



A pilot case-control study on the association between *N*-acetyl derivatives in serum and first-episode schizophrenia

Huang Ninghua^{a,1}, Cao Bing^{a,1}, Elisa Brietzke^{b,c}, Caroline Park^b, Danielle Cha^{b,d}, Zihang Pan^b, Judy Zhu^b, Liu Yaqiong^{a,e,f}, Xie Qing^{a,e,f}, Zeng Jing^{a,e,f}, Roger S. McIntyre^{b,g}, Wang Jingyu^{a,e,f,*}, Yan Lailai^{a,e,f,*}

^a Department of Laboratorial Science and Technology, School of Public Health, Peking University, Beijing 100191, PR China

^b Mood Disorders Psychopharmacology Unit, University Health Network, Toronto, Canada

^c Department of Psychiatry, Universidade Federal de São Paulo, São Paulo, Brazil

^d Faculty of Medicine, School of Medicine, University of Queensland, Brisbane, QLD, Australia

^e Beijing Key Laboratory of Toxicological Research and Risk Assessment for Food Safety, Beijing 100191, PR China

^f Peking University Medical and Health Analysis Center, Peking University, Beijing 100191, PR China

^g Brain and Cognition Discovery Foundation, Toronto, ON, Canada

ARTICLE INFO

Keywords:

N-acetyl derivatives

Schizophrenia

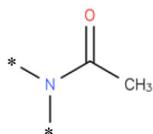
First-episode

Liquid chromatography-mass spectrometry

ABSTRACT

N-acetyl group metabolites are a general class of endogenous compounds characterized by a conjugated system consisting of an acetyl group and nitrogen moiety. The aim of our exploratory pilot case-control study is to compare the levels of five *N*-acetyl derivatives (i.e., *N*-acetyl-glutamine, *N*-acetyl-ornithine, *N*⁶-acetyl-*L*-lysine, *N*-acetyl-putrescine, and *N*-acetyl-galactosamine) in serum samples between individuals with first-episode schizophrenia and healthy controls (HC). A 1:2 age- and sex- matched pilot case-control study was performed, involving 30 cases of first-episode schizophrenia and 60 HC aged between 18 and 40 years old. The serum samples containing these *N*-acetyl derivatives from (first-episode patients with schizophrenia and HC were measured using liquid chromatography-tandem mass spectrometry (LC-MS). Results indicated that higher levels of *N*-acetyl-glutamine and lower levels of *N*⁶-acetyl-*L*-lysine may have a significant association with schizophrenia after adjusting for age, sex and BMI. *N*-acetyl-putrescine was elevated among subjects with first-episode schizophrenia when compared to HC, suggesting it as a predictor for schizophrenia onset. Further exploration of the mechanisms of *N*-acetyl group metabolites with respect to schizophrenia is warranted and may be useful for identifying novel disease markers and/or drug target molecules in schizophrenia.

1. Introduction



Schizophrenia is a complex mental illness that has been associated with elevated rates of chronicity, comorbidity, and premature mortality (Owen et al., 2016). It affects approximately 1% of the world's population and represents one of the leading causes of global health burden (Shi et al., 2009). Despite decades of research, the etiology and pathophysiology of schizophrenia is not fully understood, stagnating the discovery of disease-modifying treatments (Prabakaran et al., 2004). At

present, diagnosis of schizophrenia is mainly based on criteria from the Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DSM-5) and the International Classification of Diseases 10th Revision (ICD-10); severity evaluation is primarily dependent on the psychiatrists' subjective evaluations of symptom clusters with the Positive and Negative Syndrome Scale (PANSS) (Tamminga and Holcomb, 2005). The expansion of metabolic analysis provides an opportunity to explore metabolic determinants of schizophrenia with respect to its etiology, pathogenesis, disease progression, and treatment response.

N-acetyl group metabolites are the acetylated variant of amino acids and organic acids, which are a general class of endogenous compounds characterized by a conjugated system consisting of an acetyl group and nitrogen moiety. Two of our previous studies have already confirmed

* Corresponding authors at: School of Public Health, Peking University, Beijing 100191, P R China

E-mail addresses: wjy@bjmu.edu.cn (J. Wang), yll@bjmu.edu.cn (L. Yan).

¹ Ninghua Huang and Bing Cao contributed equally to this work.

that some of the amino acids and organic acids might be associated with the pathogenesis and progression of schizophrenia (Cao et al., 2018a,b). Also, *N*-acetyl-glucosamine has been shown to act as a nutrient sensor and mediator of insulin resistance (Wells et al., 2003). For example, preclinical studies have reported that *N*-acetyl-*L*-tryptophan exposure results in a significant increase in acetylcholinesterase activity and glutathione levels in the hippocampus (Fernandes et al., 2018); similarly, *N*-acetyl lysyltyrosylcysteine amide has been demonstrated to effectively reduce brain damage following a stroke (Yu et al., 2016). In addition, *N*-acetyl-aspartate concentrations are observed to be markedly decreased in the frontal cortex of male patients with chronic schizophrenia and bipolar disorder (Molina et al., 2007). Replicated evidence from randomized controlled trials (RCTs) has also demonstrated that supplementation of *N*-acetyl-cysteine is associated with improvements in negative symptoms and reduction of antipsychotic side effects in patients with longstanding schizophrenia (Berk et al., 2008; Carmeli et al., 2012). *N*-acetyl-cysteine inhibits inflammatory responses (Beloosesky et al., 2006). Inflammation plays a key role in the pathophysiology for major psychiatric disorders such as schizophrenia (Khandaker et al., 2015) and depression (Liu et al., 2012). Therefore, *N*-acetyl-cysteine represents an appropriate candidate for investigation in the etiology and pathogenesis of schizophrenia.

