Enhanced Intestinal Protein Fermentation Correlates With Severe Psychiatric Symptoms of Schizophrenia

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Abstract

Emerging findings highlighted the associations of mental illness to nutrition and dysbiosis in the intestinal microbiota, but the underlying mechanisms, especially in schizophrenia (SZ), remain unclarified. Here we conducted a case-control study of SZ by performing gut metagenome, fecal and plasma non-targeted metabolome, short-, medium-, and long-chain fatty acids, and targeted metabolite analysis. The results uncovered an apparent contradiction in SZ patients between inadequate protein intake and protein-fermentation-dominated intestinal microbial metabolism, which shifted from carbohydrate fermentation and protein synthesis in healthy conditions. Moreover, the extent of protein fermentation represented as the abundance of related enzymes and fecal levels of related products, usually nitrogenous and neurologically active, correlated with the severity of psychiatric symptoms. These findings provide a previously uncharacterized pathophysiological process in SZ related to the dysbiosis of gut microbiota and dysregulation in macronutrient metabolism, highlighting the importance of nutrition care and the potentials for developing microbiota-targeted therapeutics in SZ.

Introduction

Schizophrenia (SZ) affects ~ 1% of the world population and is among the top 10 global causes of disability (1). The etiology of SZ remains unknown and likely involves a wide range of environmental factors that affect the neurophysiological processes in genetically susceptible individuals (2). Although some medications of SZ are available and effective in alleviating acute psychiatric symptoms (3), their pharmacological mechanisms remain unclarified, and they are often ineffective in treating negative symptoms. Negative symptoms are a core component of SZ which refer to diminution or absence of normal behaviors related to social activity and motivation and account for the major part of long-term functional outcomes (4). As the neurological mechanisms of the negative symptoms are largely unknown, effective medications for them are scarce, leaving the huge medical need remains unmet (2, 5).

Recently, epidemiological data have highlighted the association between nutrition and mental health, which raises up more studies in the nascent field of nutritional psychiatry(6). Although diet has been reported in many studies to potentially prevent or treat mental illness, mostly major depression (7, 8), information about the causality or underlying mechanism is still unclarified. The intestinal microbiota, which not only has great and complex capacities in metabolizing various nutrients but is also in turn regulated by nutrient supply, involved deeply in the interactions between nutrition and mental illness (9). Multiple routes, such as the vagus nerve, immune system, and endocrine systems, connect the gut microbes to the brain and consequently affect human behaviors (10, 11), forming a nutrients-microbiota-brain axis. In SZ patients, metabolic dysregulation, such as abnormal blood concentrations of amino acids, has been reported (12, 13), as well as dysbiosis in the intestinal microbiome where a variety of bacterial species were found enriched or absent in patients with this disorder (14–18). However, the exact mechanisms of how these species affect nutrient metabolism and brain functions are not entirely understood in their complexity. Up to date, no population study has been reported that systemically investigated the nutrient intake, gut microbiota, fecal and plasma metabolome, and mental health status

in patients of mental illness, especially SZ, and systemic study on the associations among nutrients, microbiota, and psychiatric symptoms may help to clarify the pathophysiology of SZ.

Here, we performed a case-control study of SZ (case : control = 100 : 52) where we conducted metagenome shotgun sequencing of stool DNA and analysis of metabolome of non-targeted small molecular metabolites as well as free fatty acids of various chain-length in plasma and fecal samples to dissect the potential roles of nutrients and intestinal microbes in the pathogenesis of SZ. Our results discovered a significantly enhanced intestinal protein fermentation in patients that shifted from carbohydrate fermentation and protein synthesis in healthy conditions. The activity of protein fermentation, represented by relevant enzyme abundance, fermentation product levels, and the daily intake of protein, exhibited positive correlations to the severity of psychiatric symptoms, especially negative ones. These findings illustrated a hyperactive fermentative process where an aberrant proportion of ingested proteins were delivered to intestinal microbes and intensively catabolized into amino acids and further into nitrogenous products. As the fermentative products of protein are often neurologically active, the enhanced microbial protein fermentation in the gut provides a molecular mechanism for how gut microbes undermine the social behaviors in SZ patients.

Results

Malnutrition and altered metabolome in patients with schizophrenia

During May ~ Aug 2018, the study enrolled 100 SZ patients from a sanatorium for patients of mental illness in County Gai, Liaoning, China and 52 age- and gender-matched healthy controls who were staff members of the sanatorium or inhabitants of the same town in order to minimize the influence of food sources and dietary habits in gut microbiota (23). Demographic and clinical information, and dietary records of the recent month before enrollment, were collected in detail for each subject (Table 1). Stool and plasma samples of all subjects were collected upon informative consent and cryopreserved for subsequent shotgun sequencing and metabolome analysis.

Table 1
Demographic, clinical characteristics and dietary patterns of all subjects.

