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Original Article

MS analysis of the plasma metabolome reveals major changes of amino acid and energy metabolism for early-onset schizophrenia

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Abstract: Objectives: Early-onset schizophrenia (EOS) is a severe and chronic mental disease that manifests during childhood and adolescence. There are currently no objective biomarkers to diagnose this psychosis. Recent research has shown that metabolic disorders are closely associated with the onset of schizophrenia, but there is a lack of evidence among children and adolescent populations. This study will analyze the metabolic characteristics of patients with early-onset schizophrenia through plasma metabolomics. Methods: We analyzed plasma from 13 EOS patients and 15 healthy controls using ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF/MS) technology to identify potential biomarkers for EOS. The discriminative potential biomarkers in delineating EOS patients from controls. Results: A total of 22 different metabolites were found to be effective in differentiating EOS patients from healthy controls. EOS patients demonstrated statistically significant differences compared to the healthy control group, with 6 metabolites registering lower levels and 16 metabolites showing higher levels ($P < 0.05$). The main metabolic pathways involved include arachidonic acid metabolism, histidine metabolism, non-natural amino acid metabolism, tryptophan metabolism, and metabolism of exogenous substances mediated by cytochrome P450. Conclusions: These metabolites suggest that disturbances in amino acid and energy metabolism may be involved in the pathogenesis of EOS. The findings provide important clues for further understanding the pathogenesis of EOS and offer potential biomarkers for the diagnosis and treatment of the disease.

Keywords: Schizophrenia, biomarkers, metabolomics, LC-MS

Introduction

Schizophrenia is a severe mental disorder of unknown etiology, characterized by significant abnormalities in various aspects of mental activities, including sensation, perception, cognition, emotion, volition, and behavior. Current treatment methods generally have limited efficacy, and the prognosis of the disease is poor, which imposes significant pressure on society [1]. Early-onset schizophrenia (EOS), refers to the onset of schizophrenia before the age of 18. It accounts for approximately 14.5% of all schizophrenia cases and shares continuity with adult-onset schizophrenia. It is characterized by more pronounced functional impairments, higher comorbidity rates, and worse prognosis [2-4]. However, currently the diagnosis of EOS mainly relies on the subjective understanding of clinicians who track patients' medical history and clinical symptoms through interviews and auxiliary examinations. According to the 11th revision of the International Classification of Diseases (ICD-11) and the Diagnostic and Statistical Manual of Mental Disorders (Fifth Edition) (DSM-5), EOS is considered as the childhood-onset type of adult-onset schizophrenia, diagnosed according to the criteria for adult-onset schizophrenia, with an emphasis on hallucinations, delusions, and cognitive impairments. Therefore, the current diagnosis of EOS lacks objective support, which may lead to mis-

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diagnosis, inappropriate treatment, and poor treatment outcomes [5]. To avoid this situation, it is crucial to establish objective biological markers for the identification of EOS.

Metabolomics is a new research method following genomics, transcriptomics, and proteomics, which mainly describes the changes of small molecule metabolites in organisms, providing possibilities for revealing the pathogenesis of diseases and identifying objective biomarkers. In recent years, metabolomics has been widely used to describe the metabolic characteristics of neuro-psychiatric disorders such as autism, stroke, Parkinson's disease, multiple sclerosis, and schizophrenia [6-10]. The research findings indicate that metabolic disturbances in schizophrenia patients are manifested in aspects such as energy metabolism, lipid metabolism, amino acid metabolism, oxidative stress, and the complement system, and are correlated with clinical symptoms, functional impairments, and drug efficacy [8, 11-18]. The research subjects in the aforementioned studies were mainly focused on patients with adult-onset schizophrenia, with less attention given to EOS. Some studies have found statistically significant differences in inflammation pathway regulation, glucose and lipid metabolism-related indicators, and correlations between these indicators and clinical symptoms between early-onset and adult-onset patients [19, 20]. This suggests that patients with schizophrenia onset at different developmental stages may have different pathophysiological mechanisms.

In this study, a comprehensive metabolomics analysis was conducted using a liquid chromatography-mass spectrometry (LC-MS) platform on plasma samples from healthy subjects and individuals with EOS. Differential metabolites were further subjected to pathway analysis to gain insights into the molecular mechanisms associated with the onset of EOS (**Figure 1**). The aim is to offer potential avenues for the diagnosis and treatment of EOS.

Materials and methods

Participants and clinical assessment

Prior to the research, this project obtained approval from the Ethics Committee of Shenzhen Kangning Hospital (Ethics number: 2020-K003-02). Informed consent forms were signed by all participants and their legal guardians, providing them with information about the research objectives, methods, and risks.

