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Metabolomic Analysis of Biochemical Changes in the Plasma and Urine of First-Episode Neuroleptic-Naïve Schizophrenia Patients after Treatment with Risperidone

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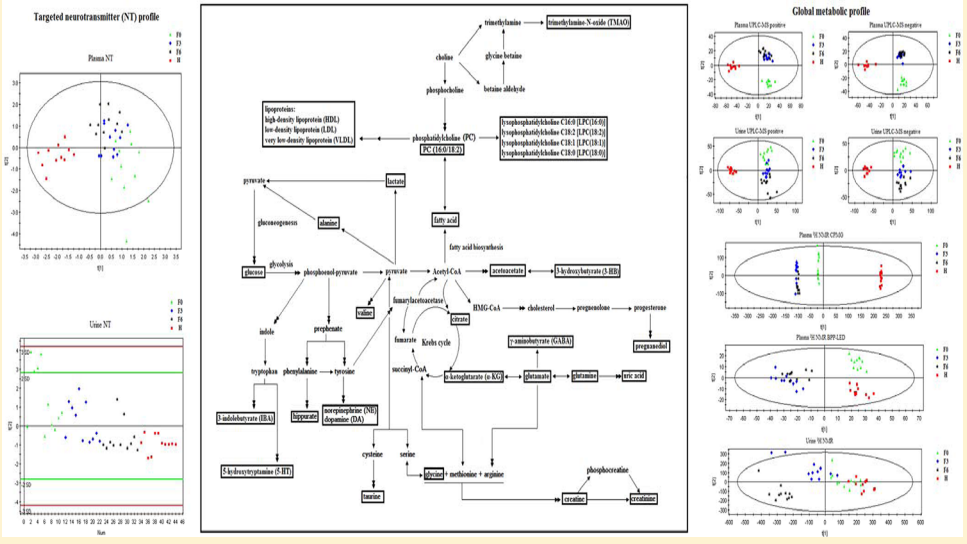
Supporting Information



ABSTRACT: Early findings propose that impaired neurotransmission in the brain plays a key role in the pathophysiology of schizophrenia. Recent advances in understanding its multiple etiologies and pathogenetic mechanisms provide more speculative hypotheses focused on even broader somatic systems. Using a targeted tandem mass spectrometry (MS/MS)-based metabolomic platform, we compared metabolic signatures consisting of monoamine and amino acid neurotransmitter (NT) metabolites in plasma/urine simultaneously between first-episode neuroleptic-naïve schizophrenia patients (FENNS) and healthy controls before and after a 6-week risperidone monotherapy, which suggest that the patient NT profiles are restoring during treatment. To detect and identify potential biomarkers associated with schizophrenia and risperidone treatment, we also performed a combined ultraperformance liquid chromatography−mass spectrometry (UPLC−MS) and ^{1}H nuclear magnetic resonance (NMR)-based metabolomic profiling of the same samples, indicating a further deviation of the patients' global metabolic profile from that of controls. The NTs and their metabolites together with the 32 identified biomarkers underpin that metabolic pathways including NT metabolism, amino acid metabolism, glucose metabolism, lipid metabolism, energy metabolism, antioxidant defense system, bowel microflora and endocrine system are disturbed in FENNS. Among them, pregnanediol, citrate and α-ketoglutarate (α-KG) were significantly associated with symptomatology of schizophrenia after Bonferroni correction and may be useful biomarkers for

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monitoring therapeutic efficacy. These findings promise to yield valuable insights into the pathophysiology of schizophrenia and may advance the approach to treatment, diagnosis and disease prevention of schizophrenia and related syndromes.

KEYWORDS: *schizophrenia, metabolomics, risperidone, biomarker, UPLC–MS/MS, NMR,*

INTRODUCTION

Schizophrenia is one of the most severe psychiatric disorders, affecting 1% of the population worldwide. Little is known about the biological mechanisms underlying the disease pathology in spite of overwhelming research in the field. Theories proposed by previous data have converged on neurotransmitters (NTs) and their receptors, and thus current therapeutic drug development has largely been targeted at dopamine, serotonin and glutamate systems. Despite the abundance of studies generally with an emphasis on investigating a single NT system, the list of reproducible findings, however, is limited. It is increasingly apparent that schizophrenia is better conceptualized as having "multineurotransmitter" pathologies since the NT systems are in fact dynamically linked. Given the significant contributions of NTs to neurological functioning, endocrinological and immunological actions, they have the potential to serve as clinically relevant biomarkers for specific disease state or to monitor treatment efficacy.[1] NTs exert primary functions within the central nervous system (CNS) and can be directly transported out of the CNS into the periphery.[1] In controlled conditions, peripheral (plasma or serum; urine) changes of NTs and their metabolites could reflect changes that occur in the brain,[2] either because the central contribution is sufficient to dominate a statistical relation to peripheral changes, or because the central and peripheral pools are correlated by systemic homeostatic mechanisms.[3] Therefore, NTs and related metabolites in the periphery should be investigated from a new holistic perspective to improve our understanding on the relationship among NT metabolism, pathophysiology of schizophrenia and response to therapy.

Traditionally, schizophrenia has been thought to be a disease merely localized in the CNS. However, recent findings and hypotheses provide the basis for an even wider "general systems" perspective on the pathophysiology of schizophrenia. Although metabolic abnormalities in schizophrenia can also be attributed to environmental determinants such as diet and lifestyle, especially intriguing is the growing number of findings indicating possible genetic underpinnings for general systems hypotheses.[4] Two of the hypotheses focused on insulin resistance and inflammation, and their interactions have become attractive for several reasons. The prevalence of diabetes in schizophrenia is 2–4 times higher than the general population rate. More than 40% of schizophrenia patients meet criteria for the metabolic syndrome, which is an important source of risk for diabetes and cardiovascular disease.[5] Additionally, the association between schizophrenia and metabolic disturbance can even present at first episode, prior to treatment,[6] suggesting that they may be an inherent part of the illness. Accumulating evidence from another angle is that widely used second-generation (atypical) antipsychotic medications worsen problems of metabolic dysregulation and disease,[7] accentuating concerns about their metabolic effects that were previously largely ignored. If such abnormalities are supported through further studies, they might encourage an enlarged conception of schizophrenia as a general somatic disorder, rather than only a brain disease. Thus, there is urgent need to scale-up knowledge in the research of schizophrenia in an attempt to understand at a systemic level the totality of biochemical changes that can contribute to the pathogenesis of the disorder.

Several metabolomic studies have been conducted in an attempt to better define metabolic perturbation in schizophrenia and its treatment,[8−10] either alone or in combination with other "-omic" platforms. These studies have identified a number of biochemical alterations that may be occurring as part of the pathogenesis of schizophrenia. However, there are several pitfalls and confounders that need to be dealt with in further studies before reliable conclusions can be made. First, almost all the metabolomic studies in schizophrenia have focused on clinical CSF or post-mortem brain tissue samples without considering the paralleled or consequential molecular changes in the periphery. Second, the majority of them have been performed using ^{1}H nuclear magnetic resonance (NMR) technology only. Last but not least, since much of the published results regarding molecular changes in schizophrenia have been obtained using samples taken from patients who have undergone various treatment at some stage of their lives, and different antipsychotics might have different mechanisms of action, one can not be sure that the alterations found are disease specific and not drug related or a consequence of chronic impairment. Therefore, longitudinal investigations on first-episode neuroleptic-naïve schizophrenia patients (FENNS) receiving antipsychotic monotherapy are needed to determine whether schizophrenia is associated with the development of certain metabolic abnormalities independently of medication and to distinguish disease biomarkers from specific treatment biomarkers. Risperidone (RIP), one of the most widely used first-line atypical antipsychotics, shows high efficacy in alleviating both positive and negative symptoms. However, the underlying molecular mechanisms of its drug action remain elusive.

Compared to CSF or tissue from CNS that is not readily accessible, plasma and urine can be routinely sampled with minimal discomfort to the patients. Furthermore, it has been suggested that for many neuropsychiatric diseases, the plasma and urine metabonome holds a great promise for the discovery of disease biomarkers.[1,11] One of the main reasons for this is that blood plasma circulates and is in dynamic molecular exchange with every tissue and organ in the whole body. Blood also carries molecules that mediate crosstalk between the brain and periphery. The circulating blood plasma is constantly filtered and concentrated by the kidneys to produce urine. Although a portion of the metabolites are biotransformed in the kidneys, some important metabolic clues in plasma regarding the disease can be amplified and become significant in urine. These media therefore can mirror both physiological and pathological processes of body in systemic level and are ideal for disease diagnosis and monitoring.

