

Metabonomics reveals peripheral and central short-chain fatty acid and amino acid dysfunction in a naturally occurring depressive model of macaques

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Purpose: Depression is a complex psychiatric disorder. Various depressive rodent models are usually constructed based on different pathogenesis hypotheses.

Materials and methods: Herein, using our previously established naturally occurring depressive (NOD) model in a non-human primate (cynomolgus monkey, *Macaca fascicularis*), we performed metabolomics analysis of cerebrospinal fluid (CSF) from NOD female macaques (N=10) and age- and gender-matched healthy controls (HCs) (N=12). Multivariate statistical analysis was used to identify the differentially expressed metabolites between the two groups. Ingenuity Pathways Analysis and MetaboAnalyst were applied for predicted pathways and biological functions analysis.

Results: Totally, 37 metabolites responsible for discriminating the two groups were identified. The NOD macaques were mainly characterized by perturbations of fatty acid biosynthesis, ABC transport system, and amino acid metabolism (eg, aspartate, glycine, serine, and threonine metabolism). Interestingly, we found that eight altered CSF metabolites belonging to short-chain fatty acids and amino acids were also observed in the serum of NOD macaques (N=13 per group).

Conclusion: Our findings suggest that peripheral and central short-chain fatty acids and amino acids are implicated in the onset of depression.

Keywords: naturally occurring depressive, depression, non-human primate, metabonomics, cerebrospinal fluid, short-chain fatty acid

Introduction

Depression is a serious mental illness worldwide, affecting more than 350 million people, equivalent to 4.4% of the world's population.^{1,2} Depression can be long-lasting or recurrent, substantially impairing not only patients' quality of life but also of their families. Moreover, depression can also lead to suicide, which is the second leading cause of death in adolescents.^{3,4} However, the underlying molecular mechanisms of depression remain largely unknown.⁵

Animal models are important tools for investigating the pathogenesis of depression. Our groups and other investigators established some rodent models to uncover the molecular mechanisms of depression.^{3,5-9} These models play an important role in understanding the pathogenesis of depression. However, it cannot be overlooked that these models are based on some kind of pathogenesis theory. Under this condition, this may limit our horizons. To address this question, we recently had successfully screened naturally depressed monkeys in a similar natural cage, designated as a naturally occurring depressive (NOD) model of a non-human primate. Similar to depressive patients, the NOD monkeys exhibit depressed behavioral phenotype, such as low levels of activity, low levels of social interaction, and anhedonia.¹⁰

Cerebrospinal fluid (CSF) is arguably the most relevant sampling substrate for the in vivo study of brain disorders as it reflects the metabolic status and the biochemistry of the brain.^{11,12} Therefore, metabolomic profiles of CSF in patients and controls have the potential to reveal protein differences linked to the pathogenesis of neuropsychiatric disorder that might have value as biomarkers.¹³ Recent studies of CSF metabolite profiles have identified initial metabolic signatures for a variety of central nervous system (CNS) disorders, including depression, bipolar disorder, and Parkinson's disease.^{11,14,15} These metabolite profiles may potentially be useful as biomarkers in neurological conditions. However, as the clinical symptoms of depression are diverse and highly heterogeneous, it is valuable to perform the CSF analysis of NOD monkey model.

Here, a non-targeted metabolomics approach based on gas chromatography–mass spectrometry (GC–MS) was used to identify differentially expressed metabolites between NOD and control macaques based on a previous study.¹⁶ To systematically analyze the role of differentially expressed metabolites, the differential metabolites identified in this study were compared with those reported in our published findings.¹⁶ Some key metabolites were detected both in the and CSF, which may provide an evidence to understand peripheral and central interactions.

Materials and methods

Subjects and ethical statement

All the cynomolgus monkeys were obtained from Xishan Zhongke Laboratory Animals Co. Ltd. (license number SYXK [Su] 2002–0032). Animals were reared in socially stable colonies with negligible rates of conflict, were provided with water ad libitum, and fed daily with fresh fruits, vegetables, and high-nutrition monkey food. Each colony was housed in an indoor free enclosure measuring 12.0×4.0×3.0 m³ (L×W×H) with 12-hour light/dark cycle (Figure 1A). Indoor pens were maintained at a temperature higher than 18°C and relative humidity of 40%–70%. The living environment and animal care procedures are detailed in a previous report.^{17–20}

All experiments in this study were approved by the Ethics Committee of Chongqing Medical University (approval no: 20100031), and all procedures were performed in accordance with the recommendations of the Weatherall report, “The use of non-human primates in research”.^{21,22} Special care was taken to minimize the number and suffering in all experiments involving non-human primates. For more details, please refer to our previous publication.^{16,20}

Behavioral observation and subject selection

In our previous study,¹⁸ we systematically documented the comprehensive systematic ethogram of *Macaca fascicularis*

