Deciphering the Gut Microbiome's Influence on Depression: A Community-Level Constraint-Based Metabolic modelling Approach

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ABSTRACT

Background: Depression is a common mental disorder worldwide, and its pathogenesis remains incompletely understood. However, increasing evidence suggests that gut microbiota play a significant role in the development of depression through the gut-brain-microbiota axis.
Results:This study utilized data from the Gmrepo database to match the gut microbiota profiles of 271 pairs of individuals with depression and healthy controls. Through systematic analysis and constrained metabolic modeling, we revealed the impact of the gut microbiome on depression. The main findings include:1.The gut microbial diversity in individuals with depression is significantly reduced compared to healthy individuals, particularly in terms of species richness and evenness.2.LEfSe analysis indicated that several species from the family Micrococcaceae are significantly enriched in patients with depression, whereas bacteria from the phylum Firmicutes are more abundant in healthy individuals.3.Metabolic pathway analysis revealed significant differences between the two groups in pathways related to fatty acid synthesis, the interconversion of pentoses and glucuronic acid, and thiamine metabolism.4.We also identified 21 differential metabolites, among which L-Dopa and copper ion metabolism showed significant differences, potentially playing an important role in the onset of depression.
Conclusions: Through systematic analysis and constrained metabolic modeling, this study revealed the potential association between gut microbiota and depression, highlighting the significant impact of microbial metabolic functions on host mental health. The findings provide potential biomarkers and metabolic targets for the future diagnosis and treatment of depression. In particular, the modulation of microbial metabolites may offer new avenues for clinical treatment of depression.

Keywords: Inflammatory bowel disease;gut microbiota markers;metagenom

INTRODUCTION

Depression is one of the most common mental illnesses today, characterized primarily by prolonged low mood, with high prevalence, high recurrence rates, and suicidal tendencies[1]. According to the World Health Organization, more than 300 million people worldwide suffer from depression, making it one of the leading causes of disability globally. Despite its significant impact on society and individuals, the pathogenesis of depression remains incompletely understood, posing major challenges for diagnosis and treatment.

The human gut hosts a vast array of microorganisms, including bacteria, viruses, archaea, and fungi, whose encoded proteins and metabolites can significantly influence human health[2,3]. Studies have shown that the gut microbiota can interact with the central nervous system through various pathways, potentially affecting the onset and progression of depression[4–6]. The gut contains a large number of neurons, and gut microbiota can communicate with the brain through the enteric nervous system.[7–9]. Over the past decade, an increasing body of evidence suggests that the brain-gut-microbiota axis plays a crucial role in the pathophysiology of depression; causal studies have shown that alterations in the gut microbiota of depressed patients influence the manifestation of depression and increase its risk[10].

A growing number of studies indicate a potential link between gut dysbiosis and depression. Although there are differences

in results across studies, a common finding is the enrichment of pro-inflammatory bacteria and the reduction of anti-inflammatory bacteria[6,11]. In depression, the phyla Firmicutes, Actinobacteria, and Bacteroidetes are the most affected[12,13], particularly with an increase in the Bacteroidetes/Firmicutes ratio. Previous studies have shown that several species of the Bacteroides genus (B. thetaiotaomicron, B. fragilis, and B. uniformis) significantly increase susceptibility to depression[14,15].

Changes in the composition of gut microbiota in patients with depression can lead to alterations in the microbial metabolome[16]. Transplanting the fecal microbiota from depressed patients into germ-free mice can induce depressive-like behaviors in these mice[12]. These mice primarily exhibit disruptions in microbial genes and host metabolites, particularly those involved in carbohydrate and amino acid metabolism[12]. Studies have shown that retinol (vitamin A), 4-hydroxycoumarin, 2-aminooctanoate, 10-undecenoate, 1-palmitoyl-2-palmitoleoyl-GPC, and 1-linoleoyl-GPA are associated with depression[16], and hippurate is considered a biomarker for depression[17]. Short-chain fatty acids (SCFAs), common microbial metabolites in the gut, are closely related to depression, with significantly lower concentrations in the feces of depressed patients compared to healthy individuals[18].

Due to the sparsity of microbiome data and significant inter-individual variability[2,19–21], directly identifying microbial features associated with depression poses significant challenges. As the final products of microbial genomes, microbial metabolites directly impact the gut environment, making metabolomic data analysis a more accurate reflection of the relationship between gut microbiota and depression. Therefore, this study utilizes species abundance data for analysis and employs constraint-based reconstruction and analysis (COBRA) methods[22,23]for metabolic flux prediction. By examining the contributions of different species to metabolic flux, a deeper understanding of the relationship between gut microbiota and depression can be achieved.

Materials and Methods

Data collection

The samples used in this study were all from GMrepo v2(https://gmrepo.humangut.info/home)Database[24]. A total of 354 depression samples and 5575 healthy samples were collected.Figure 1 provides specific details about the data.

Sample filtering and matching

In statistical analysis and scientific research, confounding factors are a significant issue as they can lead to misleading results. The observed effect may actually be caused by confounding factors rather than the independent variables under investigation[25]. For example, in studies on smoking and lung cancer, if age is not considered as a confounding factor, the impact of smoking on lung cancer may be overestimated. In the collected data, there are imbalances in age and BMI distribution between the depression and healthy sample groups.

To mitigate the effects of confounding factors such as age and BMI on the research outcomes, we designed the following algorithm, which employs different methods to match healthy samples that are similar to the depression samples based on the type of variable:

Selecting healthy samples based on different variable types:

(1) Categorical variables (e.g., nationality, gender): We select healthy samples that exactly match the depression group samples on these categorical variables. (2) Numerical variables (e.g., age, BMI): To reduce the influence of extreme values, we first calculate the 25th and 75th percentiles (i.e., the lower and upper quartiles) of these variables. Next, we compute the standard deviation within the interquartile range. Based on this standard deviation, we select healthy samples that match the depression group samples within one standard deviation for these numerical variables. Matching healthy samples to each depression sample: There are several potential outcomes for the matching process: 1) no healthy sample matches a given depression sample; 2) a single healthy sample matches a depression sample; 3) multiple healthy samples match a single depression sample; or 4) multiple depression samples match the same healthy sample.

