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ORIGINAL RESEARCH

Characteristics of Gut Microbiota and Plasma Metabolites in Patients with Post-Stroke Depression

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Purpose: The changes in gut microbiota and plasma metabolites have been proposed to play a key role in post stroke depression (PSD), but clinical study based on combined omics is still in lack. This study aimed to investigate the characteristics of gut microbiota and plasma metabolites in patients 3 months after the onset of acute ischemic stroke (AIS), compare PSD and non-PSD groups, and explore possible diagnostic biomarkers.

Patients and Methods: Seventy patients with stroke were included at 3 months after AIS onset. Plasma and fecal samples were collected. Gut microbiome was examined using 16S rRNA sequencing, and plasma metabolites were assessed via targeted liquid chromatography-mass spectrometry.

Results: Of the 70 patients with ischemic stroke, 25 (35.71%) were diagnosed with PSD. At the genus level, patients with PSD had increased abundance of *Parabacteroides, Pyramidobacter, Anaeroglobus, Haliangium, Staphylococcus, CAG−56, Shuttleworthia*, and *Epulopiscium*, and decreased levels of the *Eubacterium eligens* group and *Prevotella*. In patients with PSD, 12 plasma metabolites were altered, with cortisol and pyroglutamic acid levels increased, while 2-phosphoglyceric acid, 3-phosphoglycerate, phosphorylcholine, tryptophan, caffeine, N-methylalanine, ornithine, serotonin, theophylline, and vanillic acid were decreased. Enriched metabolic pathways included glutathione, tryptophan, and caffeine metabolism. Furthermore, significant correlations were observed between gut microbial dysregulation and major plasma metabolite alterations. The areas under the curve values of gut microbiota, plasma metabolites, and the combined dataset for PSD diagnosis were 0.704, 0.875, and 0.940, respectively.

Conclusion: This study identified the characteristics of gut microbiota and plasma metabolites as well as a panel of combined biomarkers in 3-month PSD, possibly providing a new theoretical framework for diagnosis and treatment.

Keywords: post stroke depression, metabolites, microbiome, biomarkers

Introduction

Post-stroke depression (PSD), characterized by negative mood and disinterest in activities, is a common psychiatric complication following stroke.[1] PSD adversely affects physical and psychological functioning, reduces quality of life, and increases mortality risk in stroke survivors.[2] Currently, PSD diagnosis primarily relies on symptom evaluation by physicians, which can be influenced by subjective factors. Additionally, the pathogenesis of PSD remains poorly understood, leading to inadequate assessment and treatment.[3] Although mechanisms such as increased inflammation, decreased monoamine levels, and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis have been proposed, these do not fully explain the pathogenesis and progression of PSD.[4] Therefore, further investigation is needed to uncover the mechanisms and identify potential biomarkers associated with PSD.

Gut microbiota play a significant role in the pathophysiology of the human body. As studies of the gut–brain axis have evolved, the dysbiosis of gut microbiota in PSD has been revealed.[5] Recently, based on feces collected within one week after stroke onset, a clinical study indicated that patients with PSD had higher abundance of *Streptococcus,*

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Akkermansia, and *Barnesiella*, and lower abundance of *Escherichia-Shigella, Butyricicoccus*, and *Holdemanella*.[6] Another study conducted within 2–4 weeks of stroke onset revealed increased abundance of *Escherichia coli* and *Enterococcus faecalis*, and decreased abundance of *Bifidobacterium* in patients with PSD.[7] However, these findings are inconsistent, likely because of differences in the timing of sample collection.

At the same time, plasma metabolites are influenced by gut microbiota, constituting a significant pathway for the communication of the gut–brain axis.[8] A plasma metabolomic study in acute PSD (<1 month) found alterations in lipid and amino acid metabolism.[9] Another metabolomic study at 2 weeks post-stroke indicated that the glycerophospholipid metabolism, citrate cycle, alanine, aspartate, and glutamate metabolism were associated with PSD.[10] However, these studies used non-targeted metabolomics with poor sensitivity and low detectable concentration. Therefore, high-throughput targeted approaches are needed to accurately identify and quantify metabolites in PSD. Furthermore, the aforementioned studies employed disparate specimen collection times, resulting in markedly disparate outcomes. Thus, identifying the optimal sampling time is crucial for elucidating the mechanisms of PSD.

A systematic review suggested that long-term PSD may have a different mechanism compared to early-onset PSD.[11] Three months seems to be a watershed period for PSD, as biological shifts may occur during this period compared to that in the early stage.[12] Despite this, no clinical studies have combined gut microbiota and plasma metabolomics to analyze PSD mechanisms at this time point. Understanding gut microbiota and plasma metabolite changes at 3 months post-stroke onset may provide valuable insights into PSD pathogenesis.

In this study, we used 16S rRNA sequencing and targeted metabolome analysis to compare gut microbiota and plasma metabolites between patients with and without PSD 3 months after acute ischemic stroke (AIS). We aimed to clarify the association between these two omics and identify potential diagnostic biomarkers for PSD at this time point.

Materials and Methods

Subjects

This study was conducted at Xuanwu Hospital, Capital Medical University, from June 2021 to October 2023, and was registered as a clinical trial (ChiCTR2100041895). All procedures were approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University (LYS [2020] 096). Written informed consent was obtained from all participants. A total of 86 patients with AIS were recruited within 72 hours of onset. Ultimately, 70 patients were followed up in an outpatient clinic at 3 months as shown in Figure S1.

Inclusion criteria were (1) age ≥ 18 years; and (2) AIS diagnosed with computed tomography or magnetic resonance imaging. Exclusion criteria were: (1) a history of mental disease or use of psychotropic drugs before stroke onset; (2) prior diagnosis of depression; (3) serious systemic disease such as cancer; (4) cerebral hemorrhage; (5) history of severe intestinal diseases; (6) antibiotic or probiotic use within 3 months; (7) inability to complete psychological assessments.

