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Identifying novel metabolites in children with attention-deficit hyperactivity disorder through metabolome profiling

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Metabolomics research offers promising potential for identifying key metabolites and exploring the pathophysiological underpinnings of attention-deficit hyperactivity disorder (ADHD). However, serum metabolomics in ADHD remains largely uncharted. Our study aimed to search for metabolomic biomarkers in children with ADHD. 70 drug-naïve children diagnosed with ADHD according to DSM-5 criteria and 70 sex-, age-, IQ-matched healthy controls were recruited from the National Taiwan University Hospital. All participants were assessed for clinical and ADHD symptoms using the Clinical Global Impression Severity (CGI-S) and ADHD Rating Scale-IV (ADHDRS-IV), respectively. Serum-based metabolomic profiles were obtained through liquid chromatography-mass spectrometry. We performed the Wilcoxon test for univariate analysis, the orthogonal partial least squares discriminant analysis (OPLS-DA) for multivariate analysis, and Spearman correlation analyses for the associations between identified metabolites and clinical and ADHD measures. In our study, 156 metabolites were identified in peripheral blood samples using an untargeted metabolomics approach, among which cholic acid, homoveratric acid, inosine, and nicotinuric acid were significantly different between ADHD and controls. Children with ADHD had upregulated cholic acid and homoveratric acid levels and downregulated inosine and nicotinuric acid levels compared to controls. Notably, the upregulated metabolites positively correlated, and the downregulated metabolites negatively correlated with CGI-S and ADHDRS-IV scores. These metabolites and their mechanisms suggested that the pathophysiology of ADHD might involve connections between the gut-brain axis, oxidative stress, dopaminergic pathway, and purine salvage pathway. Our findings of four novel metabolite-behavior relationships in children with ADHD enhanced our understanding of the potential pathways underlying the pathophysiological mechanisms of ADHD.

Translational Psychiatry (2025)15:180 ; https://doi.org/10.1038/s41398-025-03393-5

INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder with the hallmark symptoms of inattention, hyperactivity, and impulsivity [1]. It is also one of the most prevalent psychiatric disorders in children and adolescents, with an estimated worldwide prevalence rate ranging from 5 [2] – 10% [3]. Additionally, ADHD affects the life quality of the patients and their families significantly through various negative consequences [4], and higher injury-cause mortality [5]. These factors highlight the need to prioritize ADHD as a public health issue.

Nevertheless, numerous challenges lie in both research and clinical settings. First, despite scientific progress, diagnosing ADHD remains challenging, primarily depending on behavioral observation [2], with ongoing debates over the accuracy and objectivity of diagnosis [6, 7]. Second, the etiology of ADHD involves a complex interplay of numerous minor effects of genetic and environmental factors [8], making a deeper understanding of ADHD pathogenesis essential for treatment advancement. Biomarkers, mainly through the omics approaches including genomics, transcriptomics, proteomics, and metabolomics, offer comprehensive insights for risk assessment, diagnosis, treatment response, prognosis, and exploration of underlying mechanisms [9, 10], especially through panels or sets of biomarkers [11].

Metabolomics is particularly promising given that it collects the end products from interactions between biological factors and the environment and reflects the dynamic course of a disorder [10]. In neuropsychiatric disorders, metabolomics aids in differential diagnosis [12], exploring potential pathogenesis [13, 14], correlating with impairment severity [15], revealing interactions between gut microbiota and the pathophysiology [16] and assessing exposure to therapies [17]. Despite the emerging advantages of metabolomics studies in neuropsychiatric disorders, its application in ADHD studies remains limited. We believe the study of ADHD metabolomics may hold significant value, as previous research in animal models has yielded intriguing findings. Metabolites like amino acids, linoleic acid, amino sugar [18], cholesterol [19], oxidative stress markers [7], tryptophan, and lipids [20] linked to associate with ADHD-like behaviors, some metabolites differentiating rats with ADHD-like behaviors from controls [7]. There have been limited metabolomics studies in ADHD patients. Notably,

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Received: 27 November 2024 Revised: 30 April 2025 Accepted: 12 May 2025
Published online: 24 May 2025

one study examining urine and fecal samples from a Swedish ADHD twin cohort identified a metabolite profile linked to the gut-brain axis, with many metabolites significantly influenced by genetic factors [21]. An untargeted metabolomic investigation on urine samples from ADHD patients discovered several significant metabolites within pathways associated with leucine, tyrosine, and fatty acid metabolism. This discovery could lead to a metabolite panel with good diagnostic effectiveness [22].