To maximize the number of depression samples that can be matched to healthy samples, we convert the matching results into a bipartite graph and perform maximum bipartite matching. Maximum bipartite matching is an algorithm used to find the largest possible matching in a bipartite graph. A bipartite graph is a special type of graph where nodes can be divided into two disjoint sets, and all edges connect nodes between these two sets. The goal of maximum bipartite matching is to find the largest number of edges such that no two edges share the same node. In this study, we use the Hungarian algorithm[26,27] to perform maximum bipartite matching.

Data processing of species expression profiles

Microbial relative abundance data are considered compositional data, which reflect the proportion of each species within the total microbial community in a sample. A key characteristic of compositional data is that, for each sample, the sum of all species' relative abundances equals 1. Due to this constraint, where species abundances are interdependent, compositional data cannot be directly applied in statistical analysis methods that assume variable independence.

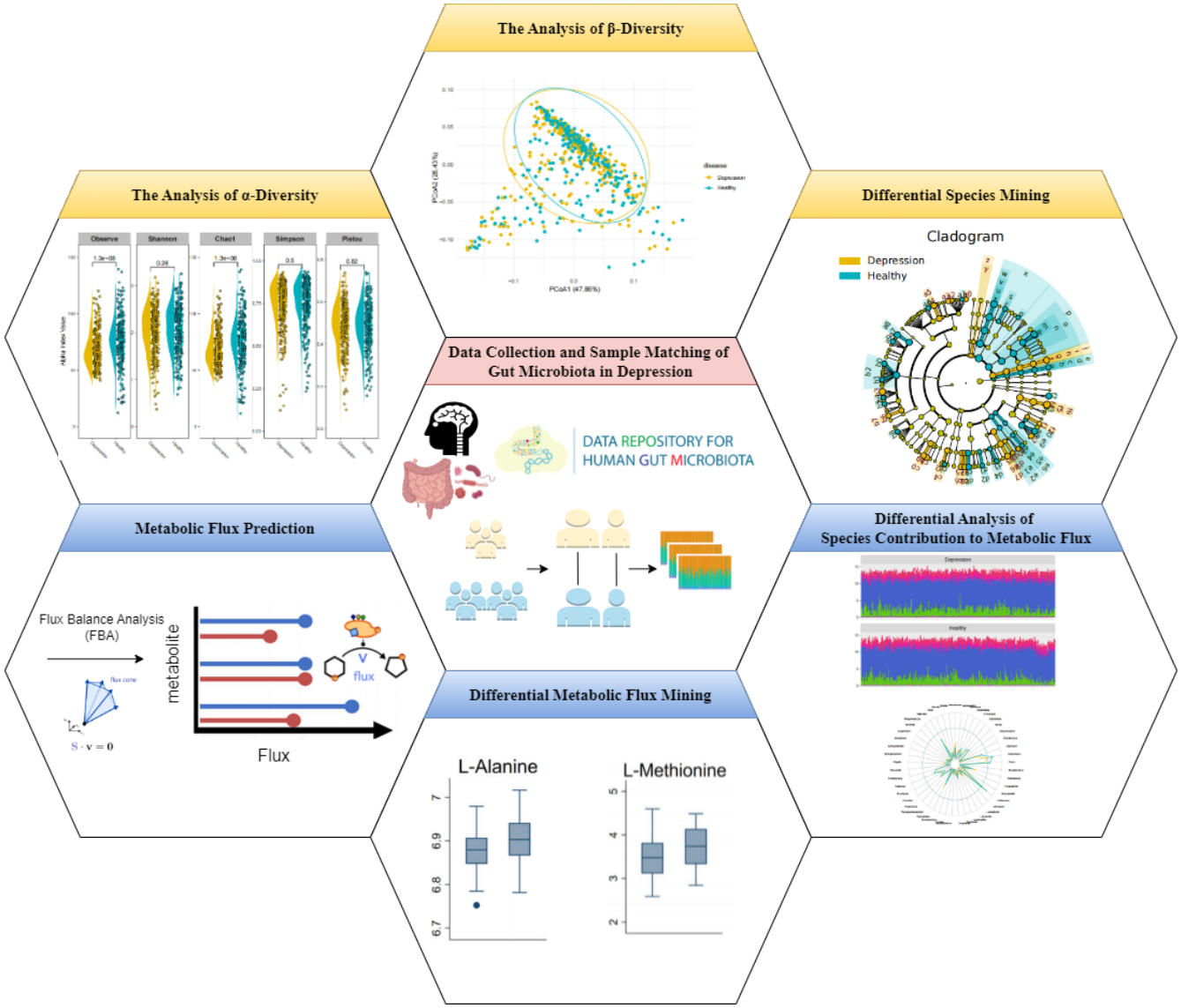

Figure 1

To address this issue, we use the centered log-ratio transformation (CLR)[28] to preprocess the microbial relative abundance data. CLR transforms the original compositional data into an unconstrained real-number space by taking the logarithm of each component relative to the geometric mean of all components in the dataset. This transformation maintains the relative relationships between variables while removing the constraint of their sum.

By applying the CLR transformation, the data become suitable for conventional statistical methods. Additionally, this transformation helps reveal patterns and relationships within the data, leading to more accurate interpretation and comparison of compositional data.

$$\mathrm{clr}(x) = \left(\ln x_i - \frac{1}{D} \sum_{j=1}^{D} \ln x_{ji} \right) \tag{1}$$

Where X_i is the value of the i-th species abundance, and D is the number of species.

Diversity analysis and differential analysis

Alpha diversity analysis

Alpha diversity measures and describes the richness and evenness of species within a sample or community. To assess the diversity differences between patients with depression and healthy individuals, we calculate several alpha diversity indices, including the Pielou index, Shannon index, and Simpson index. Statistical analysis is performed using the non-parametric Kruskal-Wallis rank sum test to compare the diversity between the two groups.