At 3 months post-AIS onset, PSD was diagnosed by two experienced psychiatrists, based on the 24-item Hamilton Depression Rating Scale (HDRS) with scores ≥ 8, along with the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) criteria.[13] The PSD group was divided into mild PSD group (8–16), moderate-severe PSD group (≥ 17) according to HAMD scores.[14]

Clinical Data and Sample Collection

Clinical data collected included age, sex, body mass index (BMI), medical history, treatment plan, Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification, lesion location, HDRS, National Institutes of Health Stroke Scale (NIHSS), Modified Rankin Scale (mRS), and Montreal Cognitive Assessment (MoCA). At the 3-month follow-up after stroke onset, plasma and fresh fecal samples were collected. Detailed sample collection methods were presented in the supplementary method 1.

16S rRNA Sequencing and Analysis

According to the supplementary method 2, DNA was extracted from fecal samples of all participants, and the V3-V4 variable region of the microbial 16S rRNA gene was amplified and sequenced. Library construction was performed using

the TruSeq Nano DNA LT Library Prep Kit (Illumina), with subsequent inspection facilitated using Bioanalyzer 2100 (Agilent) and QuantiFluor™ dsDNA System (Promega). Representative reads and an ASV abundance table were generated from the raw sequencing data after quality control using QIIME2 software. Taxonomic information was used to assess community composition, diversity, and difference analysis.

Targeted Metabolome Analysis Using Liquid Chromatography-Mass Spectrometry (LC-MS)

As mentioned in supplementary method 3, plasma samples from all participants underwent targeted LC-MS metabolome analysis using ultra-high performance liquid chromatography quadrupole trap tandem mass spectrometry. The targeted metabolome approach detected over 600 metabolites related to medical research, including carbohydrates, organic acids, amino acids, bile acids, indoles, purine nucleotides, lipids, and other metabolites.[15]

Data Analysis

Statistical analyses were performed using SPSS version 25.0. For the clinical data, continuous variables were described as mean ± standard deviation or median and quartile (Q1, Q3). The Student's *t*-test or nonparametric Mann–Whitney *U*-test was used to compare continuous variables. Categorical variables were presented as n (%) and analyzed using the chi-squared or Fisher's test.

For gut microbiota, α diversity indices (Simpson, Shannon, Chao1, and Ace) were calculated using the Wilcoxon test, and β diversity was assessed through principal coordinates analysis (PCoA) using unweighted and weighted UniFrac dissimilarity indices. System-Theoretic Accident Model and Processes (STAMP) analysis identified discriminative microbiota between the PSD and non-PSD groups.[16] STAMP can be used to compare samples from two or more groups to analyze classification and functional profiles, and assess biological relevancy by providing effect sizes and confidence intervals. Metabolomics analyses were conducted using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/), including principal component analysis (PCA) score plots and orthogonal partial least squares discriminant analysis (OPLS-DA). Metabolites with fold change < 0.83 or > 1.2, variable of interest > 1 and $P < 0.05$ were considered statistically significant.[17] Spearman's rank correlation analysis was conducted to explore associations between significant gut microbiota and plasma metabolites. Logistic regression identified biomarkers based on differential variables in each dataset, and receiver operating characteristic (ROC) curves evaluated the diagnostic efficiency of potential biomarkers. $P < 0.05$ was defined as the significance threshold.

Results

Clinical Characteristics

At the 3-month follow-up, 70 patients with AIS were investigated, including 25 (35.71%) PSD and 45 (64.29%) non-PSD. The PSD group included 16 patients with mild PSD and 9 patients with moderate-severe PSD. Clinical data are presented in Table 1. Patients with PSD had significantly higher HDRS scores than those without PSD (13 [10–22.5] vs 5 [3–6], $P < 0.001$). No significant differences were observed between the two groups in terms of age, sex, BMI, medical history, TOAST classification, treatment, lesion location, NIHSS, mRS, and MoCA scores (all $P > 0.05$).

Gut Microbiota Difference Between PSD and Non-PSD Groups at 3 months

The α diversity indices (Simpson, Shannon, Chao1, and Ace) showed no differences between both groups (all $P > 0.05$, Figure S2A–D), indicating similar species diversity in both groups. Nevertheless, β-diversity analysis revealed notable disparities between the two groups, as shown by PCoA scatterplot based on unweighted and weighted UniFrac distance, indicating distinct gut microbial composition between the two groups (Figure 1A and B).

Gut microbial composition in patients with and without PSD at phylum and genus levels is presented in Figure 2A and B. At the phylum level, both groups had gut microbiota predominantly composed of *Bacillota, Actinomycetota, Pseudomonadota, Bacteroidota*, and *Verrucomicrobiota* (Figure 2A). At the genus level, *Bifidobacterium, Faecalibacterium, Bacteroides, Prevotella, Collinsella*, and *Blautia* were the dominant genera in both groups

