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Metabolic profiling identifies phospholipids as potential serum biomarkers for schizophrenia

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ABSTRACT

Schizophrenia (SCZ) is a multifactorial psychiatric disorder. However, the molecular pathogenesis of SCZ remains largely unknown, and no reliable diagnostic test is currently available. Phospholipid metabolism is known to be disturbed during disease processes of SCZ. In this study, we used an untargeted liquid chromatography-mass spectrometry (LC-MS)-based metabolic profiling approach to measure lipid metabolites in serum samples from 119 SCZ patients and 109 healthy controls, to identify potential lipid biomarkers for the discrimination between SCZ patients and healthy controls. 51 lipid metabolites were identified to be significant for discriminating SCZ patients from healthy controls, including phosphatidylcholines (PCs), lysophosphatidylcholines (LPCs), phosphatidylethanolamines (PEs), lysophosphatidylethanolamines (LPEs) and sphingomyelins (SMs). Compared to healthy controls, most PCs and LPCs, as well as all PEs in patients were decreased, while most LPEs and all SMs were increased. A panel of six lipid metabolites could effectively discriminate SCZ patients from healthy controls with an area under the receiver-operating characteristic curve of 0.991 in the training samples and 0.980 in the test samples. These findings suggest that extensive disturbances of phospholipids may be involved in the development of SCZ. This LC-MS-based metabolic profiling approach shows potential for the identification of putative serum biomarkers for the diagnosis of SCZ.

1. Introduction

Schizophrenia (SCZ) is a multifactorial psychiatric disorder with a worldwide prevalence of approximately 0.5–1% (Saha et al., 2005), causing tremendous socioeconomic burden and negatively impacting the quality of life of patients and their families. It is characterized by psychopathology, cognitive and neurobiological abnormalities, and deficits in perception, emotions, and social behavior (Addington and Addington, 1999; Gonzalez-Liencres et al., 2014). Despite extensive research on SCZ, the underlying pathogenesis of this complex disorder remains largely unknown. Clinically, there is no reliable diagnostic test available, and current diagnosis of SCZ primarily relies on the subjective identification of clinical symptoms of patients (van Os and Kapur, 2009). Thus, it is of significant clinical value to identify molecular biomarkers sensitive to the pathological processes of SCZ, which can facilitate a better understanding of the pathogenesis and the development of objective diagnostic tools.

Lipid metabolism plays an important role in the central nervous system (CNS), as CNS has the second highest concentrations of lipids, after adipose tissue (Sethi et al., 2017a). Many neurological disorders (such as SCZ) and neurodegenerative diseases (such as Alzheimer's and Parkinson's diseases) involve dysregulated lipid metabolism (Adibhatla and Hatcher, 2007). Phospholipids are major components of neuronal membranes, and have a crucial role in neuronal and synaptic growth and remodeling (Sethi et al., 2017a). Current research on the neurological deficits of SCZ has focused on aberrations in phospholipids

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metabolism, which lead to failure of normal neurodevelopment in SCZ (Sethi et al., 2017a). Previous studies have demonstrated that SCZ is associated with alterations in phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), and sphingomyelin (SM) (Castillo et al., 2016; Misiak et al., 2017; Weber-Fahr et al., 2013). However, currently, comprehensive studies on global changes of phospholipids in SCZ are still limited.

Metabolomics enables the parallel measurement of biological samples to detect hundreds of metabolites including lipids (Chadeau-Hyam et al., 2010; Gu et al., 2012; Holmes et al., 2008). Since metabolite levels are sensitive to subtle perturbations in pathological status, metabolomics provides a powerful approach to measure global changes of metabolic profiles in response to disease (Gu et al., 2012). Over the past decade, metabolomics has resulted in rapid progress in disease biomarker discovery, and metabolomics approaches have been widely used for the integrated analysis of multiple biochemical pathways disturbed by psychiatric disorders, such as bipolar disorder, depression, and SCZ (Liu et al., 2015; Sethi and Brietzke, 2015; Sethi et al., 2017b). In fact, metabolomics studies have implicated the disturbance of phospholipid metabolism in the disease processes of SCZ. For example, Kaddurah-Daouk et al. identified significant lower levels of plasma PE lipids in SCZ patients compared to controls, and a non-significant trend toward the decrease in PCs (Kaddurah-Daouk et al., 2007). McEvoy et al. found significant changes of PC and PE lipids in the plasma of first-episode SCZ patients, both at baseline and after treatment (McEvoy et al., 2013). These changes in phospholipid metabolism indicated a metabolic vulnerability in patients with SCZ that occurs early during disease development. However, comprehensive metabolomics analyses of disturbances in phospholipid metabolic pathways for SCZ are still far from complete.

