Peripheral tissues metabolites and biological functions in Post-stroke Depression

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Abstract

Post-stroke depression (PSD) is the most common and severe neuropsychiatric complication after stroke. However, the molecular mechanism of PSD is still unclear. Previous studies have identified peripheral tissues metabolites associated with PSD using metabolomics techniques. We searched and systematically summarized metabolites that may be involved in metabolic changes in peripheral tissues of patients with PSD from the Metabolite Network of Depression Database (MENDA) and other biomedical databases. MetaboAnalyst5.0 software was used for pathway analysis and enrichment analysis of differential metabolites, and subgroup analyses were performed according to tissue types and metabolomics techniques. We identified 47 metabolites that were differentially expressed between patients with and without PSD. Five differential metabolites were found in both plasma and urine, including L-glutamic acid, pyroglutamic acid, palmitic acid, L-phenylalanine, and L-tyrosine. We integrated these metabolites into metabolic pathways, and six pathways were significantly altered. These pathways could be roughly divided into three modules including amino acid metabolism, nucleotide metabolism, and glucose metabolism. Among them, the most significantly altered pathway was “phenylalanine metabolism” and the pathway containing the most associated molecules was “aminoacyl-tRNA biosynthesis”, which deserve further study to elucidate their role in the molecular mechanism of PSD. In summary, metabolic changes in peripheral tissues are associated with PSD, especially the disruption of “phenylalanine metabolism” and “aminoacyl-tRNA biosynthesis” pathways. This study provides clues to the metabolic characteristics of patients with PSD, which may help to elucidate the molecular pathogenesis of PSD.

Introduction

Post-stroke depression (PSD) refers to a series of depressive symptoms and corresponding somatic symptoms after stroke and is the most common and most severe neuropsychiatric complication after stroke (Shi et al., 2017). Approximately 31% of stroke survivors develop depression at some time within 5 years after stroke (Hackett and Pickles, 2014). PSD negatively affects the functional outcome, recovery response, and quality of life of stroke survivors (Villa et al., 2018), and increases the burden on the family and society. In addition, PSD has been associated with an increased risk of death in stroke survivors (Cai et al., 2019). The etiology of PSD is poorly understood and is considered to be multifactorial, including biological and psychosocial factors (Das and G, 2018). Existing evidence supports mutual regulation of the neurotransmitter system, neuroinflammation, neuroendocrine activation, neuronal plasticity, and vascular factors to explain the pathogenesis of PSD (Villa et al., 2018), but its underlying molecular mechanism is still unclear. At present, the diagnosis of PSD mainly relies on depression scales and diagnostic tools. However, because many of the symptoms of depression may overlap with deficits that are a direct consequence of certain strokes, PSD can easily be missed in the clinical (Medeiros et al., 2020). Moreover, there is no objective indexes to diagnose PSD (Xiao et al., 2016), and the diagnosis results are easily affected by subjective factors of the evaluators. Therefore, screening and diagnosis of PSD require special attention (Das and G, 2018).

Metabolomics is the analysis of metabolites in cells and tissues in biological fluids and is commonly used as a tool to identify biomarkers (Johnson et al., 2016). Furthermore, metabolomics has significant diagnostic advantages compared with proteomics or transcriptomics in the diagnosis of acute stroke(Qureshi et al., 2017). The main technical methods include nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) (Bujak et al., 2015). These techniques allow measurement of metabolites in different biological samples, such as serum, plasma, urine, and cerebrospinal fluid (MacDonald et al., 2019). Many previous studies have successfully used these techniques to identify potential biomarkers of neuropsychiatric disorders such as stroke, depression, and bipolar disorder (Chen et al., 2015; Ke et al., 2019; MacDonald et al., 2019; Bot et al., 2020). A range of studies have also identified metabolites associated with PSD using metabolomics techniques. However, different studies have given contrasting results because of small sample sizes of individual studies and differences in the subjects studied and the metabolomic techniques used, limiting their clinical applicability. According to our knowledge, a systematic summary of the significant metabolites in PSD patients is currently lacking.