Beta diversity analysis

Beta diversity is a metric used to measure the differences in species composition between different samples or ecosystems. By analyzing beta diversity, we can identify species composition differences between patients with depression and healthy individuals.

Canonical Correspondence Analysis (CCA) is a commonly used multivariate statistical method in ecological studies[29]. CCA maximizes the associations between species abundances and environmental variables, such as age and BMI, in depression and healthy samples. Through this analysis, the relationships between these variables and species composition can be revealed.

Lefse(Linear Discriminant Analysis Effect Size) analysis

LEfSe (Linear Discriminant Analysis Effect Size) is a statistical method designed for high-dimensional data analysis[30]. It aims to identify and interpret features that exhibit significant differences between depression and healthy samples.

Metabolic flux prediction and reaction abundance calculation

Constructing a generic species model at the genus level

The AGORA2 database[31] contains metabolic models for 7,302 different strains, with each model encompassing a list of all known metabolic reactions occurring in a particular strain, including the stoichiometry and directionality of each reaction[32,33]. These models are typically generated from a strain's genome, corresponding genome annotations, and an extensive review of strain-specific biochemical and physiological literature[33].

Metabolic models use biomass to reflect the growth state of each strain, where the biomass reaction represents a series of reactions related to macromolecules such as nucleic acids and proteins. These metabolic models can also be visualized as networks, where edges represent the connection between two metabolites through a reaction. The network can be converted into a computer-readable format, where a sparse matrix is generated from the stoichiometric coefficients of each reaction, known as the stoichiometric matrix (S). In the S matrix, each row represents a different metabolite in the network, and each column represents a different reaction. Positive values indicate the production of a metabolite, while negative values indicate its consumption.

$$S = \begin{bmatrix} 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & -1 & -1 & -2 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & -1 \end{bmatrix}$$

Constructing individualized gut microbiota metabolic models

Building individualized gut microbiome metabolic models using genus-level pan-species models involves the following steps:
1.Mapping species abundance profiles to the pre-constructed genus-level pan-species model and removing species with zero abundance.
2.Establishing a common space for all species, where they can consume nutrients and exchange metabolites.
3.Creating independent extracellular spaces for each cell in the community, ensuring that each species has its own metabolic environment.
4.Integrating the biomass reactions of all species in the community into a single biomass reaction for the entire community.

FBA (Flux Balance Analysis) for solving metabolic reaction rates

Flux Balance Analysis (FBA) is a widely used method for analyzing metabolic networks. It predicts the steady-state flux distribution of reactions within a metabolic network under given conditions by solving a linear programming problem. The core principle of FBA is the steady-state assumption, which posits that the production rate and consumption rate of each intracellular metabolite are balanced over time, leading to no net change in metabolite concentrations(i.e.,$Sv = 0$,where S is the stoichiometric matrix and v is the vector of reaction rates).Next, we assume that each microorganism maximizes its growth rate,

represented by the biomass reaction (denoted as V_bm).This allows us to formulate the following linear programming problem:

$$\begin{aligned} \max v_{bm} \\ \text{s.t. } Sv = 0 \\ lb_i \leq v_i \leq ub_i \end{aligned} \tag{2}$$

Where lb_i and ub_i represent the lower and upper bounds for the rate of reaction i,and v_i is the flux of reaction i. In a community model with multiple species, each species has its specific abundance, denoted as a_i,The model can simulate the exchange of metabolites between species as well as between species and the environment. Based on this, we extend the equation to the following form:

$$\begin{aligned} \max \mu_c = \sum_i a_i u_i \\ \text{s.t. } \forall i : Sv = 0 \\ \mu_i = v_i^{bm} \\ lb_i \leq v_i \leq ub_i \end{aligned} \tag{3}$$

Here,a_i represents the relative abundance of species i,u_i is its growth rate, and v_i^{bm} is the biomass reaction rate for species i,The terms lb_i and ub_i denote the lower and upper bounds for the reaction rates. By solving this optimization problem, we can determine the flux of each metabolic reaction in the community model. Subsequently, by multiplying the flux vector v with the stoichiometric matrix S, we can compute the production or consumption rate of each metabolite.

Calculating the Reaction Abundance

To compute the reaction abundance of each community model, we first take the union of all reactions across the species in the community, resulting in a 1*n vector R,where n represents the total number of reactions. The reaction abundance of the community can then be expressed as:

$$R_c = \sum_i^n R_i a_i \tag{4}$$

Where n is the total number of reactions present in the community,R_i s the vector corresponding to species i,where the value in each column of R_i is 1 if the reaction exists in species i and 0 otherwise.a_i is the relative abundance of species i,Thus, the reaction abundance R_c is a weighted sum of the reaction presence vectors R_i across all species, weighted by their relative abundances.

Statistical Analysis

The Wilcoxon signed-rank test is used to compare the differences between two paired groups of data[34]. Since the data do not follow a normal distribution, the Wilcoxon signed-rank test was employed as an alternative to the paired t-test for differential analysis. The hypergeometric test is used to assess the probability of drawing a specific number of successes in a sample without replacement from a larger population[35]. This test was applied for enrichment analysis. All statistical analyses were conducted using R version 4.2.0.

RESULTS

The results of sample matching

To reduce the impact of confounding factors, we performed sample matching, resulting in 271 depression samples and their matched healthy controls. In the original data, there were significant differences in the distribution of age and BMI between the depression and healthy groups, but after matching, these differences were substantially reduced (Fig. 3).

The differences in the microbial composition between depression patients and healthy individuals

We calculated the α-diversity indices for depression and healthy samples (Fig. 2B). Only the Observed and Chao1 indices showed significant differences between the two groups, indicating that the richness and evenness of gut microbiota in depression patients were slightly reduced.

The results of Canonical Correspondence Analysis (CCA) and ANOSIM tests (Fig. 2B) revealed that there were small but significant differences in gut microbiota composition between depression patients and healthy individuals. To further explore these differences, we conducted LEfSe (Linear Discriminant Analysis Effect Size) analysis, selecting species with an LDA score greater than 2 and a p-value less than 0.005 as significantly different between the two groups (Fig. 2C).