This study recruited 13 cases of EOS in patients who were admitted to the Child and Adolescent Psychiatry Inpatient Department of Shenzhen Kangning Hospital from March 2021 to November 2022. General information such as gender, height, weight, age of onset, and medication history was collected. The Schedule for Affective Disorders Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL) was used as a diagnostic tool along with a semi-structured diagnostic examination [21]. The inclusion criteria were: diagnosis of schizophrenia spectrum disorder based on DSM-5 diagnostic criteria by at least two psychiatrists with senior titles, with onset before the age of 18. Exclusion criteria included: (a) history of traumatic brain injury or neurological diseases or serious physical illnesses; (b) metabolic diseases such as diabetes and autoimmune diseases; (c) drug treatment patients; (d) comorbid with other mental disorders such as depression, bipolar disorder, etc. For the enrolled participants, the Positive and Negative Syndrome Scale (PANSS) was used to assess the severity of psychotic symptoms. The Personal and Social Performance scale (PSP) was utilized to evaluate the extent of social dysfunction in patients.

Simultaneously, 15 healthy children matching the gender and age of EOS patients were recruited from the community. General information such as gender, height and weight were collected. The inclusion criteria were: self-reported good physical health, not meeting the diagnostic criteria for any mental disorder based on DSM-5 after a structured interview with K-SADS-PL, right-handedness, no family history of mental illness, no history of drug or alcohol abuse, and informed consent forms signed by the participants and their legal guardians.

Sample collection and laboratory measurements

In this study, 3 ml of venous blood was collected from fasting subjects in the morning and placed in EDTA anticoagulant tubes, then stored in a -25°C freezer.

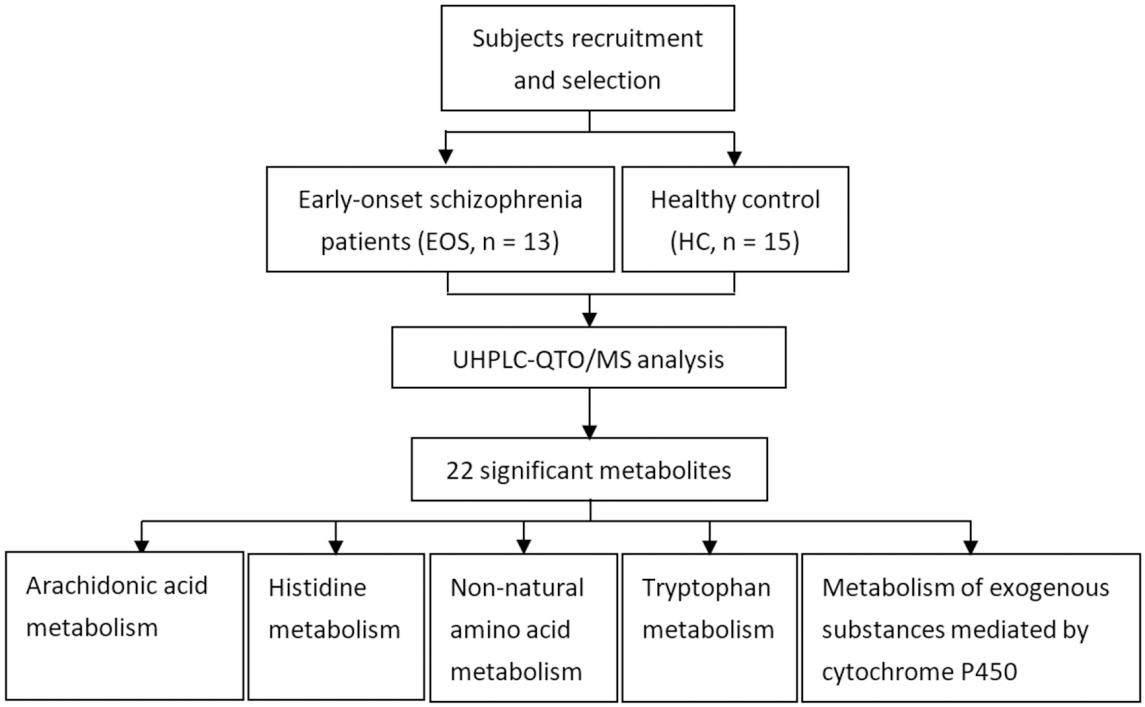

Figure 1. Flow diagram for this study. UHPLC-QTO/MS: ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry.

When conducting the test, the venous blood sample was slowly thawed on ice for sample pretreatment. Fifty μL of serum was taken and mixed with 150 μL of methanol, then vortexed for 30 seconds. The mixture was ultrasonicated at 4°C for 30 minutes and subsequently placed in a -20°C freezer for 1 hour. After centrifugation at 12,000 rpm for 15 minutes at 4°C, 100 μL of the supernatant was collected and 2.5 μL of internal standard (1 mg/ml 2,3-dichlorophenylalanine) was added, followed by thorough mixing to obtain the test plasma sample.