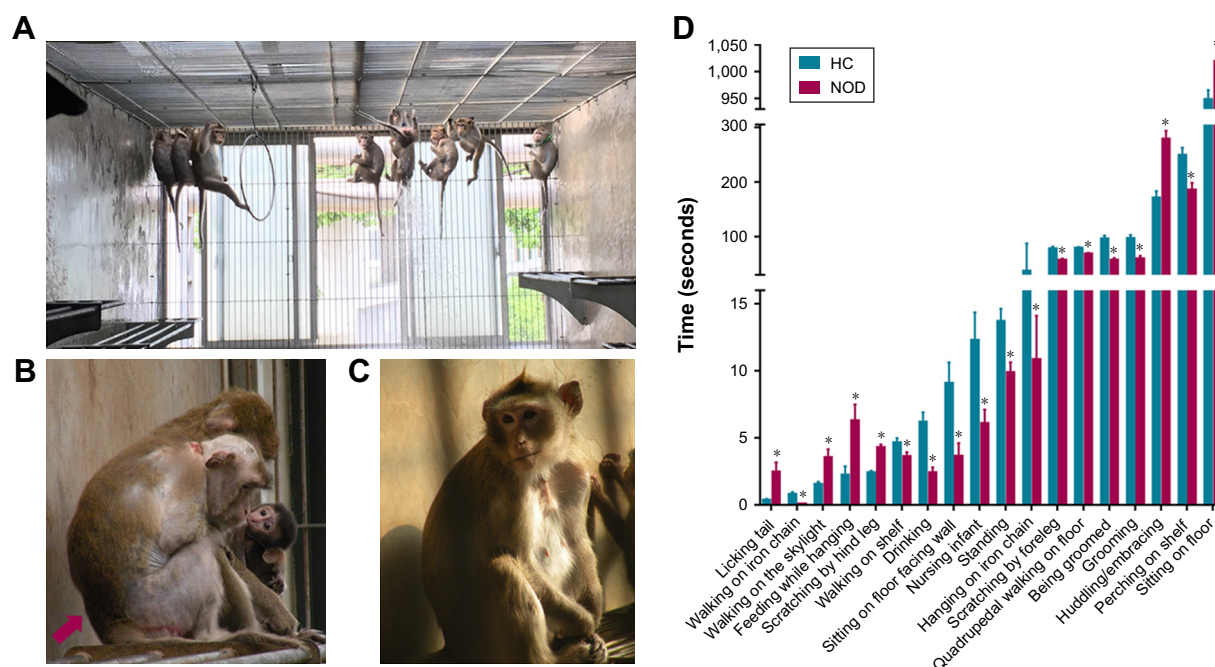


Figure 1 Behavioral observation of the cynomolgus monkeys. (A) Cynomolgus monkeys in a realistic social context. (B) Slumped or collapsed posture of a NOD macaque (red arrow). (C) A CON macaque. (D) The significant differential behavioral results between NOD macaques (n=10) and CON macaques (n=12).

Note: *Behaviors of greater duration in healthy controls relative to NOD macaques.

Abbreviations: CON, control; NOD, naturally occurring depressive; HC, healthy control.

for behavioral investigations. We classified 53 discrete behaviors into 12 behavioral categories, which were applied in the present study (Table S1). All behavior patterns of each macaque were videotaped, and the three independent observers blindly and discretely scored all behavioral items on the videotape footage using NOLDUS Observer XT software (version 10.0; Noldus Information Tech Technology, Leesburg, PA, USA).^{16,18} Based on the behavioral observation, a NOD state of *M. fascicularis* was described.¹⁸ The macaques (n=13 per group) that displayed the highest frequency of depressive behaviors over the observational period were selected in our previous study.¹⁶ Among the 13 NOD macaques, ten female macaques displayed the highest frequency of depressive behaviors and were selected for this study (Figure 1B). To match by age and sex, 12 of the 13 healthy female adult macaques were selected (controls, CON) (Figure 1C). There was no significant difference in age or weight between the NOD macaques and CON group (Table 1, $P>0.05$). Non-pregnant estrous cycles of female macaques were recorded, during which sample collection was avoided.

Sample collection and preparation for GC–MS analysis

CSF sampling was done as previously described.^{23,24} Briefly, monkeys were removed from their home pens, and anesthetized by injecting 15 mg/kg ketamine intramuscularly. Sedation was achieved in <5 minutes after returning to their respective transfer cages. A 5 cm margin was shaved laterally to the midline on each side between lumbar vertebrae L5/6 for CSF collection. Under aseptic conditions, a needle (21-gauge, 0.0285 in) was used for CSF collection. About 1.5 mL of CSF was withdrawn, initially centrifuged at 2,000 rpm for 10 minutes at 4°C, and divided into equal aliquots and stored at –80°C until further use.

About 15 µL of CSF sample was added to 10 µL of L-leucine-13C6 (0.1 mg/mL) which acts as an internal standard. After vortexing for 30 seconds, 90 µL of methanol was added. After centrifuging at 14,000 rpm for 15 minutes, 95 µL of supernatant was evaporated to dryness under a stream of

nitrogen gas. The dried residue was added to 30 µL of methoxamine hydrochloride (20 mg/mL pyridine) and incubated at 37°C for 90 minutes with continuous shaking. Subsequently, the solution was treated with 30 µL of N,O-Bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane at 70°C for 60 minutes. After derivatization and cooling to room temperature, this derivative was injected in the GC–MS for analysis.

GC–MS acquisition and data analysis

GC–MS analysis was carried out according to this group's previously published work.^{5,16} Briefly, we used an Agilent7890A/5975C GC–MS System (Agilent Technologies Inc., Santa Clara, CA, USA) and a HP-5MS capillary column (30 m ×0.25 mm ×0.25 µm; Agilent, Technologies Inc). Typical GC settings and MS parameters were used. Briefly, each 1 µL of derivative sample was injected into the system with an injector temperature of 280°C. Helium was used as a carrier gas at a constant flow rate of 6 mL/min. The column temperature was initially kept at 80°C for 2 minutes and then increased to 320°C gradually at the rate of 10°C/min, and maintained for 6 minutes. The column effluent was introduced into the ion source of mass selective detector. The MS quadrupole temperature was set at 150°C, and the ion source temperature was set at 230°C. Data acquisition was performed in the full-scan mode from 50 to 600 m/z. To avoid the influence induced by instrument signal fluctuations, a random order of continuous sample analysis was adopted.