Despite growing evidence highlighting an association between *N*-acetyl-cysteine and schizophrenia, there is a paucity of data investigating the role of *N*-acetyl-cysteine in the pathogenesis and progression of schizophrenia. Liquid chromatography column (i.e., HILIC) in combination with liquid chromatography-mass spectrometry (LC-MS) were used to minimize sample loss during extraction, chemical derivatization to enhance ionization, and sample clean-up (Pitt, 2009). By using the HILIC LC-MS approach, the current study quantified five *N*-acetyl derivatives in serum samples amongst individuals with schizophrenia and healthy controls (HC). For the five *N*-acetyl group metabolites: *N*-acetyl-glutamine, *N*-acetyl-ornithine and *N*⁶-acetyl-*L*-lysine are acetylated amino acids, whereas *N*-acetyl-putrescine and *N*-acetyl-galactosamine are acetylated organic acids. Although few evidence has indicated the relationship among the five *N*-acetyl group metabolites, it is still worth studying due to all of them including an acetyl group and nitrogen moiety. Herein, our pilot case-control study aimed to establish an association between *N*-acetyl derivatives and schizophrenia in order to enhance our understanding of the pathogenesis and progression of schizophrenia, as well as identify potential novel therapeutic targets.

2. Methods

The study protocol was reviewed and approved by the Ethics Review Committee of the Health Science Center, Peking University (IRB00001052-14071). All the subjects provided written informed consent prior to enrolment.

2.1. Study design and participants

The study was a 1:2 age- and sex- matched case-control study conducted from September 2015 to September 2016 in the Weifang Psychiatric Hospital, Shandong Province, China. Thirty drug-naïve subjects ($n = 30$) between the ages of 18–40 years' old who met the Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV) criteria for first-episode schizophrenia were recruited as cases. Sixty age-, sex-, and ethnicity-matched, antipsychotic medication-naïve individuals ($n = 60$) without any known psychiatric history were also recruited from the same district as the schizophrenia patients. All subjects (i.e., cases and HC) met the following criteria: (1) no diabetes mellitus type I/II, hyperlipidemia, cardiovascular disease, or other severe medical conditions; (2) no comorbid psychiatric mental disorders, alcohol use disorder, or other substance use disorders; (3) no pregnancy (suspected or confirmed), postpartum, and/or breastfeeding.

2.2. Reagent

The standard chemical references were used for quality assurance of *N*-acetyl group metabolites (i.e., *N*-acetyl-glutamine, *N*-acetyl-ornithine, *N*⁶-acetyl-*L*-lysine, *N*-acetyl-putrescine, and *N*-acetyl-galactosamine) (Sigma-Aldrich, Co., St. Louis, MO, USA); HPLC-grade formic acid and acetonitrile were purchased from Thermo Fisher Scientific (Waltham, MA, USA); pure water was purchased from Wahaha Co., Ltd. (Hangzhou, China).

2.3. Serum samples preparation

All fasting venous blood samples (~5 mL) for cases and controls were collected after 12 h of overnight fasting (i.e., collected between 7 and 9 am) from the forearm vein and were drawn in serum separation hoses for analyses. Serum samples were stored at -80°C until use. Prior to analysis, all serum samples were thawed in ice, vortexed and centrifuged at 12,000 rpm for 10 min. 100 μl of each serum sample was aliquoted for LC-MS analysis. For protein precipitation and metabolite extraction, 300 μl of cold acetonitrile was added to a labeled 1.5 ml microcentrifuge tube with 100 μl of each sample. Samples were vortexed and maintained at 4°C for 10 min, centrifuged at 4°C 12,000 rpm for 10 min, and then 100 μl of supernatant was transferred to a 200 μl vial insert for analysis. Equal volumes of supernatant from all samples were pooled to prepare quality control samples, which were used to monitor the stability and repeatability of the actual samples (i.e., these were analyzed once for every 10–15 study samples).

2.4. HILIC mode and UPLC-MS analysis

The UPLC separation was performed using the Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC (RSLC) system with HILIC using the gradient conditions. Both chromatographic separations were performed using a BEH Amide column (2.1 \times 100 mm, 1.7 μm , Waters, Milford, MA, USA) at 40°C . The analysis was carried out using ESI+ mode. Flow rate was 300 $\mu\text{L}/\text{min}$, autosampler temperature was 4°C , and injection volume was 1 μL . The gradient was optimized to obtain maximum separation (0–1 min: 5% B, 1–10 min: 5–50% B, 10–12 min: 50% B, 12–12.1 min: 50–5% B, 12.1–16 min: 5% B). For HILIC separation, mobile phase A was acetonitrile and mobile phase B was water; both A and B contained 0.1% formic acid and 10 mmol/L ammonium acetate.