These samples were subjected to LC-MS (Waters, UPLC; Thermo, Q Exactive) system to obtain metabolomics raw data. The analysis conditions were as follows: column, an ACQUITY UPLC HSS T3 (2.1*100 mm, 1.8 μm), with solvent A (0.05% formic acid in pure water) and solvent B (acetonitrile) as the mobile phase. The chromatographic separation and measurement employed a gradient of the mobile phase: starting at 95% A and 5% B; within 12 minutes, a linear gradient was applied to reach 5% A and 95% B, which was maintained for 1.5 minutes. Subsequently, within 0.1 minutes, the composition was adjusted to 95% A and 5.0% B, and maintained for 2.4 minutes. The flow rate was set at 0.3 mL per minute, the column temperature was set to 40°C, and the injection volume was 5 μL. The effluent was alternately connected to the high-resolution mass spectrometer (Thermo Fisher Scientific Q Exactive), and mass spectrometry analysis was performed in dynamic multiple reaction monitoring (dynamic MRM) mode.

The mass spectrometry detection parameters are as follows: the ion source type is electrospray ionization (ESI); the source temperature is 300°C; the ion spray voltage is set at 3.0 kV (ESI+)/-3.2 kV (ESI-); Sheath Gas Flow rate, 45 arb; Aux Gas Flow Rate, 15 arb; Sweep Gas Flow Rate, 1 arb; spray voltage, 3.0 KV; Capillary Temp, 350°C; S-Lens RF Level, 30% (ESI+)/60% (ESI-); the collision mode is high-energy collision dissociation (HCD); full scan at the first level (Full Scan, m/z 70-1050) and data-dependent MS2 scan (dd-MS2, TopN = 10) at the second level; the resolution is 70,000 (first-level mass spectrum) and 17,500 (second-level mass spectrum).

The laboratory measurements above were assisted by Shanghai Sensichip Biotech Co.,

Table 1. Demographic and clinical details of recruited subjects for the study

Variables	EOS (n = 13)	Control (n = 15)
Age, mean (SD)	15.31 (±1.16)	14.8 (±2.15)
Male, n (%)	37.50 (%)	47.90 (%)
Female, n (%)	62.50 (%)	52.10 (%)
Age of onset, mean (SD)	14.39 (±1.49)	N/A
PANSS Total Score, mean (SD)	46.44 (±11.90)	N/A
PANSS positive score, mean (SD)	20.22 (±5.61)	N/A
PANSS negative score, mean (SD)	26.22 (±9.01)	N/A
PSP score, mean (SD)	36.89 (±14.57)	N/A

Abbreviations: EOS: early-onset schizophrenia; PANSS: positive and negative syndrome scale; PSP: personal and social performance scale.

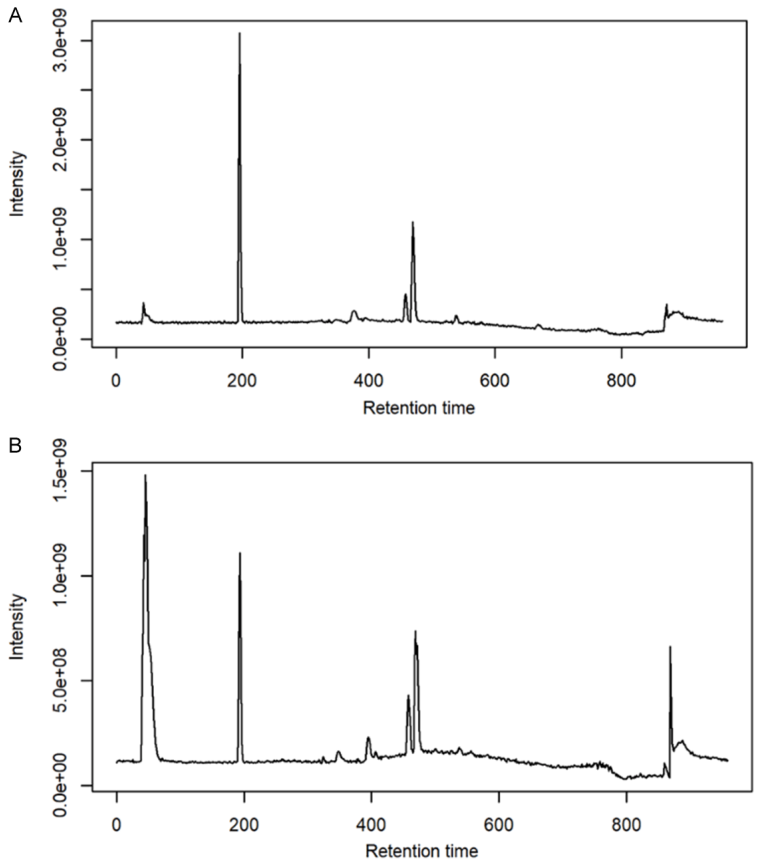

Figure 2. Typical UHPLC-QTOF/MS analysis base peak intensity spectra of plasma from healthy controls (A) and EOS patients (B) and in negative mode. UHPLC-QTO/MS: ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry; EOS: early-onset schizophrenia.

Ltd. Peak alignment, Integration, and quantification was performed in the analysis software. The concentration of metabolites was calculated based on the ratio of the peak area of the analyte to the peak area of the internal standard [17]. Finally, the results were compiled into an Excel file for further data analysis.