The chromatographic peaks in the total ion current (TIC) chromatograms represent the corresponding metabolites, and their relative concentrations can be detected using the peak area normalization method. In the present study, the TIC from the CSF samples of two groups revealed strong signals for analysis, large peak capacity, and good retention time reproducibility (Figure 3A). These peaks were annotated by comparing the accurate mass (m/z) and retention time with those provided in the National Institute of Standards and Technology online databases. The relative intensities of these metabolites were used in the subsequent multivariate statistical analysis.²⁵

Table 1 Basic background data of NOD and CON macaques

Variable	CON	NOD	P-value
Sample size (n)	12	10	–
Sex	F	F	–
Age (years)	12.50±0.66	13.06±1.90	0.475
Weight (Kg)	5.57±0.59	5.93±0.88	0.629

Note: Data are presented as mean ± SE.

Abbreviations: CON, control; F, female; NOD, naturally occurring depressive; SE, standard error.

Metabolomic data analysis

The differential metabolites were identified based on the methods described previously.^{26–28} The peak indexes (RT-m/z), sample names and normalized peak intensities, were imported into SIMCA-P 14.0 (Umetrics, Umeå, Sweden) for multivariate statistical analyses. Multivariate statistical analyses, including principal component analysis (PCA) and pair-wise orthogonal projections to latent structures discriminant analyses (OPLS-DA) with Pareto scaling spectral data, were performed to visually discriminate samples in the NOD macaques and CON group. Three parameters (R^2X , R^2Y , and Q^2Y) were used to quantify the quality of the model. R^2X and R^2Y were used to quantify the goodness-of-fit, and Q^2Y was applied to assess the predictability of the model. If the Q^2 and R^2 values resulting from the original model were higher than the corresponding values from the permutation test, the model was considered valid. The candidate metabolites were selected according to thresholds of variable importance in the projection values (variable importance in the projection [VIP] >1.0) in the OPLS-DA model and a P -value <0.05 (obtained from two-tailed Student's t -test).

Molecular pathway and network analysis

The overall workflow involved in identifying the differential metabolites in NOD macaques is summarized in Figure 2. For that significantly changed metabolites (VIP >1.0 and P <0.05), pathway analysis was performed using MetaboAnalyst 3.0 (<http://www.metaboanalyst.ca>)²⁹ and Ingenuity Pathways Analysis (IPA) software (Qiagen, NV, Venlo, the Netherlands).³⁰ MetaboAnalyst was used to generate a heat map of all the different metabolites. To identify the predicted molecular pathways and to explore the biological functions of interested molecules, identified metabolites (with Human Metabolome Database [HMDB] identifiers) and corresponding fold changes were uploaded to the IPA server. The predicted molecular pathways and biological functions were generated based on the knowledge sorted in the IPA Knowledge Base. Cytoscape software (version 3.6.0) was used to build the correlation network between these differential metabolites in CSF and serum.

Statistical analysis

Statistical analyses between the two groups were performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA). Student's t -test was used for the data that examined normal distributions and

equal variances, otherwise nonparametric Mann–Whitney U-test was used. A P -value >0.05 was considered statistically significant.

Results

Significant differences in behaviors between NOD and CON groups

The behavioral results were consistent with those reported by a previous study.¹⁶ Ten NOD macaques were selected based on our previous observations,¹⁶ and these animals displayed the highest frequency of depressive behaviors (Figure 1B). To match by age and sex, 12 healthy adult female *M. fascicularis* (aged 9–13 years) were selected (controls, CON) (Figure 1C). There were no significant differences in age or weight between the NOD macaques and CON group (P >0.05).

The 53 discrete behaviors belonging to 12 behavioral categories were used to distinguish the NOD and CON groups. Eighteen behaviors pertaining to five behavioral categories were found to be significantly altered between NOD and CON macaques (Figure 1D). Six behaviors (licking tail, walking on the skylight, sitting on floor, feeding while hanging, scratching by hind leg, and huddling/embracing) were significantly increased in NOD group relative to the CON group, while 12 behaviors (walking on iron chain, walking on shelf, drinking, sitting on floor facing wall, nursing infant, standing, hanging on iron chain, scratching by foreleg, quadrupedal walking on floor, being groomed, grooming, and perching on shelf) were significantly reduced in NOD group relative to the CON group. The behavioral results indicated that the NOD group displayed NOD behaviors compared with the CON group.

Metabolomics analysis and identification of significant metabolites from CSF samples of NOD and control macaque

In total, the GC–MS metabolomics profiling for the CSF samples produced 663 variables across the NOD and CON groups, which were used in the subsequent multivariate analysis. Representative GC–MS TIC chromatograms are shown in Figure 3A. The PCA scores plot showed clear differences between the depressed and CON groups. This analysis showed that the NOD macaques were obviously discriminated from the healthy controls (R^2X =0.754, Q^2 =0.429). The pair-wise PLS-DA score plots also revealed that the depressed macaques were statistically distinguishable from the CON group (R^2X =0.802, R^2Y =0.936, and Q^2 =0.444). The three-dimensional diagram intuitively demonstrated clear separation between the NOD and CON