After the chromatographic separation, MS ionization and data acquisition were performed using a Thermo Scientific™ Q Exactive™ hybrid quadrupole Orbitrap mass spectrometer equipped with a HESI-II probe. Positive HESI-II spray voltages were 3.7 kV, heated capillary temperature was 320°C , sheath gas pressure was 30 psi, auxiliary gas setting was 10 psi, and heated vaporizer temperature was 300°C . Both the sheath gas and auxiliary gas were nitrogen. The nitrogen collision gas was set to a pressure of 1.5 mTorr. The parameters for the full mass scan were: resolution 7×10^4 , auto gain control target $< 1.0 \times 10^6$, and m/z range 100–1500. The calibration was customized for the analysis of Q Exactive to keep the mass tolerance < 5 ppm. The parameters for MS/MS experiments were: resolution 17,500, auto gain control target $< 1.0 \times 10^5$, maximum isolation time of 50 ms, loop count of top 10 peaks, isolation window of m/z , and normalized collision energy of 30 V. The LC-MS system was controlled, collected and processed using Xcalibur 2.2 SP 1.48 software (Thermo Fisher Scientific). The Skyline (64-bit, 3.5.0.9319, MacCoss Lab, UW, USA) was used to create metabolite transitions, visualize results, integrate observed signals, and quantify all metabolites using the data detected by MS.

2.5. Statistical analysis

All statistical analyses were completed using SPSS ver. 22.0 (SPSS

Inc., Chicago, IL, USA). Body Mass Index (BMI) was calculated by the formula weight (kg) divided by the square of height (m) (kg/m^2). Each data set of *N*-acetyl group metabolites detected by LC-MS was log transformed (decimal base) prior to analysis. Descriptive statistics were performed, with continuous variables summarized as means and standard deviations (SDs) or medians and interquartile ranges (IQRs), and categorical variables summarized as frequencies and proportions. Statistical significance between various groups was tested using the χ^2 test or Fisher's exact test for categorical variables, and the *t*-test or Mann-Whitney *U* test for continuous variables. Generalized linear models (GLMs) were used to explore the association between schizophrenia and the logarithm values of *N*-acetyl group metabolites. The variables of age, sex and BMI were adjusted in the GLM models. β -values and their 95% confidence intervals (CIs) were estimated using maximum likelihood methods. Partial correlation analysis on ranks (Spearman Correlation) was used to calculate correlation coefficients among *N*-acetyl group metabolites. A two-tailed *p* value < 0.05 was considered to indicate statistical significance.

3. Results

3.1. Demographic and clinical characteristics of subjects

In total, 30 cases with DSM-IV-defined schizophrenia and 60 age- and sex-matched HC participated in the study, with an age range of 18–40 years old. The mean (SD) age in years of cases and controls were 26.8 (7.4) years and 27.1 (3.4) years, respectively. Of the total participants enrolled in the study, 43.3% of cases (M/F = 13/17) and controls (M/F = 26/34) were male. No statistically significant differences in BMI, fasting blood glucose (FBG), triglyceride (TG) and serum total cholesterol (TC) were detected between cases and HC (all *p* > 0.05). Table 1 depicts the demographic characteristics of cases and HC.

3.2. The comparison of *N*-acetyl group between cases and controls

Five types of *N*-acetyl derivatives were identified in our study. After log transforming the raw data to approximate normal distribution, the results were analyzed by GLMs. Fig. 1 depicts the distribution of the five types of *N*-acetyl group metabolites. The separate analyses using GLMs, controlling for age, sex, and BMI, revealed statistically significant differences in serum *N*-acetyl group metabolites between cases and HC for *N*-acetyl-glutamine ($\beta = 0.611$, 95% CI: 0.226, 0.996, *p* = 0.002) and *N*⁶-acetyl-*L*-lysine ($\beta = -1.723$, 95% CI: -3.118, -0.328, *p* = 0.016). The association between cases and HC with respect to *N*-acetyl-

putrescine was no longer statistically significant after age, sex, and BMI adjustments. All details are shown in Table 2.