Statistical analyses

Based on the raw data obtained above, hierarchical clustering analysis (HCA), principal component analysis (PCA), and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed using the software to study the specific accumulation of metabolites in 24 samples. Variable importance in projection (VIP) scores were obtained through OPLS-DA modeling, with variables having $P < 0.05$ and VIP > 1 considered as differentially accumulated metabolites. The differentially accumulated metabolites between healthy controls and EOS patients were studied using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database with a significance threshold of $P < 0.01$. GraphPad Prism v6.01 (GraphPad Software Inc., La Jolla, CA, USA) was used for all data visualization and plotting.

Results

Clinical features

A total of 13 EOS and 15 healthy control participants were included in this study. Their details are shown in **Table 1**. There was no significant difference between the patient and control groups in terms of gender and age ($P > 0.05$), indicating that the findings could not be attributed to demographic factors. We found that the age of diagnosis was later than the age of onset in all the patients, suggesting failure to diagnose and treat the disease in time. We also found that EOS is comorbid with neurodevelopmental disorder such as Attention Deficit Hyperactivity Disorder (ADHD).

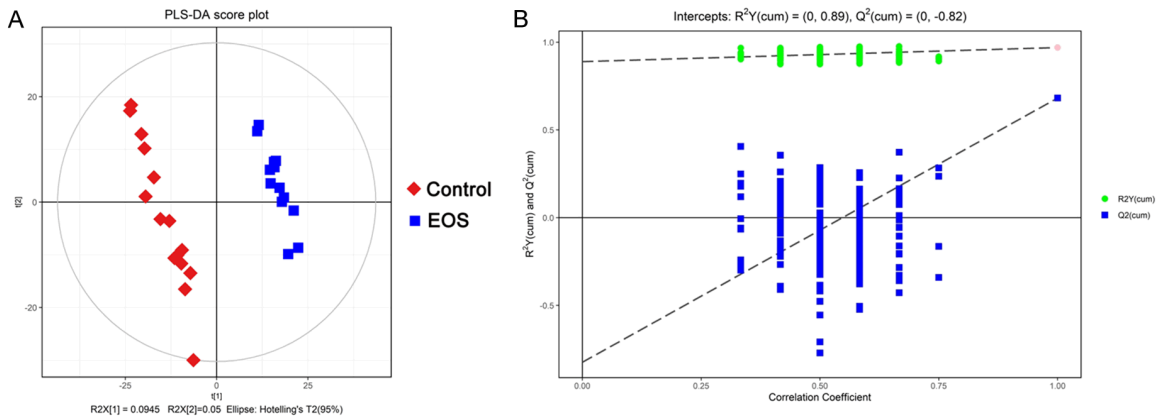

Figure 3. Metabonomic analysis of plasma Samples. A. The score plot of the PLS-DA model showing a clear discrimination between EOS subjects and healthy controls. B. Statistical validation of the PLS-DA model by permutation testing. PLS-DA: partial least squares discriminant analysis; EOS: early-onset schizophrenia.

LC-MS spectrum analysis

Typical LC-MS chromatograms of plasma samples are shown in **Figure 2**. Multiple compounds in plasma samples were identified from the LC-MS chromatograms with reference to the Human Metabolome Database (HMDB) (http://www.hmdb.ca/) and related reports. The chromatograms showed differences in the intensity of some peaks while others were present/absent.

Cluster analysis and PLS-DA model identification

The overall metabolic differences between EOS and control samples were then assessed. We first clustered these identified metabolites into a heat map. Metabolomics from LC-MS spectrum have acquired a large amount of multidimensional information and requires a range of statistical methods to obtain valid information. Using the idea of orthogonal signal correction, partial least squares discriminant analysis (PLS-DA) can filter out some random noise, better differentiate between groups, and improve the validity and analytical power of the model (see **Figure 3A**). Score plots of the PLS-DA model showed clear differentiation between the EOS patients and the healthy controls without overlap. The permutation test showed that the cumulative R2 and Q2 values were smaller than the original values, indicating that the PLS-DA model developed in this study was not overfitted (**Figure 3B**).

Identification of differentially expressed metabolites in EOS

To further analyze the differential metabolites in the EOS and control groups, we constructed a loading diagram based on the PLS-DA model (**Figure 4**). The further away from the central origin, the greater the contribution to the separation of EOS from healthy controls and the greater the likelihood of differential metabolites. The VIP value was used as an evaluation metric. Metabolites with VIP > 1 as and *p*-value less than 0.05 were considered significantly differentially expressed. The metabolites were screened against the criteria and t-tests were performed on the normalized peak areas of the metabolites using SPSS software ($P < 0.05$). It was finally found that total of 22 metabolites were identified as differential metabolites, among which Trichloroacetic acid, Monobutyl phthalate, Diphenylphosphate, Mono (2-ethylhexyl) phthalate (MEHP), Catechin, Arachidonic acid, 13-HpOTrE(r) and Erucic acid levels were significantly elevated in EOS patient, while Glutaric acid, Urocanic acid, 2-Mercaptobenzothiazole, Azelaic acid, (±) 9-HpODE and Dodecylbenzenesulfonic acid levels were significantly reduced (**Table 2**). We visualized the differences in metabolite expression and their statistical significance between the two groups using volcano and hotspot plots (**Figures 4** and **5**).