The LEfSe analysis identified 18 species significantly enriched in healthy samples and 5 species significantly enriched in depression samples. At the genus level, 16 species were found to differ, at the family level 5 species, and at the class and order levels 1 species each. The genera Auritidibacter and Kocuria under the Micrococcaceae family were significantly enriched in depression samples (LDA scores of 2.10 and 3.57, p-values of 0.001 and 0.002, respectively).

The differences in the reaction abundance between depression patients and healthy individuals

By integrating species abundance, reaction abundance, and subsystem abundance and converting them into a 0-1 matrix (Supplementary Fig. 1), it is evident that species sparsity is quite high in both depression and healthy samples. In contrast, the composition of reactions and subsystems is relatively similar across all samples, warranting further analysis based on reactions and subsystems.The alpha diversity results of reaction abundance indicate that only the Observed and Chao1 indices show significant differences between the two groups (Fig. 5A). To further explore the differences in reaction abundance between the groups, the Wilcoxon signed-rank test was performed, revealing 89 reactions with significant differences (FDR < 0.05). Fig. 5B presents boxplots of the top 20 reactions with the smallest FDR values. Subsequently, enrichment analysis of the 89 significantly different reactions showed that they were significantly enriched in 4 subsystems (Fig. 5C): Fatty acid synthesis, Pentose and glucuronate interconversions, Thiamine metabolism, and Primary amine metabolism (FDR < 0.05).

Differences in Metabolic Flux and Species Contribution between Depression Patients and Healthy Individuals

The gut microbiome can significantly impact host health, particularly through its metabolites. These metabolites can enter the bloodstream, influencing the host's physiological and pathological states. By performing a Wilcoxon signed-rank test on metabolites, 21 metabolites were identified with significant differences between depression patients and healthy individuals (FDR < 0.05).

One notable metabolite is L-Dopa, the natural form of dihydroxyphenylalanine and the direct precursor of dopamine. Unlike dopamine itself, L-Dopa can be orally administered and cross the blood-brain barrier. It is metabolized into dopamine by aromatic L-amino acid decarboxylase. The genus Enterococcus, specifically Faecalis, is believed to be a key species involved in L-Dopa metabolism. This species can metabolize L-Dopa into dopamine in the gut environment via tyrosine decarboxylase, thereby reducing the availability of L-Dopa for uptake in the brain[36–38].

DISCUSSION

Based on the α-diversity analysis of gut microbiota data from depression and healthy samples, we found that only the Observe index and Chao1 index showed significant differences between the two groups. Canonical Correspondence Analysis (CCA) was used to explore the intrinsic relationship between gut microbiota, age, and BMI. The ANOSIM analysis yielded an R value of 0.008 and a p-value of 0.004, indicating a subtle difference in microbiota composition between depression patients and healthy individuals. To further investigate the microbial differences between the two groups, LEfSe analysis was conducted, identifying 23 differential taxa at various taxonomic levels, including genera such as Auritidibacter and Kocuria (Table S1). Literature review revealed that the findings for seven taxa were consistent with previous studies, while two taxa showed opposite results (Table S1). Our data indicated that Micrococcaceae and Burkholderiaceae were enriched in healthy samples, contrary to previous studies that reported significant enrichment of these taxa in depression samples[39].
Micrococcaceae are Gram-positive bacteria widely distributed in nature. Previous studies have found significant enrichment of Micrococcaceae in depression patients[39,40]. The genus Auritidibacter contains only one species, Auritidibacter ignavus, first discovered in 2011, and isolated from the human external ear canal[41]. This species is highly associated with ear infections[42,43]. The Kocuria genus was first identified in 1995[44], but no studies have reported a direct link between Auritidibacter or Kocuria and depression so far, suggesting that these findings require further validation.

Folate deficiency specifically affects the metabolism of central monoamine neurotransmitters, leading to an increased prevalence of depression[45]. Since the human body cannot synthesize folate, it must be obtained from food sources. A healthy diet rich in fruits and vegetables can increase serum folate levels and reduce the risk of depression[46].L-Dopa, the natural form of dihydroxyphenylalanine, is a direct precursor of dopamine. Unlike dopamine itself, L-Dopa can be administered orally and cross the blood-brain barrier, where it is metabolized into dopamine by aromatic L-amino acid decarboxylase.

Due to the sparsity of species composition, while metabolic reactions and metabolites are present in almost all samples, using metabolic reaction abundance and metabolic flux provides a better reflection of sample characteristics. α-diversity and CCA analyses of metabolic reaction abundance revealed that, similar to species abundance data, only the Observe index and Chao1 index showed significant differences between depression patients and healthy individuals. However, ANOSIM analysis indicated no significant differences in metabolic reaction abundance between the two groups. Differential and enrichment analyses of metabolic reaction abundance identified 89 significantly different reactions, which were enriched in four metabolic pathways: Fatty acid synthesis, Pentose and glucuronate interconversions, Thiamine metabolism, and Primary amine metabolism. Using Flux Balance Analysis (FBA) for metabolic flux prediction, followed by analysis of metabolic flux and the

contribution of each species to the flux, we found that 37 species, although not significantly different in terms of abundance, showed significant differences in their contribution to Cu2+ flux.