Table 1 Clinical Characteristics Between PSD and Non-PSD Groups

Variables	Non-PSD (n = 45)	PSD (n = 25)	*P*
Age (Mean ± SD)	57.73 ± 12.71	63.40 ± 10.48	0.062
Sex (male), n (%)	36 (80)	15 (60)	0.071
BMI, median (Q1, Q3)	26.30(23.91, 28.77)	24.45(23.55, 27.04)	0.118
Medical history			
Hypertension (Yes), n (%)	31 (68.9)	17 (68)	0.939
Diabetes mellitus (Yes), n (%)	13 (28.9)	10 (40)	0.343
Dyslipidemia (Yes), n (%)	17 (37.8)	15 (60)	0.074
CHD (Yes), n (%)	2 (4.4)	4 (16)	0.227
Atrial fibrillation (Yes), n (%)	2 (4.4)	2 (8)	0.939
Smoking, n (%)	24 (53.3)	10 (40)	0.285
Drinking, n (%)	22 (48.9)	10 (40)	0.474
Previous stroke, n (%)	7 (15.6)	9 (36)	0.051
Number of drugs, median (Q1, Q3)	3 (3, 4)	4 (3, 5)	0.069
Treatment plan			0.922
Conservative treatment, n (%)	26 (57.8)	14 (56)	
Intravenous thrombolysis, n (%)	16 (35.6)	10 (40)	
Endovascular treatment, n (%)	3 (6.7)	1 (4)	
TOAST classification			0.660
Large-artery atherosclerosis, n (%)	32 (71.1)	16 (64)	
Small-vessel occlusion, n (%)	9 (20)	5 (20)	
Other subtypes, n (%)	4 (8.9)	4 (16)	
Lesion location			
Infarction side(left), n (%)	26 (57.8)	13 (52)	0.641
Frontal lobe (Yes), n (%)	10 (22.2)	2 (8)	0.237
Temporal lobe (Yes), n (%)	3 (6.7)	3 (12.0)	0.750
Basal ganglia region (Yes), n (%)	21 (46.7)	17 (68)	0.086
Parietal-occipital lobe (Yes), n (%)	9 (20)	6 (24)	0.696
Infratentorial region (Yes), n (%)	15 (33.3)	5 (20)	0.237
NIHSS, median (Q1, Q3)	3 (1, 4.5)	4 (2, 5)	0.187
mRS, median (Q1, Q3)	1 (1, 2)	2 (1, 2)	0.136
MoCA, median (Q1, Q3)	25 (22, 27)	24 (21, 26)	0.198
HDRS, median (Q1, Q3)	5 (3, 6)	13 (10, 22.5)	< 0.001[a]

Note: [a]$P < 0.05$.

Abbreviations: PSD, post-stroke depression; CHD, Coronary Heart Disease; NIHSS, National Institutes of Health Stroke Scale; mRS, Modified Rankin Scale; MoCA, Montreal Cognitive Assessment; HDRS, Hamilton Depression Rating Scale.

(Figure 2B). STAMP analysis identified that Synergistota was significantly more abundant in patients with PSD at the phylum level (Figure 2C). At the genus level, *Parabacteroides, Pyramidobacter, Anaeroglobus, Haliangium, Staphylococcus, CAG−56, Shuttleworthia*, and *Epulopiscium* were significantly more abundant in patients with PSD, whereas *Eubacterium eligens* group and *Prevotella* were more abundant in patients without PSD (Figure 2D).

Plasma Metabolites Difference Between PSD and Non-PSD Groups at 3 Months

PCA clustering analysis revealed a pattern of separation among certain samples and overlap in other samples (Figure 3A). OPLS-DA further clarified significant differences in plasma metabolic characteristics between the two groups (Figure 3B). Twelve altered plasma metabolites were observed in patients with PSD, with 2 metabolites (cortisol and pyroglutamic acid) upregulated and 10 metabolites (2-phosphoglyceric acid, 3-phosphoglycerate, phosphorylcholine, tryptophan, caffeine, n-methylalanine, ornithine, serotonin, theophylline, and vanillic acid) downregulated (Figure 3C).

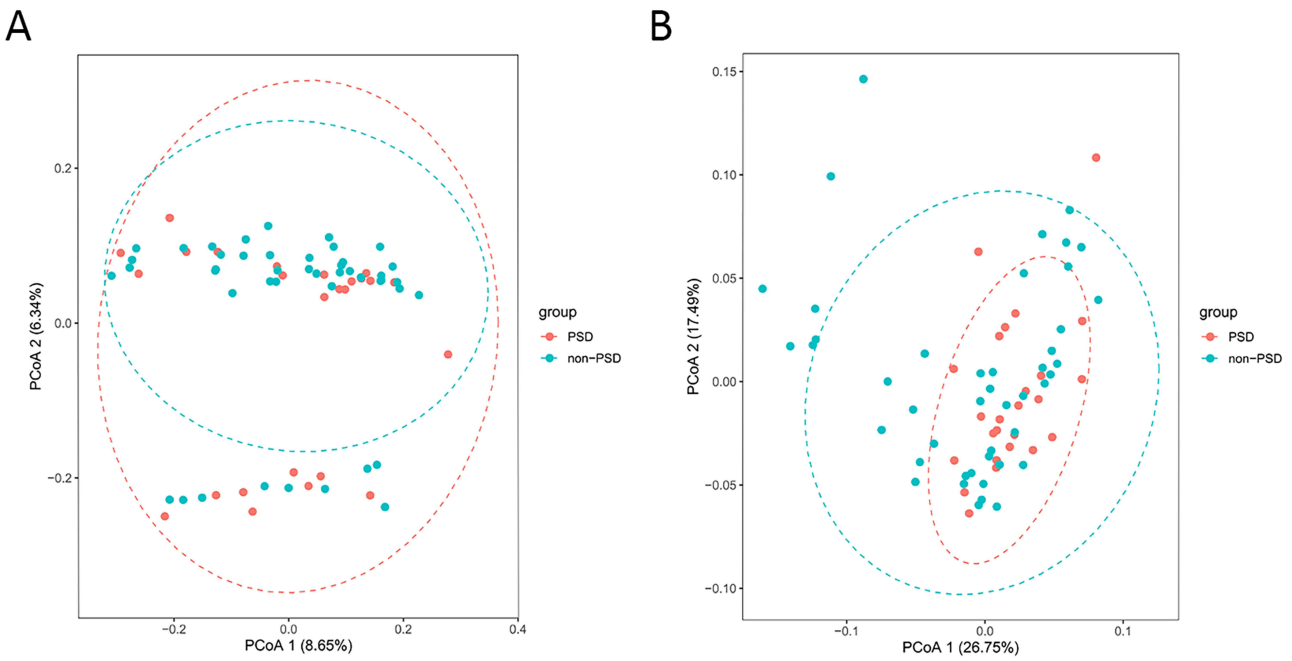

Figure 1 β Diversity of gut microbiota between PSD and non-PSD patients. (**A**) PCoA based on the unweighted UniFrac dissimilarity index. (**B**) PCoA based on the weighted UniFrac dissimilarity index.
Abbreviations: PCoA, principal coordinates analysis; PSD, post-stroke depression.