Because of the difficulty of obtaining brain tissue from patients with PSD, peripheral tissue is currently the main type of tissue examined for metabolite studies in patients with PSD. The purpose of this study is to systematically summarize the existing evidence regarding metabolic characteristics in peripheral tissues of patients with PSD, including metabolites, sources of tissue types, and metabolite expression patterns, to identify significant metabolites related to the occurrence and development of PSD. In addition, we detect significantly altered metabolic pathways and integrate all important metabolites into metabolic pathways. In conclusion, our goal is to identify metabolites and predict metabolic pathways to clarify the role of peripheral tissues metabolites in the occurrence and development of PSD.
Methods

Data source

The data were obtained from the Metabolite Network of Depression Database (MENDA) that we built previously (Pu et al., 2020) and other biomedical databases. MENDA is a comprehensive database that manually manages all available knowledge and data sets about metabolic characteristics associated with depression (Tian et al., 2020). The database includes 464 studies of metabolic changes associated with depression. In this study, only reports comparing the metabolite properties of patients with and without PSD were selected. For study subjects, only human peripheral tissue metabolites were considered. If statistical significance was found in the original study, the candidate metabolites were selected. Unique metabolites were identified by removing duplicates.

Pathway analysis and enrichment analysis

Manual data collation, statistical analysis, and metabolite map integration were carried out using Excel software (Microsoft, Ver. 2019). Then, we performed pathway analysis of differential metabolites using MetaboAnalyst5.0 online software (https://www.metaboanalyst.ca/) to integrate all important metabolites into metabolic pathways. A hypergeometric test was used for statistical analysis of the pathway, and the pathway library used was “Homo sapiens (Kyoto Encyclopedia of Genes and Genomes)”. The significance of an association between the metabolites and the typical pathway was based on the ratio of the number of matches to the number of metabolites (hits) uploaded by the user and the total number of molecules in the pathway (total) (Tian et al., 2020).

Subgroup analyses was performed according to tissue types and metabolomics techniques to ensure the reliability and completeness of the results. Tissue types were divided into blood and urine subgroups. Metabolomics techniques were divided into three subgroups: NMR, LC-MS, and GC-MS. The typical pathways of the subgroup were compared with the overall results, and a high degree of coincidence between the pathways indicated that the results had good reliability. Meanwhile, we used MetaboAnalyst5.0 to determine all enriched differential metabolites based on the Kyoto Encyclopedia of Genes and Genomes pathways. In subsequent analyses, only pathways with a nominal significance level of P < 0.05 were selected (Nurnberger et al., 2014).

Results

Search resources and identification of metabolites

A total of nine studies were obtained from MENDA and other biomedical databases. Sixty-one differential metabolites were extracted from the above studies, and 47 differential metabolites remained after removing the duplicates. Table 1 shows the characteristics of the included studies with 1,560 patients. The subjects included 763 men and 797 women, with a female proportion of 51.1%. The average age of all participants was 64.1 years old. The included studies were published between 2012 and 2020. The sample sizes of trials ranged from 20 to 385. The Hamilton Depression Rating Scale and Montgomery Asperger Depression Rating Scale were used to evaluate depression. One study included only ischemic stroke patients, and eight included both ischemic and hemorrhagic stroke patients. Two studies used LC-MS methods, two studies used NMR methods, and five studies used GC-MS methods. Two studies were conducted in Europe and seven were conducted in Asia.
Table 1
Metabolomics studies in peripheral tissues of human used as data source.