We hypothesized that various metabolic disturbances may present in FENNS and could be differentially altered by RIP antipsychotic medication. Thus we conducted a targeted NT profiling and a combined ultraperformance liquid chromatography–mass spectrometry (UPLC–MS) and ^{1}H NMR-based metabolomic study of plasma and urine from a cohort of FENNS treated with oral RIP for 6 weeks compared to matched controls.

MATERIALS AND METHODS

Subjects

We previously recruited 32 schizophrenia inpatients and 30 healthy volunteers to investigate their NT variations in response to RIP monotherapy.[12] In that group of patients, 11 were FENNS, and these and matched controls were selected for the metabolomic study. The selection criteria were briefly as follows: (1) DSM-IV-TR criteria for schizophrenia; (2) first time hospitalized for psychiatric illness and never exposed to neuroleptics; (3) without a history of substance abuse, chronic use of any other medication, CNS impairment, past or present neurological disease, or any other organic disorder; (4) aged 18−50 years (a criterion used for all patients including the FENNS and relapsed drug-free patients, but the final enrolled FENNS cohort is within a relatively young and narrow age range); and (5) from the same geographic region and of the same ethnic origin. The dose of RIP was increased progressively, and all the medications were stabilized at the target dose of 3−6 mg/d before week 3. For ethical reasons, alprazolam and benzhexol were allowed as adjunctive medications to treat anxiety and extrapyramidal side effects, respectively, when necessary. Because of the small patient sample size, controls were carefully selected to eliminate the usual confounding factors. Each patient was sex- and age-paired with a healthy volunteer enrolled from the same region and of the same ethnic origin. They were similar to the matched schizophrenia subjects in terms of their weight, height, BMI, social and economic status (of the status of their families). The controls were screened for personal or family history of psychopathology, serious medical illness, history of head trauma or history of drug/alcohol abuse. Additionally, all the participants were nonsmokers. The positive and negative syndrome scale (PANSS) was used for measuring symptom severity of patients with schizophrenia. The study protocol was approved by the ethics committee of the Second Xiangya Hospital of Central South University, and the written informed consent was obtained from the subjects or their legal guardians.

Plasma and Urine Samples

Whole blood samples were taken from all participants at around 7:00 a.m. using 10 mL sodium heparin vacutainer tubes, after at least 12 h of fasting and 15 min of rest. Plasma was isolated by centrifugation at 1200*g* for 10 min at 4 °C and then frozen at −80 °C before use. The participants were also instructed to collect their first morning midstream urine specimens at the same time. With no added preservative, the collected urine samples were immediately frozen at −80 °C pending analysis. The samples of patients were collected at baseline and weeks 3 and 6 during hospitalization. Since we initially monitored inter-individual changes over time in NTs and metabolic profiles of controls and found that they were not significant, the samples of healthy controls were collected only once following the same procedure. All samples were prepared and analyzed blindly and randomly.

Neurotransmitter Measurements

Given their unstable property and trace level presence in plasma and urine that is beyond the power of ultraperformance liquid chromatography−mass spectrometry (UPLC−MS) and ^{1}H nuclear magnetic resonance (NMR) scans, we utilized dansyl derivatization to significantly improve sensitivity in the multiple reaction monitoring (MRM) mode for quantifying these neurotransmitters (NTs) and their metabolites by MS/MS methods (for detailed information about the methodology, see Cai et al.[13,14]). The measured monoamine neurotransmitters consist of dopamine (DA), norepinephrine (NE), 5-hydroxytryptamine (5-HT) and their respective acidic metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), vanilmandelic acid (VMA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and 5-hydroxytindole-3-acetic acid (5-HIAA). The determined amino acid neurotransmitters in plasma include the most prevalent excitatory neurotransmitter glutamate (Glu) and its metabolite the most important inhibitory neurotransmitter γ-aminobutyrate (GABA). Their major precursor glutamine (Gln) was also considered in urine testing. The urine data were corrected by creatinine tested in the clinical laboratory.

Samples Preparation and UPLC−MS Analysis

To a 300 μL aliquot of plasma sample, 900 μL of cold acetonitrile was added for protein precipitation. After vortex-mixing and centrifugation at 4 °C for 10 min at 20000*g*, the supernatant was transferred into an autosampler vial maintained at 4 °C, and an aliquot of 5 μL was injected for UPLC−MS analysis. Prior to analysis, urine samples were thawed at room temperature and centrifuged at 20000*g* for 10 min at 4 °C. The supernatant liquid was placed in an autosampler vial kept at 4 °C, and a volume of 5 μL was injected into the UPLC system. Metabolic profiling of plasma or urine was conducted on ACQUITY UPLC system (Waters, Milford, MA, USA) coupled to a Micromass Quattro Premier XE tandem mass spectrometer (Waters, Manchester, U.K.). Chromatographic separation was performed on an ACQUITY UPLC HSS T3 column (2.1 × 50 mm, 1.8 μm) maintained at 40 °C. Gradient elution was achieved using the mobile phase consisting of water and acetonitrile with 0.1% formic acid in each. The gradient elution programs for plasma and urine are shown in Supporting Information Table S1. MS data were collected with a Micromass Quattro Premier XE mass spectrometer equipped with an electrospray ionization (ESI) interface operating in both positive and negative ion modes. The following parameters were set: source temperature of 120 °C and desolvation temperature of 400 °C, capillary voltage of 3.0 and 2.6 kV for positive and negative ionization modes, respectively, and cone voltage of 35 V. Nitrogen was used as the desolvation and cone gas at the flow rate of 750 and 50 L/h, respectively. Full scan mode was employed in the mass range of m/z 100−1000 amu in each assay. In the MS/MS experiments, argon was used as the collision gas and collision energy was altered between 10 and 40 eV. NaCsI was used for mass correction before and periodically during the study. Data were acquired in centroid mode.

Samples Preparation and ^{1}H NMR Experiments

All NMR experiments were performed on a Varian INOVA 600 MHz spectrometer (Varian, Inc.) operating at 300 K. The free induction decay (FID) data were collected with 64 scans and a spectra width of 8000 Hz digitized into 32 000 data points using standard 1D pulse sequence with water presaturation suppression, resulting in an acquisition time of 2 s. Saturation of water signal was applied during the relaxation delay of 2 s and the mixing time of 150 ms.

Plasma. Plasma samples were prepared by mixing 300 μL of plasma with 200 μL of deuterated water (D_2O), used as field frequency lock solvent, and 100 μL of 3-trimethylsilyl-$^2H_4$-propionic acid sodium salt (TSP) in D_2O (1 mg/mL), which served as internal chemical shift reference. After viciously vortex-mixing, samples were centrifuged at 13000*g* for 10 min, and the supernatants were transferred into 5 mm NMR tubes. ^{1}H NMR analyses of the plasma samples were carried out using the Carr−Purcell−Meiboom−Gill (CPMG) spin−echo pulse sequence

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with a total spin−spin relaxation delay of 320 ms, to emphasize the resonances from low molecular weight metabolites.[15] The FIDs were zero-filled to double size and multiplied by an exponential line-broadening factor of 0.5 Hz before Fourier transformation. Moreover, in order to observe the lipid contents of lipoproteins in plasma, diffusion-edited experiments were also carried out with bipolar pulse pair-longitudinal eddy current delay (BPP-LED) pulse sequence.[16] The gradient amplitude was set at 35.0 G/cm with a diffusion delay of 100 ms. A line-broadening factor of 3 Hz was applied to BPP-LED data sets prior to Fourier transformation.

Urine. Urine samples were produced by buffering 350 μL of urine with 350 μL of sodium phosphate buffer (0.2 M, pH = 7.4) in D_2O. The samples were then vortex-mixed and centrifuged at 13000*g* for 10 min. A volume of 600 μL of the supernatant was transferred into a 5 mm NMR tube containing 30 μL of TSP (1 mg/mL) for analysis. The ^{1}H NMR spectra of urine were collected using the first increment of a one-dimensional nuclear Overhauser effect spectroscopy (1D NOESY) pulse sequence with the relaxation delay of 2 s and the mixing time of 150 ms to achieve saturation of the water resonance. For each sample, 64 transients were collected into 32 000 data points using a spectral width of 8000 Hz. The FID signals were zero-filled and amplified by a line-broadening factor of 0.5 Hz before Fourier transformation.

Data Processing and Multivariate Analysis

NT Data. The quantified concentrations of NTs and related metabolites in plasma or urine were saved as Excel data and imported into SIMCA-P software package (V12.01, Umetrics, Umea, Sweden) for multivariate analysis. The data were unit variance scaled prior to partial least-squares discriminant analysis (PLS-DA), which was used to find systemic differences in NT profiles among the intervention and control groups (FENNS groups: F0, F3 and F6; healthy control group: H).