These metabolites primarily belonged to carboxylic acids, organooxygen compounds, steroids and indoles (Table S1). KEGG pathway enrichment analysis identified significant alterations in glutathione metabolism ($P = 0.006$), tryptophan metabolism ($P = 0.013$), and caffeine metabolism ($P = 0.045$) (Figure 3D). Furthermore, the concentration of cortisol was higher in moderate-severe PSD group than in mild PSD group (Table S2).

Correlation Between Gut Microbiota and Plasma Metabolites

Pyramidobacter was negatively correlated with phosphorylcholine and ornithine, while *Anaeroglobus* was negatively correlated with theophylline and vanillic acid. *Haliangium* was negatively correlated with caffeine and theophylline, and *Epulopiscium* was negatively correlated with serotonin. *Shuttleworthia* was negatively correlated with tryptophan, while *Eubacterium eligens* and *Prevotella* were positively correlated with tryptophan (Figure 4, Table S3). Overall, microbiota more abundant in patients with PSD were negatively correlated with downregulated metabolites, while the microbiota more abundant in the non-PSD patients were reversed. Notably, these metabolites correlated with microbiota were primarily involved in tryptophan, caffeine, and glutathione metabolism. These results revealed a significant correlation between gut microbial dysregulation and key metabolic pathway alterations in PSD.

Potential Combined Biomarkers for 3-Month PSD Diagnosis

After screening for potential biomarkers by logistic regression, *Parabacteroides* and *Staphylococcus* were included in the gut microbiota dataset, while tryptophan and theophylline were included in the plasma metabolite dataset. These datasets were further integrated into a combination dataset. ROC curve analysis demonstrated that the gut microbiota and plasma metabolite datasets had area under curve (AUC) values of 0.704 and 0.875, respectively. A combined dataset consisting of *Parabacteroides, Staphylococcus*, tryptophan, and theophylline yielded an AUC of 0.940 for diagnosing 3-month PSD (Figure 5).

Discussion

We observed significant differences in gut microbiota and plasma metabolites between PSD and non-PSD groups at 3 months. Combined omics analysis revealed significant correlation between gut microbiota dysregulation and alterations

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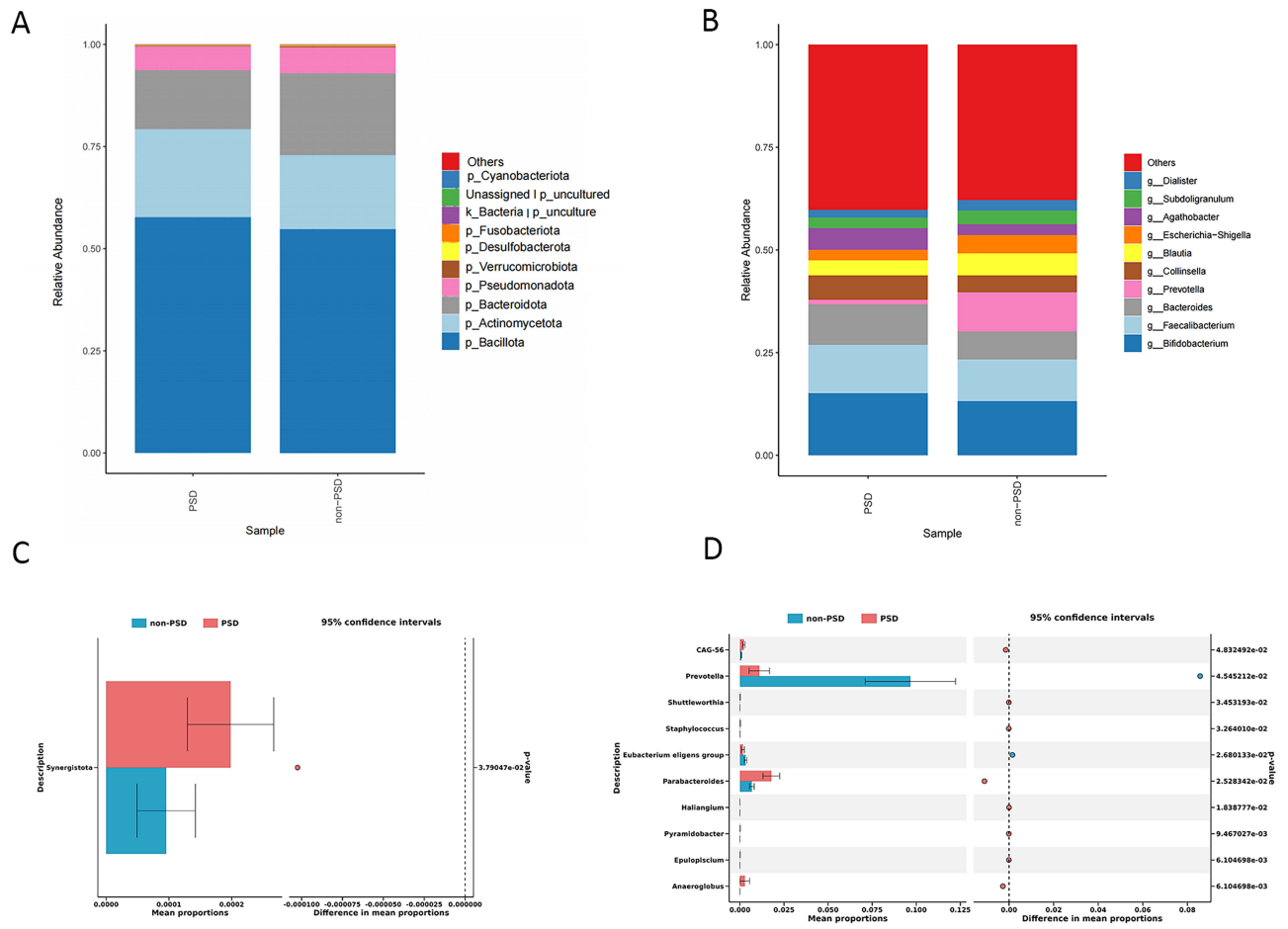