3.3. The correlation between serum levels of *N*-acetyl group metabolites in cases and HC, and between *N*-acetyl group metabolites and PANSS scores in cases

Correlation analyses of the five *N*-acetyl group metabolites were calculated with the log transformed data by using Spearman correlations for all subjects, cases and controls (Table 3). *N*-acetyl-galactosamine was significantly correlated with *N*-acetyl-ornithine ($r = -0.307$, *p* = 0.003); *N*-acetyl-putrescine was significantly correlated with *N*-acetyl-ornithine ($r = 0.259$, *p* = 0.014), *N*⁶-acetyl-*L*-lysine ($r = 0.317$, *p* = 0.002) and *N*-acetyl-glutamine ($r = 0.208$, *p* = 0.049); and *N*-acetyl-ornithine was significantly correlated with *N*⁶-acetyl-*L*-lysine ($r = 0.244$, *p* = 0.021). Spearman correlations were also calculated for the different groups (i.e., cases and HC). Details are shown in Table 3. In the case group, significant correlations were found between: *N*-acetyl-galactosamine and *N*-acetyl-ornithine ($r = -0.382$, *p* = 0.037); *N*-acetyl-putrescine and *N*-acetyl-ornithine ($r = 0.442$, *p* = 0.015), *N*-acetyl-putrescine and *N*⁶-acetyl-*L*-lysine ($r = 0.628$, *p* < 0.001). In the control group, there were significant correlations between *N*-acetyl-galactosamine and *N*-acetyl-ornithine ($r = -0.283$, *p* = 0.029), *N*-acetyl-putrescine and *N*⁶-acetyl-*L*-lysine ($r = 0.259$, *p* = 0.045), *N*-acetyl-ornithine and *N*-acetyl-glutamine ($r = 0.280$, *p* = 0.030), *N*⁶-acetyl-*L*-lysine and *N*-acetyl-glutamine ($r = 0.257$, *p* = 0.047). The Spearman correlation analysis was also performed to examine the correlation between the psychopathology (i.e., PANSS scores) with the level of *N*-acetyl group metabolites (Supplemental Table 1), significant correlations were only found between *N*-acetyl-galactosamine with general psychopathology ($r = -0.465$, *p* = 0.019), as well as *N*⁶-acetyl-*L*-lysine with general psychopathology ($r = 0.425$, *p* = 0.034).

4. Discussion

The present pilot case-control study evaluated the association between five *N*-acetyl group metabolites in serum and first-episode schizophrenia. No significant differences in BMI, FBG, TG and TC were found between cases and HC (*p* > 0.05). The results indicated that elevated *N*-acetyl-glutamine and lower levels of *N*⁶-acetyl-*L*-lysine may be associated with first episode schizophrenia after adjusting for age, sex and BMI. *N*-acetyl-putrescine was found to be elevated in cases compared to HC (*p* < 0.05) before adjusting for age, sex and BMI. No differences in levels of *N*-acetyl-ornithine and *N*-acetyl-galactosamine were found between cases and HC. The correlations between specific *N*-acetyl group metabolites were found in both cases and HC.

As an acetylated analogue of glutamine, *N*-acetyl-glutamine can decompose into *N*-acetyl-*L*-glutamic acid, *N*-(2,6-dioxo-3-piperidinyl) acetamide, pyroglutamic acid, glutamic acid, and glutamine. Due to the stability of *N*-acetyl-glutamine, it is often used as a source of glutamine for parenteral nutrition (Bergana et al., 2000). Glutamic acid and glutamine are presumed to be involved in the dysfunction of excitatory neurotransmission, plasticity, and excitotoxicity in patients with schizophrenia (Natsubori et al., 2014). Previous research has reported abnormalities in the glutamine-glutamate-GABA cycle, which may be involved in the manifestation of schizophrenia (Hashimoto, 2014). For example, a meta-analysis found a significant increase in medial frontal glutamine and a significant decrease in glutamic acid in patients with schizophrenia compared to HC (Marsman et al., 2013). In addition, a ¹H-MRS analysis revealed elevated glutamine and/or total glutamic acid + glutamine in unmedicated patient groups compared to controls (Goldstein et al., 2015). The results from these studies are consistent with our observation of higher *N*-acetyl-glutamine levels in first-episode patients with schizophrenia compared to HC. Of note, high amounts of *N*-acetyl amino acids were significantly associated with screened aminoacylase I deficiency—an inborn error of metabolism in

Table 1
Demographic and clinical characteristics of subjects at the case-control study.

Variables	Case (n = 30)	HC (n = 60)	<i>p</i> -value
Age (year); mean (SD)	26.8 (7.4)	27.1 (3.4)	0.837 ^a
Gender; n (%)			
Male	13 (43.33)	26 (43.33)	1.000 ^b
Female	17 (56.66)	34 (56.66)	
BMI (kg/m^2); mean (SD)	22.3 (3.3)	23.4 (3.6)	0.155 ^a
FBG > 6.1 mmol/L; n (%)	0 (0.0)	4 (6.7)	0.191 ^c
TG > 1.71 mmol/L; n (%)	3 (10.0)	9 (15.0)	0.382 ^c
TC > 5.7 mmol/L; n (%)	2 (6.7)	3 (5.0)	0.543 ^c
PANSS score; mean (SD)			
Total score	89.3(22.4)	—	—
Positive symptoms	22.9 (9.3)	—	—
Negative symptoms	18.9 (7.6)	—	—
General psychopathology	44.6 (15.0)	—	—

^a *p* values were calculated by two-tailed independent samples tests.

^b *p* values were calculated by chi-square tests.

^c *p* values were calculated by Fisher's exact test. BMI, body mass index; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; SD, standard deviation.