Oxidative stress can lead to the accumulation of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), causing damage to cellular macromolecules[47,48]. This phenomenon is particularly evident in the brain due to its high content of unsaturated fatty acids, high oxygen consumption per unit weight, elevated levels of key components involved in lipid peroxidation (LP), and a deficient antioxidant defense system[49]. Studies have found that copper (Cu) levels in the blood of patients with depression are significantly higher than in healthy individuals, although no significant difference is observed in hair Cu levels[50]. This finding is closely related to Cu's role as a key cofactor of antioxidant enzymes in oxidative stress. Cu/Zn superoxide dismutase (Cu/Zn-SOD), a crucial antioxidant enzyme, catalyzes the conversion of superoxide anions to hydrogen peroxide, thereby mitigating free radical-induced cellular damage.Oxidative stress is closely associated with depression, with patients typically exhibiting elevated oxidative stress levels and reduced antioxidant enzyme activity. The severity of depression is significantly positively correlated with oxidative stress levels[51]. Dysregulation of copper metabolism may lead to decreased Cu/Zn-SOD activity, exacerbating oxidative stress, which plays a key role in the pathophysiology of depression[52–54]. Under normal circumstances, the human body absorbs only a small amount of copper to maintain stable and appropriate intracellular levels. However, in patients with depression, the flux of Cu ion consumption by gut microbiota is significantly lower than that in healthy individuals, potentially leading to an excessive concentration of Cu ions in the bloodstream. This disruption of copper homeostasis could allow excess Cu ions to catalyze the Fenton reaction, resulting in the overproduction of ROS and increased oxidative stress levels in the body.Additionally, research indicates that elevated copper levels in depression are associated with significant declines in memory function, potentially by inhibiting the expression of GluN2B and PSD95 in the hippocampus, which impairs synaptic function and leads to memory deficits. Furthermore, total dietary intake of zinc, iron, copper, and selenium is negatively correlated with depression, suggesting that micronutrient intake may have a protective role in the development of depression. The PrPc protein, which contains copper-binding sites, can enhance SOD activity and thus reduce oxidative stress; mice lacking PrPc exhibit depression-like behaviors, further supporting the important role of copper in depression.

CONCLUSION

In this study, we utilized a constraint-based metabolic modeling approach to explore the relationship between the gut microbiome and depression. Significant differences in microbial composition and diversity were observed between depression patients and healthy individuals, with specific bacterial genera such as Auritidibacter and Kocuria enriched in the depression group. Metabolic pathway analysis identified notable alterations in fatty acid synthesis, pentose and glucuronate interconversions, thiamine metabolism, and primary amine metabolism. Furthermore, depression patients exhibited a reduced capacity for L-Dopa metabolism, potentially affecting dopamine levels, and lower Cu2+ consumption by gut bacteria, suggesting increased oxidative stress. These findings highlight the potential role of gut microbiota in depression pathophysiology and offer novel insights for developing microbiota-targeted therapeutic strategies.

KEY POINTS

- 1
- 2
- 3

SUPPLEMENTARY DATA

Supplementary data are available online at https://github.com/zyc134/Depression

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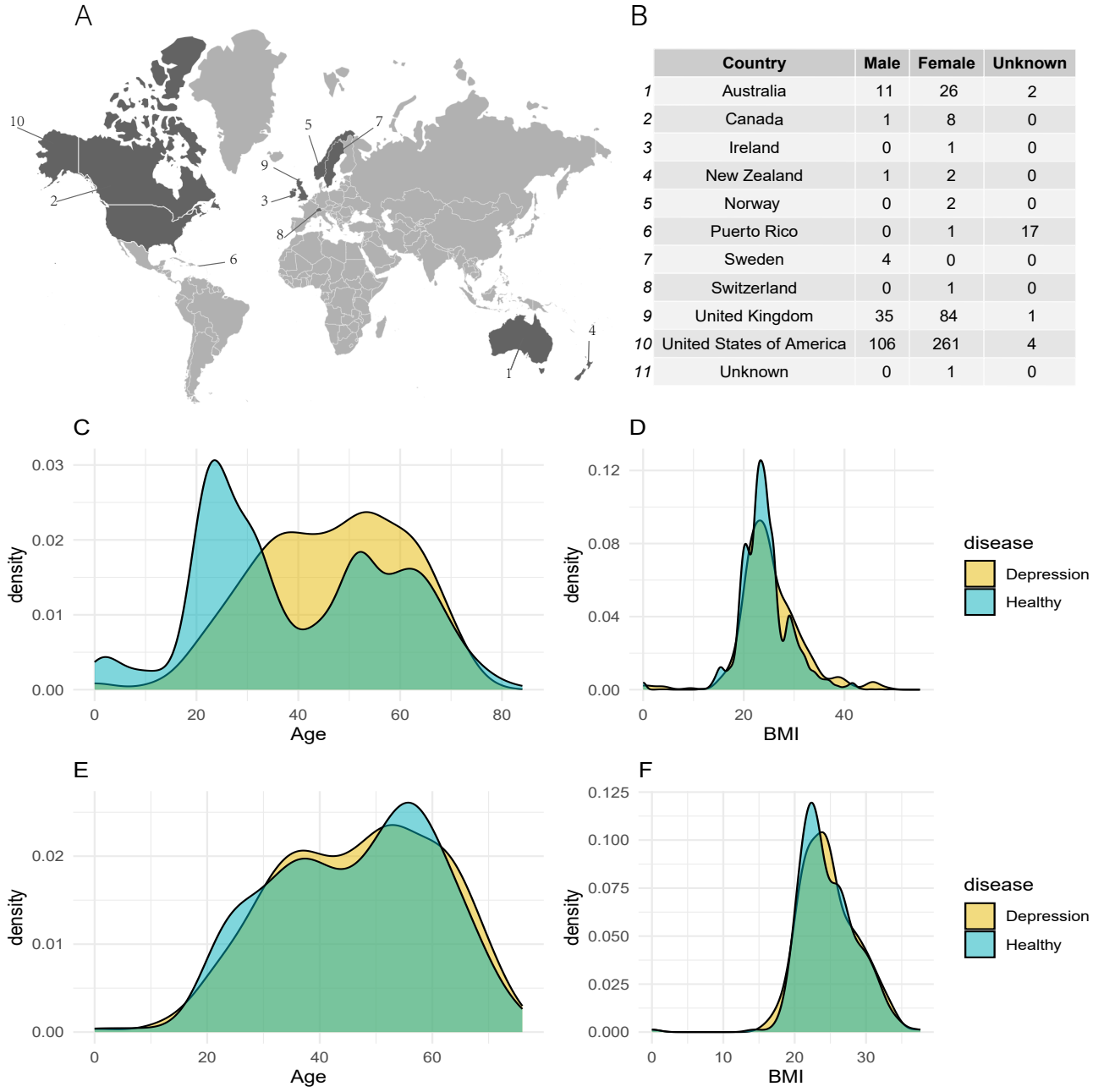