Figure 2 Taxonomic differences in gut microbiota between PSD and non-PSD groups. (**A**) Structure of gut microbiota at the dominant phylum levels. (**B**) Structure of gut microbiota at the dominant genus levels. (**C**) Significantly different phyla between PSD (red) and non-PSD (green) groups. (**D**) Significantly different genera between PSD (red) and non-PSD (green) groups.

Abbreviation: PSD, post-stroke depression.

in plasma metabolites. Furthermore, we identified a panel of combined biomarkers, including gut microbiota and plasma metabolites, for 3-month PSD diagnosis.

Gut Microbiota Difference Between PSD and Non-PSD Groups at 3 months

Our study found significant differences in β diversity, but not α diversity, between PSD and non-PSD groups at 3 months. This aligns with a meta-analysis in a Chinese population, which also reported no significant differences in α diversity between patients with PSD and healthy controls,[18] confirming significant changes in the gut microbial composition in PSD, but no difference in species diversity and richness.

At the phylum level, *Synergistota* was significantly higher in patients with PSD in our study. However, the pathogenesis of *Synergistota* in depression remains unclear. Only one cohort study in Spain found a decreased abundance of *Synergistota* phylum in individuals with depression symptoms.[19] Future research at the genus or species level may provide more robust conclusions. Furthermore, a recent study demonstrated that Synergistota was one of the predominant microbiota in saliva in patients with early-onset cryptogenic ischemic stroke and was more abundant compared with controls, indicating that this phylum may play a potential role in the development and prognosis of ischemic stroke. Future researches could focus on the potential link between Synergistota and the oral-gut-brain axis.[20]

At the genus level, we observed an elevated abundance of certain pathogenic microbiota in patients with PSD at 3 months, including *Pyramidobacter, Staphylococcus*, and *Parabacteroides*, which has also been found in the feces of humans and animals with depression previously.[6,21] A cohort study on 232 patients with AIS revealed a positive

