholic acid, THCA taurohyocholic acid, jUDCA 3j-ursodeoxycholic acid, βCDCA 3j-chenodeoxycholic acid, βDCA 3j-deoxycholic acid, βUCA β-ursodeoxycholic acid, βCA 3j-cholic acid, βMCA β-muricholic acid, UCA ursodeoxycholic acid, jUUDCA tauro β-muricholic acid, allo-LCA allolithocholic acid, iso-LCA isolithocholic acid, NorDCA 23-nordeoxycholic acid, 6-keto-LCA 6-ketolithocholic acid, 7-keto-LCA 7-ketolithocholic acid, 12-keto-LCA 12-ketolithocholic acid, apo-LCA apocholic acid, LCA-3S lithocholic acid-3-sulfate, GLCA-3S glycolithocholic acid-3-sulfate, HDCA α-hydroxydeoxycholic acid, NorCA norcholic acid, 3-DHCA 3-dehydroxycholic acid, 7-keto-DCA 7-ketodeoxycholic acid, CDCA-3Gln chenodeoxycholic acid-3-j-d-glucuronide.

*ratios were calculated from the intra-group medians of BAs between SZ and HC groups.

*p-values were calculated by Mann–Whitney U tests.

q-values were calculated by FDR adjusted p-values estimated by Mann–Whitney U tests.

VIP scores were obtained from OPLS-DA. The bold BAs indicate significantly differential BAs between SZ and HC groups.
primary BAs, was identified to discriminate SZ patients from HCs. When using their respectively best cut-off values in the two sample sets, AUC values for ROC curves were 0.758 (sensitivity: 0.695; specificity: 0.767) and 0.732 (sensitivity: 0.673; specificity: 0.708) (Fig. 5A), and for P-R curves were 0.750 (precision: 0.745; recall: 0.695) and 0.714 (precision: 0.702; recall: 0.673) (Fig. 5B) in the discovery and validation sets, respectively. And F1 scores for the two sample sets were 0.719 and 0.687, respectively. When using the best cut-off value of the discovery set to predict for the validation set, AUC values for ROC and P-R curves were 0.671 (sensitivity: 0.612; specificity: 0.729) and 0.650 (precision: 0.698; recall: 0.612), and F1 score was 0.652 in the validation set. Since BA profiles of F-SZ and R-SZ patients were similar, they could not be used to differentiate these two groups.

**DISCUSSION**

Emerging evidence indicates that BAs are present in the brain and are associated with brain diseases, such as schizophrenia, Alzheimer’s disease, and autism, highlighting the linkage between BAs and mental health. Nevertheless, data on the BA profiles in patients with SZ are scarce, which has limited our understanding of the role of BAs in the disease. The current study provides new evidence for the comprehensive characterization of circulating BA profiles in patients with SZ and increases our understanding of BA profiles and their potential role in this disease.

Circulating BAs have the ability to cross the blood-brain barrier (BBB) via either passive diffusion or active transport, and BAs and their receptors have already been identified in the human brain, alluding to BA-mediated signaling from the peripheral circulation to the central nervous system (CNS). CA and CDCA are major primary BAs synthesized from cholesterol in the liver, via the classical and alternative pathways, respectively. Since CA and CDCA can diffuse across phospholipid bilayers, they may diffuse across the BBB, and their levels in the brain are reported to correlate with those in the serum. CDCA is a potent antagonist at N-methyl-D-aspartate (NMDA) and γ-aminobutyric acid (GABA) receptors, our finding of significant reduction of CDCA levels in patients with SZ relative to HCs suggests aberrant glutamate and GABA neurotransmitter systems in SZ. CDCA generation from cholesterol was controlled by CYP27A1 via the acidic (alternative) pathway. More than 50 mutations in the gene CYP27A1 have been identified associated with cerebrotendinous xanthomatosis (CTX), a rare autosomal recessive disease of BA synthesis that presents possible psychiatric manifestations. Treatment with CDCA has been shown to improve the psychiatric symptoms of patients with CTX. As a result of a greater decrease in CDCA levels than in CA levels, we found a higher ratio of CA to CDCA in patients with SZ. Sterol 12α-hydroxylase (CYP8B1)
GCA51. Recent studies have reported decreased abundances of Collinsella, Ruminococcus genetic study identified a marked decline in BA pool size in patients with SZ. A previous study associated with SZ44, which encodes 2-methylacyl-CoA racemase, a bacterial enzyme responsible for BA deconjugation in different bacterial phylotypes. For patients with SZ, further supporting the notion that deconjugation of BAs by gut microbiota is weakened in SZ. Interestingly, BSHs exhibit distinct deconjugation selectivity on substrates. For example, BSH-T2 (Streptococcus, Enterococcus, Blautia, and Roseburia) groups display the highest specific activities with CDCA, while the T1 (Eubacterium, Blautia, Clostridium, Roseburia, Lachnospiraceae) and T4 (Bifidobacterium and Collinsella) groups display the highest specific activities with GCA41. Recent studies have reported decreased abundances of gut Streptococcus, Enterococcus, Blautia, and Roseburia in patients with SZ52,53. These findings provide clues for interpretation of our findings of decreased CDCA species-specific deconjugation potentials in SZ. Furthermore, our results of significantly lower BA deconjugation potentials in R-SZ patients than in F-SZ patients suggest that BA deconjugation by gut microbiota is likely involved in schizophrenic relapse. We therefore conjecture that dysbiotic bacteria will cause a weak deconjugative capacity for specific BAs, resulting in the depletion of unconjugated BAs, and might be further implicated in the onset risk of SZ.