Pathway analysis of candidate biomarkers

To better understand the functional importance of the differential metabolite changes, we per-

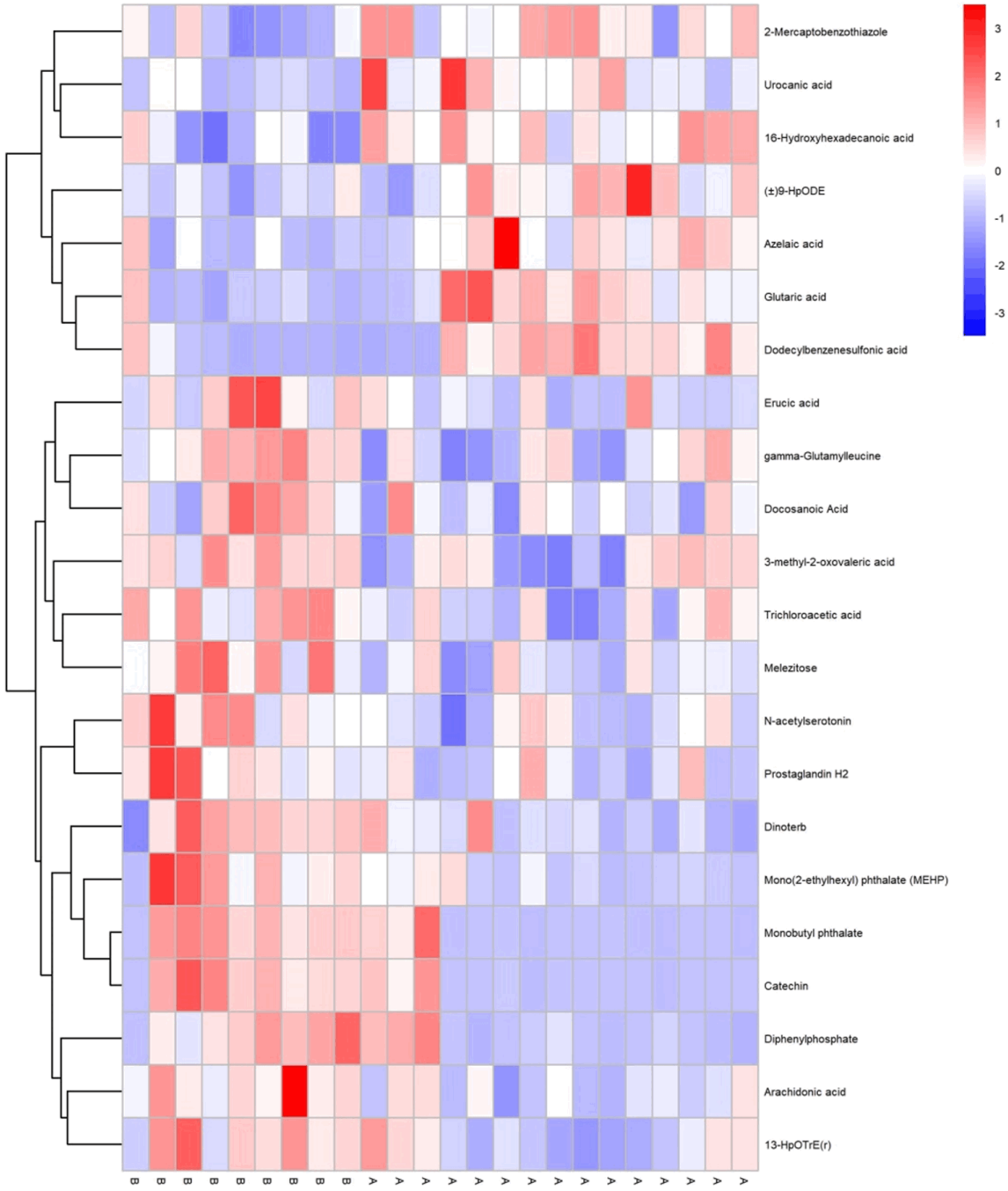

Figure 4. Clustering heatmap of the significantly different metabolites between healthy controls (A) and EOS patients (B) in the NEG model. EOS: early-onset schizophrenia; NEG: negative ion mode.

formed metabolic pathway analysis. Metabolic pathways were predicted by simply mapping metabolite changes onto known metabolic pathways. The importance of a particular pathway was expressed based on its metabolic profile. **Figure 6** visualizes the results of this analysis, showing the metabolic pathways that underwent significant changes associated with EOS. The color intensity reflects the increased importance as from white to red, while the diameter of the circle relates to the important of the pathway. By analyzing the metabolic pathways, we found that the major metabolic pathways involved in the differential metabo-