UPLC−MS Data. The raw data were analyzed using the Micromass Markerlynx applications manager within Masslynx software (V4.0, Waters). This application manager allows the detection of retention time and m/z data pairs, and intensity of the peaks eluted in each chromatogram. All data were normalized to the summed total ion intensity per chromatogram to obtain the relative intensities of metabolites. The resultant 3-dimensional data of peak number (retention time and m/z pairs), sample name and normalized ion intensity were introduced into SIMCA-P for multivariate analysis. To optimize the clustering of samples, the data were then prefiltered by orthogonal signal correction (OSC) to remove the confounding variation not related to group membership. Pareto scaling and further PLS-DA models were calculated using these filtered data.

^{1}H NMR Data. All acquired ^{1}H NMR spectra were manually phased, baseline-corrected and chemical shift referenced to the doublet signal at 1.33 ppm due to methyl group of lactate. For the CPMG spectra of plasma, each spectrum over the range of δ 0.4−4.4 was data-reduced into integrated regions of equal width (0.01 ppm). For the plasma spectra obtained using BPP-LED sequence, each spectrum over the range of δ 0−6.0 was segmented into regions of 0.04 ppm wide and integrated across the spectral regions. The region (δ 4.6−5.0) containing the resonance of residual water was excluded. For the spectra of urine, the spectral region between δ 0.2−9.4 was reduced into integrated regions of equal width (0.04 ppm), excluding the region δ 4.6−6.2 surrounding the residual water signal and the broad resonance from urea. The integral value of each spectral region was normalized to the constant sum of all intergrals in each spectrum to avoid any significant concentration differences between samples. The resulting normalized integrated data were imported into SIMCA-P and further preprocessed using OSC. The preprocessed data were mean-centered or Pareto-scaled and then subjected to PLS-DA.

For PLS-DA and OSC-PLS-DA modeling in the SIMCA-P+ software, the components necessary for modeling were automatically calculated by the software. A default 7-round cross-validation was applied to prevent overfitting, with 1/7th of the samples being excluded from the mathematical model in each round. Also, a 200 random permutations test was also performed to further validate the supervised model. The calculated R^2Y(cum) estimates the goodness of fit of the model that represents the fraction of explained Y-variation, and Q^2(cum) estimates the ability of prediction. In general, reliable models are obtained when the cumulative values of Q^2 are no less than 0.4.[17] The variable importance in the projection (VIP) values of all the peaks from 7-fold cross-validated OSC-PLS-DA model were taken as a coefficient for peak selection. VIP ranks the overall contribution of each variable to the OSC-PLS-DA model, and those variables with VIP > 1.0 were considered relevant for group discrimination.

Univariate Analysis

In parallel, univariate statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) to validate those major contributing variables from the OSC-PLS-DA models. The metabolites winnowed as contributing to differences between groups using multivariate analysis were quantified as relative ion intensities (UPLC−MS data) or integral values (^{1}H NMR data) specific for each metabolite in the normalized spectra. Because of the small numbers of subjects in the sample groups and the non-normally distributed variances, nonparametric Mann−Whitney U tests were employed to reveal the significant differences of identified metabolites between two independent groups (i.e., F0 vs H), while the Wilcoxon signed-rank tests were used for comparisons between two related groups (i.e., F6 vs F0). A change trend was defined at $p < 0.05$. Furthermore, a Bonferroni adjustment was applied to acquire the level of significance for multiple testing (given that we have 32 comparisons, the adjusted $\alpha = 0.05/32 = 0.00156$). Two-tailed Spearman rank correlation analyses were conducted to examine the relationships between the identified metabolites and psychiatric rating scores, with a Bonferroni corrected significance level of 0.00156 (32 potential biomarkers selected by multivariate methods, 0.05/32 = 0.00156). Correlations between biochemical changes and changes in symptomatology over time were also examined.

RESULTS

Demographic and Clinical Characteristics

The demographic data and clinical characteristics of the subjects are shown in Table 1. No significant changes from baseline in weight and body mass index (BMI) were observed at the end of RIP treatment. The fasting plasma glucose levels were decreased at baseline (2-tailed Mann−Whitney U test, $z = -2.04$, $p = 0.042$) but increased (2-tailed Wilcoxon signed-rank test, $z = -2.27$, $p = 0.023$) after RIP treatment. In addition, the fasting plasma insulin concentrations were higher in all groups of patients as compared to controls (2-tailed Mann−Whitney U test, F0 vs H: $z = -3.19$, $p = 0.001$; F3 vs H: $z = -3.71$, $p < 0.0001$; F6 vs H: $z = -3.71$, $p < 0.0001$). However, no significant

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Table 1. Demographic and Clinical Information of Study Participants[a]

	schizophrenia (n = 11)	control (n = 11)
sex (male/female)	6/5	6/5
age (years)	27.6 ± 9.5	27.6 ± 9.5
height (m)	1.64 ± 0.07	1.63 ± 0.05
duration of illness (years)	0.8 ± 0.6	
dose of risperidone (mg/d)	3.5 ± 0.5	
dose of alprazolam (mg/d)	0.3 ± 0.3	
dose of benzhexol (mg/d)	3.1 ± 2.1	

	schizophrenia (n = 11)			
	week 0	week 3	week 6	control (n = 11)
weight (kg)	56.3 ± 5.7	56.5 ± 5.7	56.7 ± 5.4	55.7 ± 3.7
body mass index (BMI, kg/m^2)	21.0 ± 1.7	21.0 ± 1.7	21.1 ± 1.6	21.1 ± 0.7
fasting plasma glucose (mmol/L)	4.6 ± 0.9	4.8 ± 1.2	5.0 ± 0.5	5.1 ± 0.3
fasting plasma insulin (mU/L)	10.9 ± 1.4	10.7 ± 0.9	10.9 ± 1.5	9.0 ± 0.6
PANSS subscales				
positive	17.6 ± 2.3	12.4 ± 2.5	7.0 ± 2.2	
negative	14.3 ± 7.2	9.8 ± 5.5	5.5 ± 3.6	
general psychopathology	20.6 ± 8.2	13.6 ± 6.6	8.3 ± 5.5	
PANSS subclusters				
anergia	5.4 ± 2.3	3.9 ± 2.3	2.0 ± 1.7	
thought disturbance	9.2 ± 1.5	7.2 ± 1.5	3.4 ± 2.1	
activation	2.5 ± 2.1	1.7 ± 1.7	0.9 ± 1.0	
paranoid/belligerence	7.9 ± 1.4	5.4 ± 1.0	3.2 ± 1.2	
depression	2.4 ± 3.5	1.5 ± 2.8	0.9 ± 1.7	
PANSS total	55.6 ± 16.8	38.9 ± 13.1	22.6 ± 11.2	

[a]Values are given as mean ± SD.

discrepancy was found between F6 and F0 in fasting plasma insulin.

Targeted NT Profiling

The plasma and urinary levels of NTs and their metabolites in the subjects are included in Supporting Information Table S2. PLS-DA was used to determine whether separations according to diagnostic group and treatment response can be achieved on the basis of the panel of differentially expressed NTs and their metabolites in the subjects. For plasma NT profiling of total samples, patients showed good separation from healthy controls as depicted by the PLS-DA model (Figure 1A and B), whether the patients were medicated or not. In contrast, the urine PLS-DA model for separating patients and controls was not as reliable as that constructed from plasma data (Figure 1C and D). We also produced PLS-DA models from the data of F0 vs H and F6 vs F0, seeking to identify which targeted NTs were more responsible for the differentiation according to disease per se or treatment response. The results are provided as corresponding score and loading plots in Supporting Information Figure S1.

Multivariate Analysis of UPLC–MS Data

Full scan of plasmatic or urinary metabolites was set in both positive and negative ion modes of mass spectrometry, which gave more information-rich data than a single mode. Supporting Information Figure S2 shows the positive and negative ion base peak intensity (BPI) chromatograms of representative plasma and urine samples from a healthy control. OSC-PLS-DA was employed to find out whether RIP influenced the metabolic pattern of schizophrenia patients and to identify significant metabolite changes (i.e., potential biomarker). For plasma data set, the first two components from the resulting OSC-PLS-DA model (Figure 2A and C) successfully separated the healthy control, drug-naïve (F0) and medicated patients (F3 and F6). From the first two components of urine OSC-PLS-DA, a clear separation between the schizophrenia patients and controls was observed, whereas the three variant stages of patients group were partially separated (Figure 2E and G). The possible biomarkers responsible for separation were interpreted by corresponding loading plots (Figure 2B, D, F and H). In order to clarify disease-specific and treatment-related biomarkers, respective OSC-PLS-DA models for group comparisons (F0 vs H; F6 vs F0) were also constructed (Supporting Information Figure S3). The MS/MS experiments were implemented to produce the fragmentation patterns of altered metabolites so as to recover their structural information. The procedure of biomarker identification was described in the Supporting Information Methods section briefly. The significant variables detected using UPLC–MS and their variation tendencies in comparisons are summarized in Table 2.