Our study mainly focuses on phospholipid alterations in SCZ patients. We applied an untargeted liquid chromatography-mass spectrometry (LC-MS)-based global profiling approach in conjunction with multivariate statistical analyses to compare lipid profiles in the serum samples of SCZ patients and healthy controls. Our study aimed to identify potential biomarkers associated with the diagnosis of SCZ, as well as to provide more evidence of phospholipid alterations for further focused studies on the underlying mechanisms of SCZ.

2. Methods

2.1. Ethics statement

The ethical committee of Peking University Health Science Center reviewed and approved the protocol for this study (IRB00001052-14071) and the procedures employed for sample collection. All participants signed a written informed consent before any procedure was carried out. This study was conducted in accordance with the Helsinki Declaration, as revised in 1989.

2.2. Subjects

A total of 119 inpatients meeting the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for SCZ were recruited from the inpatient clinic of the Weifang Mental Health Center (Shandong Province, China). All subjects met the following criteria: (1) age less than 40 years old; (2) no diabetes, hyperlipidemia, cardiovascular disease, or other severe physical diseases; (3) no additional mental disorders, alcoholism, or other substance abuse disorders. Of these, 28 patients were first-episode and drug-naïve, while the remaining SCZ subjects ($n = 91$) were recurrent and had not taken any antipsychotic drugs for at least 1 month before hospitalization. During the same time period, we recruited 109 healthy controls who matched the patients in age (± 5 years), gender, and ethnicity. We excluded subjects whose first degree relatives had a history of neurological diseases, psychiatric disorders, or severe physical diseases. The Positive and Negative Syndrome Scale (PANSS) was used to evaluate psychopathology of all participants.

2.3. Sample preparation

After overnight fasting of the subjects, blood samples were collected. Serum samples were separated and stored at −80 °C until use. For LC-MS analysis, 100 µl of each serum sample was aliquoted into a labeled 1.5 ml Eppendorf vial. Protein precipitation and metabolite extraction were performed by adding 300 µL methanol; the mixture was then vortexed for 15 s and stored at −20 °C for 20 min. Next, each sample was centrifuged at 12,000 × g for 10 min, and 100 µl supernatant was collected into a 200 µl vial insert for analysis. A pooled sample from all the samples was extracted using the same procedure as described above. This sample was used as the quality control (QC) sample and was analyzed once for every 10–15 study samples. A series of QC samples with 2- and 4-fold dilutions were prepared and analyzed within the experimental run to assess the linearity of extracted features.

2.4. LC-MS data acquisition

UPLC separation was performed using the Thermo Scientific Dionex UltiMate 3000 Rapid Separation LC (RSLC) system using the gradient conditions shown in supplemental Table S1. 1 µl of each sample was injected for analysis using negative and positive ionization mode, respectively. Both chromatographic separations were performed using a CSH C18 column (2.1 × 100 mm, 1.7 µm, Waters) operated at 45 °C. The flow rate was 0.3 ml/min, and the auto-sampler temperature was kept at 4 °C. Mobile phase A was methanol, and mobile phase B was water containing 0.1% formic acid.

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. The positive and negative HESI-II spray voltages were 3.7 kV and 3.5 kV, respectively. The heated capillary temperature was 320 °C, the sheath gas pressure was 30 psi, the auxiliary gas setting was 10 psi, and the heated vaporizer temperature was 300 °C. Nitrogen was used for both the sheath and the auxiliary gases. The nitrogen collision gas was set to a pressure of 1.5 mTorr. The parameters for the full mass scan were as follows: resolution 70,000, auto gain control target $< 1 \times 10^6$, and m/z range 100–1500. The calibration was customized for the analysis by the Q Exactive instrument to keep the mass tolerance < 5 ppm. Data-dependent MS/MS were acquired on a "Top 10" data-dependent mode. The parameters for MS/MS experiments were as follows: resolution 17,500, auto gain control target $< 1 \times 10^5$, isolation window 2 m/z, and normalized collision energy 30 V. The LC-MS system was controlled using Xcalibur 2.2 SP 1.48 software (Thermo Fisher Scientific, Rockford, IL, USA), and data were collected and processed with the same software.