Variable	Schizophrenia (n = 100)	healthy controls (n = 53)	p-value
Demographic characteristics			
Age, means(SD)	43.11(9.81)	48.92(12.95)	0.005
Education year, means (SD)	7.80(2.79)	9.34(3.29)	0.003
Height, means(SD)	1.64(0.07)	1.64(0.08)	0.645
weight, means(SD)	60.53(7.86)	65.04(8.20)	0.001
BMI, means (SD)	22.59(2.44)	24.11(2.45)	0.001
Female, No.(%)	34(34)	21(39.6)	0.490
Married, No.(%)	39(39)	48(90.6)	0.001
Smoking, No.(%)	0	11(20.8)	0.001
Drinking, No.(%)	0	10(18.9)	0.001
Clinical characteristics			
Fasting plasma glucos, means (SD)	4.26(0.65)	4.40(0.35)	0.252
Triglycerides, means (SD)	1.06(0.83)	1.07(0.33)	0.888
Cholesterol, means (SD)	3.80(0.74)	3.77(0.85)	0.844
Course of disease, means(SD)	13.35(8.53)	NA	NA
Monotherapy, No.(%)	81(81)	NA	NA
Equivalent dose of chlorpromazine, means(SD)	246.00(179.51)	NA	NA
positive syndrome score, means(SD)	17.45(5.56)	NA	NA
negative syndrome score, means(SD)	24.36(7.26)	NA	NA
General syndrome score, means(SD)	39.76(5.88)	NA	NA
PANSS total score, means(SD)	81.51(10.95)	NA	NA
Dietary characteristics, means(SD)			
Energy	1595.03(106.97)	1817.25(295.00)	0.001
Water	982.15(32.53)	996.84(122.75)	0.396
Protein	58.15(8.16)	97.57(19.67)	0.001

Variable	Schizophrenia (n = 100)	healthy controls (n = 53)	p-value
Fatty	82.31(8.52)	83.95(26.04)	0.658
Carbohydrate	155.27(9.74)	167.76(20.86)	0.001
Dietary fiber	12.84(1.61)	16.37(2.65)	0.001
Retinol equivalent	788.98(84.04)	841.69(143.98)	0.017
Vitamin B1	0.78(0.06)	1.13(0.27)	0.001
Vitamin B2	0.96(0.05)	1.49(0.42)	0.001
Vitamin PP	20.46(2.98)	21.32(3.98)	0.173
Vitamin E	18.56(3.95)	26.87(8.62)	0.001
Na	1260.04(188.79)	2118.78(449.25)	0.001
Ca	432.72(31.07)	543.71(134.25)	0.001
Fe	17.75(5.22)	25.58(5.32)	0.001
Vitamin C	119.39(5.48)	114.46(8.46)	0.001
Cholesterol	471.40(39.49)	805.65(277.21)	0.001

We first noticed apparent underweight in SZ patients and a significantly reduced average BMI despite adequate food supply for all patients (Fig. 1A), which observation was in concordance with previous reports from other countries (24, 25). The daily total calorie intake calculated from the dietary records in SZ patients was significantly reduced (Fig. 1B), mainly due to the almost halved protein intake (Fig. 1C) and proportion of calories provided by ingested protein (%P, Fig. 1D). This result suggested a general malnutrition status in SZ patients, especially the inadequate ingestion of food proteins.

Non-targeted metabolomics analysis for plasma and fecal samples were first performed to investigate the influence of the aberrant macronutrients on metabolism. Comparison of the plasma metabolism between cases and controls identified little differences related to protein catabolism, and among the 14 metabolites significantly altered (Wilcoxon rank-sum test, adj. $p < 0.05$, **Fig S1A**), only urea and α -hydroxyisobutyric acid (elevated in control samples) were products of protein catabolism. In contrast, the fecal metabolome revealed more extensively altered protein metabolism in SZ patients, and 12 of the 35 significantly altered metabolites (Wilcoxon rank-sum test, adj. $p < 0.05$, Fig. 1E) were products or related derivatives of protein catabolism. The most notable alterations were the elevated levels of the neuroactive dopamine and decrease in the neuroleptic γ -aminobutyric acid in SZ patients (Fig. 1F), both of which are metabolites derived from amino acids and important neurotransmitters in the pathogenesis of SZ.

We then performed metabolome-wide association studies (MWASs) using random forest-based machine learning variable selection techniques to identify fecal metabolite features that deviated SZ. The permutation importance of each metabolite showed that three out of the eight top-ranking metabolites favoring SZ were metabolites derived from amino acids, whereas none metabolites favoring control related to protein catabolism (**Fig S1B**). The apparent differences in fecal metabolism suggested potentially dysregulated protein metabolism in SZ patients, possibly the results of both aberrant protein ingestion and the intestinal microbiome dysbiosis.

To test the alteration in fatty acid metabolism in previous observation (26), we performed absolute quantification of the plasma and fecal levels of medium- and long-chain free fatty acids using GC-MS (gas chromatography-mass spectrometry). Our results confirmed significant deficiency in the plasma levels of some free fatty acids in SZ patients (Wilcoxon rank-sum test, adj. $p < 0.05$, **Fig S2**). However, the deficiency seemed not to stem from the intestinal metabolism as no difference in fatty acids was identified in the stool (Wilcoxon rank-sum test, adj. $p < 0.05$, **Fig S2**), given the fact that fats from food are primarily absorbed in the jejunum with little proportion delivered to the intestinal microbiota. Thus, the altered fatty acid profile in plasma is less likely associated with the intestinal microbiome dysbiosis.

Shifted gut microbial fermentation from carbohydrates to proteins in schizophrenia

To investigate how gut microbes participate in the metabolic disturbance in SZ, we performed shotgun-sequencing of the metagenome for all participants. In the analysis of differentially represented species (Wilcoxon rank-sum test, $p < 0.05$, **Table S1**), we noticed that 4/22 of the species enriched in patients with highest significance and fold-change were asaccharolytic, *i.e.*, *Fusobacterium mortiferum*, *Desulfovibrio piger*, *Phascolarctobacterium succinatutens*, and *Sutterella wadsworthensis*, whereas no asaccharolytic species enriched in controls. In the microbiome-wide association studies (MWASs) using random forest model to select taxonomic features of SZ, and the top three species of highest permutation importance in favor of SZ were all asaccharolytic (**Fig S3A**). In contrast, no asaccharolytic species were in the list of top permutation importance in favor of controls (**Fig S3A**). Furthermore, we combed all asaccharolytic species represented in our samples and found that most of them, especially those of higher abundance, were enriched in SZ patients, leading to the total abundance of asaccharolytic species significantly elevated in patients (Fig. 2A).