Multivariate Analysis of ^{1}H NMR Data

Typical ^{1}H NMR spectra of patient samples are shown in Supporting Information Figure S4. The results of OSC-PLS-DA of the ^{1}H NMR data of plasma and urine samples from all groups are shown in Figure 3. For plasma metabolic profiling, the score plots of CPMG and BPP-LED data showed similar clustering pattern, and the schizophrenia patients were clearly separated from controls and classified according to whether they were medicated or not (Figure 3A and C). On the other hand, OSC-PLS-DA of ^{1}H NMR data of urine samples only resulted in marginal separation of group F0 from H or F3, whereas the location of group F6 was remote from the others (Figure 3E). OSC-PLS-DA models for comparisons of F0 vs H and F6 vs F0 were further utilized to determine the specificity of metabolites in disease and drug effects (Supporting Information Figure S5). On the basis of published studies,[18,19] significant metabolites revealed by loadings were identified and labeled in the spectra, and their change trends are also listed in Table 2.

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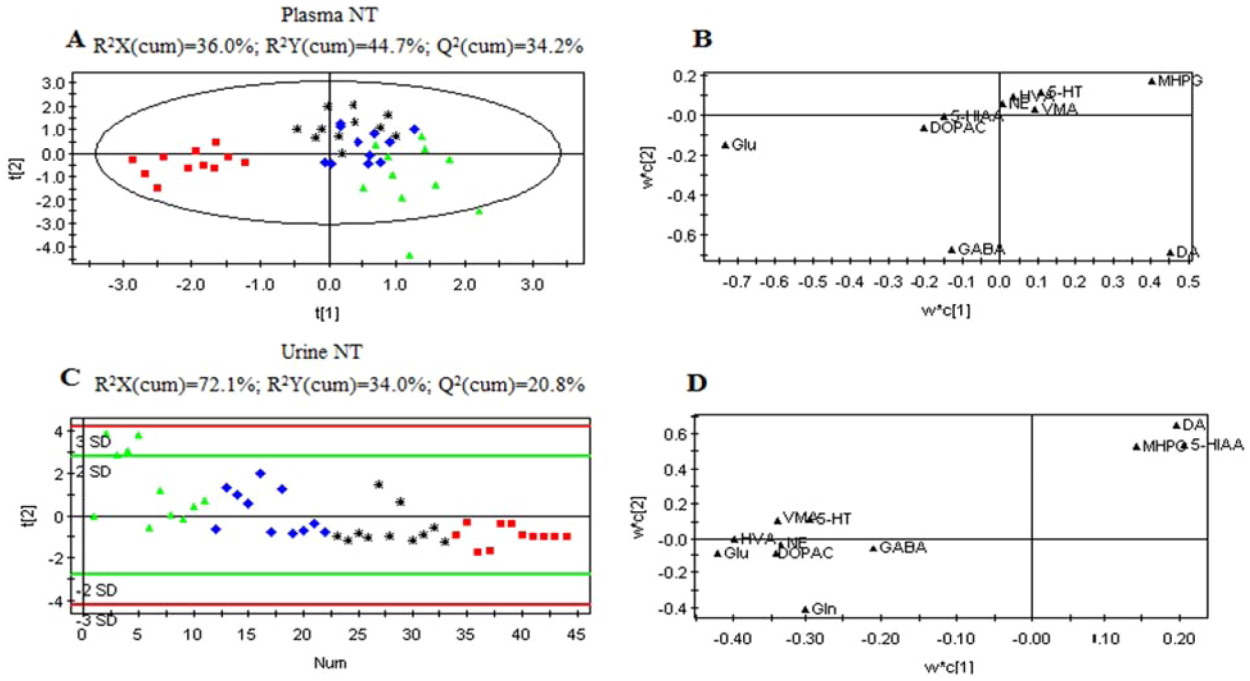

Figure 1. Partial least-squares discriminant analysis (PLS-DA) based on the neurotransmitter (NT) data of plasma and urine from the subjects. Plots on the left show the separation for the PLS-DA scores of first-episode neuroleptic-naïve schizophrenia patients (FENNS: F0, 3 and 6) vs healthy controls (H) for (A) plasma and (C) urine. Plots on the right represent the corresponding PLS-DA loading plots for (B) plasma and (D) urine. DA, dopamine; NE, norepinephrine; 5-HT, 5-hydroxytryptamine, DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; VMA, vanilmandelic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; 5-HIAA, 5-hydroxytindole-3-acetic acid; Gln, glutamine; Glu, glutamate; GABA, γ-aminobutyrate. (The group labels: H ■ (red), F0 ▲ (green), F3 ◆ (blue), F6 ∗ (black).)

Virtually all the differential metabolites associated with the division of schizophrenia, and healthy controls are also accountable for the pharmacological effects of RIP except PC (16:0/18:2) and pregnanediol, indicating a significant impact of RIP treatment on the global metabolism. Although only the changes of LPC (16:0) and hippurate remain significant after applying a stringent and conservative Bonferroni adjustment, the panel of biomarkers can indeed shed light on the pathogenesis of schizophrenia as well as the pharmacological mechanism in RIP therapy. Figure 4 provides an overview of these identified potential biomarkers and changed relevant metabolic pathways in FENNS.

Correlation Analysis

Some of the metabolic markers were validated against the clinical assessment scale PANSS. After Bonferroni correction, only the correlations of elevations in urinary pregnanediol with improvements in activation symptom cluster ($r = -0.832$, $p = 0.001$), alterations of urinary citrate with changes in depression subscores ($r = -0.842$, $p = 0.001$) and alterations of α-ketoglutarate (α-KG) with changes in anergia subscores ($r = 0.836$, $p = 0.001$) remained significant. Moreover, a significant correlation between urinary α-KG and negative symptoms was found throughout the observation (week 0: $r = -0.861$, $p = 0.001$; week 3: $r = -0.884$, $p < 0.0001$; week 6: $r = -0.843$, $p = 0.001$). No other correlations approached significance.

■ DISCUSSION

To the best of our knowledge, our study is the first work that has systematically assessed, through a combined UPLC−MS and ^{1}H NMR-based metabolomic study, the biochemical perturbations of the plasma and urine samples from FENNS receiving a 6-week RIP monotherapy and matched normal controls. Our results add to the existing knowledge, and further demonstrate the antecedent manifestation of metabolic disturbance in drug-naïve patients with first-episode illness and the significance that RIP atypical antipsychotic medication has when it comes to the modulation on neurotransmission and the development and progression of the metabolic syndrome.

Biochemical Interpretation

NT Imbalance. The patient NT profiles approached to that of controls during RIP antipsychotic treatment (Figure 1A and C), suggesting that RIP may exert regulatory effects partly by normalizing the overall NT metabolism, and restoration of the NT profiles may also be monitored in parallel with the amelioration of psychotic symptoms. It is noteworthy that the noradrenergic variables (norepinephrine, NE; vanilmandelic acid, VMA; 3-methoxy-4-hydroxyphenylglycol, MHPG) largely attributable to disease state did not seem to be affected by RIP treatment (Supporting Information Figure S1), once this one bond to α_1- and α_2-noradrenergic receptors, among others. Whether this characteristic is unique to RIP or universal for all atypical antipsychotics still needs future verification. However, it at least supports the proposal that development of new agents specifically targeting brain noradrenergic mechanisms may be a promising direction for developing novel treatments for schizophrenia.[20]

Amino Acid Metabolism Perturbation. Smeraldi et al.[21] have reported increased concentrations of various amino acids in schizophrenic patients and addressed the possibility of heterozygosity for amino acid disorder in schizophrenia. Accordingly, we found that plasma alanine, glycine and urine valine, glycine levels were increased in the FENNS at baseline measured by ^{1}H NMR. Alanine is directly involved in gluconeogenesis and the alanine−glucose cycle, functioning as an important participant in

