Alpha Psychiatry

Alpha Psychiatry **2026**; 27(6): 51602
https://doi.org/10.31083/AP51602

Original Article

Shared Plasma Metabolic Disturbances in Adolescent Autism Spectrum Disorder and Major Depressive Disorder

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Academic Editor: Tomislav Franić
Submitted: 7 March 2026 Revised: 24 April 2026 Accepted: 7 May 2026 Published: 3 November 2026

Abstract

Background: The high comorbidity between autism spectrum disorder (ASD) and major depressive disorder (MDD) during adolescence suggests the presence of shared biological mechanisms. This study aimed to identify common plasma metabolic alterations in adolescents with ASD and MDD and to evaluate their potential utility for transdiagnostic discrimination. **Methods:** Adolescents aged 8–18 years were recruited, including patients with ASD (n = 26), patients with MDD (n = 51), and healthy controls (HCs; n = 38). Targeted metabolomic profiling was performed using ultra-high-performance liquid chromatography–electrospray ionization–quadrupole linear ion trap mass spectrometry (UHPLC-ESI-QTRAP MS). Data were initially divided into a training set (70%) and an independent test set (30%) using stratified sampling. Correlation-based feature selection was conducted exclusively within the training set to identify plasma metabolites that were consistently altered in both ASD and MDD relative to HCs. A Firth-penalized logistic regression model was subsequently developed using the selected features and validated in the independent test set. **Results:** A total of 32 shared plasma metabolites were identified. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis mapped these metabolites to several key biological systems, including neurotransmitter metabolism (phenylalanine, tyrosine, and tryptophan biosynthesis), gut-brain axis-related pathways (primary bile acid biosynthesis and microbial tryptophan metabolism), and energy homeostasis pathways (branched-chain amino acid degradation, ketone body metabolism, and fatty acid degradation). Correlation-based feature selection identified the six metabolites most strongly associated with case status (Spearman $|\rho| = 0.545$–0.658; false discovery rate [FDR] = 7.05×10^{-7} to 1.11×10^{-8}), thereby refining a core metabolite panel. A Firth-penalized logistic regression model based on these six metabolites achieved an area under the receiver operating characteristic curve (AUC) of 0.842 in the independent test set. Permutation testing demonstrated that this performance was significantly greater than expected by chance (empirical $p = 0.03$). Sensitivity analyses indicated that the model's discriminatory performance remained robust after adjustment for body mass index (BMI) (AUC = 0.862) and across both medicated (AUC = 0.909) and unmedicated (AUC = 0.930) MDD subgroups. The model also demonstrated strong discriminatory performance in diagnosis-specific analyses (ASD vs. HC: AUC = 0.944; MDD vs. HC: AUC = 0.923). **Conclusions:** These findings identify a shared plasma metabolic disturbance profile in adolescents with ASD and MDD, supporting the existence of overlapping biological mechanisms across these disorders. The observed classification performance highlights the potential utility of this metabolic signature for transdiagnostic discrimination. Collectively, these results provide a foundation for future investigations into the biological basis of ASD-MDD comorbidity and the development of metabolite-based biomarkers.

Keywords: metabolomics; autism spectrum disorder; major depressive disorder; adolescence; transdiagnostic biomarker

Main Points

1. Shared plasma metabolic disturbances in adolescent autism spectrum disorder (ASD) and major depressive disorder (MDD).

2. Six-metabolite panel enables transdiagnostic discrimination (area under the curve [AUC] = 0.842).

3. Dysregulated pathways include neurotransmitter and gut-brain axis.

4. Machine learning validation underscores signature robustness.

5. Provides a metabolic framework for understanding ASD-MDD overlap.

1. Introduction

Autism spectrum disorder (ASD) and major depressive disorder (MDD) are prevalent neurodevelopmental and affective disorders in children and adolescents. They share overlapping clinical manifestations, such as impaired social functioning, social withdrawal, and anhedonia. Epidemiological data consistently show that the likelihood of developing MDD is substantially higher in individuals with ASD than in the general population [1]. This frequent co-occurrence implies that common biological pathways, rather than just symptomatic similarities, may contribute to both disorders. However, traditional psychiatric diagnostic

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systems (e.g., Diagnostic and Statistical Manual of Mental Disorders (DSM)/International Classification of Diseases (ICD)), which are based on symptomatology, struggle to reveal the biological essence of this comorbidity [2]. Thus, a metabolomic approach may help uncover shared pathophysiological mechanisms underlying ASD-MDD comorbidity.

Driven by the limitations of symptom-based diagnoses, a transdiagnostic research paradigm has emerged, seeking biological commonalities that cut across traditional diagnostic boundaries [3]. The Research Domain Criteria (RDoC) framework proposed by the National Institute of Mental Health (NIMH) is a representative example of this paradigm [4]. This approach is exemplified by metabolomic studies that transcend traditional diagnoses [5] and by computational methods that stratify patients based on biological data rather than symptom-based categories [6]. In this context, metabolomics, a key branch of systems biology, enables the systematic and unbiased assessment of endogenous small-molecule metabolites, dynamically reflecting the terminal biochemical phenotypes resulting from the interplay of genes, environment, and the microbiome. Thus, metabolomics can help reveal the biochemical basis of neuropsychiatric disorders, facilitating the discovery of biomarkers and mechanistic insights [5,7].

Research into the metabolic underpinnings of ASD has highlighted disruptions in amino acid homeostasis (e.g., tryptophan, glutamate, and branched-chain amino acids) [8,9], alongside issues with mitochondrial function and energy production [10]. In contrast, studies on MDD have often centered on monoamine neurotransmitter systems, Hypothalamic-Pituitary-Adrenal (HPA) axis dysregulation, immune activation, and shifts in tryptophan metabolism towards the kynurenine pathway [11]. Interestingly, a growing body of work points to convergent changes in both disorders, particularly within the tryptophan metabolic pathway and among metabolites implicated in gut-brain communication, such as short-chain fatty acids and bile acids [12,13,14,15,16]. These parallels offer a metabolic rationale for their clinical co-morbidity.

While these parallel findings are suggestive, direct comparisons of ASD and MDD within the same analytical framework during adolescence—a period of heightened comorbidity risk—remain limited. Most metabolomic studies to date have investigated these disorders in isolation, leaving a critical gap in our understanding of their shared metabolic architecture [17,18]. Furthermore, the validation of potential metabolic biomarkers using robust machine learning approaches in independent samples is an area requiring further exploration [19,20]. Therefore, this study aimed to directly compare the plasma metabolic profiles of ASD and MDD in a carefully characterized adolescent cohort (8–18 years) using targeted metabolomics, with the following objectives: (1) directly identify plasma metabolites and pathways that are consistently altered in both ASD and MDD compared to healthy controls; and (2) develop and validate a machine learning model based on these shared metabolites to assess their utility for transdiagnostic discrimination. We hypothesized that adolescents with ASD and MDD share a common pattern of plasma metabolic disturbances, particularly involving neurotransmitter metabolism, gut-brain axis communication, and energy homeostasis.