Comparison in metabolic pathways between cases and controls discovered that the total abundance of carbohydrate catabolism pathways, as well as two major ones, *i.e.*, starch degradation and anaerobic glycolysis, were significantly reduced in patients (**Fig S3B**). The reduction, when normalized by the proportion of calories provided by ingested carbohydrates in daily calorie intake (%C), was more remarkable (Fig. 2B), indicating the carbohydrate catabolism was hypoactive in SZ. The hypoactivity in microbial carbohydrate metabolism, together with the enrichment of asaccharolytic species in patients, implies reduced carbohydrate supply to intestinal microbes and hypoactive carbohydrate fermentation by them in the intestine of SZ patients.

Further, according to the MEROPS (database of proteolytic enzymes) and CAZymes (Carbohydrate-active enzymes database), we annotated all genes of peptidases and carbohydrate-active enzymes (CAZy), which account for the hydrolyzation of protein and carbohydrates into monomers. The initial comparison of their abundance between patients and controls resulted in no significant difference. However, when considering the great difference between the two groups in the ratio of protein to carbohydrate intake (Fig. 2C), the ratio between peptidase and CAZys was significantly reversed (Fig. 2D). Additionally, when normalized by the proportion of calories provided by ingested protein (%P) or carbohydrate (%C) in daily caloric intake, the abundance of peptidases was significantly increased in SZ (Fig. 2E), and reduced in CAZy (Fig. 2F).

To test whether more proteins were hydrolyzed in patients' gut, we performed targeted chromatographic assay to quantify the concentrations of all amino acids in the stool. The result exhibited that most amino acids' fecal concentrations increased in patients, and the total concentration of amino acids was significantly higher than controls, which were more significant when normalized by daily protein intake (Fig. 2G). Notably, we observed significantly elevated fecal concentrations of phenylalanine, tyrosine, and glutamine, which are neurotoxic at high levels in the brain (27) and neural-effective on enteric nerves (28, 29). All the above observations suggested that, in SZ patients, more undigested proteins reach the colon and are hydrolyzed by microbes there, instead of mostly being hydrolyzed and absorbed in the small intestine in normal conditions.

Enhanced microbial amino acid catabolism instead of protein biosynthesis in schizophrenia

Microbes are powerful in catabolizing amino acids into a great variety of derivatives (30, 31), many of which, including amines, NO, indole, kynurenine, quinolone, and so on, are neurologically active or affect human behavior through actions on the immune or endocrine system (32, 33). Key enzymes of amino acid catabolism fall into three categories that participate in the decarboxylation, transamination, and deamination of amino acids, respectively. We then compared their abundance and found that SZ patients harbored more enzymes in all three categories, and the difference became extremely significant when normalized by their protein intake (Fig. 3A).

Decarboxylases are the major enzymes in the generation of various amines and other derivatives. Using targeted chromatography, we quantitated the major decarboxylation derivatives of amino acids in stool and found that all derivatives were elevated in SZ patients, confirming the activated microbial decarboxylation of amino acids (Fig. 3B). Among these derivatives, indole, kynurenine, and IAA (indole-3-acetic acid), all derived from tryptophan, showed a significant increase in SZ, which was confirmed by the significantly elevated abundance of enzymes responsible for converting tryptophan into kynurenine and IAA as well (**Fig S4A**). As it has been reported that a variety of tryptophan metabolites are neurologically or immunologically active, the significant increase in tryptophan fermentation products in our study is concordant with some previous reports where tryptophan intake was associated with other brain

disorders (32, 34), supporting the potential roles of tryptophan and its metabolites in the pathogenesis of SZ.

Transaminases and deaminases, encoded by both host and microbes, account for catabolizing amino acids into ammonium and urea. The urea concentration, when quantitatively measured and normalized by protein intake, showed significantly elevated in stool in SZ patients but no difference in plasma, which indicated activated microbial production of urea in SZ (Fig. 3C). Microbial fermentation is the primary source of short-chain fatty acids (SCFAs), including branched SCFAs (BSCFAs) and straight-chain SCFAs. BSCFAs, including isobutyric and isovaleric acid, are only generated from transamination of branched-chain amino acids, *i.e.*, Ile, Leu, and Val (35), while straight-chain SCFAs derived from both amino acids (*i.e.*, lysine) and carbohydrates (*i.e.*, dietary fibers) (30). We then performed targeted chromatographic analysis to quantify the concentration of all SCFAs in both plasma and stool. The results showed that both fecal and plasma concentrations of BSCFAs significantly increased in patients (Fig. 3D). In contrast, no significant differences were detected in both plasma and fecal concentrations of SCFAs (Fig S4B), supporting the enhanced catabolism of amino acids in the gut of SZ patients.