Table 2. UPLC-QTOF/MS-detected metabolites in plasma samples of children with EOS (VIP > 1, $P < 0.05$)

MS2 name	MS2 ppm	Formula	VIP	*P*-Value	Fold change
3-methyl-2-oxovaleric acid	-8.78	C6 H10 O3	1.427528123	0.012690704	1.525485907
Glutaric acid	-8.31	C5 H8 O4	2.030477031	0.005809326	0.440886104
Urocanic acid	-9	C6 H6 N2 O2	1.658685562	0.003863028	0.545470987
Trichloroacetic acid	-8.91	C2 H Cl3 O2	1.518884063	0.00420862	1.690514718
2-Mercaptobenzothiazole	-5.65	C7 H5 N S2	1.741603573	0.003702631	0.901484478
Azelaic acid	-3.59	C9 H16 O4	1.562986848	0.029281954	0.767449797
N-acetylserotonin	-2.45	C12 H14 N2 O2	1.63303608	0.003422586	1.179888012
Monobutyl phthalate	-1.93	C12 H14 O4	2.202422724	0.001323801	3.329792627
Dinoterb	-1.4	C10 H12 N2 O5	1.631705162	0.0082021	1.310961363
Diphenylphosphate	-0.38	C12 H11 O4 P	1.885004759	0.012304696	1.802728595
gamma-Glutamylleucine	0.23	C11 H20 N2 O5	1.53877524	0.005644908	1.249136117
16-Hydroxyhexadecanoic acid	0.43	C16 H32 O3	1.886512521	0.005683087	1.54930133
Mono(2-ethylhexyl) phthalate (MEHP)	0.21	C16 H22 O4	1.949408967	0.013237786	1.961040147
Catechin	-7.69	C15 H14 O6	2.201578475	0.000599104	3.884718223
Arachidonic acid	-0.28	C20 H32 O2	1.781489312	0.020756075	1.547825174
13-HpOTrE(r)	-7.54	C18 H30 O4	1.790503086	0.00764726	1.637111318
(±)9-HpODE	0.9	C18 H32 O4	1.437809568	0.010635834	0.634877602
Dodecylbenzenesulfonic acid	-0.12	C18 H30 O3 S	1.792170635	0.004066709	0.27719486
Erucic acid	0.28	C22 H42 O2	1.357935754	0.02470896	2.017717957
Docosanoic Acid	-0.36	C22 H44 O2	1.016900278	0.047492364	1.289459842
Prostaglandin H2	9.09	C20 H32 O5	1.528683401	0.011591493	1.277025405
Melezitose	4.77	C18 H32 O16	1.687937976	0.002819608	1.302015134

Abbreviations: UHPLC-QTOF/MS: ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry; EOS: early-onset schizophrenia; MS2: second quadruple; VIP: variable importance in projection.

lites were (1) Arachidonic acid metabolism; (2) histidine metabolism; (3) Unnatural amino acid metabolism; (4) Tryptophan metabolism and; (5) Metabolism of xenobiotic by cytochrome P450.

Discussion

Identifying clinical-relevant biomarkers for heterogeneous diseases like schizophrenia is a challenging task. However, studying EOS provides a unique opportunity. EOS has a relatively homogeneous phenotype, and patients are less influenced by secondary effects of disease-related environmental changes (such as cannabis use, smoking, hospitalization), and typically show more significant genetic burden. Therefore, studying the differences in biomarkers between EOS patients and healthy control groups can facilitate the identification of biomarkers associated with schizophrenia, thus enhancing the ability for intervention in the prodromal stage or even earlier.

Compared to other metabolomics research methods, LC-MS has higher sensitivity and can analyze a wider range of compounds, making it a widely used method in metabolomics [22]. In order to understand the characteristics of metabolites in patients with EOS, this study conducted comprehensive targeted metabolomic analysis of plasma samples from both EOS patients and healthy control group using the LC-MS platform. In EOS patients and healthy control subjects, 22 differential metabolites were identified. Among EOS patients, the levels of Trichloroacetic acid, Monobutyl phthalate, Diphenylphosphate, Mono(2-ethylhexyl) phthalate (MEHP), Catechin, Arachidonic acid, 13-HpOTrE(r) and Erucic acid were significantly increased, while the levels of a Glutaric acid, Urocanic acid, 2-Mercaptobenzothiazole, Azelaic acid, (±) 9-HpODE and Dodecylbenzenesulfonic acid were significantly decreased. The main metabolic pathways involved include: (1) Arachidonic acid metabolism; (2) Histidine