2. Materials and Methods

2.1 Study Participants

This case-control study consecutively recruited participants from the Child and Adolescent Outpatient Department of Tianjin Anding Hospital. The final sample size was determined by the availability of eligible participants during the study period. Three groups of participants aged 8–18 were included: an ASD group (n = 26), an MDD group (n = 51), and a HC group (n = 38). The HC group was matched to the patient groups in terms of age distribution. The inclusion criteria for ASD patients were: (1) diagnosis by experienced psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria. The inclusion criteria for MDD patients were: (1) diagnosis according to DSM-5 criteria, further confirmed by the Mini-International Neuropsychiatric Interview for Children and Adolescents (MINI-Kid); (2) a Montgomery-Asberg Depression Rating Scale (MADRS) total score ≥22. The HC group consisted of typically developing adolescents recruited through the hospital, screened by MINI-Kid to exclude current or past psychiatric disorders. Common exclusion criteria for all participants included: (1) severe physical or neurological diseases; (2) comorbid other major psychiatric disorders; (3) history of substance or alcohol abuse in the past year; (4) severe communication impairments preventing completion of assessments. The study protocol was approved by the Ethics Committee of Tianjin Anding Hospital (Approval Nos.: 2023-13 and 2023-25). Written informed consent was obtained from the legal guardians of all participants. Given the exploratory nature of this study and the absence of prior effect size estimates, no a priori power calculation was performed. The sample size was determined by participant availability, consistent with recommendations for exploratory studies [19].

2.2 Sample Collection and Processing

Fasting venous blood (3 mL) was collected from all participants between 8:00 and 10:00 AM into EDTA anticoagulant tubes. Within 2 hours of collection, blood samples were centrifuged at 3000 rpm (approximately 1500 ×g) for 13 minutes at 4 °C to separate the upper plasma layer. The plasma was aliquoted into 300 µL tubes and immediately stored at –80 °C until metabolomic analysis.

2.3 Targeted Metabolomics Analysis

Plasma metabolites were quantified using targeted metabolomics based on ultra-performance liquid chromatography-electrospray ionization-triple quadrupole mass spectrometry (UHPLC-ESI-QTRAP MS). Briefly, metabolite extracts were separated using an Agilent 1290 Infinity LC UHPLC system (1290 Infinity LC, Agilent Technologies, Santa Clara, California, USA). To achieve broad coverage of metabolites, both hydrophilic interaction chromatography (HILIC) and reversed-phase (C18) columns (Waters UPLC BEH, 1.7 µm, 2.1 × 100 mm) were employed with optimized gradient elution programs. The separated metabolites were detected on an AB 6500 + QTRAP mass spectrometer operated in multiple reaction monitoring (MRM) mode. A pooled quality control (QC) sample, generated from all samples, was analyzed periodically throughout the sequence to monitor instrument performance and data quality [21].

2.4 Data Processing and Statistical Analysis

2.4.1 Data Preprocessing

The raw mass spectrometry data underwent a multi-step preprocessing pipeline to mitigate technical noise and ensure data integrity prior to statistical analysis. First, metabolites with a relative standard deviation (RSD) >30% in quality control samples were removed. Second, low-abundance metabolites not detected in over 50% of the samples were filtered out. For the remaining missing values, the k-nearest neighbors (KNN) algorithm was used for imputation [22]. Metabolite concentration data were then log 10-transformed and subsequently scaled using Pareto scaling to balance the contributions of high- and low-abundance metabolites [23]. Rigorous quality control was performed. The tight clustering of QC samples in Principal Component Analysis (PCA), low relative standard deviation (RSD ≤30% for >70% of metabolites), and high inter-QC correlation (Pearson's r >0.9) collectively demonstrated excellent analytical reproducibility and minimal batch effects (**Supplementary Fig. 1**). Therefore, no additional batch correction was applied.

2.4.2 Differential Metabolite Screening and Shared Feature Identification

All statistical analyses were performed in the R environment (version 4.2.3, The R Foundation for Statistical Computing, Vienna, Austria). One-way ANOVA or Kruskal-Wallis tests were used to compare continuous variables (e.g., age, body mass index (BMI)) across the three groups, while the χ^2 test was used for categorical variables (e.g., sex). Given the significant difference in sex distribution among the three groups ($p < 0.05$), subsequent differential metabolite analyses between all groups used the limma R package to fit linear models with sex and age included as covariates to control for their potential confounding effects. Pairwise comparisons were conducted for ASD vs. HC and MDD vs. HC. *p*-values were adjusted using the False Discovery Rate (FDR) method, with an FDR <0.05 set as the threshold for statistical significance. Simultaneously, Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) was performed, and the variable importance in projection (VIP) score for each metabolite was calculated. Metabolites with VIP >1 were retained as significant contributors to group separation. To visualize the overall separation trend among the three diagnostic groups, a partial least squares-discriminant analysis (PLS-DA) model was also constructed and evaluated.

To assess the potential impact of sample size imbalance, we performed an inverse probability weighting (IPW) analysis. Propensity scores were estimated using multinomial logistic regression with group membership (ASD, MDD, HC) as the outcome and age and gender as covariates. Inverse probability weights were calculated as the reciprocal of the predicted probability and then stabilized by dividing by the mean weight. The weighted data were refitted using the same limma framework with age and gender retained as covariates. An overlap ratio >80% between original and IPW-weighted results was considered evidence of robustness against sample size imbalance.

To identify metabolites consistently altered in both disorders, shared differential metabolites were defined based on the following criteria: (1) Significant in both ASD vs. HC and MDD vs. HC comparisons (FDR <0.05); (2) Exhibited consistent direction of change (both up-regulated or both down-regulated) in ASD and MDD groups compared to HC; (3) Had VIP values greater than 1 in the OPLS-DA models for both comparisons. Metabolites meeting all these criteria were defined as shared differential metabolites for ASD and MDD.

2.4.3 Metabolic Pathway Annotation

Pathway mapping was performed to interpret the biological functions of the shared metabolites. The KEGG compound identifier for each of these metabolites was used to systematically query the KEGG database to determine their primary associated metabolic pathways.

2.4.4 Machine Learning Modeling and Validation

To identify a compact metabolite panel with trans-diagnostic potential and ensure methodological rigor, we adopted a strict analytical workflow to prevent data leakage. First, the full dataset was randomly partitioned into a training set (70%) and an independent held-out test set (30%) using stratified sampling to preserve class distribution. All subsequent feature selection and model development steps were performed exclusively on the training set.

All measured metabolites were ranked within the training set only by the absolute value of their Spearman correlation coefficient (|ρ|) with the combined disease status (ASD and MDD patients vs. healthy controls). The six metabolites exhibiting the strongest correlations (|ρ| =

0.545–0.657) and highest statistical significance (FDR <7.1 × 10^{-7}) were selected as the core feature set. These six metabolites were then locked as the final feature set. A Firth-penalized logistic regression model was developed on the training set to discriminate the combined patient group from healthy controls, which helps mitigate bias due to the limited sample size. The same six metabolites were then extracted from the previously untouched test set, and model performance was evaluated on this independent held-out test set. Performance was quantified using the area under the receiver operating characteristic curve (AUC), with 95% confidence intervals estimated via bootstrap. Balanced accuracy was also reported to account for class imbalance. A 5-fold cross-validation with stratification was additionally performed within the training set [19,20].

2.4.5 Robustness Validation

To address potential concerns about small-sample instability and validate the robustness of our findings, we performed two complementary analyses. (1) Permutation testing: The case-control labels were randomly shuffled 100 times within the training set only, preserving the entire feature selection and modeling pipeline for each permutation. The empirical *p*-value was calculated as the proportion of permutations yielding a test set AUC ≥ the observed AUC. (2) Bootstrap feature stability assessment: We performed 50 bootstrap resamples (with replacement) of the training data, repeating the correlation-based feature selection for each resample. Selection frequency was recorded for each metabolite to assess the stability of the six-metabolite panel.