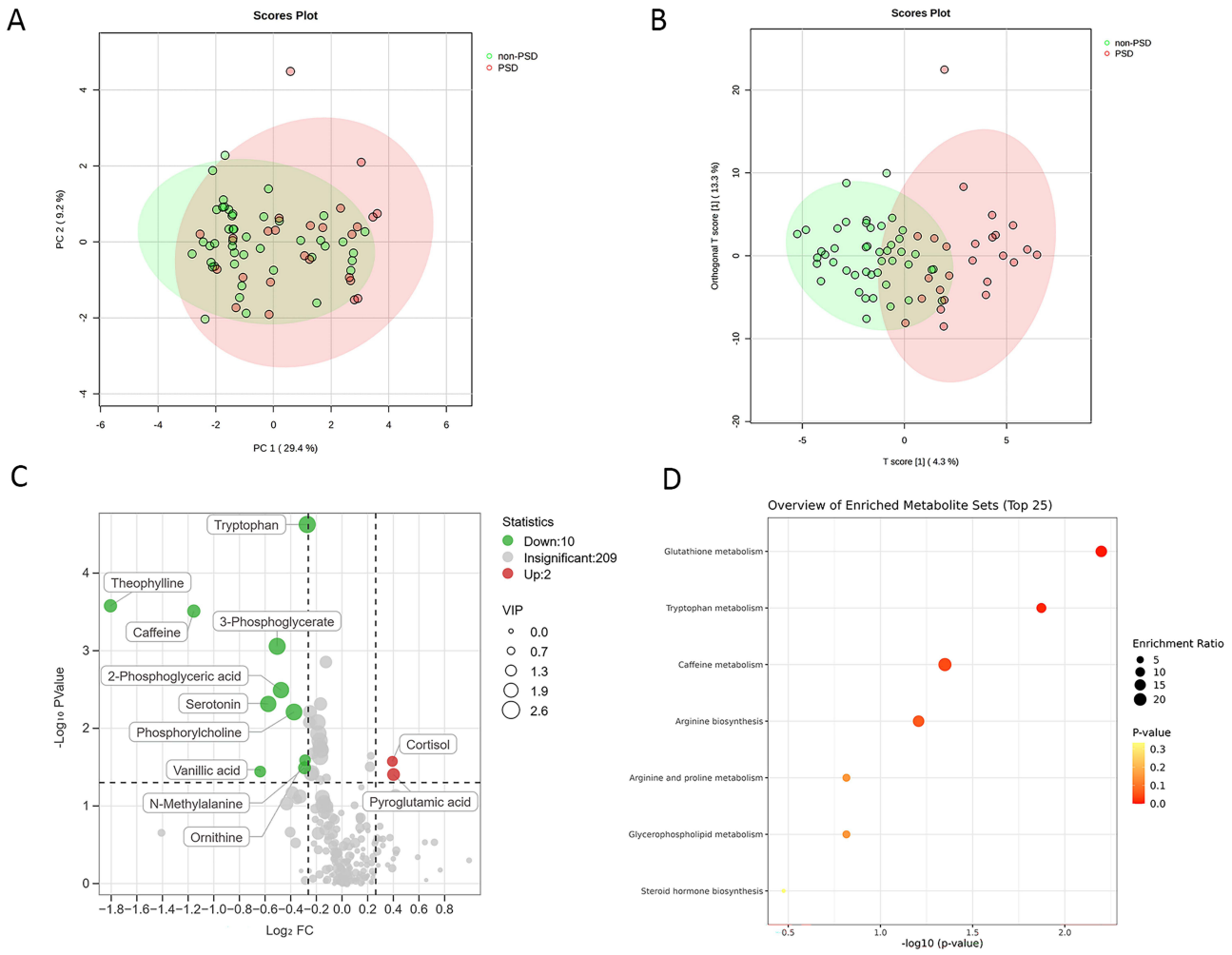

Figure 3 Altered metabolites in plasma of PSD group compared to non-PSD group. (**A**) PCA score plots of plasma metabolic profile. (**B**) OPLS-DA score plots of metabolic profiles. (**C**) The volcano plot analysis. (**D**) KEGG pathway enrichment analysis of the differential metabolites using MetaboAnalyst.
Abbreviations: PSD, post-stroke depression; PCA, principal component analysis; OPLS-DA, orthogonal partial least squares discriminant analysis.

correlation between *Pyramidobacter* and HDRS score.[6] *Pyramidobacter* has also been positively correlated with interleukin (IL)-6,[22] suggesting a pro-inflammatory role in PSD progression. Similarly, a higher abundance of *Staphylococcus* has been observed in mice exhibiting severe depressive symptoms.[23] As a common gram-positive bacterium, *Staphylococcus* can secrete various enterotoxins to stimulate inflammatory cells and promote inflammatory responses,[24] suggesting a potential influence on the development of depression. Moreover, *Parabacteroides* has been reported to disrupt the balance between indole-3-lactate and indole-3-carboxaldehyde, inducing depressive symptoms in both mice and humans.[25] Additionally, we found elevated levels of some potentially pathogenic microbiota in patients with PSD, including *Epulopiscium, Anaeroglobus*, and *Shuttleworthia*. Despite the absence of direct evidence linking *Epulopiscium, Anaeroglobus*, and PSD, their increased abundance has been associated with other neuropathological conditions such as cognitive impairment[26] and cerebral small vessel disease.[27] There is also evidence that *Shuttleworthia* can induce inflammation such as endocarditis.[28]

Furthermore, regarding patients with PSD, our study found a significant downregulation of beneficial microbiota, including *Eubacterium eligens* and *Prevotella*, which have been shown to have a strong association with depression. These microbiota have anti-inflammatory properties and can modify the gut–brain axis by regulating the synthesis of neurotransmitters and short-chain fatty acids.[29,30] An animal experiment showed that *Prevotella* could repair intestinal leakage, and inhibit inflammation by reducing levels of pro-inflammatory factors in the intestinal system and hippocampus, alleviating depressive symptoms in mice.[31] In vitro cell-based experiments proved that *Eubacterium eligens*

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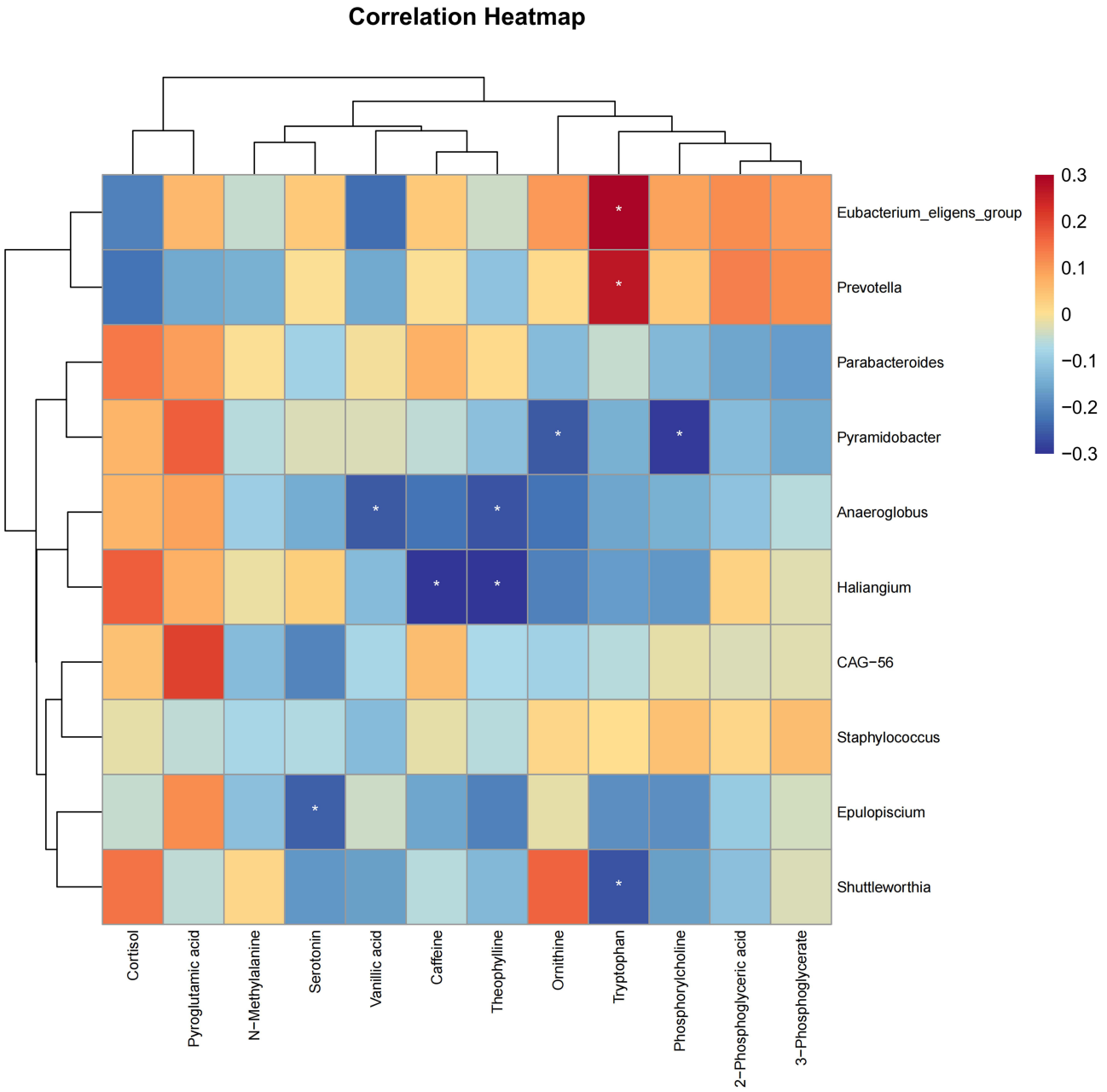