The disturbed availability of specific BAs such as CDCA influences FXR signaling, which plays a crucial role in the modulation of BA homeostasis and lipid and glucose metabolism44. Activated FXR inhibits BA production through a feedback mechanism by repressing CYP7A1 via small heterodimer partner (SHP) induction55. Our report of low FXR affinity indices is indicative of blunted activation of FXR, which would in turn enhance hepatic BA synthesis in SZ. In addition, FXR activation also decreases free fatty acids (FFAs) and triglycerides (TGs) by suppressing de novo fatty acid synthesis56,57 and promoting hepatic fatty acid oxidation58. Patients with SZ have been shown to have elevated levels of FFAs and TGs, which is in accordance with our finding of blunted activation of FXR in the disease. In addition, FXR activation facilitates the inhibition of inflammation and maintenance of intestinal epithelial barrier in inflammatory bowel disease61 and promotes the suppression of C-reactive protein (CRP) expression by interleukin-6 (IL-6)62. Our results showing decreased activation of FXR signaling may contribute to an increase in intestinal permeability63,64 and upregulation of CRP and IL-6 in patients with SZ.

The current study provides a comprehensive snapshot of serum BA profiles in patients with SZ and identifies changes in BA pool size and composition, providing new insights into the pathophysiology of SZ. The strength of this study lies in its large sample size, independent sample sets, and its high-coverage targeted metabolomics approach. Nevertheless, this study still has some limitations. First, it only demonstrates that SZ is associated with abnormal BA profiles, but cannot prove the causal relationship between BAs and SZ. Second, it lacks metagenomic data to determine the microbial enzymes catalyzing deconjugation reaction. In the future, preclinical or clinical studies on the microbiota-BA-FXR signaling pathway are warranted to determine the causality between BAs and SZ.

METHODS
Study design
We recruited a total of 216 participants in Anhui Province, China, of which 108 patients were diagnosed with SZ according to the criteria of the Schizophrenia International Research Society.

Fig. 3 Bile acid deconjugation potentials and FXR affinity index are lower in SZ than in HCs. Raincloud plots (jittered raw data, boxplots, and probability distribution of the data) for deconjugation potentials of CDCA, LCA and UDCA (A), and the FXR affinity index (B). The comparisons between the two groups were conducted by the Mann–Whitney U tests, excluding outliers. *p < 0.05; **p < 0.01; ***p < 0.001.
Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), and the remaining 108 subjects were HCs. Of the 108 patients, 62 were experiencing a first episode of psychosis, and 46 were hospitalized for relapse after at least 1 month without any antipsychotics. Patients who met the criteria for any other axis I disorder or who received any form of mood-stabilizing drugs in the recent 2 weeks before blood sample collection were excluded from the study. In both SZ and HC groups, participants with metabolic disorders (such as type I or type II diabetes) and/or with heavy consumption of alcohol were also excluded from this study. All patients with SZ were antipsychotics free and all participants and/or with heavy consumption of alcohol were also excluded from this study. In both SZ and HC groups, participants were assigned into the discovery data set (59 SZ and 60 HC) or the validation data set (49 SZ and 48 HC), consistent with our previous studies \(^2\), \(^5\). This study was approved by the local ethics committee of the Fourth People’s Hospital of Wuhu and was carried out in accordance with the Declaration of Helsinki. All participants signed written informed consent forms before undergoing any procedures.