2.4.6 Sensitivity Analysis

Given the heterogeneity of psychotropic medications (including Selective Serotonin Reuptake Inhibitors (SSRIs), atypical antipsychotics, benzodiazepines, and mood stabilizers, often in combination) and the limited sample size per drug class, we performed a preliminary analysis by grouping MDD patients into binary categories ("medicated" vs. "unmedicated"). To assess the potential impact of psychotropic medication, we compared the plasma levels of the six core metabolites between medicated (n = 25) and unmedicated (n = 26) MDD patients using two-sided *t*-tests. We further evaluated the diagnostic performance of the metabolite panel separately in the medicated and unmedicated subgroups against HC.

To evaluate the potential influence of BMI as a metabolic confounder, we conducted an additional analysis. The residual of each metabolite after regressing out BMI was calculated to represent BMI-adjusted metabolite levels. The entire machine learning pipeline—including correlation-based feature selection, model training with Firth logistic regression, and validation—was then repeated using these adjusted data to assess the robustness of the core metabolic signature.

3. Results

3.1 Demographic Data

The three participant groups (ASD, MDD, HC) showed no significant differences in baseline demographic characteristics such as age and body mass index ($p > 0.05$), as shown in Table 1. Due to the unbalanced sex distribution among the groups ($p < 0.05$), sex was included as a covariate in all subsequent metabolomic differential analyses.

Inverse probability weighting (IPW) analysis was conducted to evaluate the potential impact of sample size imbalance. The overlap ratio between original and IPW-weighted analyses was 100% (26/26) for the ASD group and 93.5% (58/62) for the MDD group. Crucially, all 32 shared metabolites, including the six core biomarkers, were entirely robust (100% retention). An overlap ratio >80% indicates strong robustness against sample size imbalance. These results confirm that our findings are not substantially biased by the imbalanced sample sizes (**Supplementary Table 1**).

3.2 Transdiagnostic Differential Metabolites and Pathway Mapping

PLS-DA revealed a clear overall separation among the ASD, MDD, and HC groups (Fig. 1A), indicating distinct global metabolic profiles for each diagnostic cat-

Table 1. Demographic and clinical characteristics of the study participants.

Characteristic	ASD (n = 26)	MDD (n = 51)	HC (n = 38)	*p*-value
Age (years), mean ± SD	13.31 ± 3.16	14.61 ± 1.33	14.37 ± 2.89	0.263
BMI (kg/m^2), mean ± SD	22.15 ± 5.02	20.23 ± 4.07	21.94 ± 3.88	0.061
Gender (F/M)	6/20	31/20	22/16	0.005
Current psychotropic medication, n (%)				
Medicated (within 2 weeks)	-	25 (49.0%)	-	-
Unmedicated	-	26 (51.0%)	-	-

Data are presented as mean ± standard deviation or n (%). Abbreviations: ASD, autism spectrum disorder; MDD, major depressive disorder; HC, healthy controls; BMI, body mass index. A medication status was assessed for the MDD group. Current psychotropic medication refers to any psychotropic medication (e.g., antidepressants, anxiolytics) being taken within the two weeks prior to sample collection. *p*-values were derived from one-way ANOVA (Age, BMI) or χ^2 test (Gender).

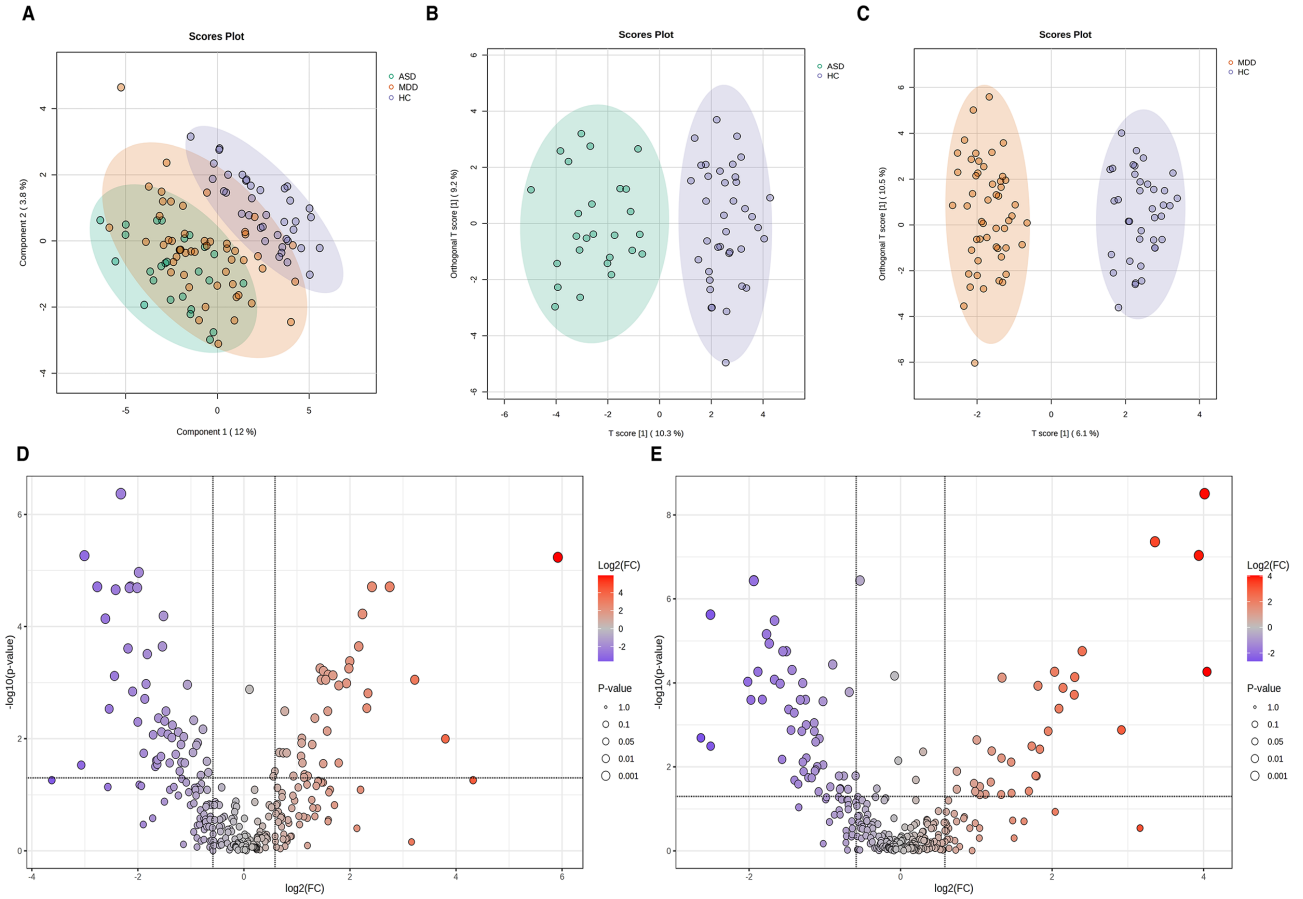


Fig. 1. Multivariate analysis of plasma metabolic profiles reveals distinct clustering and differential metabolites. (A) Scores plot from the three-group partial least squares-discriminant analysis (PLS-DA) model showing the overall separation between adolescent groups: autism spectrum disorder (ASD, n = 26), major depressive disorder (MDD, n = 51), and healthy controls (HC, n = 38). (B,C) Orthogonal partial least squares-discriminant analysis (OPLS-DA) scores plots demonstrating distinct separation in the pairwise comparisons of (B) ASD vs. HC and (C) MDD vs. HC. (D,E) Volcano plots displaying the significance versus magnitude of metabolite changes in the comparisons of (D) ASD vs. HC and (E) MDD vs. HC. Significantly upregulated (red) and downregulated (blue) metabolites (FDR <0.05) are highlighted. FDR, False Discovery Rate.