| Study ID  | Metabolomics techniques | PSD Sample size (M/F) | Age (Year)a | Non-PSD Sample size (M/F) | Age (Year)a | HC Sample size (M/F) | Age (Year)a | Recruitment region | Stroke type | Depression scale |
|-----------|-------------------------|----------------------|-------------|---------------------------|-------------|---------------------|-------------|-------------------|-------------|------------------|
| Cheng 2014 | LC-MS                   | 70 (34/36)           | 69.7 ± 13.5 | 139 (95/44)               | 61.2 ± 9.6  | NA                  | NA          | China             | IS          | HAMD-17          |
| Ding 2015  | GC-MS                   | 28 (15/13)           | 52.5 ± 14.5 | 27 (18/9)                 | 55.5 ± 11.3 | 32 (20/12)         | 57.4 ± 14.3 | China             | IS&HS      | HAMD, MADRS      |
| Hu 2019    | NMR                     | 42 (23/19)           | 62.2 ± 8.4  | 46 (26/20)                | 60.9 ± 8.7  | 46 (20/26)         | 59.8 ± 5.0  | China             | IS&HS      | HDRS-17          |
| Michaela 2012 | GC-MS                | 149 (52/97)          | 81.0 ± 1.4  | NA                        | NA          | NA                  | NA          | Swedish           | IS&HS      | MADRS            |
| Michaela 2015 | GC-MS                | 149 (52/97)          | 81.0 ± 1.4  | NA                        | NA          | NA                  | NA          | Swedish           | IS&HS      | MADRS            |
| Wang 2020  | LC-MS                   | 10 (7/3)             | 61.1 ± 14.4 | 10 (9/1)                  | 57.9 ± 9.7  | NA                  | NA          | China             | IS&HS      | HDRS             |
| Xiao 2016  | NMR                     | 94 (43/51)           | 61.2 ± 5.9  | 78 (41/37)                | 61.1 ± 8.2  | 74 (38/36)         | 59.4 ± 5.9  | China             | IS&HS      | HDRS             |
| Xie 2020   | GC-MS                   | 92 (48/44)           | 54.2 ± 4.1  | 89 (45/44)                | 52.1 ± 5.0  | NA                  | NA          | China             | IS&HS      | HDRS             |
| Zhang 2015 | GC-MS                   | 130 (61/69)          | 62.0 ± 7.2  | 128 (60/68)               | 61.8 ± 8.4  | 61.8 ± 8.4         | 61.5 ± 5.9  | China             | IS&HS      | HDRS-17          |

Notes: a Continuous variables are presented as mean ± SD.

Abbreviations: PSD, post-stroke depression; Non-PSD, post-stroke without depression; HC, health controls; M, male; F, female; LC-MS, liquid chromatography-mass spectrometry; GC-MS, gas chromatography-mass spectrometry; NMR, nuclear magnetic resonance; NA, not available; IS, ischemic stroke; HS, hemorrhagic stroke; HAMD, Hamilton depression rating scale; HDRS, Hamilton Depression Rating Scale.

Outcomes

In total, we found that 47 metabolites were differentially expressed between patients with and without PSD, as shown in Table 2. Eighteen differential metabolites were found in plasma, 32 were found in urine, and three were found in serum. Five differential metabolites were found in both plasma and urine, including L-glutamic acid, pyroglutamic acid, palmitic acid, L-phenylalanine, and L-tyrosine. We generated a metabolite atlas to show the expression patterns of all significant metabolites in the relevant tissues (Fig. 1). All significant metabolites are represented by white circles in this diagram and are linked by lines of different colors and thicknesses to their associated tissue types, represented by black circles. The blue lines indicate down-regulated metabolite expression in the tissue, and the red lines indicate up-regulated metabolite expression. The width of the lines was positively correlated with the number of supporting studies. The size of the black circle is positively correlated with the frequency of literature reports.
Table 2  
Summary of metabolites in post-stroke depression patients.