**Serum sample collection**

Overnight fasting whole blood samples were collected from all subjects and were stored at room temperature, which clotted naturally after approximately 1 h. Serum samples were then obtained after centrifugation at 10,000 rpm for 10 min. All samples were immediately aliquoted and stored at \(-80\) °C until further analysis.

**Quantification of bile acids**

Reference standards of 61 BAs were purchased from Steraloids Inc. (Newport, RI, USA) and TRC Chemicals (Toronto, ON, Canada). Ten stable isotope-labeled standards (CA-D4, UDCA-D4, DCA-D4, LCA-D4, BCA-D5, GCA-D4, TCA-D4, GCDCA-D4, TCDCA-D9, and GDCA-D4) were obtained from C/D/N Isotopes Inc. (Quebec, Canada) and Steraloids Inc. (Newport, RI, USA). Methanol (Optima LC-MS), acetonitrile (Optima LC-MS), isopropanol (Optima LC-MS), and formic acid (Optima LC-MS) were obtained from Thermo-Fisher Scientific (Fairlawn, NJ, USA). Ultrapure water was produced with a Milli-Q Reference system equipped with an LC-MS Pak filter (Millipore, Billerica, MA, USA). The standards and stable isotope-labeled standards were accurately weighed and prepared in methanol to obtain individual stock solutions at 5.0 mM concentration. Depending on the solubility of the standard, either water or methanol was used to prepare the stock solution. All stock solutions were stored at \(-80\) °C. The individual BA stock solutions were mixed and prepared in a BA-free serum matrix to obtain a series of BA standards at final concentrations of 2500, 500, 250, 50, 10, 2.5, and 1 nM. Quality control samples were prepared at concentrations of 1500, 150, and 5 nM. Internal standard (IS) concentrations were kept constant at all calibration points (150 nM for 10 stable isotope-labeled standards).

A Waters ACQUITY UPLC system equipped with a binary solvent delivery manager and sample manager (Waters, Milford, MA) was used in this study. The mass spectrometer was a Waters XEVO TQ-S instrument with an ESI source (Waters). The LC-MS system was controlled using the MassLynx 4.1 software. The chromatographic separations were performed using an ACQUITY UPLC CORTECS C18 1.6 μM VanGuard pre-column (2.1 × 5 mm) and ACQUITY UPLC CORTECS C18 1.6 μM analytical column (2.1 × 100 mm). A 20-μL aliquot of serum sample was added to 180 μL of acetonitrile/methanol (8:2) containing 10 internal standards, and the mixtures were vortexed for 2 min and allowed to stand for 10 min before being centrifuged at 20,000 g for 10 min at 4 °C. A 160-μL aliquot of the supernatant was transferred to a clean tube and vacuum-dried. The residue was redissolved with equal amounts of acetonitrile/methanol (80/20, v/v) and ultrapure water (0.1% formic acid) to a final volume of 40 μL, and was then centrifuged at 13,500 g for 20 min at 4 °C. After centrifugation, the supernatant was used for UPLC–MS/MS analysis. The injection volume of all samples was 5 μL. The mobile phases were water with formic acid (pH = 3.25) (A) and acetonitrile/methanol (v/v = 80/20) (B), with a flow rate of 0.4 mL/min. The elution gradient conditions were as follows: 0–1 min (5% B), 1–3 min (5–30% B), 3–8 min (30–40% B), 8–15 min (40–100% B)

**Fig. 4 Conjugated BAs and BA deconjugation potentials differ in SZ patients with first (F-SZ) versus recurrent episodes (R-SZ).** Scatter box plots for total conjugated BAs, glycine conjugated BAs and taurine conjugated BAs (A), and deconjugation potentials of CA, CDCA, and UDCA (B). The comparisons between the two groups were conducted by the Mann–Whitney U tests, excluding outliers. \(^* p < 0.05\).
B), 15–16 min (100–5% B), and 16–17 min (5% B). The mass spectrometer was operated in negative ion mode with the following optimal conditions: capillary voltage of 2 kV, source temperature of 120 °C, desolvation temperature of 550 °C, and desolvation gas flow rate of 1000 L/h.

To diminish analytical bias within the entire analytical process, QC samples (pooled biological samples), calibrators, and blank samples were analyzed across the entire sample set and the serum samples from SZ and HC groups were alternately injected. The calibrators consisted of a blank sample (without IS), zero sample (with IS), and series of 7 concentrations covering the expected range for BAs present in the samples. The calibration curve and the corresponding regression coefficients were obtained using IS adjustment. QC samples and IS were used for quality control of BA analysis. The UPLC-TQMS raw data files were processed and quantified using the TargetLynx Applications Manager (version 4.1, Waters). Finally, using 61 BA standards to identify and quantify the BA concentrations, 40 BAs were identified and quantified in real samples.