	Country	Male	Female	Unknown
1	Australia	11	26	2
2	Canada	1	8	0
3	Ireland	0	1	0
4	New Zealand	1	2	0
5	Norway	0	2	0
6	Puerto Rico	0	1	17
7	Sweden	4	0	0
8	Switzerland	0	1	0
9	United Kingdom	35	84	1
10	United States of America	106	261	4
11	Unknown	0	1	0

Figure 2. A,B.the distribution and details of samples;The distribution of age (C) and BMI (D) between depression patients and healthy individuals before sample matching. E, F. The distribution of age (E) and BMI (F) between depression patients and healthy individuals after sample matching.

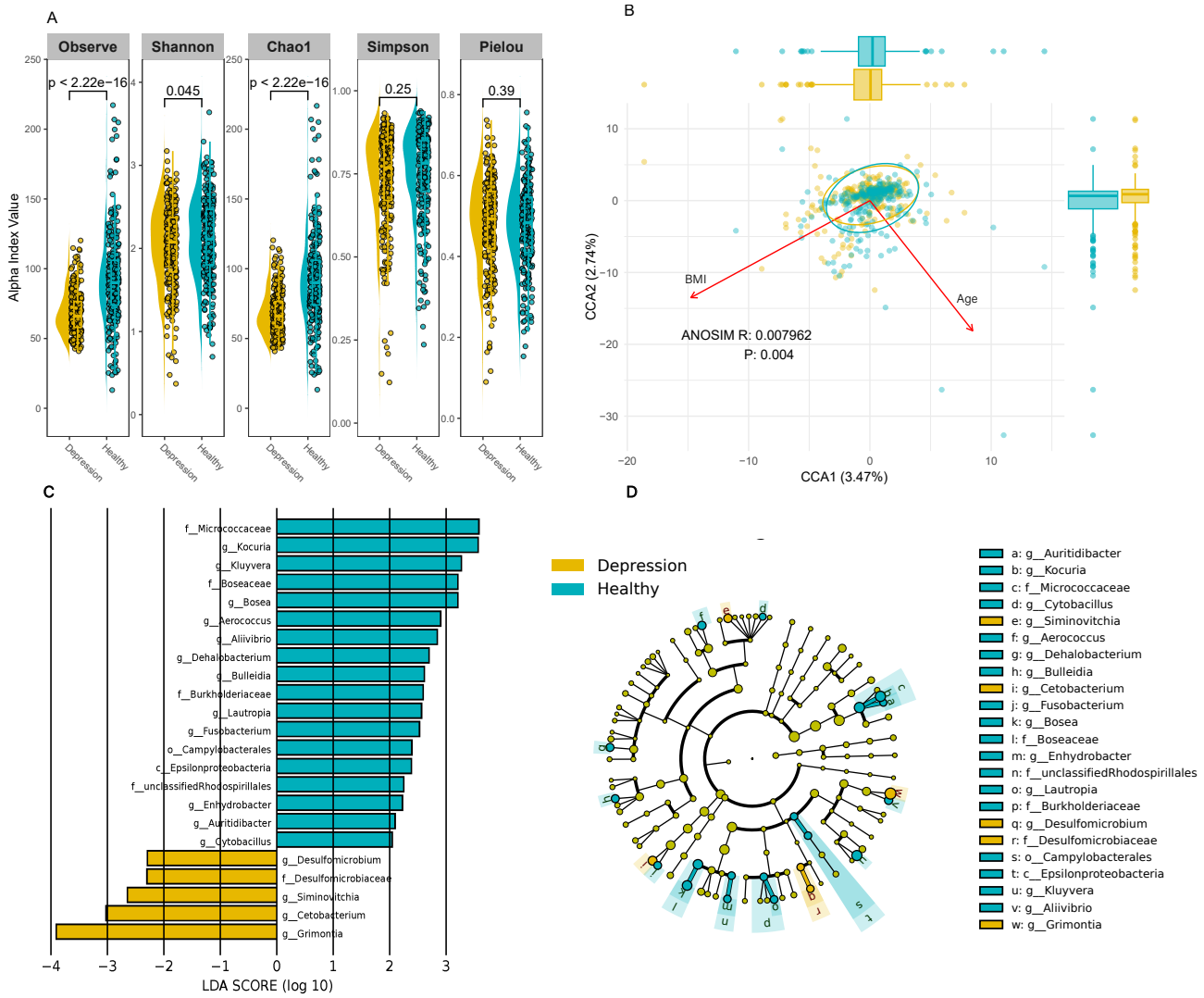

Figure 3. Species Diversity Analysis and LEfSe Results. A. Alpha diversity analysis of species abundance between depression and healthy samples, showing differences in the Observed, Shannon, and Chao1 indices. B. CCA (Canonical Correspondence Analysis) results, ANOSIM R: 0.007962, p: 0.004. C. LDA scores of significantly different species. D. Cladogram of differential species.