| Metabolite                          | Tissue Type | Expression |
|------------------------------------|-------------|------------|
| L-Glutamic acid                    | Plasma      | Up         |
|                                    | Urine       | Down       |
| L-Proline                          | Plasma      | Up         |
| Pyroglutamic acid                  | Plasma      | Up         |
|                                    | Urine       | Up         |
|                                    | Urine       | Down       |
| Palmitic acid                      | Plasma      | Up         |
|                                    | Plasma      | Down       |
|                                    | Urine       | Up         |
| Oleic acid                         | Plasma      | Up         |
| Linoleic acid                      | Plasma      | Up         |
| Oxalic acid                        | Plasma      | Down       |
| Rhamnose                           | Plasma      | Up         |
| 1-Methylhistidine                  | Plasma      | Up         |
| 3-Methylhistidine                  | Plasma      | Up         |
| LDL                                | Plasma      | Up         |
| L-Phenylalanine                    | Plasma      | Up         |
|                                    | Urine       | Up         |
|                                    | Urine       | Down       |
| L-Tyrosine                         | Plasma      | Down       |
|                                    | Urine       | Down       |
| Homocysteine                       | serum       | Up         |
| Methylmalonic acid                 | serum       | Up         |
| Albumin                            | serum       | Down       |
| Mulberrofuran M                    | Plasma      | Up         |
| Phenylacetyl glutamine             | Plasma      | Up         |
| Dihydroxyacetone                   | Plasma      | Up         |
| Betaine                            | Plasma      | Down       |
| 3-Methoxy-4-Hydroxyphenylglycol sulfate | Plasma | Down |
| 2-Hydroxybutyric acid              | Urine       | Down       |
| Sarcosine                          | Urine       | Down       |
| Acetamide                          | Urine       | Down       |
| Camosine                           | Urine       | Up         |
| L-Arabinol                         | Urine       | Up         |
| Alpha-D-Glucose                    | Urine       | Up         |
| Metabolite                     | Tissue Type | Expression |
|-------------------------------|-------------|------------|
| Formic acid                   | Urine       | Up         |
| Hydroxylamine                 | Urine       | Up         |
| Myristic acid                 | Urine       | Up         |
| D-Glucose                    | Urine       | Up         |
| D-Fructose                   | Urine       | Up         |
| Glyceric acid                | Urine       | Up         |
| L-Lactic acid                | Urine       | Up         |
| Azelaic acid                 | Urine       | Up         |
| D-Alfa-aminobutyric acid     | Urine       | Up         |
| Uric acid                    | Urine       | Up         |
| Pseudouridine                | Urine       | Up         |
| Sucrose                      | Urine       | Up         |
| 5-Hydroxyhexanoic acid       | Urine       | Up         |
| Hippuric acid                | Urine       | Down       |
| Indoxyl sulfate              | Urine       | Down       |
| (S)-3-Hydroxyisobutyric acid | Urine       | Down       |
| 3-Aminoisobutanoic acid      | Urine       | Down       |
| D-Ribose                     | Urine       | Down       |
| Hypoxanthine                 | Urine       | Down       |
| L-Leucine                     | Urine       | Down       |

Among all included patients, metabolic pathway analysis using MetaboAnalyst5.0 identified six significantly altered pathways, as shown in Table 3. The most significantly altered pathway was “phenylalanine metabolism” (P = 0.0016867, impact = 0.35714), which consists of three metabolites (L-phenylalanine, hippurate, and L-tyrosine). In addition, “aminoacyl-tRNA biosynthesis” contained the most associated molecules, with five metabolites (L-phenylalanine, L-leucine, L-tyrosine, L-proline, and L-glutamate acid). Among the six metabolic pathways, L-phenylalanine, L-tyrosine, and L-glutamate acid were enriched in at least three pathways. These pathways could be roughly divided into three modules. The first module was mainly composed of amino acid metabolism pathways such as “phenylalanine metabolism”, “phenylalanine, tyrosine and tryptophan biosynthesis”, and “histidine metabolism”. The second module consisted mainly of nucleotide metabolism pathways, such as “aminoacyl-tRNA biosynthesis”. The third module was mainly composed of glucose metabolism pathways, such as “glycolysis/gluconeogenesis” and “glyoxylate and dicarboxylate metabolism”. The metabolites were classified according to tissue type (plasma and urine), and six significantly altered metabolic pathways were identified (Table 4). Four metabolic pathways, “phenylalanine, tyrosine and tryptophan biosynthesis”, “aminoacyl-tRNA biosynthesis”, “phenylalanine metabolism”, and “histidine metabolism”, were significantly altered in different tissues. The metabolites were classified according to the metabolomic techniques used, including NMR, LC-MS, and GC-MS, and eight, four, and five significantly altered metabolic pathways were obtained, respectively (Table 5). “Nitrogen metabolism” and “D-glutamine and D-glutamate metabolism” were the metabolic pathways that were significantly altered according to metabolite detection using NMR and LC-MS. “Phenylalanine, tyrosine and tryptophan biosynthesis”, “phenylalanine metabolism”, and “aminoacyl-tRNA biosynthesis” were the metabolic pathways that were significantly altered according to metabolite detection using NMR and GC-MS. In the subgroup analyses, the metabolic pathways in each subgroup were similar to the overall results. MetaboAnalyst5.0 was used for the enrichment analysis of all metabolites, and a total of 37 enriched pathways were identified. The first 25 pathways are shown in Fig. 2.
Table 3
Differentially expressed metabolites involved in six significantly altered signaling pathways in post-stroke depression patients.