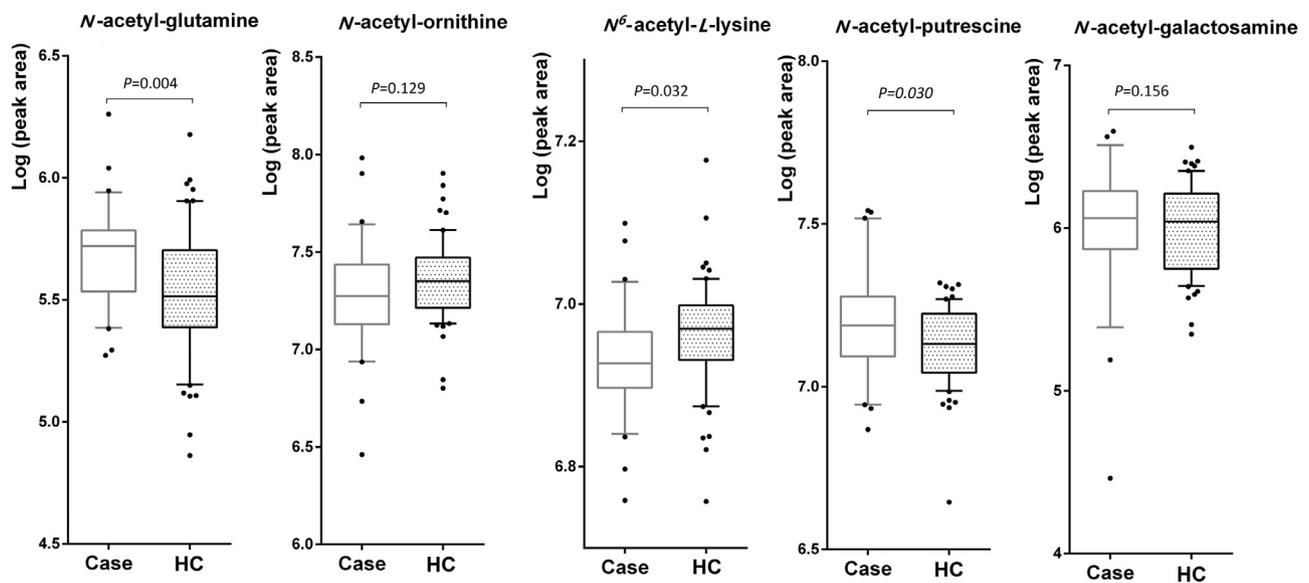


Fig. 1. The boxplots of N-acetyl group in cases and HCs.

Table 2

The comparison of N-acetyl group between cases and HCs.

Variables	Crude β (95% CI)	<i>p</i>	Adjusted* β (95% CI)	<i>p</i>
N-acetyl-glutamine	0.538 (0.176, 0.900)	0.004	0.611 (0.226, 0.996)	0.002
N-acetyl-ornithine	-0.308 (-0.707, 0.090)	0.129	-0.329 (-0.751, 0.094)	0.127
N ⁶ -acetyl-L-lysine	-1.530 (-2.927, -0.134)	0.032	-1.723 (-3.118, -0.328)	0.016
N-acetyl-putrescine	0.739 (0.074, 1.405)	0.030	0.665 (-0.002, 1.333)	0.051
N-acetyl-galactosamine	-0.130 (-0.310, 0.050)	0.156	-0.176 (-0.362, 0.010)	0.063

* Adjusted β and 95% CI were calculated by adjusting age, sex and BMI.

Table 3

Spearman correlations of N-acetyl group metabolites in cases and HCs.

	N-acetyl-galactosamine	N-acetyl-putrescine	N-acetyl-ornithine	N ⁶ -acetyl-L-lysine	N-acetyl-glutamine
Total					
N-acetyl-galactosamine	1.000	-0.160	-0.307**	-0.146	0.028
N-acetyl-putrescine		1.000	0.259*	0.317**	0.208*
N-acetyl-ornithine			1.000	0.244*	0.207
N ⁶ -acetyl-L-lysine				1.000	0.178
N-acetyl-glutamine					1.000
Cases					
N-acetyl-galactosamine	1.000	-0.273	-0.382*	-0.176	0.141
N-acetyl-putrescine		1.000	0.442*	0.628**	0.313
N-acetyl-ornithine			1.000	0.317	0.233
N ⁶ -acetyl-L-lysine				1.000	0.298
N-acetyl-glutamine					1.000
Controls					
N-acetyl-galactosamine	1.000	-0.069	-0.283*	-0.108	-0.062
N-acetyl-putrescine		1.000	0.181	0.259*	0.072
N-acetyl-ornithine			1.000	0.172	0.280*
N ⁶ -acetyl-L-lysine				1.000	0.257*
N-acetyl-glutamine					1.000

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

patients caused by gene mutation (Engelke et al., 2008), indicating these metabolites have the potential as biomarkers of specific diseases.