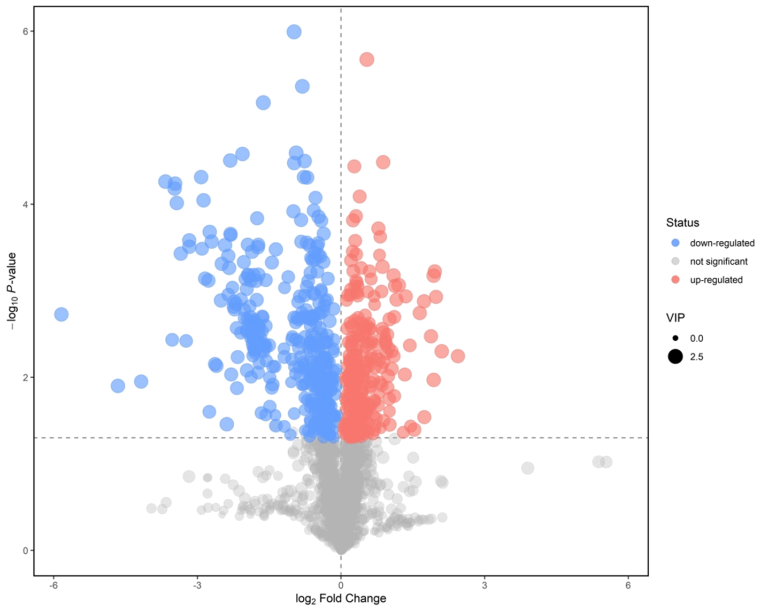

Figure 5. Volcano plot showing the differential expressed metabolites, red indicates upward adjustment, blue indicates downward adjustment. Volcano plots for differential metabolite analysis. Each point in the volcano plot represents a metabolite, VIP is represented by the vertical axis, and the ventral axis represents the logarithm of the multiplicity of quantitative differences in metabolites in the EOS and normal groups. Red dots represent metabolites with up-regulated, blue dots represent metabolites with reduced differences, and gray dots represent metabolites with detected but no significant differences. VIP: variable importance in projection; EOS: early-onset schizophrenia.

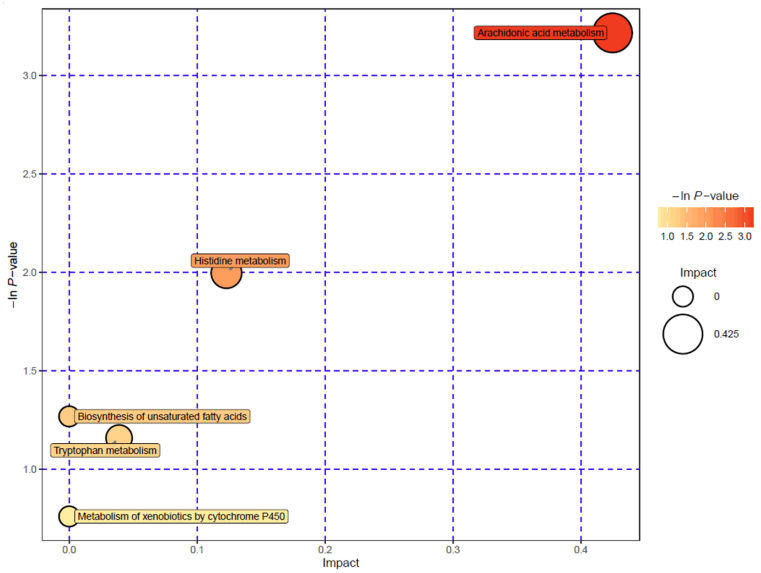

Figure 6. Metabolic pathway analysis of identified metabolites showing metabolic pathways represented as nodes. The size of the diameter of the circular nodes represents the impact associated with the pathway (based on pathway topology analysis). Color intensity (from white to red) indicates statistically significant increase in importance (based on pathway enrichment analysis).

metabolism metabolism; (3) Unnatural amino acid metabolism; (4) Tryptophan metabolism and; (5) Metabolism of xenobiotic by cytochrome P450.

When conducting metabolomics studies on adult schizophrenia patients, we have recently found evidence of abnormalities in amino acid and lipid metabolism, which is consistent with our research findings. Mednova et al. indicate disrupted amino acid metabolism in patients with schizophrenia, with reduced levels of valine, aspartate, arginine, glutamine, glycine, and ornithine. Additionally, there are abnormal lipid metabolism patterns, evidenced by increased concentrations of various fatty acids with distinct structural compositions [17]. According to a recent review, 18 group-specific compounds associated with schizophrenia were identified, and many of these compounds are associated with the conversion of tyrosine and steroids, with a particular emphasis on androgens [8]. Su et al. and Liu et al. both found the level of phosphatidylcholine may have the potential to distinguish between drug-naïve schizophrenia patients and healthy individuals [13, 23]. A study of saliva metabolomics found that patients with clinical high risk for psychosis had abnormal metabolism of aromatic amino acids, glutamine and nucleotides, and these abnormalities persisted into the development of psychosis [24]. The metabolic pathways involving these metabolites are not merely closely related to neurological functioning, but also associated with several physiological pro-

cesses including inflammation response, immune regulation, and antioxidant reaction. While these compounds are thought to play a role in the development of schizophrenia by affecting amino acid and lipid metabolism, there is considerable heterogeneity in the specific metabolites identified across different studies. Further research involving larger and more diverse populations, including individuals of different ages, is needed to better understand the mechanisms and significance of these compounds in the disease.