A recent systemic review identified 229 potential biomarkers for ADHD from previous studies, mainly comprising cell counts, metals, and markers of inflammation or neural growth, with only a few being novel metabolites [10]. To our knowledge, only one study [23], has conducted an untargeted metabolomic analysis on the plasma samples of ADHD children, identifying nine significant metabolites from 58 ADHD children and 38 controls. However, these preliminary findings from studies with small sample sizes need validation in larger, independent samples. Investigating the relationships between these metabolites and behavioral phenotypes is required to understand their underlying mechanisms. Hence, our study aims to identify metabolites that could serve as biomarkers and provide further insights into the pathogenesis of ADHD by comparing the variations in plasma metabolome profile between individuals with ADHD and healthy controls. Additionally, we investigate the relationships between these identified metabolites and the severity of ADHD symptom and their ability to distinguish between the two groups by the metabolites.

METHODS

Participants

All participants were recruited from the Children's Mental Health Center, Department of Psychiatry, National Taiwan University Hospital (NTUH), Taipei, Taiwan, from November 2016 to July 2021. ADHD patients who met all inclusion criteria were recruited, including a clinical diagnosis of ADHD according to DSM-5 criteria as well as confirmed by the Chinese version of the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Epidemiological Version (K-SADS-E), aged 7–18 years, a Clinical Global Impressions-ADHD-Severity (CGI-ADHD-S) score > 4 at baseline, FIQ score of 80 or more, and psychotropic drug-naïve. Healthy controls, who did not have any DSM-5 psychiatric disorder based on the K-SADS-E interviews, with matched age, sex, and FIQ, were enrolled. Subjects who met any of the exclusion criteria were excluded, including a diagnosis of schizophrenia, schizoaffective disorder, organic psychosis, bipolar disorders, or autism spectrum disorder, a history of epilepsy, substance abuse, cardiovascular disease, serious suicidal risk, or taking Chinese medicine or health-food supplements with central nervous system activity. A total of 70 ADHD participants and 70 healthy controls were enrolled. An explanation of the purpose and procedure of this study was conducted, and written informed consent was obtained from all subjects' parents or legal representatives before the recruitment. All the procedures have been approved by the Research Ethics Committee (approval numbers: 201601035RINA and 201912085RINA) of the NTUH.

Clinical measurements

ADHD rating scale-IV–parent version: investigator-administered and scored (ADHDRS-IV). To evaluate ADHD symptom severity, all participants underwent assessment with the ADHDRS-IV, administered by the investigator through a semi-structured interview with each participant's parent. The ADHDRS-IV, a well-established instrument, includes 18 items reflecting the DSM-IV diagnostic criteria for ADHD, 9 items focusing on inattention (IA), and 9 on hyperactivity/impulsivity (HI), as detailed by Shang, et al. [24].

Clinical global impression severity scale (CGI-S). The investigator assessed the global clinical function of each participant by using the CGI-S, a single-item measurement on a seven-point scale. The CGI-S was rated based on the clinical experiences of the investigator with other ADHD patients [24–26].

Metabolomic profiling

All participants were instructed to fast for eight hours prior to blood sample collection. The collected samples were then sent to the Metabolomics Core Laboratory at National Taiwan University (NTU) for further metabolomics experiments [27].

Sample preparation. For each 100 µL of serum, extraction solvents (1:4) was added. The mixture was subjected to mechanical agitation using a Geno Grinder 2010 (SPEX, Pittsburg, CA, USA) at a speed of 1000 rpm for 2 min, followed by centrifugation at 15000 g for 5 min at a temperature of 4 °C. The resulting supernatant was put into a centrifugal vaporizer (EYELA, Tokyo, Japan) and evaporated for 2 h. 200 µL of 50% methanol was then added to the residue, and the mixture was centrifuged at 15000 g for 5 min. The supernatant was collected and underwent filtering with a 0.2 µm Minisart RC 4 filter (Sartorius, Goettingen, Germany). The final residue was ready for HPLC-Q-TOF-MS (high-pressure liquid chromatography coupled with quadrupole time-of-flight mass spectrometry) analysis.