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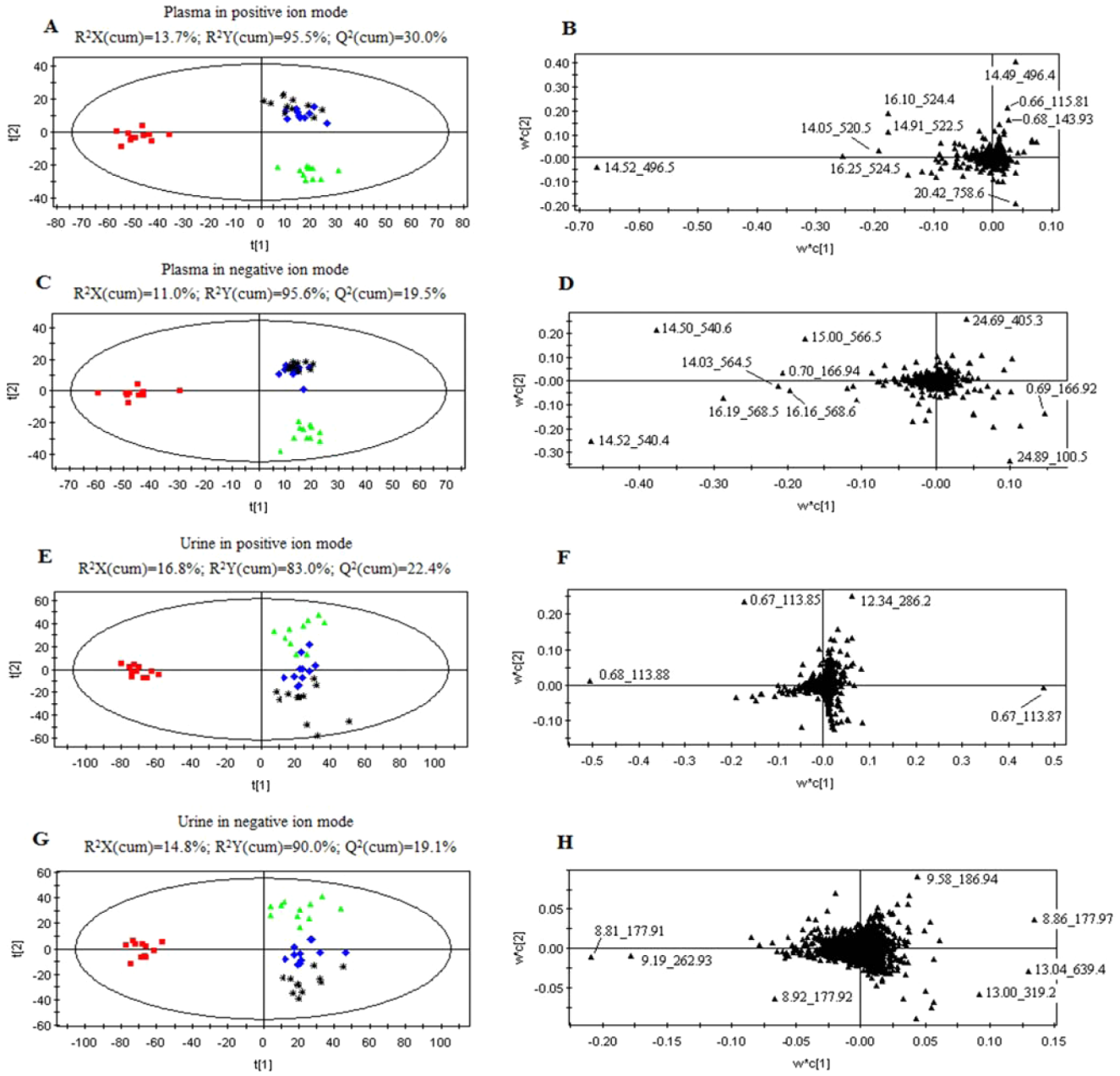

Figure 2. Score plots (A, C, E and G) and corresponding loading plots (B, D, F and H) derived from orthogonal signal correction partial least-squares discriminant analysis (OSC-PLS-DA) of ultraperformance liquid chromatography−mass spectrometry (UPLC−MS) data in positive and negative ion modes from the plasma/urine of first-episode neuroleptic-naïve schizophrenia patients (FENNS: F0, 3 and 6) and healthy controls (H). (The group labels: H ■ (red), F0 ▲ (green), F3 ◆ (blue), F6 ∗ (black).)

glucose metabolism. The fact that plasma alanine levels inversely paralleled plasma glucose levels may indicate an obstructed glucose generation in the FENNS. Notably, increased expression of proteins associated with the glycolysis pathway has been demonstrated in FENNS.[22] An increased glycolysis may also aggravate hypoglycemia and elevate levels of valine and glycine.[23] On the other hand, glycine is a coagonist with glutamate for NMDA receptors and can be beneficial in the management of schizophrenia.[24] Chronically medicated schizophrenia patients have also shown higher plasma glycine levels than controls.[25] Although the mechanism is unclear, 6-week RIP therapy markedly increased plasma glycine, and it may be related to the improvements in psychosis.

Glucose Metabolism Impairment. The fasting plasma glucose level of the present cohort of FENNS was relatively lower than that of controls partly because of increased glycolysis. Another possible cause is that elevated plasma insulin, which was repeatedly proved in FENNS,[6,22] can stimulate glucose uptake before disease manifestation. A 25-year follow-up study showed that insulin secretion tended to be increased rather than decreased in the prediabetic phase and was appropriate for the level of insulin resistance.[26] Therefore, it is presumed that elevated insulin may also be responsible for normoglycemia or even relative hypoglycemia in schizophrenia patients at an early stage of their first episode. It is out of expectation that elevated urine glucose was found coincided with reduced level of plasma glucose since the kidneys usually respond to high levels of

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Table 2. Biomarkers Identified by UPLC−MS/MS (A) and ^{1}H NMR (B) and Their Change Trends[a]

(A) UPLC−MS/MS		*m/z*			relative intensity[c]				group comparison	
matrix	retention time (min)	positive	negative	metabolites	F0	F3	F6	H	F0 vs H (*z* and *p* values)[d]	F6 vs F0 (*z* and *p* values)[e]
plasma	0.7	143.9, 115.8		IBA fragments	18.3 ± 3.2	20.1 ± 4.8	28.3 ± 3.2	10.8 ± 2.6	↑ ($z = -2.01$, $p = 0.045$)	↑ ($z = -2.40$, $p = 0.017$)
	14.5	496.5	540.5	LPC (16:0)[b]	346 ± 18.0	365 ± 21.1	390 ± 23.6	199 ± 17.2	↑* ($z = -3.84$, $p < 0.0001$)	↑ ($z = -2.46$, $p = 0.014$)
	14.1	520.5	564.5	LPC (18:2)[b]	195 ± 12.0	205 ± 17.5	233 ± 14.4	143 ± 8.1	↑ ($z = -3.12$, $p = 0.002$)	↑ ($z = -2.20$, $p = 0.028$)
	15.0	522.5	566.6	LPC (18:1)[b]	120 ± 6.8	135 ± 8.0	169 ± 11.8	93.8 ± 4.0	↑ ($z = -2.59$, $p = 0.009$)	↑ ($z = -2.01$, $p = 0.045$)
	16.3	524.5	568.5	LPC (18:0)[b]	122 ± 10.2	143 ± 13.1	176 ± 6.6	86.3 ± 5.5	↑ ($z = -2.60$, $p = 0.009$)	↑ ($z = -2.02$, $p = 0.045$)
	20.4	758.6		PC (16:0/18:2)	28.1 ± 3.6	41.3 ± 4.7	56.7 ± 4.7	36.9 ± 4.1	↓ ($z = -2.08$, $p = 0.038$)	↑ ($z = -2.67$, $p = 0.008$)
	0.7		166.9	uric acid	98.4 ± 6.7	80.1 ± 10.3	71.1 ± 5.1	122 ± 15.7	↓ ($z = -2.27$, $p = 0.023$)	↓ ($z = -2.05$, $p = 0.041$)
urine	0.7	113.9		creatinine	95.1 ± 11.9	81.0 ± 19.0	72.9 ± 18.3	147 ± 28.9	↓ ($z = -2.07$, $p = 0.039$)	↓ ($z = -2.00$, $p = 0.045$)
	1.4		166.9	uric acid	28.2 ± 3.1	34.5 ± 5.1	52.5 ± 10.4	19.2 ± 4.1	↑ ($z = -2.13$, $p = 0.033$)	↑ ($z = -2.52$, $p = 0.012$)
	8.8		177.9	hippurate	63.5 ± 26.9	65.7 ± 17.5	70.0 ± 38.7	334 ± 40.2	↓* ($z = -3.84$, $p < 0.0001$)	− ($z = -0.800$, $p = 0.424$)
	13.0		639.4, 319.2	pregnanediol	41.3 ± 11.0	58.4 ± 27.6	77.2 ± 25.0	26.3 ± 2.9	↑ ($z = -2.13$, $p = 0.033$)	↑ ($z = -2.61$, $p = 0.009$)