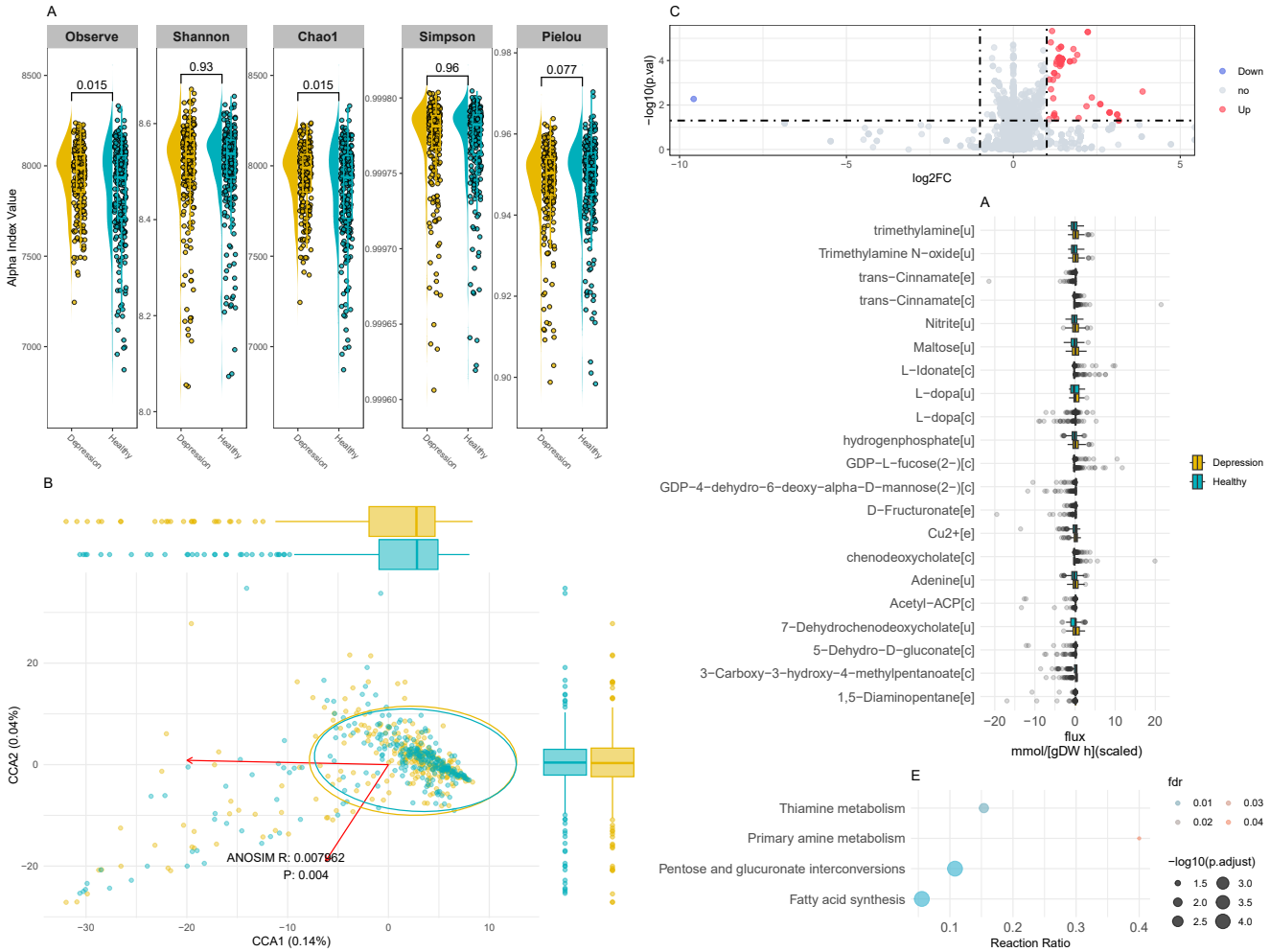

Figure 4. The analysis of reactions.A. Alpha diversity analysis of reaction abundance between depression and healthy samples, showing significant differences in the Observed and Chao1 indices.B. Canonical Correspondence Analysis (CCA) results, ANOSIM R: -0.0003651, p: 0.493, indicating no significant differences in reaction abundance composition between the two groups.C, D. Reactions with significant differences between the two groups, identified through Wilcoxon signed-rank test ($p < 0.05$).E. Enrichment of 89 significantly different reactions into 4 subsystems. The size of the points represents the number of different reactions within each subsystem, and the color represents the FDR value.