egory. To pinpoint the specific metabolites driving these separations, we performed pairwise OPLS-DA analyses, which confirmed distinct metabolic distinctions for both ASD vs. HC (Fig. 1B) and MDD vs. HC (Fig. 1C) comparisons. Volcano plots revealed numerous significant differential metabolites in both ASD (Fig. 1D) and MDD (Fig. 1E) compared to HC. We identified 32 shared differential metabolites (**Supplementary Table 2**). These shared disturbances spanned several key biological systems, including aromatic amino acid metabolism (e.g., tryptophanamide, N-acetyltryptophan), fatty acid and ketone body metabolism (e.g., suberic acid), bile acid synthesis (e.g., apocholic acid), and catecholamine pathway precursors (e.g., norepinephrine, N-acetylphenylalanine). A heatmap was generated to visually display the distinct expression patterns of these 32 metabolites across the sample groups, clearly clustering the diagnostic categories (Fig. 2).

To understand the biological context of these shared metabolic disturbances, we performed systematic KEGG pathway mapping. Systematic KEGG pathway enrichment analysis revealed that the 32 shared metabolites were significantly mapped to several discrete biochemical pathways (Fig. 3), primarily including phenylalanine, tyrosine and tryptophan biosynthesis; tryptophan metabolism; glycerophospholipid metabolism; primary bile acid biosynthesis; valine, leucine and isoleucine degradation; and ketone body metabolism.

3.3 Core Shared Metabolic Panel

From these 32 shared metabolites, a correlation-based feature selection strategy applied exclusively to the training set (70% of the data) identified a compact panel of six metabolites that exhibited the strongest associations with the combined disease status (ASD + MDD vs. HC). These six core metabolites, their pathway associations, statistical strengths, and direction of change are detailed in Table 2. The panel consisted of norepinephrine, N-acetylphenylalanine, suberic acid, tryptophanamide, apocholic acid, and 3,4-dihydroxyhydrocinnamic acid. Functionally, these metabolites are integral to the key disturbed



Fig. 2. Heatmap of the 32 shared differential metabolites. The heatmap visually displays the distinct expression patterns of the 32 metabolites identified as consistently altered in both ASD and MDD compared to HC. Each row represents a metabolite, and each column represents an individual sample. Metabolite levels are z-score normalized, with red indicating up-regulation and blue indicating down-regulation relative to the mean. The clear clustering of samples by diagnostic category (ASD, MDD, HC) demonstrates the robustness of the shared metabolic signature.

pathways (Fig. 3) including neurotransmitter and tryptophan metabolism. The distinct distribution patterns of these six core metabolites across the ASD, MDD, and HC groups, which underpin their discriminatory power, are visually summarized in **Supplementary Fig. 2**.

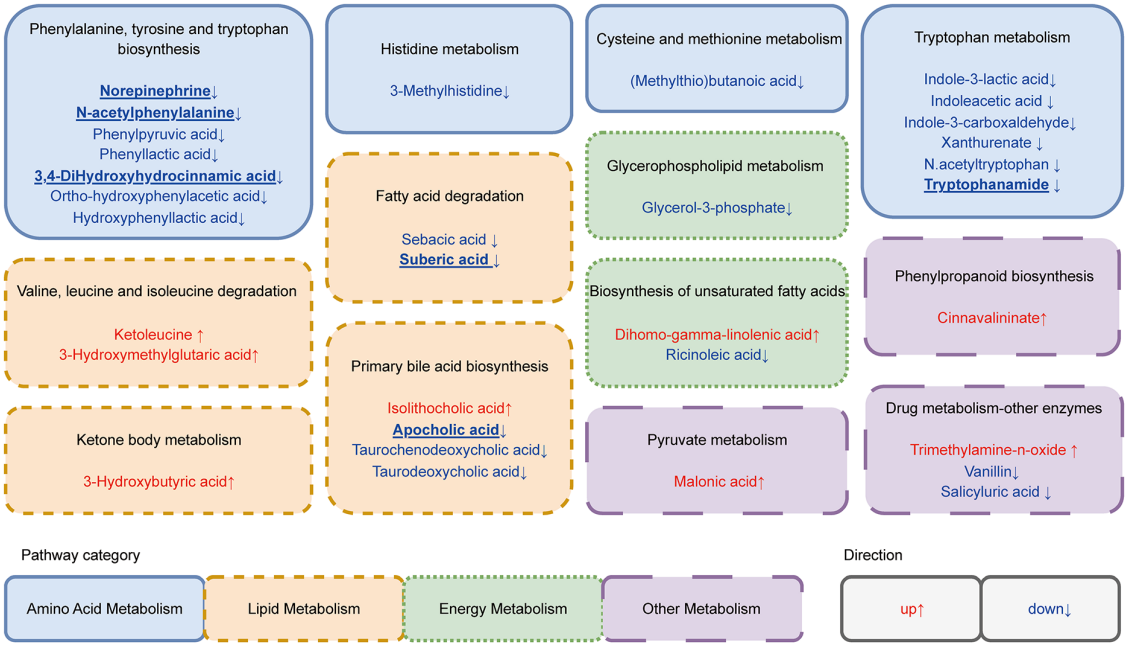


Fig. 3. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway mapping of the 32 shared plasma metabolites. The 32 shared differential metabolites are mapped to their primary biochemical pathways according to the KEGG database. Metabolites are grouped by canonical KEGG pathway categories (as indicated by the colored background boxes). The direction of change for each metabolite is indicated by its font color: red text denotes up-regulated and blue text denotes down-regulated in both ASD and MDD groups compared to healthy controls. The six machine learning-selected core metabolites are highlighted in the figure.

Table 2. Six plasma metabolites constituting the core shared biomarker panel between ASD and MDD.

Metabolite	Pathway	Spearman ρ	Direction	OR (95% CI)	ASD vs. HC		MDD vs. HC	
					FDR	VIP	FDR	VIP
3,4-DiHydroxyhydrocinnamic acid	Phenylalanine, tyrosine and tryptophan biosynthesis	–0.545	Down	1.191 (0.231–2.298)	<0.001	1.297	<0.001	1.299
Apocholic acid	Primary bile acid biosynthesis	–0.557	Down	0.586 (0.210–1.627)	<0.001	1.529	<0.001	1.801
N-acetylphenylalanine	Phenylalanine, tyrosine and tryptophan biosynthesis	–0.615	Down	0.886 (0.069–11.453)	<0.001	2.683	<0.001	3.361
Norepinephrine	Phenylalanine metabolism	–0.658	Down	0.164 (0.013–1.109)	<0.001	2.047	<0.001	2.410
Suberic acid	Fatty acid degradation	–0.615	Down	0.923 (0.520–2.509)	0.006	1.530	0.013	1.477
Tryptophanamide	Tryptophan metabolism	–0.558	Down	0.119 (0.017–0.498)	<0.001	1.912	<0.001	1.982

VIP, Variable Importance in Projection from OPLS-DA; FDR, false discovery rate; OR, odds ratio; CI, confidence interval. Spearman's ρ correlation with the combined disease status (ASD + MDD vs. HC). Direction of change in both patient groups compared to HC. The Odds Ratio (OR) and 95% Confidence Interval (CI) are from the final Firth logistic regression.