Calculation of bile acid deconjugation potentials and FXR affinity index

According to the deconjugation process of BAs, BA deconjugation potentials were analyzed using 6 ratios of unconjugated to conjugated BAs, including CA/(GCA and TCA), CDCA/(GCDCA and TCDDA), DCA/(GDCA and TDCDA), UDCA/(GUDCA and TUDCA), LCA/(GLCA and TLCA), and HCA/(GHCA and THCA). As in a previous study, an FXR affinity index was calculated based on the following formula: FXR affinity index = CA (nM) × 0.81 + CDCA × 1 + DCA × 0.4 + LCA × 0.04.

Statistical analysis

For multivariate analysis, PCA and OPLS-DA were conducted using MetaboAnalyst version 5.0 and obtained the corresponding VIP value for each BA. For univariate analysis, the Shapiro-Wilk test was first used to examine the distribution of continuous variables. A Student's t-test was then carried out to investigate differences in normally distributed variables between groups. The Mann-Whitney U test was performed to investigate differences in BA measurements between groups, excluding outliers less than Q1 – 1.5 × IQR or greater than Q3 + 1.5 × IQR. The resultant p values for BAs were subsequently adjusted to account for multiple testing by the false discovery rate (FDR) method. Logistic regression models were constructed based on the BA concentrations, and AUCs of ROC and P-R curves as well as F1 scores were calculated to evaluate the performance of the fitted logistic regression models. A p-value less than 0.05 was considered statistically significant. Both of the discovery and validation sets followed the above analytical procedures. All analyses were carried out using SPSS 24 and R 4.1.0.

DATA AVAILABILITY

The mass spectrometry raw data associated with this study have been deposited on the open metabolomics database MetaboLights (ID: MTBLS5535).

CODE AVAILABILITY

The R code that supports the findings of this study are available from the corresponding authors upon reasonable request.

Received: 7 June 2022; Accepted: 1 August 2022; Published online: 18 August 2022

REFERENCES

1. Ferrari, A. J. et al. Global, regional, and national burden of 12 mental disorders in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Psychiatry 9, 137–150 (2022).
2. Wang, C. J. et al. Serum free Fatty acids and glucose metabolism, insulin resistance in schizophrenia with chronic antipsychotics. Biol. Psychiatry 70, 1309–1313 (2006).
3. Muller, N. Inflammation in Schizophrenia: pathogenetic aspects and therapeutic considerations. Schizophr. Bull. 44, 973–982 (2018).
4. Chavez-Talavera, O., Tailleux, A., Lefebvre, P. & Staels, B. Bile acid control of metabolism and inflammation in obesity, Type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. Gastroenterology 152, 1679–1694.e3 (2017).
5. Guo, C. S. et al. Bile acids control inflammation and metabolic disorder through inhibition of NLPR3 inflammasome. Immunity 45, 802–816 (2016).
6. Tao, Y. L., Zheng, F., Cui, D. H., Huang, F. & Wu, X. J. A combination of three plasma bile acids as a putative biomarker for schizophrenia. Acta Neuropsychiatr. 33, 51–54 (2021).
7. Russell, D. W. The enzymes, regulation, and genetics of bile acid synthesis. Annu. Rev. Biochem. 72, 137–174 (2003).
ACKNOWLEDGEMENTS

This work was supported by the Natural Science Foundation of Shanghai (20ZR1426700), Startup Fund for Youngman Research at SJTU (19X100040033), Interdisciplinary Program of Shanghai Jiao Tong University (YG2019QNA03, YG2019QNB35), and Science and Technology Plan Project of Wuhu, Anhui Province (2020ms3-19). We appreciate all participants for their contributions.

AUTHOR CONTRIBUTIONS

Y.Q. and W.J. supervised the study. Y.Q. and P.W. (Pengkun Wang) designed the study. L.H., P.W. (Peng Wang), K.L., and Z.X. contributed to recruiting the subjects and collecting serum samples and clinical information. Y.Q., P.W. (Pengkun Wang), G.C., and J.Z. performed the experiments. Y.Q. and P.W. (Pengkun Wang) contributed to the data analysis. Y.Q. wrote the manuscript. All authors approved the final manuscript. These authors contributed equally: Y.Q. and P.W. (Pengkun Wang).

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41537-022-00273-5.

Correspondence and requests for materials should be addressed to Ying Qing or Wei Jia.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.