| Pathway name                        | Metabolites involved | P-value     | Metabolites included in the pathway                      |
|-------------------------------------|----------------------|-------------|----------------------------------------------------------|
| Phenylalanine metabolism            | 3                    | 0.0016867   | L-Phenylalanine, Hippurate, L-Tyrosine                   |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 2                    | 0.003772    | L-Phenylalanine, L-Tyrosine                             |
| Aminoacyl-tRNA biosynthesis         | 5                    | 0.0067073   | L-Phenylalanine, L-Leucine, L-Tyrosine, L-Proline, L-Glutamate |
| Histidine metabolism                | 3                    | 0.0070664   | L-Glutamate, Carnosine, N(pi)-Methyl-L-histidine         |
| Glycolysis / Gluconeogenesis        | 3                    | 0.027444    | (S)-Lactate, beta-D-Glucose, alpha-D-Glucose            |
| Glyoxylate and dicarboxylate metabolism | 3                    | 0.047071    | L-Glutamate, D-Glycerate, Formate                        |

Table 4
Significant changes in metabolic pathways classified by tissue type.

| Pathway name                              | P-value     |
|-------------------------------------------|-------------|
| Plasma metabolites pathway                | < 0.001     |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 0.001122   |
| Aminoacyl-tRNA biosynthesis               | 0.004286    |
| Phenylalanine metabolism                  | 0.0052379   |
| Biosynthesis of unsaturated fatty acids   | 0.011023    |
| Histidine metabolism                      | 0.032306    |
| Glutathione metabolism                    | < 0.001     |
| Urine metabolites pathway                 | 0.0019823   |
| Phenylalanine metabolism                  | 0.010884    |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 0.011469  |
| Aminoacyl-tRNA biosynthesis               | 0.020292    |
| Glycolysis / Gluconeogenesis              | 0.034492    |
| Glyoxylate and dicarboxylate metabolism   |             |
| Histidine metabolism                      |             |
Table 5
Significant changes in metabolic pathways classified according to metabolomic techniques.

| Pathway name                                                | P-value         |
|-------------------------------------------------------------|-----------------|
| NMR metabolites pathway                                     |                 |
| Histidine metabolism                                        | < 0.001        |
| Phenylalanine, tyrosine and tryptophan biosynthesis          | < 0.001        |
| Phenylalanine metabolism                                    | 0.0023901      |
| Aminoacyl-tRNA biosynthesis                                 | 0.0050408      |
| Glycolysis / Gluconeogenesis                                 | 0.016111       |
| Glyoxylate and dicarboxylate metabolism                     | 0.023961       |
| Nitrogen metabolism                                         | 0.045634       |
| D-Glutamine and D-glutamate metabolism                      | 0.045634       |
| LC-MS metabolites pathway                                   |                 |
| Nitrogen metabolism                                         | 0.01923        |
| D-Glutamine and D-glutamate metabolism                      | 0.01923        |
| Arginine biosynthesis                                       | 0.044409       |
| Butanoate metabolism                                        | 0.04752        |
| GC-MS metabolites pathway                                   |                 |
| Phenylalanine metabolism                                    | < 0.001        |
| Phenylalanine, tyrosine and tryptophan biosynthesis          | 0.0018472      |
| Aminoacyl-tRNA biosynthesis                                 | 0.0095982      |
| Biosynthesis of unsaturated fatty acids                     | 0.025307       |
| Valine, leucine and isoleucine degradation                  | 0.033372       |