3.4 Machine Learning Validation of the Metabolic Panel

A Firth logistic regression model constructed with these six metabolites was then subjected to rigorous val-

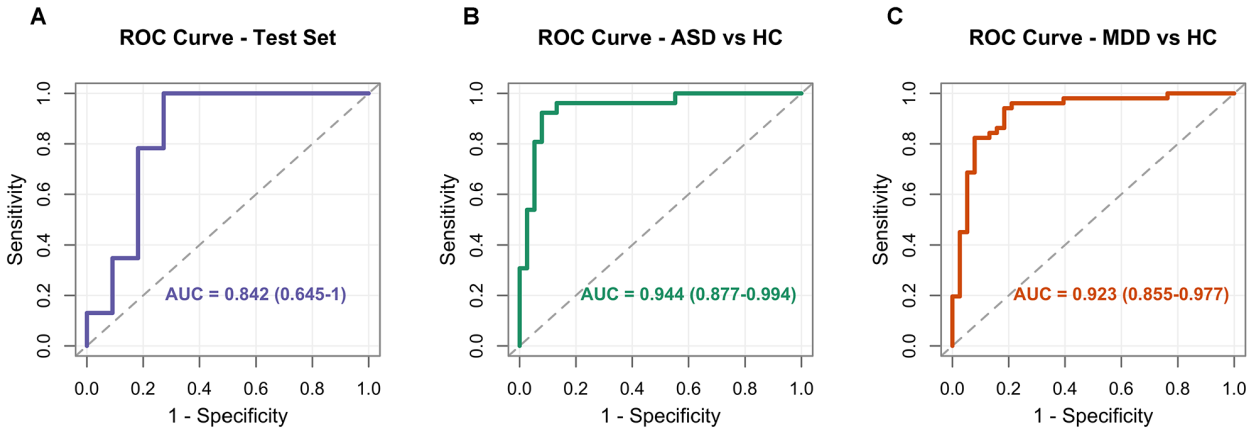


Fig. 4. A six-metabolite panel demonstrates robust diagnostic accuracy for ASD and MDD. Receiver operating characteristic (ROC) curves validate the performance of the Firth logistic regression model. (A) Independent test set (AUC = 0.842, 95% CI: 0.645–1.000). (B) ASD vs. HC (AUC = 0.944, 95% CI: 0.877–0.994). (C) MDD vs. HC (AUC = 0.923, 95% CI: 0.855–0.977). The consistently high AUC values across all comparisons underscore the clinical potential of this metabolic signature for distinguishing neuropsychiatric disorders from healthy controls.

idation. On the independent held-out test set, the model achieved an area under the curve (AUC) of 0.842 (95% CI: 0.645–1.000), with a sensitivity of 0.870, specificity of 0.727, and balanced accuracy of 0.798 (**Supplementary Table 3**). The model demonstrated strong discriminatory power in diagnostic subgroup analyses, yielding an AUC of 0.944 (95% CI: 0.877–0.994) for classifying ASD vs. HC and 0.923 (95% CI: 0.855–0.977) for MDD vs. HC (Fig. 4A–C). The stability of the model was further supported by 5-fold cross-validation, which produced a mean AUC of 0.924 (95% CI: 0.867–0.982) (**Supplementary Table 3**).

Analysis of the model coefficients provided insight into the relative contribution of each metabolite (**Supplementary Table 4**). Tryptophanamide (odds ratio, OR = 0.119, 95% CI: 0.017–0.498, $p = 0.001$) emerged as a statistically significant predictor. Norepinephrine showed a trend toward significance (OR = 0.164, 95% CI: 0.013–1.109, $p = 0.065$), while the other metabolites were not significant predictors in the model. Despite the lack of individual significance for most metabolites, their combined inclusion in the model was crucial for achieving high discriminatory performance, supporting a systems-level view where the overall pattern of metabolic disturbance, rather than any single molecule, is most informative. These findings indicate that tryptophanamide may be particularly central to the shared metabolic profile of ASD and MDD, while the collective contribution of all six metabolites was essential for achieving optimal discriminatory power.

3.5 Robustness Validation

Permutation testing confirmed that the observed model performance (AUC = 0.842) was significantly better than chance (empirical $p = 0.03$; only 3 of 100 random permutations achieved AUC ≥0.842). Bootstrap analysis revealed differential stability among the six core metabolites: norepinephrine (selected in 100% of resamples), suberic acid (86%), and N-acetylphenylalanine (84%) showed high stability; tryptophanamide (46%) and apocholic acid (44%) showed moderate stability; while 3,4-dihydroxyhydrocinnamic acid (24%) exhibited lower selection frequency. These results are detailed in **Supplementary Table 5** and visualized in **Supplementary Fig. 3**.

3.6 Sensitivity Analysis

The plasma levels of the six core metabolites did not differ significantly between medicated and unmedicated MDD patients (**Supplementary Table 6**). Critically, the logistic regression model demonstrated nearly identical and high discriminatory power in both unmedicated (AUC = 0.93, 95% CI: 0.856–1) and medicated (AUC = 0.909, 95% CI: 0.831–0.988) patients when compared to HC (**Supplementary Fig. 4** and **Supplementary Table 7**).

Analysis using BMI-adjusted metabolite data yielded comparable results. The model performance remained robust (AUC = 0.862, 95% CI: 0.684–0.99) on the independent test set, with minimal change from the original model (ΔAUC = 0.02). Furthermore, feature selection stability was high, with five of the six original core metabolites (83.3%) being re-selected after BMI adjustment. These results are summarized alongside medication analysis in **Supplementary Table 8**.

4. Discussion

Our direct comparison of plasma metabolic profiles in an age-matched adolescent cohort revealed a shared pattern of metabolic disturbances between ASD and MDD.

The distinct clustering of ASD, MDD, and HC groups supports the presence of systemic metabolic dysregulation in both disorders. We identified a compact panel of plasma metabolites that effectively discriminated patients from HC, highlighting the value of a transdiagnostic approach [3,24]. KEGG pathway analysis indicated that these metabolites are involved in interconnected physiological systems: neurotransmitter metabolism and neuroimmunity, gut-brain axis communication, and cellular energy homeostasis. Dysregulation in these systems provides a plausible biological basis for core symptoms common to both disorders, such as social withdrawal, anhedonia, and fatigue, offering a metabolic framework for understanding their frequent co-occurrence.

Within these shared metabolic features, tryptophan metabolism emerged as a central hub. The kynurenine pathway of tryptophan degradation, in particular, is increasingly recognized as a common node in the pathophysiology of diverse neuropsychiatric disorders [25]. Within the broader set of 32 shared metabolites, three were tryptophan-related (N-acetyltryptophan, tryptophanamide, and the gut microbiota-derived indole-3-lactic acid), underscoring the centrality of this pathway. Notably, tryptophanamide was further selected into the core six-metabolite panel. Our observation of disrupted tryptophan metabolites aligns with prior reports of tryptophan pathway alterations in children with ASD [8] and is a well-established component of the metabolic signature of MDD [11].