Chromatographic and mass spectrometric analysis. All samples were analyzed using an Agilent 1290 UHPLC system coupled to a 6540-QTOF (Agilent Technologies, Santa Clara, CA, USA). For sample separation, 2 µL of each sample was inserted into an ACQUITY UPLC HSS T3 column (100 × 2.1 mm, 1.8 µm; Waters, Milford, MA, USA), with the flow rate controlled at 0.3 mL/min and column oven maintained at 40 °C. A Jet Stream electrospray ionization source was applied to ionize samples, setting 4 kV capillary voltage in positive mode and 3.5 kV capillary voltage in negative mode. The MS parameters were established with the following settings: a dry gas temperature of 325 °C, a dry gas flow rate of 5 L/min, a nebulizer pressure of 40 psi, a sheath gas temperature of 325 °C, a sheath gas flow rate of 10 L/min, and a fragmentor voltage of 120 V. A scan range of 50–1700 m/z was encompassed.

Data processing

Peak detection was performed using MZmine3 with ADAP algorithm [28, 29] on the acquired data. This process involved matching m/z and retention time to an established in-house database: the NTU MetaCore Metabolomics Chemical Standard Library. Subsequent data processing for potential metabolites involved missing value replacement using random forest techniques [30, 31], normalization by sum, logarithmic transformation, and no scaling by the web-based tool MetaboAnalyst 5.0 [32], setting the stage for further statistical analysis.

Statistical analysis

We performed independent t-tests for the demographic and clinical characteristics between ADHD patients and controls. We performed the non-parametric Wilcoxon rank-sum test and orthogonal partial least squares discriminant analysis (OPLS-DA) to identify significant metabolites for ADHD by using the web-based tool MetaboAnalyst 5.0 (www.metaboanalyst.ca/MetaboAnalyst/ModuleView.xhtml). The following criteria were employed to select significant metabolites: (1) p value < 0.05, (2) the absolute value of the fold change ($|FC|$) > 0, and (3) variable importance in the projection (VIP) value > 1.0 [33, 34]. The Spearman correlation was applied with R Studio (http://www.posit.co/.) to determine the relationship between specific metabolites and the severity of ADHD symptoms.

RESULTS

Sample characteristics

Table 1 presents the demographics, IQ profiles, and clinical characteristics of the ADHD and control group. Both groups are matched on age-, sex-, and FIQ-matched participants. 71.4% of participants are males, with a mean age of 8.4 in the ADHD group and 8.8 in the control group. Demographic characteristics, including weight, height, and Body Mass Index (BMI), exhibit no difference between groups. The ADHD group has higher scores in CGI-S and ADHDRS-IV, including total scores, IA subscale, and HI subscale.

Identification of potential metabolic biomarkers

In this study, 156 metabolites were identified in peripheral blood samples using an untargeted metabolomics approach. Two statistical analyses were employed to identify differentially expressed metabolites between groups. The first involved calculating fold change (FC) and p values using the Wilcoxon

Table 1. Demographics, intelligence profiles, and clinical characteristics for the ADHD and control groups.

	ADHD ($n = 70$)	Control ($n = 70$)	t	p value
Demographic characteristics				
Male, n (%)	50 (71.4%)	50 (71.4%)	0.00	1.000
Age, years	8.4 ± 1.4	8.8 ± 1.4	−1.67	0.098
Weight (kg)	31.1 ± 7.7	31.3 ± 8.9	−0.09	0.927
Height (m)	1.3 ± 0.1	1.3 ± 0.1	−0.28	0.779
BMI	17.2 ± 2.6	17.1 ± 3.3	0.10	0.922
Intelligence				
FIQ	109.3 ± 10.2	112.1 ± 10.4	−1.61	0.110
PIQ	106.9 ± 11.3	109.1 ± 11.9	−1.35	0.180
VIQ	112.5 ± 9.6	113.5 ± 10.3	−0.78	0.437
Clinical characteristics				
ADHD-RS-IV				
Total	33.6 ± 9.0	4.7 ± 5.0	23.51	<0.001
Inattention	21.8 ± 3.6	3.3 ± 3.2	32.64	<0.001
Hyperactivity/Impulsivity	11.8 ± 7.1	1.4 ± 2.3	11.69	<0.001
CGI	5.2 ± 0.6	1.3 ± 0.4	42.32	<0.001

Data are presented as mean ± SD or n (%).
BMI body mass index, Intelligence was assessed by the WISC-III, the Wechsler Intelligence Scale for Children–Third Edition, *FIQ* full intelligence quotient, *PIQ* performance intelligence quotient, *VIQ* verbal intelligence quotient, *ADHD-RS-IV*,ADHD rating scale-IV–parent version: Investigator-Administered and Scored for ADHD, with scores on Inattention subscale, Hyperactivity/Impulsivity subscale, and Total of the ADHD-RS-IV, *CGI* the clinical global impressions scale.