Our study found decreased N⁶-acetyl-L-lysine in first-episode patients with schizophrenia compared to HC. Research indicates that N⁶-acetyl-L-lysine plays an essential role in regulating gene transcription, cell-cycle progression, apoptosis, DNA repair, and cytoskeletal organization (i.e., which is increasingly being recognized as an intracellular signalling mechanism). To our knowledge, no previous studies have

reported on the association between N⁶-acetyl-L-lysine and schizophrenia. L-lysine interferes with the brain's nitric oxide (NO) signalling system and may represent a novel therapeutic target in schizophrenia. Two clinical trials indicated that L-lysine could act as an adjunctive treatment in patients with schizophrenia (Wass et al., 2011; Zeinoddini et al., 2014). Under normal circumstances, the histone acetyltransferase (HAT) enzyme transfers an acetyl group from acetyl-coenzyme A (acetyl-CoA) to the amino group of lysine side chains in the basic N-

terminal tail region of histones. The abnormal N^6 -acetyl-*L*-lysine levels in patients with first-episode schizophrenia may be indicative of dysfunctional enzymes associated with energy metabolism.

N-acetyl-putrescine is the most abundant polyamine (Lee et al., 1998) and is produced by the breakdown of amino acids. Considerable evidence has reported that the polyamine system plays important roles in disparate biological processes, including apoptosis and inflammation (Chen et al., 2011). Mounting evidence suggests that the polyamine system may also participate in the etiology and pathology of mental disorders (Fiori and Turecki, 2008), including schizophrenia. Higher levels of *N*-acetyl-putrescine was found in first-episode patients with schizophrenia compared to HC in our study. Notably, large doses of *N*-acetyl-putrescine have been demonstrated to be toxic. Spermidine/spermine- N^1 -acetyltransferase is the key enzyme in the polyamine catabolic pathway, and is down-regulated in the brains of patients with depressive symptoms such as suicidal ideation (Gross and Turecki, 2013). Therefore, abnormalities in *N*-acetyl-putrescine in first-episode patients with schizophrenia may be associated with a dysregulation of enzymatic function.

Correlation analyses revealed an association between *N*-acetyl groups and mental health status (i.e., case vs. HC), indicating that the normal induction effect between cytokine networks may be damaged in individuals with schizophrenia. Other psychotropic medications such as antidepressants have demonstrated strong effect in reduction of pro-inflammatory cytokines over a period of time (Lu et al., 2017). Preliminary studies have shown that antipsychotic drug such as risperidone reduce pro-inflammatory cytokines over the course of treatment (Song et al., 2014). Further research is required to evaluate the longitudinal relationship between of *N*-acetyl groups and pro-inflammatory cytokines. No statistically significant differences were observed between cases and controls for *N*-acetyl-ornithine or *N*-acetyl-galactosamine. The examination of the correlations between the psychopathology with the level of *N*-acetyl group metabolites in cases indicated that low *N*-acetyl-galactosamine and high N^6 -acetyl-*L*-lysine levels may be related to general psychopathology, which needs to be verified in future pathway analyses.

4.1. Limitations

There are some limitations to consider when interpreting these results. Firstly, due to the naturalistic nature and case-control design of the study, no conclusions can be made regarding causality between *N*-acetyl group metabolites and schizophrenia. Secondly, despite controlling for potential confounds such as age, sex and BMI, the impact of unmeasured factors cannot be disregarded (e.g., cigarette smoking and alcohol use may influence *N*-acetyl group metabolites levels). In addition, the small sample size of the pilot study design may limit the extrapolation of the current research results; further larger studies are required to confirm these results (Thabane et al., 2010). Finally, the presentation and characterization of schizophrenia is highly heterogeneous, which limits the generalizability of our findings. While the exact role of *N*-acetyl derivatives in serum metabolic pathways remains unclear, our findings provide a rationale for exploring *N*-acetyl group metabolites in other schizophrenia populations and conducting longer studies in larger sample cohorts.

4.2. Conclusions

Using the HILIC UPLC-MS platform, the association between serum levels of five *N*-acetyl group metabolites (i.e., *N*-acetyl-glutamine, *N*-acetyl-ornithine, N^6 -acetyl-*L*-lysine, *N*-acetyl-putrescine, and *N*-acetyl-galactosamine) in serum samples and first-episode schizophrenia was investigated. The current study found that higher levels of *N*-acetyl-glutamine and lower levels of N^6 -acetyl-*L*-lysine may be associated with schizophrenia, which indicates that there is an association between *N*-acetyl derivatives and schizophrenia. Based on the results reported

herein, further exploration of the mechanisms of *N*-acetyl group metabolites and schizophrenia is warranted.

Conflict of interests

The authors declare no conflict of interests.

Authors' contribution

Wang JY, Huang NH and Cao B conceived and designed the study; Cao B, Huang NH, Zeng J, Liu YQ and Xie Q collected the data and performed the statistical analysis; Huang NH, Cao B, Pan ZH, Brietzke E, McIntyre RS, Wang JY, Park C and Cha D contributed to the discussion; Wang JY, McIntyre RS, Park C, Cha D, Brietzke E, Zhu J, Liu YQ and Xie Q revised the paper. All authors have read and approved the final version of this article.