To define the deviations in the metabolic profiles between SZ and controls, we utilized a recently developed method for metagenome analysis—Quasi-paired cohort (21). In the list of differential metabolic pathways identified by the Quasi-paired cohort (Wilcoxon signed-rank test for paired samples, $FDR < 0.001$, mean abundance in control $> 10^{-5}$, **Table S2**), among pathways enriched in controls, 8/31(26%) were in the category of amino acid biosynthesis, whereas none in this category overrepresented in SZ. Notably, we also compared the abundance of aminoacyl-tRNA synthases (EC 6.1.1) between the paired SZ-control samples, and 12 out of the 22 synthases were significantly enriched in controls (Wilcoxon signed-rank test for paired samples, $FDR < 0.05$) with the other ten enzymes showed no difference in-between. Comparing the total abundance of the 38 amino acid biosynthesis pathways and the 22 aminoacyl-tRNA transferases revealed extremely significant deficiency in the functional potential of protein synthesis in SZ patients (Fig. 3E).

We further constructed random forest classifiers based on the abundance of the 38 amino acid biosynthesis pathways and the 22 aminoacyl-tRNA transferases, respectively, and the models performed excellently in discriminating cases and controls and achieved AUC (area under the curve) of 0.85 and 0.91, respectively, when evaluated with ROC (receiver operating characteristic) curve (Fig. 3F). This result indicated that a shift from protein synthesis in normal conditions to protein catabolism was a major deviation in the intestinal metabolism in SZ patients.

Associations between gut protein fermentation and the impairment of social behaviors

Finally, we investigated whether the protein fermentation in SZ patients correlated to the severity of their psychiatric symptoms, which was reassessed by the PANNS scale (20) for all patients. For each patient, the total score of negative symptoms (N), positive symptoms (P), general symptoms (G), and total score of all symptoms (T) were calculated and used as indicators for the severity of the disease.

First, we investigated the correlations of amino acid catabolizing enzymes to clinical scores and observed that most enzymes exhibited positive correlations to N, G, and T, but slight negative correlations to P (Fig. 4A). Supportively, the fecal concentration of amino acids and their derivatives also presented similar correlations to clinical scores (Fig. 4B). The irrelevance of positive symptoms to intestinal protein fermentation is not surprising as many cofactors such as apastia (food refusal) and psychiatric medications might alleviate positive symptoms (36) and conceal the exacerbating effects of protein fermentation on them. These results suggested the association of intestinal microbial protein fermentation to the severity of psychiatric symptoms, especially the negative ones, which representing the unsolved central pathogenesis of SZ.

In concordance with the hypothesis that the enhanced intestinal protein fermentation might impair human behaviors, we even observed positive correlations of daily protein intake to N, G, and T, but not to P (Fig. 4C). In contrast, the daily carbohydrate intake showed no significant correlation to any psychiatric symptoms (**Fig S5**). Therefore, it is the food supply of excess proteins but not carbohydrates associated with severe symptoms, highlighting the role of macronutrient intake in the pathogenesis of SZ. In this point of view, the reduction in the daily protein intake in SZ patients might protect against harmful protein fermentation products from the gut in case of ordinary food supplies.

Discussion

Our findings uncovered an aberrant microbial metabolism in the gut of SZ patients characterized as a shift from carbohydrate fermentation to protein fermentation. Although the shift is also reported in other ill-conditions, such as constipation (37) and colon-rectal cancer (38), the deviation towards protein fermentation in SZ seems much more salient and correlated with the severity of symptoms in our study, suggesting its potential role in the pathogenesis of schizophrenia. Various metabolites generated from protein fermentation have been reported to be active in regulating neural, immune, or endocrine activities, and they often have detrimental effects on brain disorders via the gut-brain axis (32, 39). It is conceivable that the altered milieu of protein fermentation products in the intestinal lumen may send abnormal signals to the brain, which results in abnormalities in behaviors and arouse psychiatric symptoms in genetically susceptible individuals.

The intestinal microbes are genetically diverse in fermenting proteins and capable of producing a broad range of metabolites. Although previous studies have reported the effects of some specific microbial metabolites on behavior (11, 40), our clinical study only observed a general association of the catabolism of many amino acids, especially those of neurologically active, to the severity of psychiatric symptoms. It seems that molecular mechanisms of how protein catabolism involves in the pathogenesis of SZ are more complex or heterogeneous than currently understood.

Our findings also underscore the importance of nutrition care in SZ patients who often exhibit malnutrition characterized by inadequate protein intake. However, the food supply of common proteins seems detrimental to psychiatric symptoms. For unknown reasons, the digestion and absorption of

macronutrients in SZ patients is severely disordered, leading to an excess supply of undigested proteins and hyperactivated protein fermentation in gut microbiota. In this regard, the reduced protein intakes seems to bring a protective effect for SZ patients, although unintendedly. Therefore the nutrition care for SZ patients is fragile and paradoxical: maintain the supply of essential amino acids for the biosynthesis of neurotransmitters in one aspect and avoid excessive delivery of undigested proteins to the gut microbes in the other, a situation similar to that in hepatic encephalopathy.

Diet plan has been demonstrated critical in treating hepatic encephalopathy and seizure (41), and therapeutic strategies aiming to rectify the intestinal dysbiosis and callback the deviated gut signals to the brain seem promising (42). Relative strategies may include: increase carbohydrates that can only be digested by microbes, such as dietary fiber and lactulose, to flourish carbohydrate-fermenting microbes, and supply hydrolyzed proteins or amino acids instead of ordinary proteins to facilitate the absorption in the small intestine and reduce the delivery of undigested proteins to gut protein-fermenting species. However, nutrition care is still under investigation and attracts inadequate attention in the care of SZ patients (43). Given that abnormally enhanced protein fermentation participates in the pathogenesis of SZ, delicate diet management may improve the long-term prognosis and open a new avenue for the treatment of the disease.

Conclusion

In conclusion, our findings suggest a potential role of gut dysbiosis and enhanced protein fermentation in the pathophysiology of SZ, highlighting the importance of nutrition care in treating SZ patients and the potentials of microbiota-targeted therapeutics for SZ.