Discussion

In this study, we performed an integrated analysis of the metabolic profiles of the peripheral system of patients with and without PSD. We found 47 metabolites that were differentially expressed between patients with and without PSD. Of these identified metabolites, 14 (L-glutamic acid, D-glucose, uric acid, L-lactic acid, 2-Hydroxybutyric acid, L-phenylalanine, formic acid, L-arabitol, azelaic acid, glyceric acid, pseudouridine, 5-Hydroxyhexanoic acid, L-tyrosine, and homocysteine) have also been reported in previous studies as potential biomarkers for PSD (Tang et al., 2016; Levada and Troyan, 2018). Residual metabolites were first reported as differentially expressed metabolites between patients with and without PSD. We found that the expression patterns of metabolites were inconsistent or even opposite among different studies. This may be related to the small sample size, differences in the subjects included, different sources of tissue types of metabolites and different metabolomic techniques used of previous studies. We have integrated these 47 metabolites into biological signaling pathways to investigate the biological functions associated with these metabolic alterations, and the results showed that they are mainly involved in the disruption of amino acid metabolism, particularly "phenylalanine metabolism" and "phenylalanine, tyrosine and tryptophan biosynthesis". L-glutamic acid, L-phenylalanine, and L-tyrosine were enriched in at least three of the six metabolic pathways identified. In addition, metabolites were classified according to tissue types and metabolomic techniques, and pathway analysis was used to investigate the metabolites. The subgroup and the overall results were similar, indicating good reliability of the results.

Glutamate is a major excitatory neurotransmitter in the central nervous system (Murrough et al., 2017), the most abundant free amino acid in the brain, involved in a variety of metabolic pathways in the body (Zhou and Danbolt, 2014), and a key transmitter in the degeneration of signaling neurons after stroke (Lai et al., 2014). Glutamate is associated with various aspects of the...
pathophysiological processes associated with depression (Murrough et al., 2017) and is increasingly recognized for its role in stress-related illnesses, including anxiety and depression (Ashe et al., 2019). Additionally, stroke was independently associated with glutamate levels (Lai et al., 2014; Frank et al., 2019). PSD is accompanied by changes in frontal glutamate levels, which may reflect abnormal glutamate transmission immediately after stroke (Glodzik-Sobanska et al., 2006; Wang et al., 2012). In a study of rats with middle cerebral artery occlusion, Frank et al. (Frank et al., 2019) found changes in glutamate levels in rats with PSD. Li et al. (Li et al., 2019) also found significant changes in glutamate content in rats with PSD. Furthermore, glutamate was one of 25 metabolites that differed between PSD rats and control and stroke rats (Jiang et al., 2021). Glutamate levels have also been shown to be independently associated with PSD in clinical studies (Cheng et al., 2014). Moreover, early PSD after acute ischemic stroke was independently associated with glutamate levels (Geng et al., 2017). This study also found that glutamate was differentially expressed in the peripheral system of patients with and without PSD.