2.5. Metabolomics data analysis and biomarker identification

The data analysis steps are shown in supplemental Fig. S1. All obtained LC-MS raw data were imported into Progenesis QI data analysis software (Nonlinear Dynamics, Newcastle, UK) for data processing. Peak deconvolution, alignment, and picking were carried out to produce a list of mass-to-charge ratio (m/z) and retention time (RT) pairs with the corresponding peak intensities. To reduce the false positive results that might be either spectral noise or not biologically relevant (Karaman, 2017), the features were then selected based on their coefficients of variation (CVs) of the QC injections. Features with CVs of all QC injections larger than 20% were eliminated. Also, the dilution factors were regressed against the corresponding intensities of each feature in serially diluted QC samples. Features with low R^2 and with negative beta coefficients were thus removed from the data table. Furthermore, features missing in over 20% of samples in both patient and control

Table 1
Demographic and clinical characteristics of SCZ patients and healthy controls.

Variables	Training set[a]			Test set[b]		
	SCZ-BL (N = 84)	Controls (N = 77)	$p1$	SCZ-BL (N = 35)	Controls (N = 32)	$p2$
Age (years)	29.13 (6.05)	30.61 (4.87)	0.091*	27.93 (6.67)	30.5 (3.94)	0.087*
Gender; male n (%)	37 (44.04)	25 (32.47)	0.071#	13 (37.14)	12 (37.50)	0.976#
BMI (kg/m^2)	23.54 (3.92)	23.06 (3.21)	0.389*	23.90 (3.80)	23.94 (3.73)	0.945*
Current smoker; n (%)	10 (11.90)	5 (6.49)	0.315#	3 (8.57)	0 (0.00)	0.085#
Current drinker; n (%)	5 (6.00)	10 (12.99)	0.292#	3 (8.57)	6 (18.75)	0.435#
Psychiatric family history; n (%)	15 (17.86)	0 (0.00)	<0.001#	3 (8.57)	0 (0.00)	0.240#
Fasting glucose (mmol/L)†	5.09 (4.95–5.23)	5.29 (5.18–5.41)	0.256‡	5.18 (5.01–5.37)	5.44 (5.24–5.63)	0.107‡
TG (mmol/l)†	1.03 (0.91–1.18)	0.92 (0.82–1.04)	0.158‡	0.94 (0.77–1.14)	1.00 (0.81–1.22)	0.912‡
TC (mmol/l)†	4.49 (4.26–4.73)	4.20 (4.01–4.41)	0.006‡	4.32 (4.04–4.63)	4.43 (4.16–4.72)	0.544‡
VLDL (mmol/l)†	0.48 (0.42–0.54)	0.42 (0.37–0.47)	0.129‡	0.43 (0.36–0.52)	0.46 (0.37–0.56)	0.865‡
Age of onset (years)†	22.06 (20.90–23.29)	–	–	21.50 (19.54–23.66)	–	–
Duration of illness (years)†	4.20 (3.24–5.43)	–	–	3.73 (2.39–5.83)	–	–
First-episode patients; n (%)	20 (23.81)	–	–	8 (22.86)	–	–
PANSS scores						
Total score†	86.58 (82.52–90.85)	–	–	86.16 (80.10–92.69)	–	–
Positive symptoms†	19.56 (17.60–21.75)		–	20.17 (17.55–23.12)	–	–
Negative symptoms†	17.85 (15.81–20.14)		–	20.36 (18.68–22.62)	–	–
General psychopathology†	38.11 (33.43–43.45)	–	–	44.02 (40.67–47.51)	–	–

Values are the arithmetic means (SD) unless otherwise stated.
† Values are geometric means (95% CI).
* p values were calculated by two-tailed t test.
# p values were calculated using the chi-square test.
‡ p values were calculated using GLM adjusting for age, gender, BMI, smoking, drinking and psychiatric family history. BMI, body mass index; TG, triglyceride; TC, total cholesterol; VLDL, very low density lipoprotein; PANSS, Positive and Negative Syndrome Scale.

groups were removed (Bijlsma et al., 2006), and the remaining missing values in the data set were estimated using a k-nearest neighbor imputation method (Karaman, 2017).