Our findings also highlight the coordinated involvement of central neurotransmitter systems and peripheral gut-brain communication. This integrated dysregulation is quantitatively captured by the core metabolite panel: norepinephrine, derived from phenylalanine/tyrosine metabolism; apocholic acid, a primary bile acid; and the microbiota-related metabolites 3,4-dihydroxyhydrocinnamic acid and N-acetylphenylalanine. Lower plasma norepinephrine may suggest a state of dysregulated sympathetic tone. Previous studies have established that norepinephrine modulates reward circuitry and motivated behavior via projections from the locus coeruleus to the nucleus accumbens [26]. Dysfunction of the noradrenergic system is closely linked to depressive symptoms such as anhedonia and fatigue [11], which are commonly observed in both ASD and MDD. Thus, the observed reduction in plasma norepinephrine may reflect a peripheral correlate of central noradrenergic dysregulation, although this hypothesis requires direct validation. Elevated apocholic acid indicates an alteration in host-microbiome communication, Gut microbiota dysregulation has been well-documented in both MDD [27] and ASD [28].

Neuroinflammation and mitochondrial dysfunction are increasingly recognized as transdiagnostic mechanisms in psychiatric disorders, and both were reflected in the observed metabolic disturbances. Alterations in the tryptophan-kynurenine pathway represent a common node in the pathophysiology of diverse neuropsychiatric disorders [25]. Activation of this pathway can lead to the accumulation of neuroactive metabolites such as quinolinic acid (an N-methyl-D-aspartate (NMDA) receptor agonist) and kynurenic acid (an NMDA receptor antagonist), which may contribute to excitotoxicity or neuroprotection, respectively. Gut microbial metabolites such as bile acids can influence central neuroinflammation via immune pathways, including Toll-like receptor activation and microglial modulation [29], with prior evidence in both MDD [27] and ASD [28]. Mitochondrial dysfunction, reflected by suberic acid accumulation, has been clearly linked to both ASD [17] and MDD [30]. Impaired mitochondrial function may lead to reduced ATP production, increased oxidative stress, and altered synaptic plasticity, all of which have been implicated in the pathophysiology of both disorders. More recently, neurovascular coupling—the mechanism that links neuronal activity to local cerebral blood flow—has emerged as another potential shared pathway, with disruption reported in both ASD and MDD. Collectively, these pathways may form interconnected networks linking peripheral metabolic disturbances to central nervous system pathology.

Notably, among the six-metabolite panel, only tryptophanamide reached statistical significance in the regression model. However, this does not mean the other five metabolites lack value. Norepinephrine showed a trend toward significance and is biosynthetically linked to N-acetylphenylalanine and 3,4-dihydroxyhydrocinnamic acid via the phenylalanine/tyrosine pathway. Suberic acid and apocholic acid, while not significant in the model, have established biological relevance in the literature.

Furthermore, we observed the consistent alteration of suberic acid—an intermediate in fatty acid degradation—in ASD and MDD. As medium-chain dicarboxylic acids involved in mitochondrial β-oxidation, their accumulation is a recognized marker of impaired fatty acid metabolism and a shift towards ω-oxidation, which often occurs under conditions of metabolic stress. This finding provides direct metabolomic evidence for disrupted cellular energy homeostasis in both disorders, which may contribute to the neurodevelopmental and affective symptoms observed in ASD and MDD, given the established links between mitochondrial dysfunction and both ASD [17] and MDD [30]. The concurrent alterations in these pathways, like tryptophanamide and suberic acid, suggest a broad disruption of metabolic equilibrium. This dysregulation may influence serotonin production, immune signaling, and energy metabolism—all implicated in ASD and MDD [31,32].

Importantly, our six-metabolite panel reflects dysregulation across multiple, interconnected physiological systems, supporting a systems-level view of shared pathology. Norepinephrine and N-acetylphenylalanine both derive from phenylalanine/tyrosine metabolism, suggesting a broader disruption in catecholamine precursor availabil-

ity. This could be further influenced by mitochondrial stress indicated by suberic acid accumulation, as energy deficits may impair neurotransmitter synthesis. Concurrently, alterations in apocholic acid (a bile acid) and tryptophanamide (a tryptophan derivative) point to a disrupted gut-brain axis. These pathways are not independent but likely interact bidirectionally. For example, mitochondrial dysfunction may impair neurotransmitter synthesis, while gut-derived signals could influence central neurotransmission via inflammation or neural pathways. Furthermore, aromatic amino acids compete for blood-brain barrier transporters [33], meaning a disturbance in one pathway can affect others. Therefore, although tryptophanamide was the only individually significant predictor in the regression model, the collective pattern of all six metabolites provides a more comprehensive and systems-oriented view of the shared metabolic landscape. The lack of individual significance for most metabolites does not compromise model interpretability; rather, it reflects multicollinearity among metabolites and underscores the systems-level nature of the shared metabolic pathology. The model performed consistently across validation frameworks, supporting the utility of this metabolic signature [19,20].

Previous metabolomic studies have linked each of the six metabolites to either ASD or MDD. Norepinephrine alterations are well-documented in MDD [11]. Tryptophanamide and related tryptophan metabolites have been linked to both ASD [8] and MDD [25]. Gut-brain axis dysfunction has been implicated in both ASD [28] and MDD [27], consistent with our observation of altered apocholic acid levels. Suberic acid has been reported as a marker of mitochondrial dysfunction in both disorders [17,30]. N-acetylphenylalanine and 3,4-dihydroxyhydrocinnamic acid are derived from the phenylalanine/tyrosine pathway, a pathway known to be disrupted in ASD [9] and MDD [11]. The key contribution of our study is the direct demonstration of overlap—within the same adolescent cohort and analytical framework—of these metabolic disturbances across ASD and MDD, providing a biological rationale for their clinical comorbidity.

Translating this finding into clinical practice faces practical challenges. While the six-metabolite panel shows promise as a transdiagnostic biomarker, its widespread adoption is not yet feasible. LC-MS/MS-based targeted metabolomics requires specialized instrumentation and technical expertise, which may not be readily available in routine clinical settings. Additionally, the need for fasting blood collection and strict sample handling protocols may pose logistical challenges. Thus, while this metabolic signature represents a proof-of-concept, large-scale, multicenter validation studies are needed prior to clinical adoption.

Nonetheless, our findings provide metabolomic support for a transdiagnostic approach in adolescent psychiatry [3,24]. By demonstrating that ASD and MDD share coherent disturbances in core physiological systems, this work offers biological evidence for dimensional frameworks like RDoC and provides a rationale for exploring targeted interventions (e.g., nutritional or microbiome-based) for adolescents with this shared metabolic profile [28]. Our findings align with the perspective that transdiagnostic biological approaches may help elucidate comorbidity mechanisms beyond traditional diagnostic boundaries.

Limitations

However, several limitations warrant acknowledgment. First, the sample size is modest and imbalanced, particularly the small ASD group (n = 26). This imbalance may affect our findings in four ways: (1) The model may achieve high overall accuracy by predicting the larger MDD group well while performing poorly on the smaller ASD group. (2) Machine learning feature selection may be biased toward larger groups. (3) Reduced statistical power may increase the risk of false negatives. (4) And the small ASD sample limits generalizability to the broader ASD population. Although we used statistical methods such as inverse probability weighting and bootstrap resampling to assess robustness, and our machine learning model performed well in internal validation, these results should be interpreted with caution. This strongly underscores the need for replication in larger, balanced cohorts, particularly with oversampling of ASD individuals, in future research [19,20].