Table 2. Four metabolites showed significant group differences based on the Wilcoxon Test and OPLS-DA.

Metabolites	p value	VIP score	$\log_2$ ratio of Control/ADHD
Cholic acid	0.001	3.209	−0.468
Inosine	0.028	1.128	0.111
Nicotinuric acid	0.022	1.987	0.068
Homoveratric acid	0.016	2.013	−0.077

OPLS-DA orthogonal partial least squares discriminant analysis, *VIP* variable importance in projection.

test, which revealed that among the 156 metabolites, four metabolites showed statistical significance with p values < 0.05 and |FC| > 0. The second method involved selecting these four metabolites based on their variable importance in projection (VIP) scores >1.0 in the multivariate OPLS-DA model analysis. The VIP score was a key metric in metabolomics studies, used to assess the contribution of individual variables in partial least squares discriminant analysis (PLS-DA) models. By identifying variables with VIP scores greater than 1, researchers could prioritize features that significantly influence group differentiation or explain data variance. Variables with high VIP scores were considered more influential in the model [33–36]. These four metabolites met the criteria for further investigation. A metabolite must simultaneously satisfy the conditions of VIP > 1, p value < 0.05, and |FC| > 0 to be considered a potential metabolic biomarker. Among the significant metabolites, cholic acid and homoveratric acid were upregulated, while inosine and nicotinuric acid were downregulated in the ADHD group (Table 2, Fig. 1).

Correlation of the four metabolites with ADHD symptoms and severity

Spearman's correlation analyses were used to examine the associations between the four metabolites and the severity of ADHD symptoms. The respective correlation coefficients and p values are listed in Table 3. The two upregulated metabolites (i.e., cholic acid, and homoveratric acid) showed positive correlations with IA, HI, total scores of ADHDRS-IV and CGI. The other two downregulated metabolites, inosine and nicotinuric acid, are negatively correlated with IA, HI, total scores of ADHDRS-IV, and CGI.

Discrimination of ADHD patients and healthy controls by an OPLS-DA model

OPLS-DA were applied to explore the possibility of metabolomic profiling to differentiate ADHD patients from healthy controls and search for important features. To validate the performance of the model, we performed 100 permutation tests. The results of the permutation tests revealed the Q2 value of 0.128 ($p < 0.01$) and the R2Y value of 0.171 ($p < 0.01$). The result is presented in Supplementary Fig. 1.

DISCUSSION

To our knowledge, our study is distinguished for being among the few metabolomic investigations into ADHD and it also includes the largest cohort on this topic to date. Four significant metabolites were identified, including upregulated cholic acid and homoveratric acid, and downregulated nicotinuric acid and inosine in patients with ADHD. Correlational analyses revealed that upregulated metabolites had positive correlations, while the downregulated ones had negative correlations with the clinical severity of ADHD symptoms.

Although there is no known direct evidence linking cholic acid to ADHD, some studies have highlighted metabolites associated with cholic acid, such as choline, and related pathways like cholesterol metabolism with connections to ADHD [19, 37], suggesting a potential role for these compounds in the disorder. Cholic acid, a primary bile acid from cholesterol catabolism, is involved in fat digestion and cholesterol homeostasis [38]. Bile acid balance is important at the peripheral level, including maintaining gut health [39], and at the brain level [40]. Both excessive and insufficient levels of cholic acid have been associated with various diseases, including cerebrotendinous xanthomatosis (CTX), Zellweger syndrome, and familial