The resulting data were normalized by MS total useful signal (MSTUS) which is the sum of the intensities of those features that are detected (Chen et al., 2013), and $\log_{10}$-transformed to approximate normality. These features were compared between SCZ patients and healthy controls in the training set by generalized linear models (GLMs) with adjustment for age, gender, body mass Index (BMI), smoking, drinking and psychiatric family history using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). The Benjamini-Hochberg false discovery rate (FDR) control was implemented to correct for multiple comparisons, and the q values are FDR corrections for p values calculated from GLMs. In this study, the threshold of FDR q-value for significant markers was set at 0.05. Then the data were imported into the Simca-P 14.1 software (Umetrics, Umeå, Sweden) for multivariate analysis. The enrolled subjects were randomly separated into a training set (70% of the samples in each group) and a test set (30% of the samples in each group), shown in Table 1. The training set included 84 patients and 77 controls; the remaining patients and controls made up the test set. Principal component analysis (PCA) was first utilized to get an overview of systematic variations and general clustering among all subjects in the training set. To identify differential metabolites, partial least-squares discriminant analysis (PLS-DA) was performed using the training set data with auto-scaling to construct classification models. An internal 7-fold cross-validation was carried out to estimate the performance of PLS-DA models. The quality of the PLS-DA models was described by three parameters (R^2X, R^2Y, and Q^2Y). The calculated R^2X and R^2Y were used to estimate the goodness-of-fit of the model that represent the fractions of explained X and Y-variation, respectively; Q^2Y was applied to assess the predictability of the models (Mahadevan et al., 2008). Excellent models are obtained when the values of R^2Y and Q^2 are above 0.8 (Xuan et al., 2011). Model validations were also performed using 300-iteration permutation tests that could evaluate whether the specific classification of the individuals in the two designed groups is significantly better than any other random classification in two arbitrary groups (Westerhuis et al., 2008). If the values of Q^2 and R^2 resulting from the original model were higher than the corresponding values from the permutation test, the model was considered valid (Mahadevan et al., 2008). Based on the variable importance in projection (VIP) values from the cross-validated models which summarize the importance of the variables (Chong and Jun, 2005; Mehmood et al., 2012), a number of significant variables were obtained for the separation of different groups. Finally, the differential metabolic features were obtained based on VIP values (VIP >1) taken from PLS-DA model and significant q-values ($q < 0.05$) derived from corrected GLM analyses. These differential features were initially identified by comparing the m/z with the human metabolome database (HMDB, http://www.hmdb.ca/)(Wishart et al., 2007) and LIPID MAPS (http://www.lipidmaps.org/)(Fahy et al., 2007) with the mass tolerance 5 ppm. Further, manual identification was performed by matching MS/MS spectra of each feature with the commonly occurring product ions for lipids in Mass Spectrometry Analysis Tools of LIPID MAPS (http://www.lipidmaps.org/resources/tools/index.php) (Fahy et al., 2007), and matching with MS spectra and retention times of pure chemical standards in-house.

Areas under the receiver-operating characteristic (ROC) curves (AUROCs) of the identified differential lipids were calculated to evaluate their individual classification accuracy using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Lipid metabolites with AUROCs >0.850 were then selected for further exploration of a simplified set of lipid biomarkers for SCZ. In the training set samples, backward stepwise logistic regression model based on Akaike's information criterion (AIC) (Zheng et al., 2013) was performed by SAS 9.4 (SAS Institute, INC., Cary, NC, USA) to optimize the metabolite biomarker combination for the discrimination between SCZ patients and healthy controls. Finally, the optimized panel of biomarkers was used to construct a new PLS-DA model for discrimination between above two groups in the training set. AUROCs were then calculated to evaluate the classification performance of the PLS-DA model in both training and test sets.

For demographic and clinical characteristics, the chi-square test was used to test the statistical significance between patient and control groups for categorical variables. For continuous variables, the Student's t test was used for comparisons between the two groups. Partial correlation analysis was used to calculate correlation coefficients among identified lipid metabolites, as well as of metabolites with clinical measurements with adjustment for age, gender, BMI, smoking, drinking and psychiatric family history. Two-tailed p-value of less than 0.05 was

considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics

A total of 119 SCZ patients and 109 healthy controls were included in the study. The total cholesterol of patients in the training set was higher than that of controls. There was no statistically significant difference of other demographic and clinical characteristics between patients and healthy controls in both training and test sets (Table 1). We then separated the patient group into two subgroups: first episode and recurrent patients. Detailed basic characteristics of these two groups are shown in supplemental Table S2. It was shown that none of PANSS positive symptom, negative symptom, general psychopathology scores, or total scores had any significant difference between first episode patients and recurrent ones.