In this study, the pathway was divided into three modules. The first module was mainly composed of amino acid metabolism pathways. This study showed that “phenylalanine metabolism” (metabolites: L-phenylalanine, hippurate, and L-tyrosine) was the most significantly altered pathway. “Phenylalanine metabolism” was also identified as one of the three pathways most associated with PSD in a previous study (Chen et al., 2021) and has been identified as an important pathway in the acute phase of ischemic stroke (Sidorov et al., 2020). As one of eight essential amino acids, phenylalanine may have the potential to relieve pain and depression (Chen et al., 2021). Phenylalanine metabolism was also found to be associated with depression in a depressive rat model (Xu et al., 2019; Yang et al., 2020a). Imbalance of central and peripheral phenylalanine metabolism was found in rats subjected to chronic unpredictable mild stress (Han et al., 2019). Plasma phenylalanine levels showed an association with the Diagnostic and Statistical Manual of Mental Disorders, Third Edition depression subgroup (dysthymic disorders, major recurrent depression, and bipolar depression) (Chironi et al., 1990). Urine metabolic phenotypes of patients with MDD were analyzed, and phenylalanine metabolism was significantly affected in middle-aged patients with MDD (Chen et al., 2019). The determination of phenylalanine metabolism can provide a reasonable biochemical pathway for the pathogenesis of neuropsychiatric disorders, support individualized treatment, and predict the outcome (Strasser et al., 2017). In vivo, phenylalanine is mainly catalyzed by phenylalanine hydroxylase, which produces tyrosine (Xu et al., 2019). A recent meta-analysis of 15,428 participants showed a significant association between tyrosine and depression (Bot et al., 2020). Ke et al. (Ke et al., 2019) identified tyrosine as a consistent biomarker associated with PSD. An animal study also showed that tyrosine is associated with the development of depression (Han et al., 2019). Changes in central and peripheral tyrosine and phenylalanine concentrations are thought to be related to the pathogenesis of depression (Ogawa et al., 2018). In addition, Ormstad et al. (Ormstad et al., 2016) found that tyrosine and phenylalanine were biomarkers for the diagnosis of acute ischemic stroke. Phenylalanine and tyrosine are essential for synthesis of the neurotransmitters dopamine, norepinephrine, and epinephrine (Teraishi et al., 2018; Wang et al., 2020) and are directly linked to the development of depression. Chen et al. (Chen et al., 2021) also found that “phenylalanine metabolism” and “phenylalanine, tyrosine and tryptophan biosynthesis”, two pathways related to phenylalanine metabolism, were significantly affected in patients with PSD, which was consistent with the results of our study. In addition, phenylalanine and tyrosine are metabolic by-products of intestinal microbiota. In recent years, the relationship between intestinal microbiota and depression has become a focus of research (Zheng et al., 2016), and the results have suggested that the occurrence and development of PSD may also be related to the disturbance of intestinal microflora. The second module consisted mainly of nucleotide metabolism pathways. Among them, the “aminoacyl-tRNA biosynthesis” pathway contained the most associated molecules. Simultaneously, “aminoacyl-tRNA biosynthesis” is an important metabolic pathway in human psychiatric disorders such as MDD and attention-deficit/hyperactivity disorder (Yang et al., 2020b). A combined analysis of feces, serum, liver and hippocampal metabolites from mice that underwent fecal microbiota transplantation from MDD patients showed significant changes in “aminoacyl-tRNA biosynthesis” (Li et al., 2018). The third module was mainly composed of glucose metabolism pathways. Depression is well known to be linked to disorders of glucose metabolism. A study of mice with MDD found that the “glycolysis/gluconeogenesis” pathway in the hippocampus of mice was affected after drug use, as shown by changes in metabolite levels and connected metabolite level ratios (Weckmann et al., 2014).

This study has several obvious limitations. First, only a small number of studies were available in the database, and the data available for analysis were limited. Therefore, the results identifying significant metabolites should be interpreted with caution. Second, a small amount of metabolite information may be lost in the standardized naming process of candidate metabolites because of the non-standard naming methods used in some studies. Third, both ischemic stroke and hemorrhagic stroke patients were included in this study, but these two types of patients were not analyzed separately. The metabolites associated with ischemic stroke and hemorrhagic stroke may differ because of their different pathophysiological mechanisms. Therefore, future studies should analyze these two groups of patients separately. Fourth, we did not verify the relevant pathways, which should be further verified in future studies. Finally,
we only analyzed metabolites in peripheral tissues (plasma, serum, and urine), and future studies should further analyze the metabolites in the brain.

In conclusion, by analyzing the metabolite levels of patients with and without PSD, this study found that 47 metabolites were differentially expressed in the peripheral tissues. We also predicted six signaling pathways that may be involved in the development of PSD, among which the most significantly altered pathway is phenylalanine metabolism. These pathways were roughly divided into three modules, which are involved in amino acid metabolism, nucleotide metabolism, and glucose metabolism. Subgroup analyses indicated that the results have good reliability. Our findings may contribute to elucidate the molecular mechanism of PSD and may provide clues for the early objective diagnosis of PSD. However, because of limited evidence and multiple confounding factors in the sample, more large sample studies are needed to determine the strength of these associations.

Declarations

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Availability of data and material: Datasets from this study are available from public database.

Code availability: Not applicable.

Authors’ contributions: Haiyan Liu drafting and refining the manuscript. Haiyan Liu and Qinxiang Zhou collected the data. Haiyan Liu and Juncai Pu analyzed the data. Lining Yang, Juncai Pu and Dingqun Bai: critical reading of the manuscript. All of the authors have read and approved the manuscript.

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Figures
Figure 1

Metabolite map of post-stroke depression (PSD). A comparative study of metabolites in PSD patients and non-PSD stroke patients was conducted. The thickness of the lines is positively correlated with the number of references. The size of the circles is positively correlated with the frequency of literature reports.
**Figure 2**

Enrichment of metabolite signaling pathways in post-stroke depression (PSD). The bar on the right shows the P value.