Funding

This work was sponsored by the interdisciplinary medicine Seed Fund (BMU20140435, BMU2018MX006) of Peking University. The supporting foundation had no involvement in study design, the collection, analysis, or interpretation of data, writing of the report; or in the decision to submit the article for publication.

Institutional review board statement

This study was reviewed and approved by the Ethics Review Committee of Public Health at Peking University Health Science Center (IRB00001052-14071).

Acknowledgments

This work was supported by Peking University Health Science Center Medical Cross Disciplinary Fund (BMU20140435). We thank Dr Chuanbo Zhang and other medical staff from Weifang Mental Health Center for their assistance with subject recruitment, follow up and blood sample collection. Beijing Omics Bio-tech Co., Ltd for their kind help in data collection for this study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2018.11.064.

References

- Beloosesky, R., Gayle, D.A., Amidi, F., Nunez, S.E., Babu, J., Desai, M., Ross, M.G., 2006. *N*-acetyl-cysteine suppresses amniotic fluid and placenta inflammatory cytokine responses to lipopolysaccharide in rats. *Am. J. Obstet. Gynecol.* 194 (1), 268–273.
- Bergana, M.M., Holton, J.D., Reyzer, I.L., Snowden, M.K., Baxter, J.H., Pound, V.L., 2000. NMR and MS analysis of decomposition compounds produced from *N*-acetyl-*L*-glutamine at low pH. *J. Agric. Food Chem.* 48 (12), 6003–6010.
- Berk, M., Copolov, D., Dean, O., Lu, K., Jeavons, S., Schapkaity, I., Anderson-Hunt, M., Judd, F., Katz, F., Katz, P., Ording-Jespersen, S., Little, J., Conus, P., Cuenod, M., Do, K.Q., Bush, A.L., 2008. *N*-acetyl cysteine as a glutathione precursor for schizophrenia—a double-blind, randomized, placebo-controlled trial. *Biol. Psychiatry* 64 (5), 361–368.
- Cao, B., Jin, M., Brietzke, E., McIntyre, R.S., Wang, D., Rosenblat, J.D., Raguett, R.M., Zhang, C., Sun, X., Rong, C., Wang, J., 2018a. Serum metabolic profiling using small molecular water-soluble metabolites in individuals with schizophrenia: a longitudinal study using a pre-post treatment design. *Psychiatry Clin. Neurosci.*
- Cao, B., Wang, D., Brietzke, E., McIntyre, R.S., Pan, Z., Cha, D., Rosenblat, J.D., Zuckerman, H., Liu, Y., Xie, Q., Wang, J., 2018b. Characterizing amino-acid bio-signatures amongst individuals with schizophrenia: a case-control study. *Amino Acids* 50 (8), 1013–1023.
- Carmeli, C., Knyazeva, M.G., Cuenod, M., Do, K.Q., 2012. Glutathione precursor *N*-acetyl-cysteine modulates EEG synchronization in schizophrenia patients: a double-blind, randomized, placebo-controlled trial. *PLoS One* 7 (2), e29341.
- Chen, G.G., Fiori, L.M., Mamer, O.A., Turecki, G., 2011. High-resolution capillary gas chromatography in combination with mass spectrometry for quantification of three