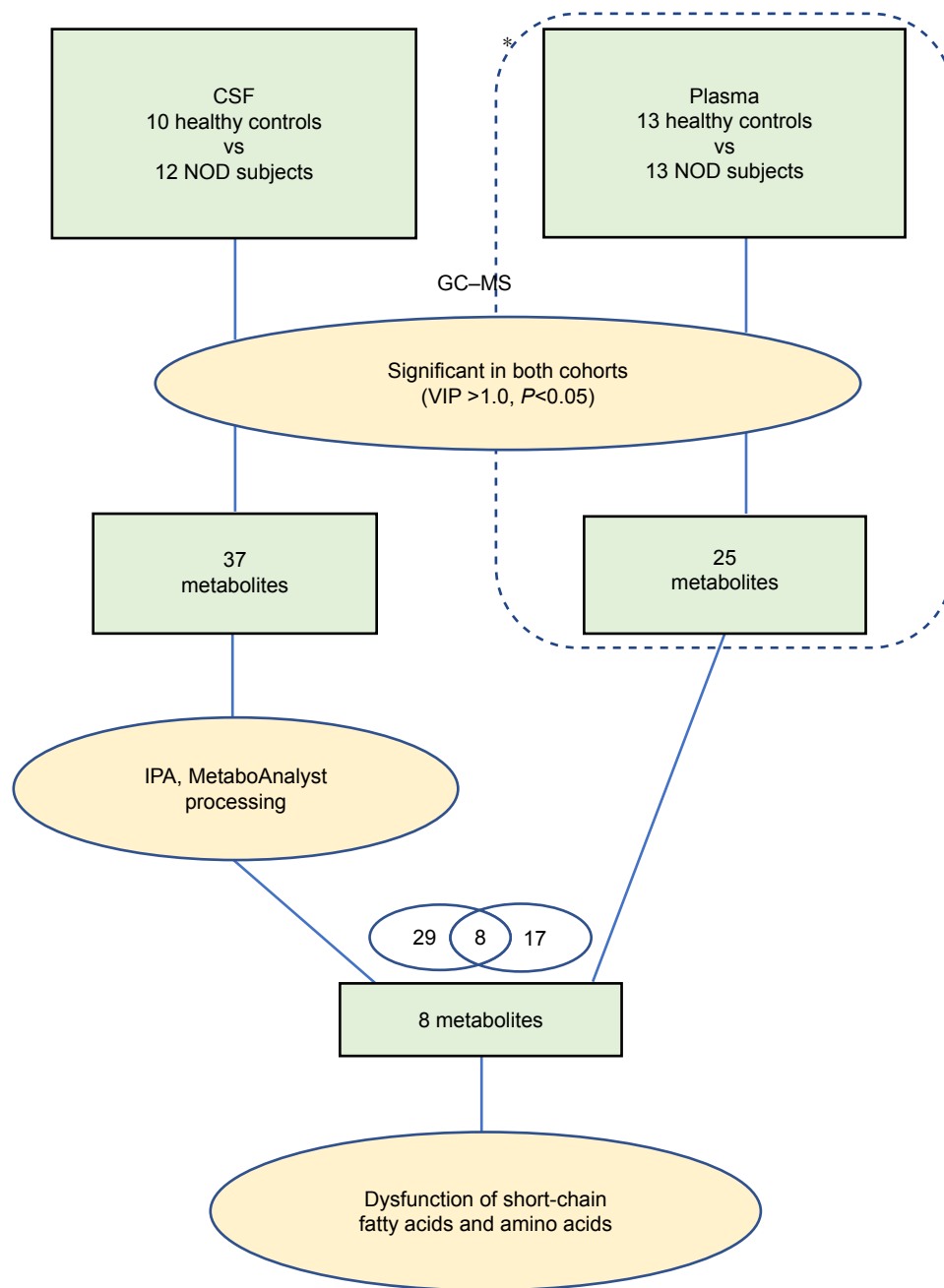


Figure 2 An overview of the GC-MS-based metabolomic workflow identifying differentially expressed CSF metabolites in NOD macaques.

Note: *The data was from our published work (Xu et al¹⁶).

Abbreviations: CON, control; NOD, naturally occurring depressive; GC-MS, gas chromatography–mass spectrometry; CSF, cerebrospinal fluid; VIP, variable importance in the projection; IPA, Ingenuity Pathways Analysis.

groups (Figure 3B). R^2X and R^2Y represent the cumulative model variations in X and Y, respectively, and Q^2 is the cumulative predicted variation. The values of these parameters approaching 1.0 indicate a stable model with predictive reliability.

According to the PLS-DA analysis, significant differential metabolites between the NOD group and the CON group were identified in the CSF samples. Using a VIP >1.0 in

the PLS-DA models and $P < 0.05$ using two-tailed Student's *t*-test, 37 differential metabolites were identified between the two groups (Table 2, Figure 4A). When compared to healthy controls, NOD macaques were characterized by higher levels of propanoic acid, acetic acid, hydroxylamine, propanedioic acid, butanoic acid, proline, methanamine, glycine, isothiurea, nonanoic acid, carbamic acid, threonine,