(B) ^{1}H NMR				relative integral[c]				group comparison	
matrix	pulse sequence	chemical shift (ppm)	metabolites	F0	F3	F6	H	F0 vs H (*z* and *p* values)[d]	F6 vs F0 (*z* and *p* values)[e]
plasma	CPMG	0.84−0.90, 1.26−1.30	lipoprotein	458 ± 41.4	573 ± 70.1	639 ± 48.8	508 ± 44.0	↓ ($z = -2.08$, $p = 0.038$)	↑ ($z = -2.22$, $p = 0.026$)
		1.19−1.20	3-HB	33.2 ± 4.6	21.5 ± 3.6	15.1 ± 2.2	51.2 ± 17.7	↓ ($z = -2.53$, $p = 0.011$)	↓ ($z = -2.76$, $p = 0.006$)
		1.32−1.34, 4.09−4.10	lactate	1.19e^3 ± 81.4	1.24e^3 ± 103	1.33e^3 ± 125	855 ± 49.2	↑ ($z = -2.59$, $p = 0.009$)	↑ ($z = -2.43$, $p = 0.015$)
		1.46−1.48	alanine	225 ± 14.1	221 ± 16.1	226 ± 13.6	202 ± 5.7	↑ ($z = -1.99$, $p = 0.047$)	− ($z = -0.533$, $p = 0.594$)
		2.22	acetoacetate	22.0 ± 3.5	10.8 ± 2.1	8.6 ± 2.7	28.5 ± 6.4	↓ ($z = -2.43$, $p = 0.015$)	↓ ($z = -2.67$, $p = 0.008$)
		3.40−4.00	glucose	4.41e^3 ± 243	4.53e^3 ± 207	4.67e^3 ± 86.5	4.75e^3 ± 63.2	↓ ($z = -2.01$, $p = 0.045$)	↑ ($z = -2.18$, $p = 0.029$)
		3.55	glycine	51.8 ± 11.3	67.0 ± 9.8	102 ± 10.0	30.9 ± 4.3	↑ ($z = -2.53$, $p = 0.011$)	↑ ($z = -2.85$, $p = 0.004$)
	BPP-LED	0.82, 0.86	LDL	804 ± 32.4	924 ± 17.8	928 ± 16.3	848 ± 21.2	↓ ($z = -2.07$, $p = 0.039$)	↑ ($z = -2.93$, $p = 0.003$)
		1.30, 1.34	VLDL/LDL	741 ± 37.2	790 ± 59.0	814 ± 35.4	885 ± 51.7	↓ ($z = -2.61$, $p = 0.009$)	↑ ($z = -2.23$, $p = 0.026$)
		1.18, 1.22	HDL	662 ± 24.6	820 ± 24.0	840 ± 22.4	742 ± 19.1	↓ ($z = -2.42$, $p = 0.015$)	↑ ($z = -2.93$, $p = 0.003$)
		1.26	VLDL	755 ± 27.9	887 ± 45.3	930 ± 29.6	807 ± 41.7	↓ ($z = -2.33$, $p = 0.020$)	↑ ($z = -2.61$, $p = 0.009$)
		1.58	lipid	119 ± 3.5	110 ± 2.9	100. ± 4.3	172 ± 3.6	↓ ($z = -2.59$, $p = 0.009$)	↓ ($z = -2.22$, $p = 0.026$)
		5.26, 5.30	UFA	227 ± 7.6	260 ± 7.7	280 ± 9.6	245 ± 5.1	↓ ($z = -2.46$, $p = 0.014$)	↑ ($z = -2.93$, $p = 0.003$)
urine	1D NOESY	3.06, 3.94	creatine	1.39e^3 ± 74.9	1.20e^3 ± 50.5	1.20e^3 ± 51.1	1.50e^3 ± 53.8	↓ ($z = -2.07$, $p = 0.039$)	↓ ($z = -2.49$, $p = 0.013$)
		3.06, 4.06	creatinine	2.05e^3 ± 131	1.77e^3 ± 94.7	1.66e^3 ± 103	2.18e^3 ± 94.7	↓ ($z = -2.46$, $p = 0.014$)	↓ ($z = -2.49$, $p = 0.013$)
		0.90, 0.94	valine	104 ± 16.6	112 ± 23.0	126 ± 20.6	73.3 ± 8.6	↑ ($z = -2.53$, $p = 0.011$)	− ($z = -1.51$, $p = 0.131$)
		2.54, 2.66	citrate	209 ± 36.0	196 ± 28.2	187 ± 19.3	274 ± 35.3	↓ ($z = -2.00$, $p = 0.045$)	− ($z = -0.622$, $p = 0.534$)
		2.46, 3.02	α-KG	155 ± 14.9	155 ± 14.6	150 ± 15.8	182 ± 14.0	↓ ($z = -2.22$, $p = 0.026$)	− ($z = -0.533$, $p = 0.594$)
		3.54	glycine	94.3 ± 12.3	150 ± 34.7	173 ± 49.6	80.1 ± 11.9	↑ ($z = -2.46$, $p = 0.014$)	↑ ($z = -2.52$, $p = 0.012$)
		3.40−4.00	glucose	4.76e^3 ± 83.3	4.81e^3 ± 56.2	4.92e^3 ± 136	4.57e^3 ± 90.5	↑ ($z = -2.40$, $p = 0.017$)	↑ ($z = -2.42$, $p = 0.015$)

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Table 2. continued

(B) ^{1}H NMR				relative integral[c]				group comparison	
matrix	pulse sequence	chemical shift (ppm)	metabolites	F0	F3	F6	H	F0 vs H (z and p values)[d]	F6 vs F0 (z and p values)[e]
		3.26, 3.42	taurine	327 ± 34.5	283 ± 39.2	250 ± 17.9	393 ± 19.3	↓ (z = −2.49, p = 0.013)	↓ (z = −2.59, p = 0.009)
		3.26	TMAO	209 ± 14.2	179 ± 14.0	175 ± 11.3	237 ± 13.4	↓ (z = −2.20, p = 0.028)	↓ (z = −2.40, p = 0.016)
		7.54, 7.66, 7.82	hippurate	103 ± 30.5	121 ± 38.5	129 ± 28.7	177 ± 28.0	↓* (z = −3.41, p = 0.001)	− (z = −1.16, p = 0.248)

[a]Abbreviations: UPLC−MS/MS, ultra-performance liquid chromatography−tandem mass spectrometry; IBA, 3-indolebutyrate; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; ^{1}H NMR, ^{1}H nuclear magnetic resonance; CPMG, Carr−Purcell−Meiboom−Gill; BPP-LED, bipolar pulse pair-longitudinal eddy current delay; 3-HB, 3-hydroxybutyrate; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; HDL, high-density lipoprotein; 1D NOESY, one-dimensional nuclear Overhauser effect spectroscopy; UFA, unsaturated fatty acids, α-KG, α-ketoglutarate; TMAO, trimethylamine-*N*-oxide. F0, 3 and 6: first-episode neuroleptic-naïve schizophrenia patients at weeks 0, 3 and 6, respectively. H: healthy controls. Symbol indications: ↑, increased ($p < 0.05$); ↓, decreased ($p < 0.05$); −, basically unchanged ($p > 0.05$); *, $p < 0.00156$ after Bonferroni adjustment. [b]The relative intensity of each LPC is the sum of relative intensities at positive and negative ion modes. [c]Values are expressed as mean ± SD. [d]F0 vs H, two-tailed Mann−Whitney U test. [e]F6 vs F0, two-tailed Wilcoxon signed-rank test.