Materials And Methods

Subject recruitment

Schizophrenic patients in the age of 18 ~ 65 were recruited from the sanatorium and were reassessed with the Mini International Neuropsychiatric Interview (M.I.N.I.) according to the diagnostic criteria of the international classification of diseases (ICD-10). Healthy controls matched in age and gender were recruited from the faculty of the sanatorium or surrounding communities. Subjects in one or more of the following conditions were excluded: (1) was diagnosed as other mental disorders, or complicated with other chronic diseases; (2) had taken antibiotics, probiotics, prebiotics, glucocorticoids, immunosuppressants, and gastrointestinal examination in the past six months; (3) had a history of gastrointestinal or hepatobiliary surgery in recent five years; (4) BMI < 18.5 or > 27.9 kg/m²; (5) significantly changed dietary habits in recent month.

Clinical information collection

Clinical information was collected for each subject through questionnaires, which included gender, age, medical history, body height, and weight. Information on dietary in the past month was collected for all

subjects using the Food Frequency Questionnaire (FFQ) (19), adjusted to 117 items and 15 food types according to Chinese dietary habits. The questionnaire adopts a closed survey method, and each closed question provides nine answer options. Open-ended questions were added at the end of the questionnaire to gather information about other foods not listed. Daily intake of carbohydrates, protein, fat, and other nutrients content was calculated according to the standard amount consumed by food category and the total amount of food in the last month. The severity of psychiatric symptoms was reassessed using the Positive And Negative Symptom Scale (PANSS) (20) for each patient.

Fecal and plasma samples collection

For each subject, a fresh stool sample was collected in a clean and dry container and immediately transferred to the laboratory in the hospital. Each ~ 400 mg sample from the central part of the sample was transferred into a sterile tube in triplicate. The tubes were immediately put into liquid nitrogen for storage. Blood samples were collected in 1.5 mL heparin anticoagulant tubes, let stand at room temperature for 1 h, and then centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and separated in triplicate sterile tubes, then put into liquid nitrogen for storage. All samples were transported in liquid nitrogen and stored at -80°C.

Metagenome sequencing and annotation

The total DNA of the stool sample was extracted using EZNA® Stool DNA Kit (Omega Bio-Tek Inc.), and the concentration and purity of extracted DNA were assessed by Qubit 2.0 fluorometer (Invitrogen) and NanoDrop 2000 (Thermo Fisher Scientific). Paired-end libraries were constructed using a KAPA HyperPrep Kit (Roche) for Illumina platforms, and paired-end sequencing was performed on Illumina HiSeq X Ten platform (Annoroad, Beijing, China) at 150bp×2 and average 10Gb raw reads for each sample. Quality control, annotation of taxonomy profile and metabolic functions, and reconstruction of quasi-paired cohort were performed routinely or as previously described (21), refer to Supplementary Methods for details.

Metabolome analysis of fecal and plasma samples

The analysis of short-chain fatty acids, medium- and long-chain fatty acids, and non-targeted metabolome were performed following routine operations by Tinygene Bio-Tech (shanghai) Co. Ltd. For each subject, three aliquots of 100 µl plasma were respectively applied to the analysis of short-chain fatty acids, medium- and long-chain fatty acids, and non-targeted metabolome, and fecal samples (~ 400 mg) went for the same analyses after pretreatment of grinding and sonication. Refer to Supplementary Methods for details. For targeted fecal metabolomic detection, internal standard references of amino acids and related metabolites were purchased from Cambridge Isotope Laboratories (U.S), and the methods of metabolites extraction, instrument and data analyzing of target metabolomic detection was conducted as previously described (22) with modification, and refer to Supplemental Methods for details.

Statistical analysis

SPSS 19.0 was used to analyze clinical and dietary data. The results were expressed as mean $\pm$ standard deviation or percentage. The t-test (double-tailed) and the Wilcoxon rank-sum test were used to determine significant differences between the two groups, and the Chi-square test was used to compare the differences between the two groups for classification variables. P-value < 0.05 was considered to be significant.

The significance of the difference in nutrient intake, CAZys, peptidases, species, SCFAs, and metabolites were calculated based on the Wilcoxon rank-sum test. Differential pathways between quasi-paired cohorts were performed using the Wilcoxon signed-rank test for paired samples. Correlations of species, enzymes, metabolites, and symptom scores were conducted using Spearman's rho. Features selection of metabolites and species was performed using Boruta package (<https://cran.r-project.org/web/packages/Boruta/>) with the default parameters, in which a shuffled data matrix was built, and Random Forest classifier was used to compute the importance score for each feature in the original data and the shuffled data, and features were reported as important by comparing the importance scores (100 iterations, $p < 0.01$).

The abundance of 34 amino acid biosynthesis pathways or 22 aminoacyl-tRNA transferases were used to construct random forest classifiers with the packages of caret [<https://cran.r-project.org/web/packages/caret/>] and randomForest [<https://cran.r-project.org/web/packages/randomForest/>] in R. The model was trained with 2/3 of all samples and tested by the other 1/3 samples with 1000 times of bootstrapping. The performance of the model was evaluated by the Receiver operating characteristic curve (ROC) analysis with Scikit-learn v0.21.2 (https://scikit-learn.org/stable/whats_new/v0.21.html#version-0-21-2), and ROC curve was plotted using matplotlib 3.0.3. Curve fitting and statistical calculation were performed using ggplot2 (<https://ggplot2.tidyverse.org>) package. All these data procession and calculation were performed using R.