The results of metabolomics studies yield valuable insights in identifying potential biomarkers for disease discrimination. A Mendelian randomization study identified eight metabolites associated with the risk of mental disorders. Specifically, 3-Hydroxybutyrate was considered to be associated with major depressive disorder, butyrylcarnitine with schizophrenia, and sphingomyelins with anorexia nervosa [25]. Wang et al. identified 111 lipid species between the patients with schizophrenia or major depressive disorder, which could distinguished them reliably [26]. In a study using a liquid chromatography electrochemical array platform, the researchers found that patients with schizophrenia had lower 5-hydroxytryptophan and higher glutathione comparing to bipolar disorder, but there were no noticeable differences in metabolite concentration between them [27]. Another study also found there were no significant metabolites that could differentiate patients with schizophrenia, bipolar disorder and healthy individuals [28].

Integrating the findings of this study with previous literature, both neurodevelopmental disorders and EOS demonstrate metabolomic abnormalities in amino acid metabolism and oxidative stress. Ahrens et al.'s study reveals complex associations between ADHD and alterations in amino acid metabolism, neurotransmitter dysregulation, oxidative stress, and the kynurenine pathway [29]. Wang et al. found differential metabolites enriched in the pathway of tryptophan metabolism and metabolism of xenobiotic by cytochrome P450 in individuals with Autism Spectrum Disorder (ASD) [30]. Serotonin, oxytocin, and immune system dysregulation have been implicated in the pathogenesis of ASD [31]. However, these analyses lack disease specificity. Currently, there is a lack of metabolomics-based discrimination between EOS and other neurodevelopmental disorders such as autism spectrum disorder, attention-deficit hyperactivity disorder, and intellectual disabilities. However, comorbidities with neurodevelopmental disorders are not uncommon in EOS patients [5]. According to the clinical evidence and recently published articles [32], the symptoms of schizophrenia in children and adults were share several characteristics with those of ASD, ADHD, and bipolar disorders. The underlying mechanisms are unclear, and treatment presents significant challenges for clinical diagnosis and management. Subsequent screening and identification of differential metabolites between neurodevelopmental disorders and EOS may provide initial insights into the physiological mechanisms of these diseases.

Metabolomics research can also provide invaluable guidance for assessing treatment effectiveness and functional prognosis. Almeida et al. suggested that phosphatidylserines levels could be a potential biomarker for poor response to atypical antipsychotic treatments [33]. The conclusion of Li et al. has described that the ratio of enzymes and different types of fatty acids in the pathway of fatty acid metabolism on the erythrocyte membrane were correlated with PANSS total score [12]. Su et al. indicated that phosphatidylcholines exhibited a positive correlation with the PANSS positive symptom sub score, whereas cholic acid demonstrated a positive association with the PANSS negative symptom sub score in drug-naïve schizophrenia patients [23]. An additional study observed a relationship between linoleoyl carnitine levels and a decrease in PANSS positive symptom sub score subsequent to Olanzapine treatment [34]. Of interest, the differential level of some amino acids, glutaric acid, Vanillylmandelic acid, and L-sorbose may serve as potential predictors of violent behavior in patients with schizophrenia [18]. The research on the correlation between metabolomics and clinical characteristics as well as treatment response in schizophrenia holds significant implications for guiding clinical practice. Identifying the key factors that influence outcomes may provide valuable insights and guidance for clinicians in their real-world work.

The limitation of this study lies in the relatively small sample size, considering the significant heterogeneity of schizophrenia itself and the

identification of biomarkers remains a complex process, requiring comprehensive consideration of genetic, environmental, and other interacting factors. A larger sample size may provide a more robust theoretical basis for different phenotypes of schizophrenia. Furthermore, we did not include adult schizophrenia patients in this study, the inclusion of the adult schizophrenia may provide further insights into the differences between the pathogenesis of EOS patients and adult patients, and provide mechanistic references to the pathogenesis of schizophrenia. Besides, the present study did not choose to exclude EOS patients with comorbid ADHD. Perhaps when subsequent studies are able to obtain a larger sample size, patients with and without comorbidities can be analyzed to obtain more credible findings. The identification of biomarkers may aid clinicians in enhancing the diagnostic process. But metabolomics is still in the early stages of clinical practice, and further research is needed to facilitate its transition from clinical studies to routine diagnostics. Further in-depth exploration and validation in larger sample sizes are required for biomarker research in schizophrenia and other complex diseases.

Conclusions

This study utilized LC-MS metabolomics technology to analyze the metabolic characteristics of EOS. The results indicated that a total of 22 different metabolites could effectively differentiate patients from healthy controls. These metabolites suggest that disturbances in amino acid and energy metabolism may be associated with the pathogenesis of EOS. This research approach may help identify potential biomarkers for early detection of schizophrenia, providing an opportunity for earlier therapeutic intervention and improving patient prognosis.

Acknowledgements

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Disclosure of conflict of interest

None.

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