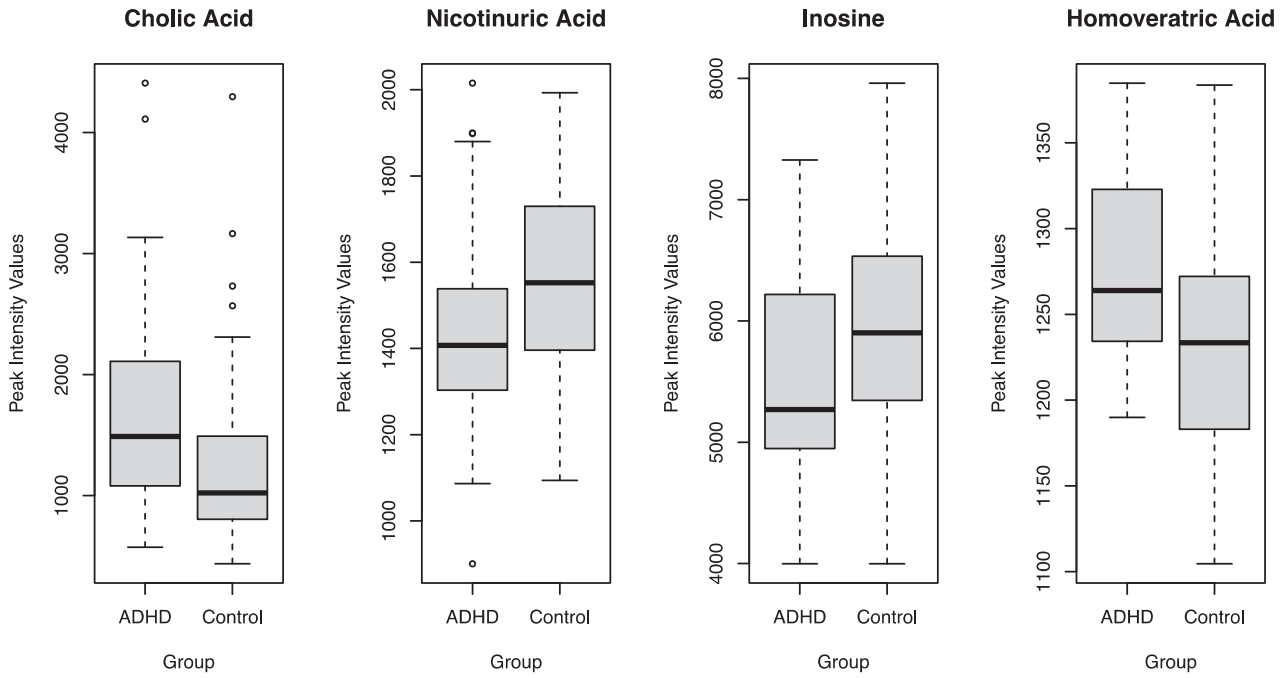

Fig. 1 Box plots of the peak intensity values of the four metabolites significantly differed between the ADHD and control groups. Samples containing outliers were removed from the box plot. Outliers are defined as values below Q1 (first quartile) − 1.5 IQR (interquartile range) or above Q3 (third quartile) + 1.5 IQR (interquartile range).

Table 3. The correlations between the four metabolites and ADHD symptom severity.

	CGI	ADHD-RS-IV Inattention	ADHD-RS-IV Hyperactivity	ADHD-RS-IV Total
Cholic acid				
rho value	0.224	0.206	0.172	0.195
p value	0.008	0.014	0.043	0.021
Inosine				
rho value	−0.265	−0.266	−0.300	−0.280
p value	0.002	0.002	<0.001	0.001
Nicotinuric acid				
rho value	−0.151	−0.180	−0.198	−0.189
p value	0.074	0.034	0.019	0.025
Homoveratric acid				
rho value	0.323	0.351	0.326	0.329
p value	<0.001	<0.001	<0.001	<0.001

rho value, Spearman's correlation coefficient. Investigator-Administered and Scored for ADHD, with scores on Inattention subscale, Hyperactivity/Impulsivity subscale, and Total of the ADHD-RS-IV.
CGI the clinical global impressions scale, *ADHD-RS-IV* ADHD rating scale-IV–parent version.

hypercholanemia (FHCA) [38]. Some patients of CTX showed behavioral disturbance [41], and the pathophysiology might be associated with disrupted biosynthesis from cholesterol to cholic acid [42]. Animal studies of FXR (Farnesoid X receptor) knockout mice with increased concentrations of cholic acids in serum and in the brain showed symptoms similar to ADHD [43]. Another potential connection of cholic acid with ADHD was through the gut-brain axis. In mouse models colonized with microbiota from ADHD mice, the gut microbiota drove the change in brain structure, function, and behavior [44]. Previous studies supplying rats with excessive cholic acid resulted in gut microbiota dysbiosis, with decreased bacterial diversity in microbiota and expansion in the secondary bile acid-producing bacteria [39]. Further studies are needed to examine the pathophysiology of upregulated cholic acid in ADHD children.