Figure 4 Spearman correlation between gut microbiota and the concentration of PSD-related differential metabolites in plasma.
Note: *$P < 0.05$.
Abbreviation: PSD, post-stroke depression.

promoted the synthesis of IL-10, an anti-inflammatory cytokine.[32] *Eubacterium* also produces butyric and propionic acids, improving the intestinal barrier integrity and inhibiting inflammation.[33] Overall, our findings indicate that PSD is associated with gut microbiota dysbiosis, characterized by an elevated abundance of potentially pathogenic and pro-inflammatory microbiota and a decreased abundance of anti-inflammatory microbiota. This imbalance may be a vital factor in the etiology of PSD. The differential microbiota in PSD patients at three months was not entirely consistent with the early PSD fecal samples. However, the results at both time points demonstrated an increase in opportunistic pathogens and a decrease in beneficial bacteria.[6] Dysbiosis and increased intestinal permeability may result in a systemic low-grade inflammatory response. Pro-inflammatory cytokines or bacterial metabolites can cross the blood-brain barrier and alter neurotransmitter metabolism.[34] Fecal microbiota transplantation introduces healthy microbiota into

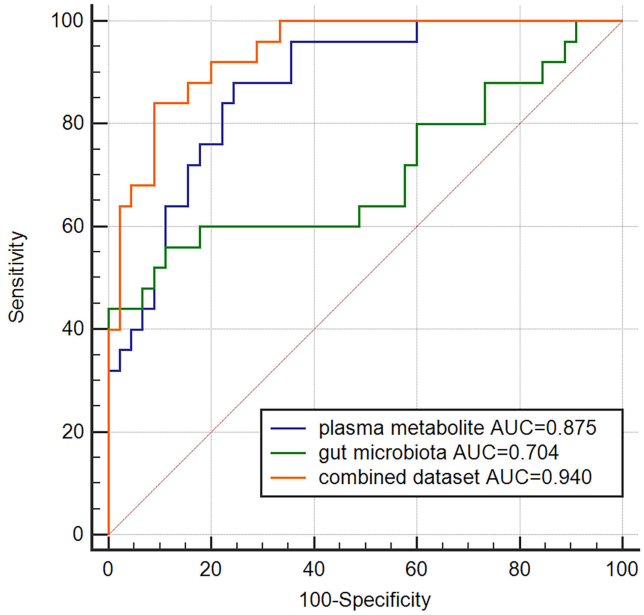

Figure 5 ROC curves representing the diagnostic ability in each dataset.
Abbreviation: ROC, receiver operating characteristic.

the gastrointestinal tract and helps promote recovery from ecological dysbiosis. Supplementation with probiotics may act on inflammatory markers that play a role in the pathogenesis of depression, thereby ameliorating depressive symptoms.[35]

Plasma Metabolites Difference Between PSD and Non-PSD Groups at 3 Months

Recent advances in neuropsychiatric research have highlighted the critical role of metabolites in mediating these disorders.[36] In our study, we observed remarkable disparities in plasma metabolites between PSD and non-PSD groups at 3 months. Notably, cortisol and pyroglutamic acid levels were elevated, while tryptophan, serotonin, and caffeine levels were reduced in patients with PSD. These alterations primarily involved tryptophan, glutathione, and caffeine metabolism pathways. However, the changes of plasma metabolomics in PSD patients within 1 month were mainly concentrated in lipids, glycerophospholipids, alanine, aspartic acid and glutamate, which may indicate differences in the metabolism characteristics between early and late PSD.[9,10]

Tryptophan is an essential amino acid that must be obtained from the diet. Imbalances in neurotransmitters within the tryptophan pathway, particularly a decrease in serotonin, are proven to underlie the pathophysiology of PSD.[37] The availability of tryptophan in the blood significantly determines serotonin synthesis in the brain.[38] A machine learning model of plasma protein data indicated that serotonin pathway activity in PSD patients was reduced by alterations of kynureninase and quinoid dihydropteridine reductase.[39] Additionally, the inflammatory response following a stroke may reduce serotonin bioavailability by increasing the conversion of tryptophan to kynurenine.[40] Our findings of reduced plasma levels of tryptophan and serotonin in patients with PSD suggest that dysregulation of tryptophan metabolism is a significant contributing factor to PSD development.