Declarations

Ethics approval and consent to participate: All experimental protocols were approved by the ethics committee of Gaizhou Renkang Hospital, a sanatorium for patients with mental illness (No. 201801). The study design complied with all relevant ethical regulations and aligned with the Declaration of Helsinki. All participants gave their informed consent.

Consent for publication: Not applicable

Availability of data and material: The raw metagenome sequencing data reported in this paper have been deposited in the Genome Sequence Archive in BIG Data Center, Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under accession numbers CRA003445 at <http://bigd.big.ac.cn/gsa/s/2gi5Ka06>.

Competing interests: The authors declare no competing interests.

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Authors' contributions: Y.L. and Y.K. conceived and designed the project. Y.L., Y.S., X.S., and Z.H. were in charge of participant enrollment and sample collection. Y.C., X.S., J.W. and C.S carried out DNA extraction, PCR and microbiome sequencing. X.S. and J.C. completed all the bioinformatic and statistical analyses of the microbiota. Y.K. and X.S. conducted data acquiring and processing as well as figure predations. Y.K. draft and revised manuscript. All the authors have revised and approved the manuscript submission.

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References

1. Charlson FJ, Ferrari AJ, Santomauro DF, Diminic S, Stockings E, Scott JG, McGrath JJ, Whiteford HA. Global Epidemiology and Burden of Schizophrenia: Findings From the Global Burden of Disease Study 2016. *Schizophr Bull.* 2018;44:1195-1203.
2. Marder SR, Cannon TD. Schizophrenia. *The New England journal of medicine.* 2019;381:1753-1761.
3. Stepnicki P, Kondej M, Kaczor AA. Current Concepts and Treatments of Schizophrenia. *Molecules.* 2018;23.
4. Dollfus S, Lyne J. Negative symptoms: History of the concept and their position in diagnosis of schizophrenia. *Schizophr Res.* 2017;186:3-7.
5. Correll CU, Schooler NR. Negative Symptoms in Schizophrenia: A Review and Clinical Guide for Recognition, Assessment, and Treatment. *Neuropsychiatr Dis Treat.* 2020;16:519-534.
6. Sarris J, Logan AC, Akbaraly TN, Paul Amminger G, Balanzá-Martínez V, Freeman MP, Hibbeln J, Matsuoka Y, Mischoulon D, Mizoue T, Nanri A, Nishi D, Parletta N, Ramsey D, Rucklidge JJ, Sanchez-Villegas A, Scholey A, Su KP, Jacka FN. International Society for Nutritional Psychiatry Research consensus position statement: nutritional medicine in modern psychiatry. *World psychiatry : official journal of the World Psychiatric Association (WPA).* 2015;14:370-371.
7. Lassale C, Batty GD, Baghdadli A, Jacka F, Sánchez-Villegas A, Kivimäki M, Akbaraly T. Healthy dietary indices and risk of depressive outcomes: a systematic review and meta-analysis of observational studies. *Molecular psychiatry.* 2019;24:965-986.
8. Psaltopoulou T, Sergentanis TN, Panagiotakos DB, Sergentanis IN, Kosti R, Scarmeas N. Mediterranean diet, stroke, cognitive impairment, and depression: A meta-analysis. *Annals of neurology.* 2013;74:580-591.
9. Visconti A, Le Roy CI, Rosa F, Rossi N, Martin TC, Mohny RP, Li W, de Rinaldis E, Bell JT, Venter JC, Nelson KE, Spector TD, Falchi M. Interplay between the human gut microbiome and host metabolism. *Nature communications.* 2019;10:4505.

10. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, Codagnone MG, Cussotto S, Fulling C, Golubeva AV, Guzzetta KE, Jaggar M, Long-Smith CM, Lyte JM, Martin JA, Molinero-Perez A, Moloney G, Morelli E, Morillas E, O'Connor R, Cruz-Pereira JS, Peterson VL, Rea K, Ritz NL, Sherwin E, Spichak S, Teichman EM, van de Wouw M, Ventura-Silva AP, Wallace-Fitzsimons SE, Hyland N, Clarke G, Dinan TG. The Microbiota-Gut-Brain Axis. *Physiological reviews*. 2019;99:1877-2013.
11. Johnson KV, Foster KR. Why does the microbiome affect behaviour? *Nature reviews Microbiology*. 2018;16:647-655.
12. Macciardi F, Lucca A, Catalano M, Marino C, Zanardi R, Smeraldi E. Amino acid patterns in schizophrenia: some new findings. *Psychiatry Res*. 1990;32:63-70.
13. He Y, Yu Z, Giegling I, Xie L, Hartmann AM, Prehn C, Adamski J, Kahn R, Li Y, Illig T, Wang-Sattler R, Rujescu D. Schizophrenia shows a unique metabolomics signature in plasma. *Transl Psychiat*. 2012;2.
14. Xu R, Wu B, Liang J, He F, Gu W, Li K, Luo Y, Chen J, Gao Y, Wu Z, Wang Y, Zhou W, Wang M. Altered gut microbiota and mucosal immunity in patients with schizophrenia. *Brain Behav Immun*. 2020;85:120-127.
15. Shen Y, Xu J, Li Z, Huang Y, Yuan Y, Wang J, Zhang M, Hu S, Liang Y. Analysis of gut microbiota diversity and auxiliary diagnosis as a biomarker in patients with schizophrenia: A cross-sectional study. *Schizophr Res*. 2018;197:470-477.
16. Zhu F, Ju Y, Wang W, Wang Q, Guo R, Ma Q, Sun Q, Fan Y, Xie Y, Yang Z, Jie Z, Zhao B, Xiao L, Yang L, Zhang T, Feng J, Guo L, He X, Chen Y, Chen C, Gao C, Xu X, Yang H, Wang J, Dang Y, Madsen L, Brix S, Kristiansen K, Jia H, Ma X. Metagenome-wide association of gut microbiome features for schizophrenia. *Nat Commun*. 2020;11:1612.
17. Zheng P, Zeng B, Liu M, Chen J, Pan J, Han Y, Liu Y, Cheng K, Zhou C, Wang H, Zhou X, Gui S, Perry SW, Wong ML, Licinio J, Wei H, Xie P. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. *Science advances*. 2019;5:eaau8317.
18. Zhu F, Guo R, Wang W, Ju Y, Wang Q, Ma Q, Sun Q, Fan Y, Xie Y, Yang Z, Jie Z, Zhao B, Xiao L, Yang L, Zhang T, Liu B, Guo L, He X, Chen Y, Chen C, Gao C, Xu X, Yang H, Wang J, Dang Y, Madsen L, Brix S, Kristiansen K, Jia H, Ma X. Transplantation of microbiota from drug-free patients with schizophrenia causes schizophrenia-like abnormal behaviors and dysregulated kynurenine metabolism in mice. *Mol Psychiatry*. 2019.
19. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol*. 1985;122:51-65.
20. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull*. 1987;13:261-276.