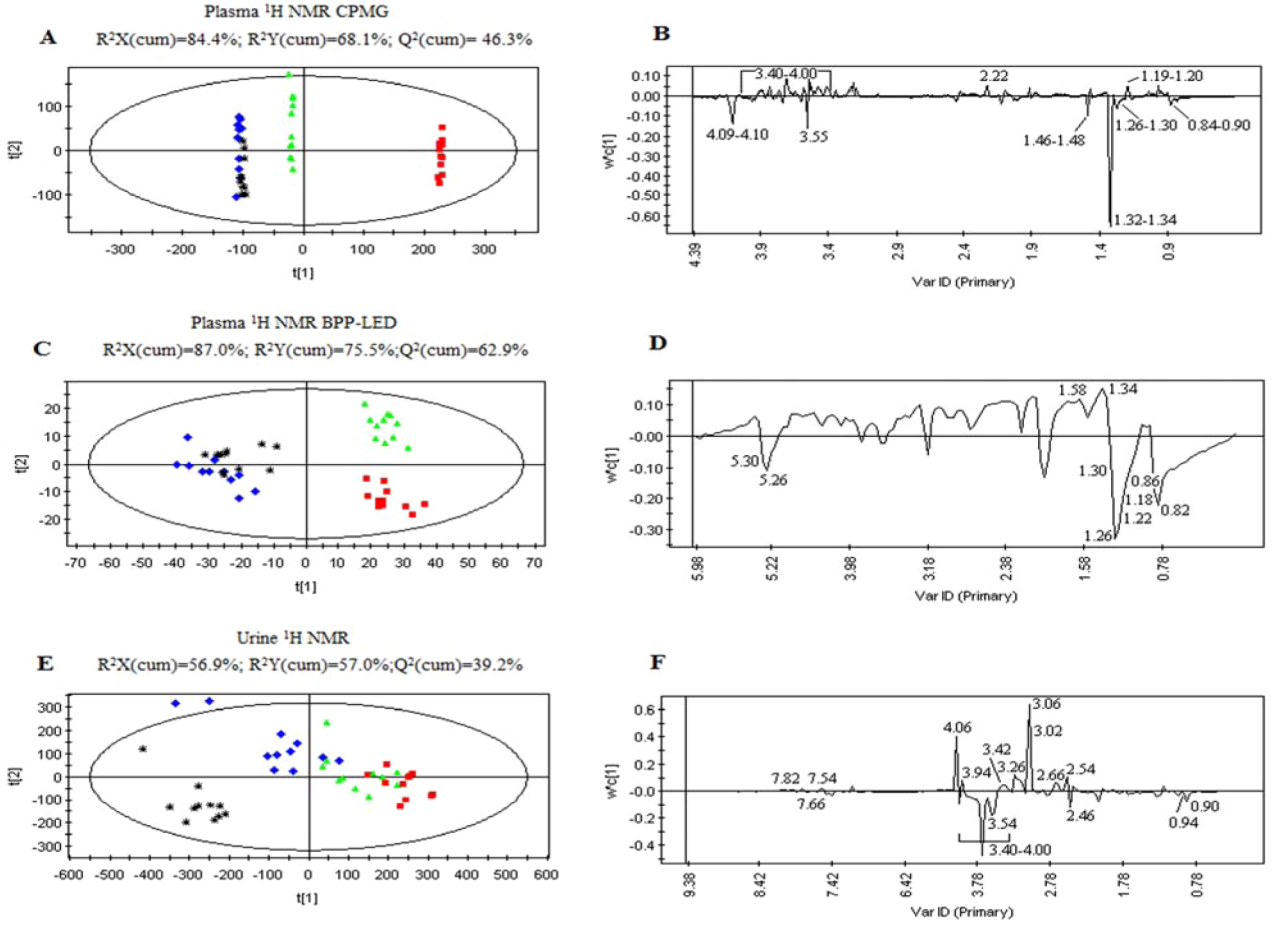

Figure 3. Orthogonal signal correction partial least-squares discriminant analysis (OSC-PLS-DA) of high-resolution ^{1}H NMR Carr−Purcell−Meiboom−Gill (CPMG), bipolar pulse pair-longitudinal eddy current delay (BPP-LED) data of plasma samples as well as ^{1}H NMR data generated from urine samples of all the four groups (first-episode neuroleptic-naïve schizophrenia patients, FENNS: F0, 3 and 6; healthy controls: H): score plots (A, C and E) and corresponding loading plots (B, D and F). (The group labels: H ■ (red), F0 ▲ (green), F3 ◆ (blue), F6 ∗ (black).)

glucose in the blood by flushing out the extra glucose in urine. The paradoxical trends are presumably due to the different metabolic/excretion rates in different biological compartments and indicate that an increased excretion of glucose may also contribute to plasma glucose depletion. RIP treatment, however, induced increments of circulating and excreting glucose levels in the patients in accordance with its ability to inhibit glucose uptake.[27]

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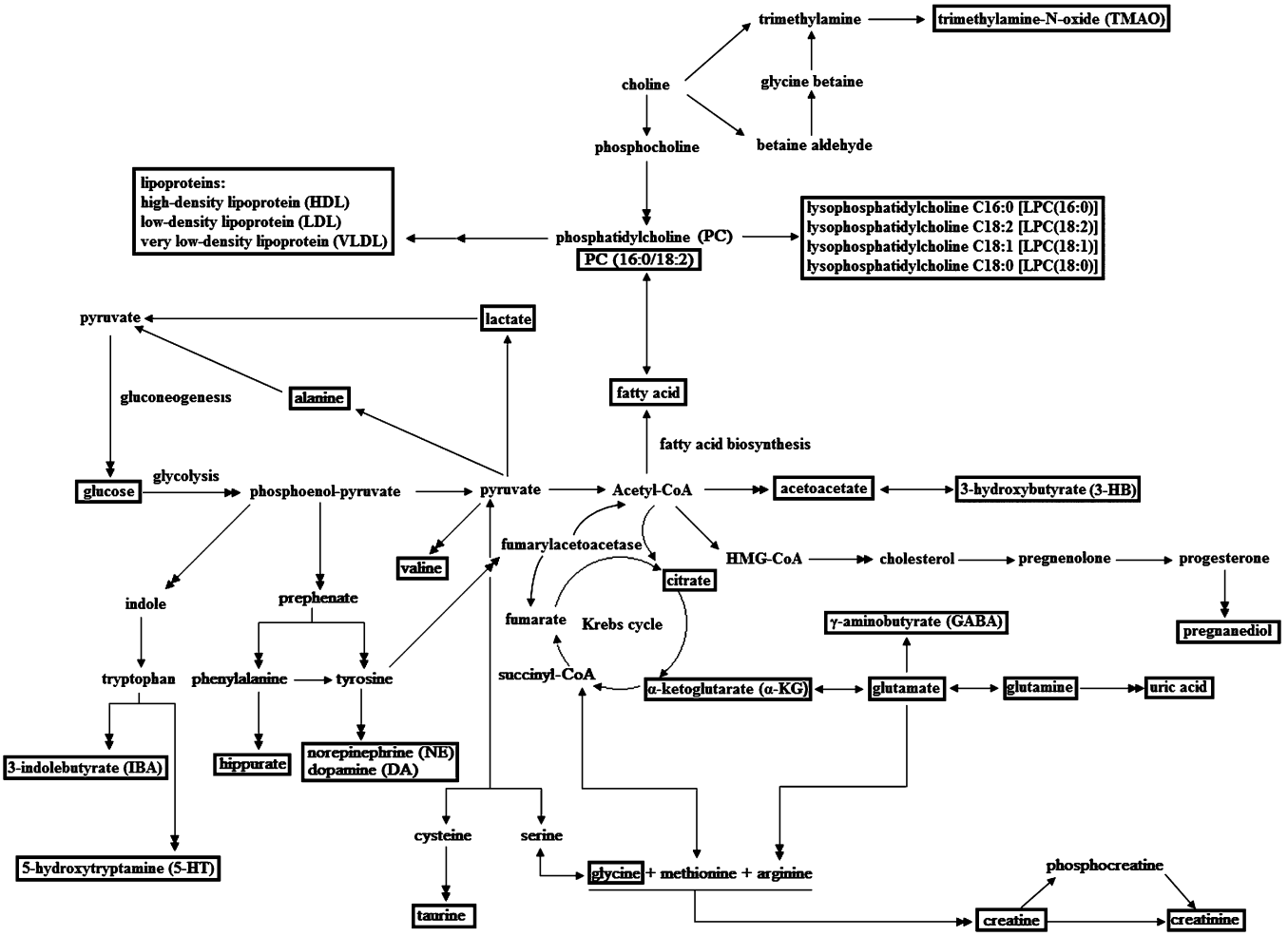

Figure 4. The summarized potential disease and treatment biomarkers and changed relevant metabolic pathways in first-episode schizophrenia patients. Bold rectangle denotes the identified metabolites.