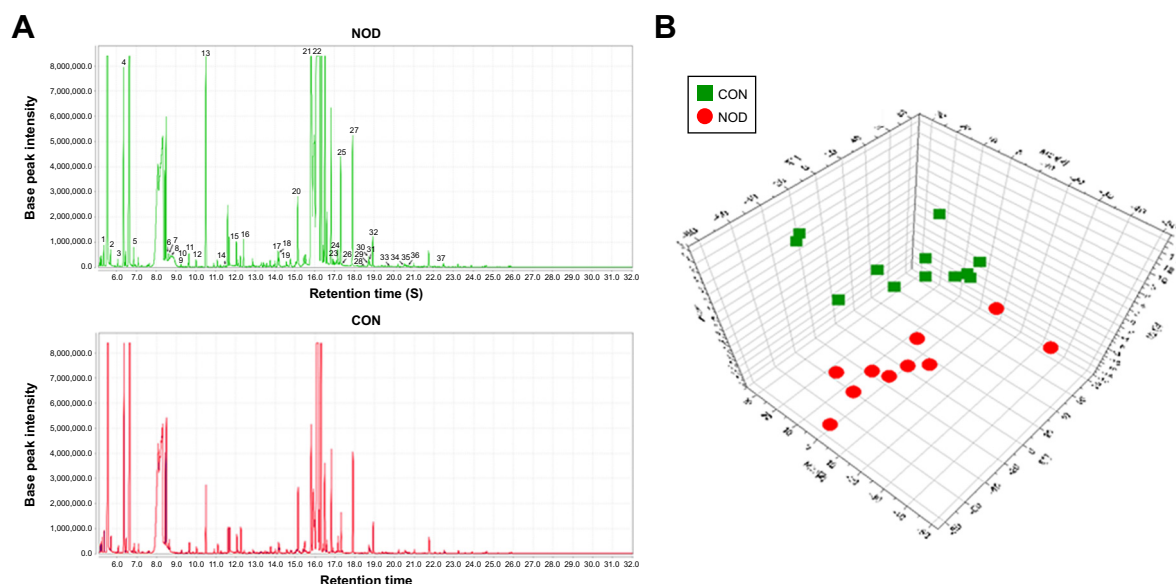


Figure 3 (A) Representative GC-MS total ion chromatograms (TIC) of the CON and NOD group. (B) Partial least squares-discriminate analysis (PLS-DA) scores plot derived from GC-MS spectra of the CON and NOD macaques.

Abbreviations: CON, control; NOD, naturally occurring depressive; GC-MS, gas chromatography-mass spectrometry.

beta-alanine, threitol, erythronic acid, L-aspartic acid, xylitol, ribitol, 2-keto-D-gluconic acid, 1,4-butanediamine, D-fructose, myoinositol, glucaric acid, hexadecanoic acid, scyllitol, gulose, heptadecanoic acid, linolelaidic acid, trans-9-octadecenoic acid, oleic acid, octadecanoic acid, N-acetyl-D-glucosamine, D-glycero-D-galacto-heptitol, galactitol, 5-phenylvaleric acid, in addition to lower levels of benzoic acid and 1H-indole-2-carboxylic acid.

Metabolic pathway and function analyses

Using the HMDB classification, among the 37 metabolites, over 35.1% were sub-grouped as carbohydrates and carbohydrate conjugates; about 32.4% as lipids; and about 21.6% as amino acids, peptides, and analogues (Figure 4B). These metabolites were primarily located in cytoplasm (29.7%), extracellular (27.0%), membrane (16.2%), mitochondria (10.8%), and lysosome (8.1%) (Figure 4C).

To understand the molecular functional roles of the differential metabolites, they were mapped into the IPA Knowledge Base. The NOD macaques were characterized by disturbances in the following top five canonical pathways: 1) fatty acid biosynthesis, 2) ABC transport system, 3) aminoacyl-tRNA biosynthesis, 4) aspartate metabolism, and 5) threonine degradation. The detailed results of pathway analysis are shown in Table 3. The differential metabolites found in NOD monkeys were predicted to be significantly involved in the biological functions like biosynthesis of lipids and uptake of amino acids (Figure 4D–E).

Relationship between peripheral and central metabolic disturbances in NOD and control macaques

To develop an integrated understanding of the central and peripheral metabolic disturbances that occurred in NOD macaques, we compared the differential metabolites identified in this study with those of our published findings. A correlation network was built to show the trend of metabolic disturbances in serum and CSF (Figure 5). Among these differential metabolites, eight metabolites (butanoic acid, l-proline, l-threonine, threitol, 2-keto-D-gluconic acid, 1,4-butanediamine, D-fructose, and myo-inositol) were altered both in the serum and CSF of NOD macaques. The eight altered metabolites were mainly identified as short-chain fatty acids and amino acids. These findings suggest that perturbations of short-chain fatty acid and amino acid metabolism in serum and CSF are implicated in the onset of depression.

Discussion

In this study, we for the first time characterized the metabolic changes of CSF samples from NOD cynomolgus monkeys relative to matched controls. We found that the NOD macaques were mainly characterized by perturbations in fatty acid biosynthesis, ABC transport system, and amino acid metabolism. Interestingly, the eight altered CSF metabolites, belonging to short-chain fatty acid and amino acids, were

Table 2 Differential metabolites of cerebrospinal fluid (CSF) between NOD and CON macaques

No	Metabolite	HMDB ID	Ret (min)	m/z	VIP	P-value	Log (FC)
1	Propanoic acid	HMDB00237	5.3654	174	1.26	0.029	0.103
2	Acetic acid	HMDB00042	5.7046	205	1.32	0.022	0.137
3	Hydroxylamine	HMDB03338	6.2860	154	1.16	0.046	0.074
4	Propanedioic acid	HMDB00691	6.4586	218	1.15	0.049	0.089
5	Butanoic acid	HMDB00039	6.9491	145	1.36	0.017	0.143
6	L-proline	HMDB00162	8.7842	142	1.21	0.038	0.105
7	Methanamine	HMDB00164	8.8266	160	1.33	0.021	0.086
8	Glycine	HMDB00123	8.9144	174	1.65	0.003	0.169
9	Isothiourea	HMDB34155	9.3807	245	1.17	0.046	0.081
10	Nonanoic acid	HMDB00847	9.5321	215	1.32	0.021	0.108
11	Carbamic acid	HMDB03551	9.6229	278	1.38	0.016	0.142
12	Threonine	HMDB00167	10.0166	117	1.33	0.020	0.097
13	Beta-alanine	HMDB00056	10.5041	73	1.51	0.007	0.165
14	Threitol	HMDB04136	11.5125	217	1.76	0.001	0.155
15	Erythronic acid	HMDB00613	12.0575	205	1.93	0.000	0.164
16	L-aspartic acid	HMDB00191	12.4118	147	1.16	0.046	0.238
17	Xylitol	HMDB02917	14.1409	103	1.59	0.004	0.146
18	Ribitol	HMDB00508	14.1984	117	1.19	0.040	0.116
19	2-keto-D-gluconic acid	METPA0825	14.6496	292	1.29	0.024	0.126
20	1,4-butanediamine	HMDB01414	15.3885	86	1.20	0.038	0.095
21	D-fructose	HMDB00660	15.7973	73	1.46	0.009	0.166
22	Myoinositol	HMDB00211	16.0304	265	1.19	0.040	0.128
23	Glucaric acid	HMDB00663	17.0993	333	1.35	0.018	0.223
24	Hexadecanoic acid	HMDB00220	17.1599	73	1.21	0.038	0.086
25	Scyllitol	HMDB06088	17.2326	318	1.49	0.008	0.160
26	Gulose	HMDB12326	17.3052	204	1.38	0.016	0.219
27	Heptadecanoic acid	HMDB02259	18.0653	327	1.15	0.048	0.092
28	Benzeneacetic acid	HMDB00209	18.5165	295	1.21	0.037	-0.055
29	Linolelaic acid	HMDB06270	18.6800	337	1.37	0.016	0.117
30	Trans-9-octadecenoic acid	HMDB00573	18.7194	341	1.26	0.030	0.126
31	Oleic acid	HMDB00207	18.7799	68	1.17	0.044	0.104
32	Octadecanoic acid	HMDB00827	18.9374	257	1.17	0.044	0.085
33	1H-indole-2-carboxylic acid	HMDB02285	19.7853	369	1.29	0.025	-0.051
34	N-acetyl-D-glucosamine	HMDB00215	20.5362	73	2.05	0.000	0.176
35	D-glycero-D-galacto-heptitol	HMDB33750	20.5907	262	2.05	0.000	0.192
36	Galactitol	HMDB00107	20.6634	307	1.87	0.000	0.191
37	5-phenylvaleric acid	HMDB02043	22.3046	334	1.50	0.007	0.179