21. Zhang M, Chu Y, Meng Q, Ding R, Shi X, Wang Z, He Y, Zhang J, Liu J, Zhang J, Yu J, Kang Y, Wang J. A quasi-paired cohort strategy reveals the impaired detoxifying function of microbes in the gut of autistic children. *Science advances*. 2020;6:eaba3760.
22. Yuan M, Breitkopf SB, Yang X, Asara JM. A positive/negative ion-switching, targeted mass spectrometry-based metabolomics platform for bodily fluids, cells, and fresh and fixed tissue. *Nat Protoc*. 2012;7:872-881.
23. Gentile CL, Weir TL. The gut microbiota at the intersection of diet and human health. *Science*. 2018;362:776-780.
24. Bushe CJ, Slooff CJ, Haddad PM, Karagianis JL. Weight change by baseline BMI from three-year observational data: findings from the Worldwide Schizophrenia Outpatient Health Outcomes Database. *J Psychopharmacol*. 2013;27:358-365.
25. Sugai T, Suzuki Y, Yamazaki M, Shimoda K, Mori T, Ozeki Y, Matsuda H, Sugawara N, Yasui-Furukori N, Minami Y, Okamoto K, Sagae T, Someya T. High prevalence of underweight and undernutrition in Japanese inpatients with schizophrenia: a nationwide survey. *BMJ open*. 2015;5:e008720.
26. Davison J, O'Gorman A, Brennan L, Cotter DR. A systematic review of metabolite biomarkers of schizophrenia. *Schizophrenia research*. 2018;195:32-50.
27. Albrecht J, Norenberg MD. Glutamine: a Trojan horse in ammonia neurotoxicity. *Hepatology*. 2006;44:788-794.
28. McRoberts JA, Coutinho SV, Marvizon JC, Grady EF, Tognetto M, Sengupta JN, Ennes HS, Chaban VV, Amadesi S, Creminon C, Lanthorn T, Geppetti P, Bunnett NW, Mayer EA. Role of peripheral N-methyl-D-aspartate (NMDA) receptors in visceral nociception in rats. *Gastroenterology*. 2001;120:1737-1748.
29. Baj A, Moro E, Bistoletti M, Orlandi V, Crema F, Giaroni C. Glutamatergic Signaling Along The Microbiota-Gut-Brain Axis. *International journal of molecular sciences*. 2019;20.
30. Oliphant K, Allen-Vercoe E. Macronutrient metabolism by the human gut microbiome: major fermentation by-products and their impact on host health. *Microbiome*. 2019;7:91.
31. Portune KJ, Beaumont M, Davila A-M, Tomé D, Blachier F, Sanz Y. Gut microbiota role in dietary protein metabolism and health-related outcomes: The two sides of the coin. *Trends in Food Science & Technology*. 2016;57:213-232.
32. Agus A, Planchais J, Sokol H. Gut Microbiota Regulation of Tryptophan Metabolism in Health and Disease. *Cell Host Microbe*. 2018;23:716-724.
33. Bugda Gwilt K, Gonzalez DP, Olliffe N, Oller H, Hoffing R, Puzan M, El Aidy S, Miller GM. Actions of Trace Amines in the Brain-Gut-Microbiome Axis via Trace Amine-Associated Receptor-1 (TAAR1). *Cell Mol Neurobiol*. 2020;40:191-201.
34. Kanchanatawan B, Sirivichayakul S, Ruxrungtham K, Carvalho AF, Geffard M, Ormstad H, Anderson G, Maes M. Deficit, but Not Nondeficit, Schizophrenia Is Characterized by Mucosa-Associated Activation of the Tryptophan Catabolite (TRYCAT) Pathway with Highly Specific Increases in IgA Responses Directed to Picolinic, Xanthurenic, and Quinolinic Acid. *Molecular neurobiology*. 2018;55:1524-1536.