Homoveratric acid, (3,4-dimethoxyphenylacetic acid), has been identified as a key urinary metabolite potentially linked to dopamine metabolism [45]. It has been found in schizophrenia patients' urine [46], with potential neurotoxic effect involved in this disorder [47, 48]. Later studies also suspected its role in the pathogenesis of Parkinson's disease [49]. Increased urinary excretion of homoveratric acid might reflect altered metabolism of endogenous compounds and could serve as an indicator of neurological disease progression. Other studies reported association of homoveratric acid with brain mitochondrial respiration and oxidative stress through the monoamine oxidase/H2O2-dependent or non-dependent mechanism [50]. Oxidative stress might play an essential role in the pathophysiology of ADHD [51, 52]. Further investigation on detecting, quantifying, and exploring the metabolite is needed.

Nicotinuric acid, a primary metabolite from the catabolism of nicotinic acid and nicotinamide [53, 54], was reported with down regulation in the brain tissue of animal models for Parkinson's disease [55]. Nicotinic acid, serving as an essential constituent of many vital enzymes and coenzymes [56], was predominantly eliminated as the glycine-conjugated nicotinuric acid in the urine [57]. Given that the association between ADHD and nicotinuric acid or nicotinic acid remains largely uninvestigated [58], further studies are needed to validate our findings.

Inosine, an intermediate product in the purine salvage pathway [38], is recognized to have neuroprotective effects under hypoxia, inflammation, and ischemic injury in various in vivo and in vitro studies [59]. Associated mechanisms include the promotion of axonal growth [60], prevention of cell death [59], interaction with receptors like the G-protein coupled receptors and adenosine receptor [61], ERK phosphorylation, and A2AR expression [62]. Exogenously supplement of inosine is demonstrated to slow the progression of Parkinson's disease [62, 63], protect against autoimmune encephalomyelitis [62], promote axonal rewiring after stroke [64], and reduce lesions in multiple sclerosis [65]. Additionally, inosine might reduce anxiety and depressive symptoms in animal models [66] via the gut-brain axis [67]. Despite the growing recognition of inosine's role and potential therapeutic effects in various neuropsychiatric disorders, research specifically focused on inosine in ADHD is still scarce. The only study linked to ADHD is the study of juvenile rats treated with the psychostimulant methylphenidate, with detected inosine in different doses of methylphenidate [68]. Our observation of the downregulation of inosine in ADHD individuals suggests a diminished neuroprotective function in these individuals. Further investigations on the mechanisms behind this phenomenon is promising for future research. This exploration could illuminate the neurobiological underpinnings of ADHD and lead a new therapeutic pathway.

Studies on the untargeted metabolomics of ADHD are still scare, with a recent review reporting only a handful of metabolites in ADHD, among which only one were untargeted metabolomics study with discovery of novel metabolomic markers [10]. Other untargeted metabolomic studies in ADHD have further identified several metabolites with significant difference between ADHD and controls. However, these metabolites were often not consistently identified across different studies [21–23]. The four significant metabolites identified in our study have not been reported in these studies previously as well. It was common for identified metabolites in untargeted metabolomics studies to not be detected in other studies. The reasons for this variability included differences in experimental conditions, such as sample collection, preparation methods, and analytical platforms used (e.g., GC-MS vs LC-MS), and different data analysis strategies leading to variations in metabolite identification and quantification. These factors, along with the inherent complexity of metabolite profiles and the sensitivity of detection techniques, contributed to the challenges of achieving consistent findings across different studies [69–71]. Nevertheless, integration of the results of the metabolomic studies might reveal important pathways and mechanisms. The four metabolites identified in our studies and its known mechanisms suggested a connection between ADHD and pathways such as the gut-brain axis, oxidative stress, the dopaminergic system, and the purine salvage pathway, aligning with recent insights presented in a systematic review of ADHD-related metabolomic markers [72], further supporting the relevance of these biological pathways in ADHD pathophysiology. This correspondence underscored the potential of metabolomics to uncover consistent and biologically meaningful markers for ADHD. Untargeted metabolomic studies were explorative in nature, and therefore it was important to conduct further investigation on identified metabolites.