The cross-sectional design limits causal inference. Prospective longitudinal studies are needed to determine whether the identified metabolic disturbances are antecedents, concomitants, or consequences of the disorders [34].

Unmeasured lifestyle confounders exist. We did not collect data on diet, physical activity, or circadian rhythms, all of which are known to influence plasma metabolite profiles. Diet, particularly tryptophan and fatty acid intake, can directly affect the levels of tryptophan metabolites and fatty acids—two key categories in our findings. Physical activity influences energy metabolism and fatty acid oxidation, which are relevant to suberic acid and energy homeostasis. Circadian rhythms regulate hormone secretion and metabolic processes, and the timing of blood collection (morning) was standardized, but we could not assess individual circadian variations. Thus, the observed metabolic differences may partially reflect these unmeasured confounders rather than disease status alone. Future studies incorporating detailed lifestyle assessments are needed to disentangle these effects.

Heterogeneity of medication effects cannot be fully excluded. While our preliminary analysis suggested that medication status did not substantially alter the core metabolic signature at the group level, the heterogeneity of psychotropic regimens (SSRIs, atypical antipsychotics, benzodiazepines, mood stabilizers, often in combination) precludes definitive conclusions regarding medication ef-

fects. Future investigations in medication-naïve populations are crucial [35].

Two important study design limitations exist. First, the absence of individuals with comorbid ASD and MDD in our cohort limits our ability to answer an important question: whether the six-metabolite signature identified in this study is additive, synergistic, or qualitatively distinct in individuals with both conditions. Therefore, we cannot determine whether this metabolic signature is amplified or altered in the comorbid state. Future studies should include a comorbid ASD and MDD group to directly examine how this shared metabolic signature manifests in comorbidity. Second, our study focused on plasma metabolomics, which provides a systemic view but has inherent limitations. Plasma metabolites reflect a mixture of host metabolism, gut microbial activity, and dietary inputs, making it difficult to determine the origin of the observed changes. Therefore, our findings cannot fully elucidate the directionality of gut-brain axis mechanisms. Future multi-omics studies integrating metagenomics, metabolomics of other biofluids, and possibly proteomics will be valuable to dissect the origin, directionality, and tissue-specificity of the observed alterations.

Finally, while our study identifies associations between specific metabolites and disease status, the underlying causal mechanisms remain to be elucidated. Future studies using cell-based assays or animal models are needed to validate the functional roles of these metabolites in ASD and MDD pathophysiology.

In summary, our study identifies a shared pattern of plasma metabolic disturbances in adolescent ASD and MDD, involving interconnected physiological systems such as neurotransmitter metabolism, gut-brain axis communication, and energy homeostasis. The identified metabolite panel demonstrates promising discriminatory power, supporting its potential as a transdiagnostic biomarker signature. These findings refine the understanding of the shared metabolic basis of ASD-MDD comorbidity. Future research should focus on validating these biomarkers in larger, independent cohorts and exploring their utility in guiding personalized intervention strategies.

5. Conclusions

In this study, we identified a shared plasma metabolic signature in adolescents with ASD and MDD, characterized by disturbances in neurotransmitter metabolism, gut-brain axis communication, and energy homeostasis. A six-metabolite panel demonstrated robust transdiagnostic discriminatory ability, highlighting its potential as a biomarker for these commonly co-occurring disorders. These findings provide a metabolic framework for understanding ASD-MDD comorbidity and lay the groundwork for future investigations into early detection and mechanism-based interventions.

Availability of Data and Materials

The datasets generated during the current study are not publicly available due to institutional ethics guidelines and the protection of participant confidentiality, but are available from the corresponding author on reasonable request.

Author Contributions

FW: conceptualization, formal analysis, investigation, writing-original draft. PL: investigation, resources, funding acquisition, writing-review & editing. TL: investigation, data curation, writing-original draft. LS: investigation, resources, writing-review & editing. BZ: conceptualization, funding acquisition, project administration, supervision, writing-review & editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was carried out in accordance with the guidelines of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Tianjin Anding Hospital (Approval Nos.: 2023-13 and 2023-25). Written informed consent was obtained from the legal guardians of all participants.

Acknowledgment

We thank all participants and their families for their contribution to this study.

Funding

This work was supported by the Tianjin Key Medical Discipline Construction Project (Grant No. TJYXZDXK-3-015B), the Tianjin Municipal Education Commission (Grant No. 2024ZD041), and the Tianjin Municipal Health Commission (Grant No. TJWJ2023MS038 and TJWJ2023QN086).

Conflicts of Interest

The authors declare no conflicts of interest. Dr. Bin Zhang is serving as an Editorial Board member of this journal. He had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to the Tomislav Franić.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the corresponding author used DeepSeek-V3.1 to check spelling and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Supplementary Material

Supplementary material associated with this article can be found in the online version, at https://doi.org/10.31083/AP51602.

References

[1] Hudson CC, Hall L, Harkness KL. Prevalence of Depressive Disorders in Individuals with Autism Spectrum Disorder: a Meta-Analysis. Journal of Abnormal Child Psychology. 2019; 47: 165–175. https://doi.org/10.1007/s10802-018-0402-1

[2] Hawks ZW, Constantino JN. Neuropsychiatric "Comorbidity" as Causal Influence in Autism. Journal of the American Academy of Child and Adolescent Psychiatry. 2020; 59: 229–235. https://doi.org/10.1016/j.jaac.2019.07.008

[3] Dalgleish T, Black M, Johnston D, Bevan A. Transdiagnostic approaches to mental health problems: Current status and future directions. Journal of Consulting and Clinical Psychology. 2020; 88: 179–195. https://doi.org/10.1037/ccp0000482

[4] Insel T, Cuthbert B, Garvey M, Heinssen R, Pine DS, Quinn K, et al. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. The American Journal of Psychiatry. 2010; 167: 748–751. https://doi.org/10.1176/appi.ajp.2010.09091379

[5] Davison J, O'Gorman A, Brennan L, Cotter DR. A systematic review of metabolite biomarkers of schizophrenia. Schizophrenia Research. 2018; 195: 32–50. https://doi.org/10.1016/j.schres.2017.09.021

[6] Marquand AF, Wolfers T, Mennes M, Buitelaar J, Beckmann CF. Beyond Lumping and Splitting: A Review of Computational Approaches for Stratifying Psychiatric Disorders. Biological Psychiatry. Cognitive Neuroscience and Neuroimaging. 2016; 1: 433–447. https://doi.org/10.1016/j.bpsc.2016.04.002

[7] Chen CJ, Lee DY, Yu J, Lin YN, Lin TM. Recent advances in LC-MS-based metabolomics for clinical biomarker discovery. Mass Spectrometry Reviews. 2023; 42: 2349–2378. https://doi.org/10.1002/mas.21785

[8] Likhitweerawong N, Thonusin C, Boonchooduang N, Louthrenoo O, Nookaew I, Chattipakorn N, et al. Profiles of urine and blood metabolomics in autism spectrum disorders. Metabolic Brain Disease. 2021; 36: 1641–1671. https://doi.org/10.1007/s11011-021-00788-3