- major polyamines in postmortem brain cortex. *Methods Mol. Biol.* 720, 427–436.
- Engelke, U.F., Sass, J.O., Van Coster, R.N., Gerlo, E., Olbrich, H., Krywawych, S., Calvin, J., Hart, C., Omran, H., Wevers, R.A., 2008. NMR spectroscopy of aminoacylase 1 deficiency, a novel inborn error of metabolism. *NMR Biomed.* 21 (2), 138–147.
- Fernandes, J., Mudgal, J., Rao, C.M., Arora, D., Basu Mallik, S., Pai, K.S.R., Nampoothiri, M., 2018. *N*-acetyl-*L*-tryptophan, a substance-P receptor antagonist attenuates aluminum-induced spatial memory deficit in rats. *Toxicol. Mech. Methods* 28 (5), 328–334.
- Fiori, L.M., Turecki, G., 2008. Implication of the polyamine system in mental disorders. *J. Psychiatry Neurosci.* 33 (2), 102–110.
- Goldstein, M.E., Anderson, V.M., Pillai, A., Kydd, R.R., Russell, B.R., 2015. Glutamatergic neurometabolites in clozapine-responsive and -resistant schizophrenia. *Int. J. Neuropsychopharmacol.* 18 (6).
- Gross, J.A., Turecki, G., 2013. Suicide and the polyamine system. *CNS Neurol. Disord. Drug Targets* 12 (7), 980–988.
- Hashimoto, K., 2014. Abnormalities of the glutamine-glutamate-GABA cycle in the schizophrenia brain. *Schizophr. Res.* 156 (2-3), 281–282.
- Khandaker, G.M., Cousins, L., Deakin, J., Lennox, B.R., Yolken, R., Jones, P.B., 2015. Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. *Lancet. Psychiatry* 2 (3), 258–270.
- Lee, S.H., Suh, J.W., Chung, B.C., Kim, S.O., 1998. Polyamine profiles in the urine of patients with leukemia. *Cancer Lett.* 122 (1-2), 1–8.
- Liu, Y., Ho, R.C., Mak, A., 2012. Interleukin (IL)-6, tumour necrosis factor alpha (TNF- α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. *J. Affect. Disord.* 139 (3), 230–239.
- Lu, Y., Ho, C.S., Liu, X., Chua, A.N., Wang, W., McIntyre, R.S., Ho, R.C., 2017. Chronic administration of fluoxetine and pro-inflammatory cytokine change in a rat model of depression. *PLoS One* 12 (10), e0186700.
- Marsman, A., van den Heuvel, M.P., Klomp, D.W., Kahn, R.S., Luijten, P.R., Hulshoff Pol, H.E., 2013. Glutamate in schizophrenia: a focused review and meta-analysis of (1)H-MRS studies. *Schizophr. Bull.* 39 (1), 120–129.
- Molina, V., Sanchez, J., Sanz, J., Reig, S., Benito, C., Leal, I., Sarramea, F., Rebolledo, R., Palomo, T., Desco, M., 2007. Dorsolateral prefrontal *N*-acetyl-aspartate concentration in male patients with chronic schizophrenia and with chronic bipolar disorder. *Eur. Psychiatry* 22 (8), 505–512.
- Natsubori, T., Inoue, H., Abe, O., Takano, Y., Iwashiro, N., Aoki, Y., Koike, S., Yahata, N., Katsura, M., Gono, W., Sasaki, H., Takao, H., Kasai, K., Yamasue, H., 2014. Reduced frontal glutamate + glutamine and *N*-acetylaspartate levels in patients with chronic schizophrenia but not in those at clinical high risk for psychosis or with first-episode schizophrenia. *Schizophr. Bull.* 40 (5), 1128–1139.
- Owen, M.J., Sawa, A., Mortensen, P.B., 2016. Schizophrenia. *Lancet* 388 (10039), 86–97.
- Pitt, J.J., 2009. Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. *Clin. Biochem. Rev.* 30 (1), 19–34.
- Prabakaran, S., Swatton, J.E., Ryan, M.M., Huffaker, S.J., Huang, J.T., Griffin, J.L., Wayland, M., Freeman, T., Dudbridge, F., Lilley, K.S., Karp, N.A., Hester, S., Tkachev, D., Mimmack, M.L., Yolken, R.H., Webster, M.J., Torrey, E.F., Bahn, S., 2004. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol. Psychiatry* 9 (7), 684–697.
- Shi, J., Levinson, D.F., Duan, J., Sanders, A.R., Zheng, Y., Pe'er, I., Dudbridge, F., Holmans, P.A., Whittemore, A.S., Mowry, B.J., Olincy, A., Amin, F., Cloninger, C.R., Silverman, J.M., Buccola, N.G., Byerley, W.F., Black, D.W., Crowe, R.R., Oksenberg, J.R., Mirel, D.B., Kendler, K.S., Freedman, R., Gejman, P.V., 2009. Common variants on chromosome 6p22.1 are associated with schizophrenia. *Nature* 460 (7256), 753–757.
- Song, X., Fan, X., Li, X., Zhang, W., Gao, J., Zhao, J., Harrington, A., Ziedonis, D., Lv, L., 2014. Changes in pro-inflammatory cytokines and body weight during 6-month risperidone treatment in drug naive, first-episode schizophrenia. *Psychopharmacology (Berl)* 231 (2), 319–325.
- Tamminga, C.A., Holcomb, H.H., 2005. Phenotype of schizophrenia: a review and formulation. *Mol. Psychiatry* 10 (1), 27–39.
- Thabane, L., Ma, J., Chu, R., Cheng, J., Ismail, A., Rios, L.P., Robson, R., Thabane, M., Giangregorio, L., Goldsmith, C.H., 2010. A tutorial on pilot studies: the what, why and how. *BMC Med. Res. Methodol.* 10, 1.
- Wass, C., Klamer, D., Katsarogiannis, E., Palsson, E., Svensson, L., Fejgin, K., Bogren, I.B., Engel, J.A., Rembeck, B., 2011. *L*-lysine as adjunctive treatment in patients with schizophrenia: a single-blinded, randomized, cross-over pilot study. *BMC Med.* 9, 40.
- Wells, L., Vosseller, K., Hart, G.W., 2003. A role for *N*-acetylglucosamine as a nutrient sensor and mediator of insulin resistance. *Cell Mol. Life Sci.* 60 (2), 222–228.
- Yu, G., Liang, Y., Huang, Z., Jones, D.W., Pritchard Jr., K.A., Zhang, H., 2016. Inhibition of myeloperoxidase oxidant production by *N*-acetyl lysyltyrosylcysteine amide reduces brain damage in a murine model of stroke. *J. Neuroinflammation* 13 (1), 119.
- Zeinoddini, A., Ahadi, M., Farokhnia, M., Rezaei, F., Tabrizi, M., Akhondzadeh, S., 2014. *L*-lysine as an adjunct to risperidone in patients with chronic schizophrenia: a double-blind, placebo-controlled, randomized trial. *J. Psychiatr. Res.* 59, 125–131.