Notes: Obtained from PLS-DA with a threshold of 1.0. A positive \log_2 (FC) indicates significantly higher levels in NOD group compared with CON group. A negative \log_2 (FC) indicates significantly lower levels in NOD group compared with CON group.

Abbreviations: VIP, variable importance in the projection; CON, control; NOD, naturally occurring depressive; Ret, retention time; FC, fold change; PLS-DA, partial least squares-discriminate analysis.

also observed in the serum sample of NOD model. These findings suggest that perturbations in peripheral and central short-chain fatty acids and amino acids are implicated in the onset of depression. Perturbations in lipid and amino acid metabolism were also reported in depressive patients^{31,32} and rodent models.^{6,33,34}

Higher primates like cynomolgus monkeys appear to be particularly appropriate as a model for mental disorder.³⁵ The cynomolgus monkey model described in our study better resembles the etiological development of depression compared to traditional rodent models, as these depressive monkeys were screened from an approximately natural

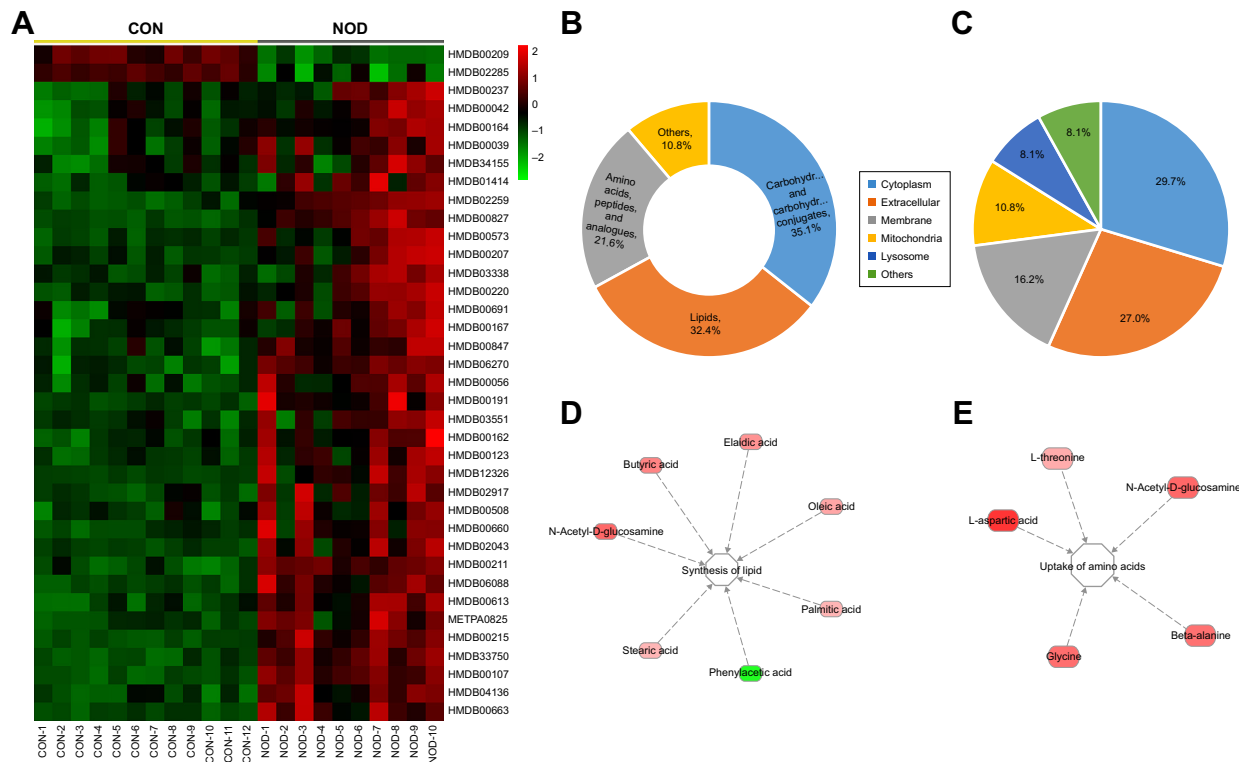


Figure 4 (A) Heat map of differentially expressed CSF metabolites in CON and NOD macaques. (B) Chemical taxonomy and (C) cellular locations based on the annotations of Human Metabolome Database (HMDB). The predicted biological functions were significantly involved in the biosynthesis of lipid (D) and uptake of amino acids (E). **Abbreviations:** CON, control; NOD, naturally occurring depressive; CSF, cerebrospinal fluid.

environment. In this condition, the depressed monkeys demonstrated superior validity without any human intervention, which may better imitate the psychosocial stressors encountered by adult humans living in a resource-based competitive society.