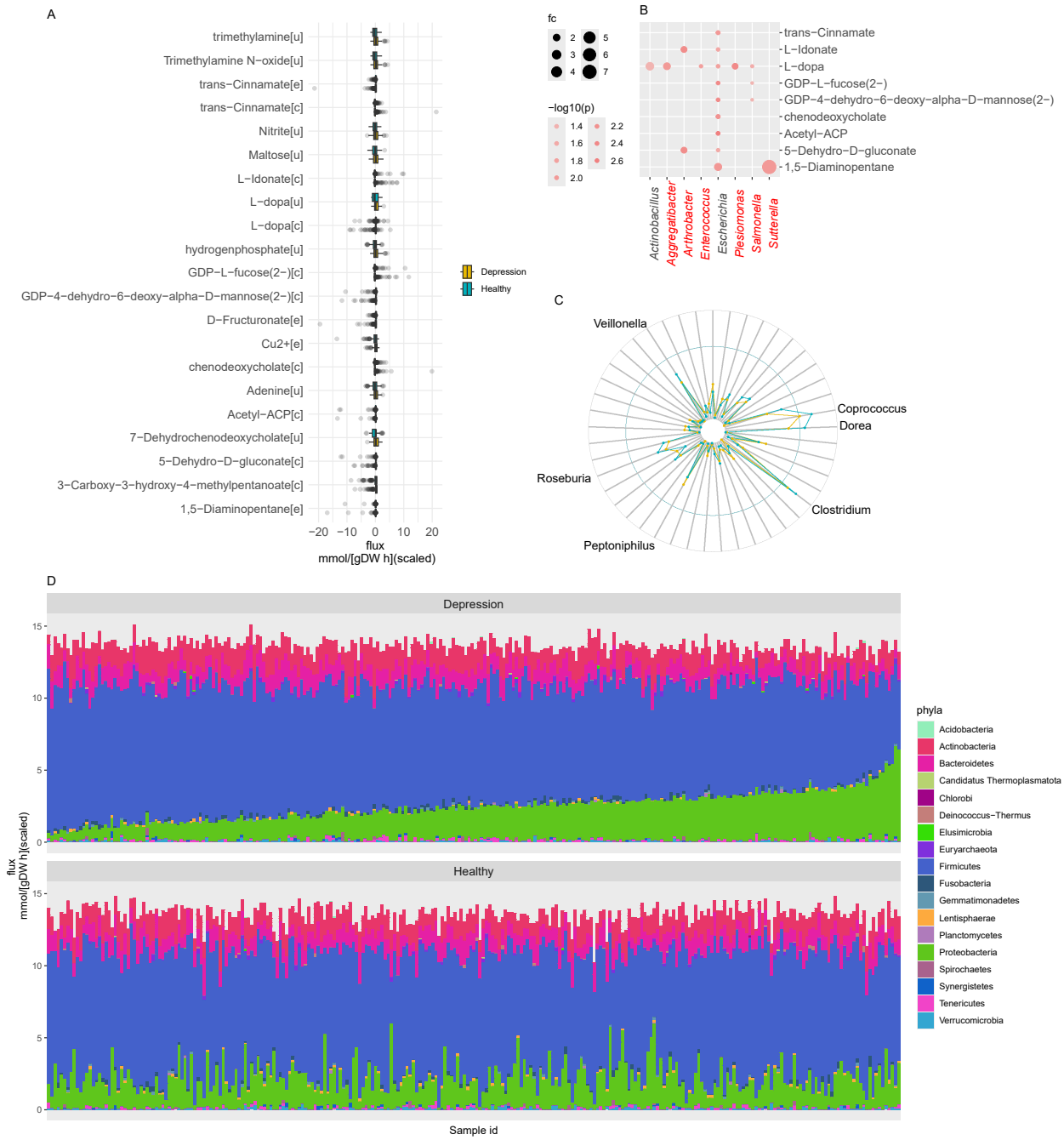

Figure 5. A. Significant differences in metabolic flux between depression patients and healthy individuals (Wilcoxon signed-rank test, $p < 0.05$).B. Differences in species contribution to metabolic flux, with dot size representing fold change (FC) and color indicating p-values. Species highlighted in red show no abundance difference between the two groups, but exhibit significant differences in their contribution to metabolic flux.C,D. Cu^2 consumption flux differences between genus(C) and phyla(D) in the two groups.

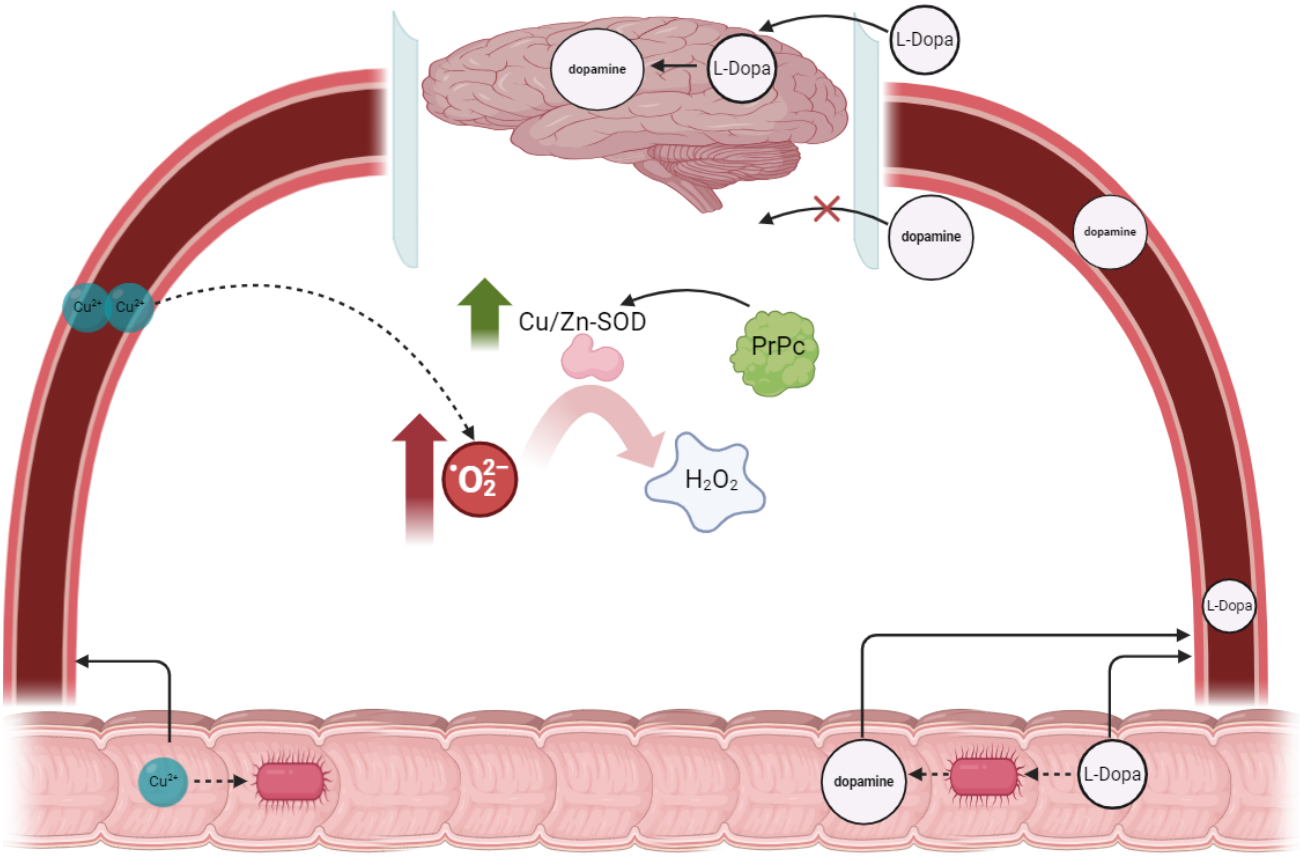
L-Dopa
dopamine
L-Dopa
dopamine
dopamine
Cu/Zn-SOD
PrPc
Cu^{2+}
Cu^{2+}
$^{\bullet}O_2^{2-}$
H_2O_2
L-Dopa
Cu^{2+}
dopamine
L-Dopa

Figure 6