35. Allison MJ. Production of branched-chain volatile fatty acids by certain anaerobic bacteria. *Appl Environ Microbiol.* 1978;35:872-877.
36. Fusar-Poli P, Papanastasiou E, Stahl D, Rocchetti M, Carpenter W, Shergill S, McGuire P. Treatments of Negative Symptoms in Schizophrenia: Meta-Analysis of 168 Randomized Placebo-Controlled Trials. *Schizophr Bull.* 2015;41:892-899.
37. Roager HM, Hansen LB, Bahl MI, Frandsen HL, Carvalho V, Gobel RJ, Dalgaard MD, Plichta DR, Sparholt MH, Vestergaard H, Hansen T, Sicheritz-Ponten T, Nielsen HB, Pedersen O, Lauritzen L, Kristensen M, Gupta R, Licht TR. Colonic transit time is related to bacterial metabolism and mucosal turnover in the gut. *Nature microbiology.* 2016;1:16093.
38. Thomas AM, Manghi P, Asnicar F, Pasolli E, Armanini F, Zolfo M, Beghini F, Manara S, Karcher N, Pozzi C, Gandini S, Serrano D, Tarallo S, Francavilla A, Gallo G, Trompetto M, Ferrero G, Mizutani S, Shiroma H, Shiba S, Shibata T, Yachida S, Yamada T, Wirbel J, Schrotz-King P, Ulrich CM, Brenner H, Arumugam M, Bork P, Zeller G, Cordero F, Dias-Neto E, Setubal JC, Tett A, Pardini B, Rescigno M, Waldron L, Naccarati A, Segata N. Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation. *Nature medicine.* 2019;25:667-678.
39. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, Cryan JF. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A.* 2011;108:16050-16055.
40. Needham BD, Kaddurah-Daouk R, Mazmanian SK. Gut microbial molecules in behavioural and neurodegenerative conditions. *Nature reviews Neuroscience.* 2020.
41. Olson CA, Vuong HE, Yano JM, Liang QY, Nusbaum DJ, Hsiao EY. The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. *Cell.* 2018;173:1728-1741.e1713.
42. Firth J, Teasdale SB, Allott K, Siskind D, Marx W, Cotter J, Veronese N, Schuch F, Smith L, Solmi M, Carvalho AF, Vancampfort D, Berk M, Stubbs B, Sarris J. The efficacy and safety of nutrient supplements in the treatment of mental disorders: a meta-review of meta-analyses of randomized controlled trials. *World psychiatry : official journal of the World Psychiatric Association (WPA).* 2019;18:308-324.
43. Marx W, Moseley G, Berk M, Jacka F. Nutritional psychiatry: the present state of the evidence. *The Proceedings of the Nutrition Society.* 2017;76:427-436.

Figures

Figure 1

Malnutrition and dysregulated intestinal metabolism in SZ patients. A-D. Differences in BMI (A), daily calorie intake (B), protein intake (C), and the percentage of calories provided by ingested protein (%P) (D) between HC and SZ groups. E. Volcano plot of the differential metabolites in stool. F. Fecal concentrations of GABA and dopamine between HC and SZ groups. HC, healthy controls; SZ, patients with schizophrenia; GABA, γ-aminobutyric acid. *, $p < 0.05$, ***, $p < 0.001$, Wilcoxon rank-sum test.

Figure 2

Intestinal metabolism shift from carbohydrate fermentation to proteolysis in SZ patients. A. The abundance of each asaccharolytic species (left panel) and their sum (right panel) in HC and SZ samples. B. The relative abundance of each carbohydrate catabolism pathways (left panel) and their sum (right panel) in HC and SZ samples normalized by %P. C. The ratio of protein to carbohydrate intake in HC than SZ subjects. D. The ratio of peptidase to CAZys abundance in HC and SZ samples. E. and F. The total

abundance of peptidases normalized by %P (E.) and CAZys normalized by %C (F.) in HC and SZ samples. G. The fecal concentration of each amino acids and their sum (boxes on the far left) in HC and SZ samples normalized by daily protein intake. HC, healthy controls; SZ, patients with schizophrenia; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, Wilcoxon rank-sum test.

Figure 3

Enhanced amino acids catabolism in SZ patients. A. The relative abundance of each enzyme accounting for amino acid decarboxylation, transamination, and deamination (left panel) and the sum abundance of each category (right panel) normalization by %P in SZ and HC samples. The color in the heatmap is normalized by Z-score. B. The fecal concentrations of amino acids derivatives normalized by daily protein intake in SZ and HC samples. C. and D. Plasma and fecal concentrations of urea (C) and BSCFAs (D) normalized by daily protein intake in HC and SZ samples. E. The total abundance of amino acids biosynthesis pathways (left panel) and aminoacyl-tRNA synthases (right panel) in HC and SZ samples. ****, $p < 0.0001$, Wilcoxon signed-rank test. F. ROC curve of the diagnostic models based on the abundance of amino acids biosynthesis pathways (left panel) and aminoacyl-tRNA synthases (right panel). KYN, kynurenine; IAA, indole-3-acetic acid; IPA, indole-3-propionic acid; GABA, γ -aminobutyric acid; BSCFA, branched short-chain fatty acid. HC, healthy controls; SZ, patients with schizophrenia; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, Wilcoxon rank-sum test.

Figure 4

The correlation between microbial protein fermentation and the severity of psychiatric symptoms. A. The correlations between the relative abundance of amino acids catabolizing enzymes and the clinical score of total score of psychiatric symptoms (T), score of general symptoms (G), negative symptoms (N), and positive symptoms (P). B. The correlation between the fecal concentrations of amino acids and their derivatives and each of the clinical scores of psychiatric symptoms. The color bar indicates the rho value

of Spearman's rank correlation coefficient; red, positive correlation; blue, negative correlation. C. The correlation of daily protein intake with the clinical scores of psychiatric symptoms. R, Pearson correlation coefficient.

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