In this study, we found that fatty acid biosynthesis was significantly disturbed in CSF of NOD macaques relative to control. Fatty acids influence a number of cellular functions at different levels of incorporation, including cell membrane fluidity, membrane protein-mediated responses, eicosanoid generation, gene expression, and cell signaling. Through these mechanisms, fatty acids influence cell and tissue

physiology, and the way cells and tissues respond to external signals in both the serum and CSF.^{36,37} Evidence suggests that in appropriate quantities, both saturated fatty acids and their derivatives are required for brain health. In a recent study³⁸ using human frontal cortex tissue demonstrated that lipid rafts, membrane structures intimately associated with cell signalling, are predominately composed of the saturated fatty acids palmitic and stearic acid. Oleic acid, formed from the desaturation of stearic acid, has been shown to promote axonogenesis in the striatum during brain development,³⁹ and is used as a cerebral energy source when glucose availability diminishes. Consistent with these studies, our

Table 3 Top five affected ingenuity canonical pathways enriched in CSF between NOD and CON macaques

Pathway name	Total	Hits	P-value	FDR	Matching IDs
Fatty acid biosynthesis	16	6	2.98e-06	1.01e-04	HMDB00042 HMDB00691 HMDB00039 HMDB00220 HMDB00207 HMDB00827
ABC transporters	22	5	5.43e-05	1.36e-03	HMDB00123 HMDB00167 HMDB00191 HMDB01414 HMDB00215
Aminoacyl-tRNA biosynthesis	20	4	1.63e-04	5.22e-03	HMDB00162 HMDB00123 HMDB00167 HMDB00191
Aspartate metabolism	16	3	5.69e-04	9.67e-03	HMDB00691 HMDB00056 HMDB00191
Threonine degradation	17	3	1.69e-04	1.46e-02	HMDB00237 HMDB00123 HMDB00167

Abbreviations: CON, control; NOD, naturally occurring depressive; CSF, cerebrospinal fluid; FDR, false discovery rate.

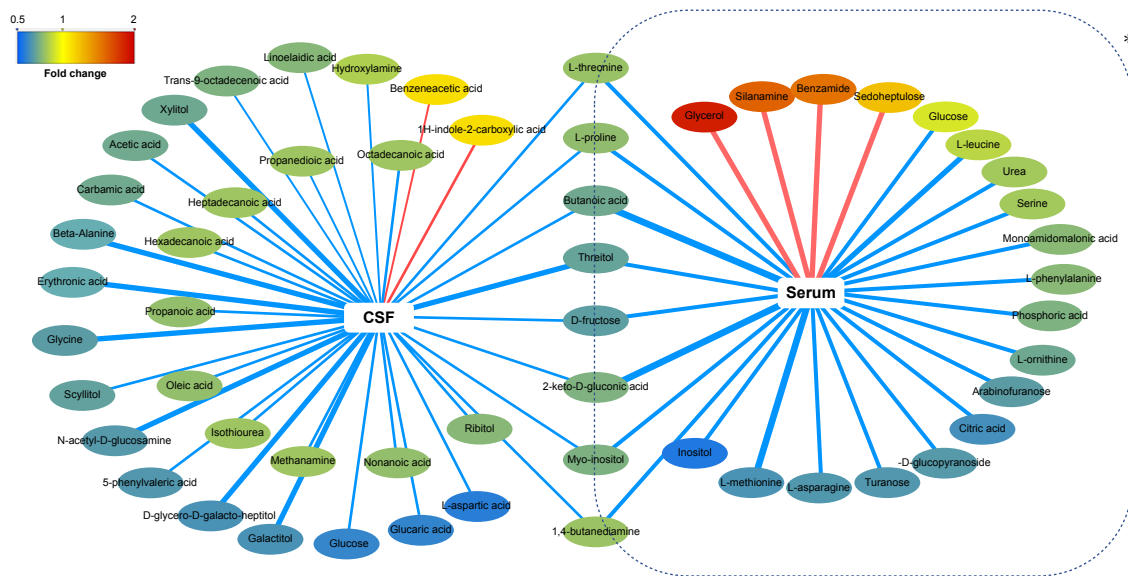


Figure 5 Network analysis of differentially expressed metabolites in CSF and serum of NOD macaques.

Notes: *The data was from our published work (Xu et al¹⁶). Red-colored boxes and red-colored lines indicate upregulation in NOD macaques compared with control (CON), while green-colored boxes and green-colored lines indicate downregulation. The width of the line represents the VIP scores of differential metabolites.

Abbreviations: NOD, naturally occurring depressive; CSF, cerebrospinal fluid; VIP, variable importance in the projection.

results indicated that oleic acid, octadecanoic acid, and hexadecanoic acid were markedly perturbed in NOD macaques compared with control. In addition, short-chain fatty acids (acetic acid, propanedioic acid, and butyric acid) were also found to be disturbed in CSF of NOD macaques. These short-chain fatty acids are important metabolites for the gut microbiota, which are important for host health, as they provide the preferential energy source for intestinal epithelium, stimulate the production of regulatory T cells, inhibit inflammation, and regulate gene expression as a histone deacetylase inhibitor.⁴⁰ The entire amount of butyrate that our body needs is produced by butyrate-producing bacteria living in our gut.⁴¹ In the present study, butyrate level was found to be significantly different both in the serum and CSF samples of NOD macaques.