The study cohort was subjected to a rigorous selection process that ensured participants were sex-, age-, and IQ-matched and had not been treated with drugs, aiming to minimize the potential confounding effects. However, our study does have several limitations. First, recruiting participants from an outpatient clinic at a medical center may limit the generalizability of our findings to broader community populations. Second, the analytic data came from peripheral blood samples may not fully capture the biochemical environment within the brain. The detected metabolites in the blood may not necessarily correspond to those in the central nervous system (CNS) levels. However, recent research has shown significant correlations between blood and CNS metabolite levels [73]. Third, the cross-sectional study design does not allow for detecting the dynamic fluctuations of metabolic profiles over time, potentially impacting the interpretation of the results. Fourth, although this study employed an untargeted metabolomic approach, identifying detected peaks was still contingent upon comparison with an existing metabolomic library. The size of the library could restrict the number of metabolites identified, as not all peaks may be represented in the database, requiring manual checks. Furthermore, during untargeted analysis, it is possible for a single peak to correspond to multiple metabolites with similar mass-to-charge ratios due to overlapping signals or co-elution of different metabolites. Pathway enrichment analysis offers valuable insights into the relationships between observed metabolites and underlying biological mechanisms. However, due to the limited number of identified metabolites in our dataset, we were unable to conduct a meaningful analysis. To address this limitation, increasing the number of identified metabolites is essential. This can be achieved through several strategies, including the integration of complementary techniques such as GC- and LC-MS [71, 74, 75]. GC-MS is highly effective for analyzing volatile and semi-volatile compounds, while LC-MS is particularly suited for non-volatile and thermally labile compounds. When used together, these techniques provide complementary strengths, enabling a broader and more comprehensive analysis of the metabolome. This combination enhances the ability to detect a wider range of metabolites [74, 75]. Lastly, permutation testing, although practical for assessing model robustness, does not provide direct evidence of external validity. To strengthen the validation of the metabolomic biomarkers identified in this study and their potential links to ADHD or its symptoms, future research should consider approaches such as replication in independent cohorts [21, 72] to confirm the reliability and generalizability of these biomarkers, longitudinal studies [72] to examine the stability of these biomarkers over time and assess their value for ADHD onset or progression, and multi-omics integration [76] to provide a more comprehensive understanding of the biological mechanisms underlying ADHD and improve diagnostic accuracy.

CONCLUSION

Our study is an ADHD metabolomic study with the largest sample size thus far, comprising a cohort of 140 drug naïve children and adolescents, carefully matched across sexes, ages, and IQs between the two groups. We identified four novel metabolites-cholic acids, homoveratric acid, inosine, and nicotinuric acid, which exhibited significant differences in expression levels between children with ADHD and their healthy counterparts. Notably, cholic acid and homoveratric acid were found to be upregulated and positively correlated with ADHD symptoms and general clinical severity. In contrast, inosine and nicotinuric acid were downregulated and negatively correlated. Although these metabolites have received limited attention in ADHD research, our findings highlight their promising potential as avenues for further exploration into the pathophysiologic mechanisms underlying ADHD.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request and subject to approval by the institutional ethics committee. Due to ethical restrictions and participant confidentiality, the data are not publicly available.

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ACKNOWLEDGEMENTS

This work was supported by grants from the National Science and Technology Council, Taiwan [MOST 105-2314-B-002-060-MY3 and MOST 109-2314-B-002-169-MY3] to SCY, and National Health Research Institute, Taiwan [NHRI-EX110-11002PI, NHRI-EX111-11002PI] and Chen-Yung Foundation to SSG. We thank all the research assistants for your data collection and all the participants and their parents for contributing to the study.

AUTHOR CONTRIBUTIONS

Yi-An Hung: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. Tien-Chueh Kuo: Conceptualization, Formal analysis, Writing - review & editing. Yufeng Jane Tseng: Conceptualization, Formal analysis, Writing - review & editing. Susan Shur-Fen Gau: Conceptualization, Writing – review & editing. Chi-Yung Shang: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. SS Gau is the senior author of this paper.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

All methods were performed in accordance with the relevant guidelines and regulations. The study protocol was reviewed and approved by the Research Ethics

Committee (approval numbers: 201601035RINA and 201912085RINA) of the NTUH. Informed consent was obtained from all participants and their legal guardians prior to participation.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41398-025-03393-5.

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