[9] Galineau L, Arlicot N, Dupont AC, Briend F, Houy-Durand E, Tauber C, et al. Glutamatergic synapse in autism: a complex story for a complex disorder. Molecular Psychiatry. 2023; 28: 801–809. https://doi.org/10.1038/s41380-022-01860-9

[10] Tang X, Feng C, Zhao Y, Zhang H, Gao Y, Cao X, et al. A study of genetic heterogeneity in autism spectrum disorders based on plasma proteomic and metabolomic analysis: multiomics study of autism heterogeneity. MedComm. 2023; 4: e380. https://doi.org/10.1002/mco2.380

[11] Gururajan A, Clarke G, Dinan TG, Cryan JF. Molecular biomarkers of depression. Neuroscience and Biobehavioral Reviews. 2016; 64: 101–133. https://doi.org/10.1016/j.neubiorev.2016.02.011

[12] Roth W, Zadeh K, Vekariya R, Ge Y, Mohamadzadeh M. Tryptophan Metabolism and Gut-Brain Homeostasis. International Journal of Molecular Sciences. 2021; 22: 2973. https://doi.org/10.3390/ijms22062973

[13] Chen Y, Xu J, Chen Y. Regulation of Neurotransmitters by the Gut Microbiota and Effects on Cognition in Neurological Disorders. Nutrients. 2021; 13: 2099. https://doi.org/10.3390/nu13062099

[14] Morton JT, Jin DM, Mills RH, Shao Y, Rahman G, McDonald D, et al. Multi-level analysis of the gut-brain axis shows autism

spectrum disorder-associated molecular and microbial profiles. Nature Neuroscience. 2023; 26: 1208–1217. https://doi.org/10.1038/s41593-023-01361-0

[15] Góralczyk-Bińkowska A, Szmajda-Krygier D, Kozłowska E. The Microbiota-Gut-Brain Axis in Psychiatric Disorders. International Journal of Molecular Sciences. 2022; 23: 11245. https://doi.org/10.3390/ijms231911245

[16] Liu G, Yu Q, Tan B, Ke X, Zhang C, Li H, et al. Gut dysbiosis impairs hippocampal plasticity and behaviors by remodeling serum metabolome. Gut Microbes. 2022; 14: 2104089. https://doi.org/10.1080/19490976.2022.2104089

[17] Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. Molecular Psychiatry. 2012; 17: 290–314. https://doi.org/10.1038/mp.2010.136

[18] Chen ZS, Kulkarni PP, Galatzer-Levy IR, Bigio B, Nasca C, Zhang Y. Modern views of machine learning for precision psychiatry. Patterns (New York, N.Y.). 2022; 3: 100602. https://doi.org/10.1016/j.patter.2022.100602

[19] Vabalas A, Gowen E, Poliakoff E, Casson AJ. Machine learning algorithm validation with a limited sample size. PloS One. 2019; 14: e0224365. https://doi.org/10.1371/journal.pone.0224365

[20] Woo CW, Chang LJ, Lindquist MA, Wager TD. Building better biomarkers: brain models in translational neuroimaging. Nature Neuroscience. 2017; 20: 365–377. https://doi.org/10.1038/nn.4478

[21] Sugimoto M, Aizawa Y, Tomita A. Data Processing and Analysis in Liquid Chromatography-Mass Spectrometry-Based Targeted Metabolomics. Methods in Molecular Biology (Clifton, N.J.). 2023; 2571: 241–255. https://doi.org/10.1007/978-1-0716-2699-3_21

[22] Troyanskaya O, Cantor M, Sherlock G, Brown P, Hastie T, Tibshirani R, et al. Missing value estimation methods for DNA microarrays. Bioinformatics (Oxford, England). 2001; 17: 520–525. https://doi.org/10.1093/bioinformatics/17.6.520

[23] van den Berg RA, Hoefsloot HCJ, Westerhuis JA, Smilde AK, van der Werf MJ. Centering, scaling, and transformations: improving the biological information content of metabolomics data. BMC Genomics. 2006; 7: 142. https://doi.org/10.1186/1471-2164-7-142

[24] McQuaid RJ. Transdiagnostic biomarker approaches to mental health disorders: Consideration of symptom complexity, comorbidity and context. Brain, Behavior, & Immunity - Health. 2021; 16: 100303. https://doi.org/10.1016/j.bbih.2021.100303

[25] Oxenkrug GF. Metabolic syndrome, age-associated neuroendocrine disorders, and dysregulation of tryptophan-kynurenine metabolism. Annals of the New York Academy of Sciences. 2010; 1199: 1–14. https://doi.org/10.1111/j.1749-6632.2009.05356.x

[26] Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Research. Brain Research Reviews. 2003; 42: 33–84. https://doi.org/10.1016/s0165-0173(03)00143-7

[27] Jiang H, Ling Z, Zhang Y, Mao H, Ma Z, Yin Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. Brain, Behavior, and Immunity. 2015; 48: 186–194. https://doi.org/10.1016/j.bbi.2015.03.016

[28] Kang DW, Adams JB, Coleman DM, Pollard EL, Maldonado J, McDonough-Means S, et al. Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. Scientific Reports. 2019; 9: 5821. https://doi.org/10.1038/s41598-019-42183-0

[29] Darmanto AG, Yen TL, Jan JS, Linh TTD, Taliyan R, Yang CH, et al. Beyond metabolic messengers: Bile acids and TGR5 as pharmacotherapeutic intervention for psychiatric disorders. Pharmacological Research. 2025; 211: 107564. https://doi.org/10.1016/j.phrs.2024.107564

[30] Karabatsiakis A, Böck C, Salinas-Manrique J, Kolassa S, Calzia E, Dietrich DE, et al. Mitochondrial respiration in peripheral blood mononuclear cells correlates with depressive subsymptoms and severity of major depression. Translational Psychiatry. 2014; 4: e397. https://doi.org/10.1038/tp.2014.44

[31] Miura H, Ozaki N, Sawada M, Isobe K, Ohta T, Nagatsu T. A link between stress and depression: shifts in the balance between the kynurenine and serotonin pathways of tryptophan metabolism and the etiology and pathophysiology of depression. Stress (Amsterdam, Netherlands). 2008; 11: 198–209. https://doi.org/10.1080/10253890701754068

[32] Teng T, Clarke G, Maes M, Jiang Y, Wang J, Li X, et al. Biogeography of the large intestinal mucosal and luminal microbiome in cynomolgus macaques with depressive-like behavior. Molecular Psychiatry. Molecular Psychiatry. 2022; 27: 1059–1067. https://doi.org/10.1038/s41380-021-01366-w

[33] Fernstrom JD. Large neutral amino acids: dietary effects on brain neurochemistry and function. Amino Acids. 2013; 45: 419–430. https://doi.org/10.1007/s00726-012-1330-y

[34] Nees F, Deserno L, Holz NE, Romanos M, Banaschewski T. Prediction Along a Developmental Perspective in Psychiatry: How Far Might We Go? Frontiers in Systems Neuroscience. 2021; 15: 670404. https://doi.org/10.3389/fnsys.2021.670404

[35] Gan X, Li X, Cai Y, Yin B, Pan Q, Teng T, et al. Metabolic features of adolescent major depressive disorder: A comparative study between treatment-resistant depression and first-episode drug-naive depression. Psychoneuroendocrinology. 2024; 167: 107086. https://doi.org/10.1016/j.psyneuen.2024.107086