Lipid Metabolism Disturbance. In our study, the disturbances of lipid metabolism in plasma of the FENNS at baseline were reflected by (a) reduced levels of high-, low- and very low-density lipoproteins (HDL, LDL and VLDL); (b) decrements in unsaturated fatty acids (UFA) and lipid; and (c) elevations of lysophosphatidylcholines (LPC) (16:0, 18:2, 18:1, and 18:0) and a reduction of phosphatidylcholine (PC) (16:0/18:2) when compared with controls. These results are consistent with and substantially add to the previous reports of decrease in phospholipid content and increase in lipid peroxide levels in the periphery of FENNS.[28] There is also convincing evidence for aberrant central phospholipid metabolism in FENNS.[29] Remarkably, associations between brain levels of certain membrane phospholipid catabolites and erythrocyte membrane phospholipid UFA content have been reported not only in healthy human subjects[30] but also in patients with schizophrenia,[31] suggesting peripheral and central membrane dynamic may be coupled. Taken together, these findings imply that a lipid disorder expressing throughout the body may be potentially involved in schizophrenia. In the present study, RIP medication resulted in elevations of VLDL, LDL, HDL and PC (16:0/18:2) at higher extension. However, long-term RIP-treated patients with chronic schizophrenia did not reveal significant changes in LDL and HDL.[32] A possible explanation is that chronically treated schizophrenia patients may have developed tolerance to the effects of RIP on increasing lipoproteins levels, which FENNS may still be sensitive to. PC (16:0/18:2) was only revealed by F6 vs F0 comparison of plasma UPLC−MS data (Supporting Information Figure S3D), highlighting the effects of RIP on regulating pathways for biosynthesis of n6 UFA in PC that illustrates the possible increase of Δ6 desaturase in schizophrenia patients.[8]

Energy Metabolism Compromise. Citrate, α-KG, creatine and creatinine contributing to the separation between patient group and control group are directly associated with energy metabolism. In the present work, decreased levels of them suggest the dysfunctions of Krebs cycle and creatine-phosphocreatine shuttle system in the FENNS. The blood ketone bodies, acetoacetate and 3-hydroxybutyrate (3-HB), were shown to be reduced in the FENNS group before treatment, indicative of increased utilization of ketone bodies rather than decreased metabolism of fatty acids in vivo since plasma lipid was also reduced in the FENNS. Ketone bodies derived from incomplete oxidation of lipid act as an alternate fuel source for the CNS in response to insufficient blood glucose. Thus further decreases of them in the patients group during RIP treatment also imply an excessive consumption of ketone bodies resulting from intrinsic inhibited glucose metabolism and a severely diminishing energy supply to the schizophrenic brain. Concordantly, a recent animal study found that atypical antipsychotics could rapidly and inappropriately switch energy utilization from glucose to lipid, impairing metabolic flexibility.[33] As a consequence, the

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perturbations of normal energy metabolism levels in schizophrenia may be expected to adversely affect brain function, leading to psychiatric symptoms.[34] In the present work, the corresponding associations between parameters of citrate, α-KG and negative symptom domains in support of this view were also confirmed. Alternatively, since it has been suggested that the schizophrenic brain may preferentially utilize lactate over glucose as a primary energy substrate,[9] the elevated plasma lactate level in our FENNS and its subsequent increase induced by RIP therapy may serve as a compensatory mechanism for energy deficiency in the short term. However, the adaptive increase of lactate can harmfully lower cellular pH in the long run, which is correlated with oxidative stress and a series of other pathological conditions in the schizophrenia syndrome.[35]

Oxidative Stress. Reduced levels of antioxidants uric acid and taurine were found in the FENNS, off and on RIP. It suggests that a dysregulation of the antioxidant defense system, which may lead to oxidative damage, occurs early in the course of schizophrenia and is independent of treatment effects. More enlighteningly, the LPCs that are converted from the PCs during LDL oxidation were first found to be increased in schizophrenia compared with controls, indicating an elevation of oxidative stress. It is reported that LPC possesses a wide variety of proatherogenic properties:[36] chemotactic activity for circulating monocytes and T-lymphocytes, impairment of endothelium-dependent arterial relaxation, mitogenic effect of macrophages and smooth muscle cells, induction in coronary artery smooth muscle cell migration, and inhibition of endothelial cell motility. Thereafter, close relations of increased LPC contents in LDL with type 2 diabetes mellitus have been reported as well.[37] Given the essential role LPCs play in the development of cardiovascular disease and diabetes, they may be a kind of pivotal biomarkers bridging schizophrenia and the metabolic syndrome.

Microflora Variation. In the patients group, hippurate and trimethylamine-*N*-oxide (TMAO) were decreased and 3-indolebutyrate (IBA) was increased. These new biomarkers are respectively the metabolic products of phenylalanine, choline and tryptophan by the gut microflora. Their changes strongly suggest that there are variations in the gut microflora in response to schizophrenia. Moreover, alterations of intestinal flora might lead to gastrointestinal symptoms as well as the comorbidity of schizophrenia and irritable bowel syndrome (IBS). This is consistent with the finding of a significant prevalence of IBS among patients with schizophrenia.[38] Although IBS is rarely a precursor to schizophrenia, this psychiatric syndrome usually antedates IBS. Accumulating evidence has demonstrated a bidirectional communication between the brain and gut, including neural, immune, and endocrine pathways.[39] This bidirectional pathway may provide a link between schizophrenia and the disorder of gut microflora, and a novel therapeutic target for schizophrenia.

Endocrine Dyscrasia. Pregnanediol, a major endogenous human progesterone metabolite, is first reported as a biomarker for schizophrenia. Considering the positive relationship between urinary pregnanediol and plasma/serum progesterone,[40] the elevation in urinary pregnanediol found in the FENNS should readily indicate an increase of their plasma progesterone, which is a sign of metabolic stress and hypothalamic−pituitary−adrenal (HPA) axis activation in schizophrenia.[41] However, considerable evidence accumulates suggesting antipsychotic-like properties of progesterone in animals and humans.[42] In addition, marker information of urinary pregnanediol was only shown in the loadings of F6 vs F0 (Supporting Information Figure S3P), emphasizing the therapeutic effects of RIP on the further increase in plasma progesterone. Consistently, elevated urinary pregnanediol was found associated with improvements in PANSS activation scores during RIP monotherapy. Pregnanediol may therefore offer a useful biomarker for monitoring therapeutic efficacy. Nevertheless, steady increase of progesterone caused by RIP also manifests the overactivity in HPA axis, which can later cause endocrine side effects in schizophrenia patients.[43]

Differential Metabolic Impact of RIP on the CNS versus the Periphery

The synergetic effect of symptomatic therapy was not taken into account and could be a limitation of this study. Nonetheless, an interesting finding is that targeted NT profile (getting close to control) and global metabolic profile (further deviating from control) of the same FENNS are on opposite trajectories under RIP treatment for 6 weeks. Logically they may represent the therapeutic effects on the CNS and the metabolic side effects on the periphery related to RIP medication, respectively, describing the dilemma of current atypical medication from a metabolic perspective; that is, RIP may exacerbate the already existing disruptions in their system health condition when alleviating clinical mental symptom dimensions. Not merely imbalanced neurotransmission, RIP could exert its neuroprotective function against some other pathophysiological processes occurred in the schizophrenic brain such as energy metabolism,[10] oxidative stress and membrane lipid peroxidation.[44] However, our data suggest that some of the same metabolic dysfunctions also existing in the periphery have been unaffected or even aggravated by RIP administration. By comparing all groups of the metabolites, plasma glucose, PC (16:0/18:2), lipoproteins, LDL, VLDL, HDL and UFA with a distinct response (decrease before but increase after treatment) particularly indicate that glucose and lipid metabolism are influenced at higher extension. If the periphery is the original place where these metabolic perturbations begin, they may again result in "metabolic injury" in the CNS with a variety of possible causes,[45] finally leading to the recurrence of psychosis after discontinuation of antipsychotic medications.

■ CONCLUSION

We used the UPLC−MS/MS and ^{1}H NMR analytical platforms to longitudinally characterize the targeted NT profiles and global metabolic profiles of plasma and urine from FENNS during a 6-week RIP monotherapy. Collectively, biomarkers identified from the metabolomic analysis suggest disturbances of NT metabolism, amino acid metabolism, glucose metabolism, lipid metabolism, energy metabolism, antioxidant defense system, bowel microflora and endocrine system in schizophrenia patients, which could be therapeutically or adversely affected by the RIP treatment. Some of the metabolic markers are correlated with the symtomatology in schizophrenia and may have potential for monitoring treatment efficacy. The plasma and urine profiling approaches show their potential to identify noninvasive biomarkers for diagnostics and therapeutics of schizophrenia. Further refinement and validation of these biomarkers in larger cohorts of patients and with longer durations of follow-up would be considerably interesting.

■ ASSOCIATED CONTENT

Supporting Information

Table S1 lists the gradient elution programs of UPLC−MS for plasma and urine. Table S2 includes the plasma and urinary levels of neurotransmitters and their metabolites in the subjects.

Methods provide the procedure for biomarker identification by UPLC−MS/MS. Figure S1 depicts the results of multivariate comparative analysis of NT data (F0 vs H; F6 vs F0). Figure S2 displays UPLC−MS positive and negative ion base peak intensity (BPI) chromatograms of representative plasma and urine samples from a healthy control. Figure S3 represents the corresponding OSC-PLS-DA score and loading plots of UPLC−MS data (F0 vs H; F6 vs F0). Figure S4 shows typical ^{1}H NMR spectra of plasma and urine samples from a healthy control. Figure S5 represents the corresponding OSC-PLS-DA score and loading plots of NMR data (F0 vs H; F6 vs F0). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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