Furthermore, our study observed elevated plasma level of cortisol in patients with PSD. Stress injury of hypothalamus and pituitary gland after stroke may lead to dysregulation of HPA axis.[41] Glucocorticoids in the HPA axis can activate the inflammatory response,[42] leading to depressive symptoms through neuroendocrine-immune interactions. An animal experiment indicated an elevation in serum cortisone levels in PSD mice,[43] whereas antidepressant drugs significantly alleviate depression by inhibiting HPA axis activation in rats with stroke.[44] Additionally, our study observed the enrichment of glutathione and caffeine metabolism, suggesting their potential effect on PSD development. Previous studies have demonstrated the dysregulation of these two metabolic pathways in depression.[45,46] Dysregulation of glutathione metabolism has been demonstrated in major depressive disorder patients, with significantly lower activities

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of glutathione peroxidase and glutathione reductase.[45] Glutathione has been identified as a potential marker of early depression,[47] with impaired synaptic plasticity being associated with low levels of glutathione.[48] Moreover, caffeine has neuroprotective and anti-inflammatory properties, which may reduce the risk of depression.[46] Another animal experiment indicated that caffeine inhibited the production of lipopolysaccharide-induced nitric oxide and reduced the expression of pro-inflammatory genes including IL-3, IL-6, IL-12, inducible nitric oxide synthase and cyclooxygenase-2.[49]

Correlation Between Gut Microbiota and Plasma Metabolites

There is growing evidence that gut microbiota exert a substantial influence on host physiology and behavior by modulating metabolites in the blood.[36] Depression-like behavior is associated with multiple metabolic pathways co-regulated by both the host and microbiota.[50] In our study, correlation analyses between plasma metabolites and gut microbiota demonstrated that pro-inflammatory microbiota were negatively correlated with metabolites that counter depression, but the results were reversed for anti-inflammatory microbiota. Our results indicate that gut microbial dysregulation may contribute to PSD through the modulation of plasma metabolites, particularly those involved in tryptophan metabolism.

Although gut microbiota can directly affect host tryptophan metabolism through microbial metabolites, their indirect effect, including regulatory effect on the immune system, cannot be ignored. Gut microbiota metabolize tryptophan through three pathways: the indole pathway, kynurenine pathway, and serotonin pathway.[51] The dysregulation of pro- and anti-inflammatory microbiota can trigger pro-inflammatory cascade reactions, effectively activating indoleamine 2,3 - dioxygenase (IDO). IDO prompts the conversion of tryptophan to the kynurenine pathway and generates cytotoxic quinolinic acid, which leads to the occurrence of depression.[52,53] Activation of IDO also promotes serotonin catabolism.[52] An animal experiment confirmed that pro-inflammatory microbiota indirectly stimulated intestinal kynurenine synthesis and increased the circulating kynurenine/tryptophan ratio by inducing an immune response.[54] In contrast, probiotics with anti-inflammatory effects can increase peripheral levels of tryptophan[55] and serotonin.[56] Therefore, gut microbiota dysregulation may contribute to PSD development by influencing tryptophan metabolism.

Potential Combined Biomarkers for 3-Month PSD Diagnosis

There is an urgent need for objective diagnostic tools for PSD to improve detection and intervention.[57] A plasma metabolomic study determined a panel consisting of three metabolites with an AUC value of 0.894 as potential biomarkers for PSD at 2 weeks.[10] Using feces collected within one week post-stroke onset, another study identified a combination of seven microbiota to distinguish PSD from non-PSD with an AUC value of 0.705.[6] However, these studies only investigated biomarkers based on a single dimension and concentrated on biological characteristics at the acute or subacute stage of stroke, in which the pathophysiologic conditions of patients were still unstable Consequently, potential biomarkers of PSD may not have been adequately and precisely identified. In our study, based on combined omics data collected at 3 months post-stroke onset, we screened potential biomarkers and established a classification model. Notably, a panel of combined biomarkers, including *Parabacteroides, Staphylococcus*, tryptophan, and theophylline showed excellent diagnostic ability for 3-month PSD, with an AUC value of 0.940.

Advantages and Limitations

Based on combined omics approaches, this study is the first to investigate the structures of both gut microbiota and plasma metabolites in PSD at 3 months after AIS onset. The three-month period represents a critical juncture in the evolution of PSD, with biomarkers potentially exhibiting distinctive characteristics compared to earlier stages.[12] Our investigation substantiates the observation that the biomarkers of PSD at the three-month exhibit certain divergences from those observed in previous early-onset PSD cases. By demonstrating the association between the two omics, we provided more comprehensive evidence for PSD pathogenesis. Furthermore, we integrated gut microbiota and plasma metabolites to identify diagnostic biomarkers for 3-month PSD. However, this study has several limitations. Firstly, it is a cross-sectional study with a limited sample size. Validations in vitro and vivo are necessary for further exploring the mechanisms. Secondly, the 16S rRNA sequencing used in our study had a relatively low resolution and was only capable of accurately obtaining results at the genus level, with some important species-level information being lost, which made

it challenging to distinguish closely related microbiota at the species level. Future studies could use metagenomics to identify the specific species and functional attributes of the gut microbiota in PSD. Finally, smoking affects the gut microbiome by altering immune homeostasis, biofilm formation, or direct exposure to microorganisms in tobacco, thereby influencing a variety of diseases.[58] Furthermore, it has been demonstrated that changes in the gut microbiome of patients with depression are sex-specific, and that a panel of sex-specific biomarkers offers a superior diagnostic performance.[59] Future studies should adjust for these factors when analyzing the gut microbiota of patients with PSD.

Conclusions

In this study, we characterized gut microbiota and plasma metabolites in patients with PSD 3 months after AIS onset, demonstrating the significant correlation between gut microbiota dysregulation and alterations in plasma metabolites. Furthermore, we established a panel of combined biomarkers derived from gut microbiota and plasma metabolites for 3-month PSD diagnosis, providing a novel theoretical framework for future detection and intervention.

Data Sharing Statement

The datasets used during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

All procedures were approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University (LYS [2020] 096). Written informed consent was obtained from all participants. Our study adheres to the principles of the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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