In addition to the reduced short-chain fatty acids, two microbiotic metabolites – methanamine and benzeneacetic acid – were decreased in NOD macaques relative to HCs. These metabolites are uniquely produced by microbial metabolism in the intestinal tract, indicating that major depressive disorder (MDD) may be associated with variations in intestinal microflora. Consistent with this result, our previous studies^{3,42} showed that several altered metabolites in MDD macaques are the metabolic byproducts of gut microbiota. This is the first study to detect microbial-specific metabolites both in the periphery and brain of the same non-human primate model of depression. These findings may contribute to a better understanding of the microbiota–gut–brain axis.

ABC transporters, which are important components of the blood–brain barrier (BBB), function by either preventing the entry of or expelling numerous molecules including toxins, drugs, and other xenobiotics.⁴³ In the present study, five differential metabolites, involved in ABC transport system, were observed in NOD macaques. The disturbed ABC transport system may affect BBB permeability, leading to some neuropsychiatric disorders.⁴⁴ It has been shown that various physical or psychological stressors can impact the BBB's ability to selectively restrict the passage of substances from the blood to the brain.⁴⁵ Deranged BBB permeability has been reported in MDD which implies that protein exchange may occur between the brain and peripheral circulation. The ABC transporters have been recognized to play an important role in neurological diseases like Alzheimer's and Parkinson's, depression, schizophrenia, and epilepsy.⁴⁶ Some studies suggested that ABC transporters family and their genetic polymorphisms may play a role in mediating antidepressant response.^{47,48}

Aminoacyl-tRNA biosynthesis and a panel of amino acids were significantly disturbed in CSF of NOD macaques compared to HCs. The aminoacyl-tRNA synthetases are prominently known for their classic function in the first step of protein synthesis, where they bear the responsibility of setting the genetic code.⁴⁹ Disturbed amino acid metabolism was regarded as an important factor in depression. Some clinical evidence^{50,51} suggested that the plasma levels of aspartic acid and glycine were significantly downregulated

in depression macaques. In animal models of depression, proline was also found to be remarkably decreased.⁵² Consistent with these studies, our results showed that the levels of proline, glycine, threonine, and aspartic acid decreased in NOD macaques, with perturbed pathway of aspartate metabolism; threonine degradation; glycine, serine, and threonine metabolism; and arginine and proline metabolism. Our published studies also found that disturbance in the metabolism of certain amino acids was an important factor contributing to MDD in serum and CSF.^{5,6,34,42,53}

Limitations

There are some limitations in this study that should be recognized. Firstly, the absolute value of our sample size was not particularly large because of the high cost and ethical regulations involved in the use of monkeys for animal experiments. Secondly, only female NOD macaques were employed. It is required to determine whether the altered metabolites exhibit gender specificity. Thirdly, we have no results with regard to metabolic changes in the brain. Further studies that can capture the metabolic changes in key brain regions of depressive monkeys are required.

Conclusion

Employing a GC–MS metabolomic approach, we found that metabolic profiling of CSF sample from NOD macaques was significantly different from that of CON macaques. The NOD group was mainly associated with disturbances in fatty acid biosynthesis, ABC transport system, and amino acid metabolism. Moreover, some CSF metabolites belonging to short-chain fatty acids and amino acids were found to be changed in the serum of NOD model. Our findings suggest that changes in peripheral and central short-chain fatty acid and amino acid metabolism are implicated in the onset of depression.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 The included behaviors of 12 behavior categories

Behavioral categories	Behavioral items
Ingestion behaviors	Searching, feeding while squatting, feeding while hanging, feeding while sitting, drinking, chewing, licking residue from floor, picking remaining food, feeding while perched, suckling
Thermo-regulatory behaviors	Huddling, quivering, embracing
Rutting and estrous behaviors	Licking genital area, presenting buttocks, peri-vulvar discoloration, sniffing anogenital area, sniffing urine, rolling tongue, homosexual roaring, homosexual mounting, tail arching
Mating behaviors	Mounting, copulating, post-copulation guarding, post-copulation standing, ejaculating, masturbating
Resting behaviors	Sitting on floor, sitting on floor facing wall, perching on shelf (socially dominant), sitting on ring, lying on floor, lying on shelf, hanging on window or door (non-socially dominant), hanging on skylight, hanging on iron chain, hanging on ventilator, sitting and sleeping
Parental behaviors	Nursing infant, holding infant, defending infant, licking anus of infant, checking anus of infant
Amicable behaviors	Grooming, being groomed, embracing
Conflict behaviors	Driving (socially dominant, aggressive), attacking (socially dominant, aggressive), fleeing (non-socially dominant), pulling foreleg, pulling hind leg, protracting ears, threatening (socially dominant, aggressive), being threatened (non-socially dominant), being attacked (non-socially dominant), parallel pacing, biting (socially dominant, aggressive)
Vigilance behaviors	Shifting position, alarmed jumping, watching company, alarmed calling, miscellaneous calling, shaking cage
Locomotion behaviors	Galloping, walking on shelf (socially dominant), quadrupedal walking on floor (non-socially dominant), moving, climbing, walking on iron chain, walking on skylight, standing, stepping, trotting
Communication behaviors	Lip smacking, sniffing, voiding, alarmed calling, miscellaneous calling
Miscellaneous behaviors	Huddling (solitary), shaking body, playing (solitary), licking hair, scratching by hind leg, scratching by foreleg, yawning, licking hand, catching pest, digging anus, rubbing palm on floor, licking tail, shaking ID card

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