3.2. Serum lipid profiles of SCZ patients and healthy controls

In the obtained LC-MS raw data, there were in total 758 features detected in positive and negative mode after peak deconvolution, alignment, and picking. A total of 391 features were generated after data preprocessing (supplementary Fig. S1). After normalized by MSTUS, the median coefficient of variation (CV) of QC was 10.3%, ranging from 2.3 to 21.4%, with 82% of metabolites having CV $<15\%$ in QC sample (supplementary Fig. S2).

These 391 features were used for subsequent multivariate analysis. The PCA model derived from LC-MS spectra of all samples showed separation trend between controls and patients with SCZ (R^2X (cum) $=0.831$, Q^2 (cum) $= 0.632$; supplementary Fig. S3). The PLS-DA model was further employed to explore intrinsic differences in lipid profiles between patients and controls in the training set. As presented in Fig. 1A, the PLS-DA score plot (Okada et al., 2010; Qi et al., 2014) showed a clear discrimination between these two groups (R^2X (cum) $=0.400$, R^2Y (cum) $= 0.923$, Q^2 (cum) $= 0.857$), suggesting that lipid perturbations under the pathological condition were evident in the patients. In the training set, according to the VIP values (VIP > 1) taken from the PLS-DA model and significant q-values ($q < 0.05$) derived from FDR control for GLM analyses, 151 statistically differential features were obtained, among which there were 51 lipid metabolites identified by searching HMDB and LIPID MAPS databases with MS and MS/MS spectra, and 30 lipid metabolites validated using pure chemical standards in-house (Table 2). These 51 metabolites included PCs, lysophosphatidylcholines (LPCs), PEs, lysophosphatidylethanolamines (LPEs) and SMs. A permutation test with 300 iterations was performed, and the results showed that the PLS-DA model was valid, as the true-class Q^2 and R^2 values to the right were significantly higher than the corresponding permutated values to the left (Fig. 1B). A heatmap of the levels for the 51 significant lipid metabolites that differentiated patients from healthy controls is shown in supplementary Fig. S4. As shown in Table 2 and Fig. 2, the changes of 21 differential PCs were complex, including 6 that increased and 15 that decreased. Most of the differentiating LPCs, which were either saturated or monounsaturated, were decreased, while the one polyunsaturated LPC (22:6) was increased compared to healthy controls. The levels of all PEs were decreased, while all SMs were elevated in SCZ patients. For LPEs, 16:0 and 20:4 were significantly higher, but 20:0 was lower in patients compared to controls.

To assess the metabolic phenotypes of first episode and recurrent SCZ patients, the above PLS-DA model was reconstructed using data from 64 recurrent patients and 77 healthy controls in the training set (Fig. 1C). This regenerated PLS-DA model was then used to predict the class membership of 20 first-episode and drug-naïve SCZ patients in the training set. The T-predicted scatter plot from the reconstructed PLS-DA model demonstrates that 19 of the 20 recurrent patients were correctly classified (Fig. 1D). The GLM analysis also indicated that all features showed no statistical differences between recurrent and first episode patients with all q-values >0.05 after FDR correction in the training set (data not shown). These results demonstrated that the recurrent patients after at least 1 month without any antipsychotic drugs showed similar lipid profiles to that of first-episode patients.

3.3. Associations among the potential biomarkers and clinical characteristics

The correlations were calculated among individual lipid metabolites and clinical characteristics (Fig. 3). In SCZ patients, although most individual lipids were correlated with one another, the highest correlation coefficients ($|r| \geq 0.7$) were clustered among LPCs and SMs, and most individual LPCs were inversely correlated with individual SMs. Most lipid metabolites showed no significant correlation or weak correlation ($|r| \leq 0.4$) with fasting glucose, TG, TC, VLDL, onset age of disease, duration of disease, and PANSS scores (negative symptom, positive symptom, general psychopathology, and total scores).

3.4. Global alterations of lipid classes

Five major phospholipid classes, PC, LPC, PE, LPE and SM were detected in this study. We next evaluated the differences of these overall lipid classes between patients and controls by integrating the signals of the individual lipid metabolites within the same class. As shown in Fig. 4, SCZ patients had significantly higher total levels of LPE and SM compared to healthy controls after adjusting for covariates. On the other hand, the overall levels of LPC and PE in patients were significantly lower than those in controls. Overall PCs showed similar levels between patients and controls.

3.5. Biomarker panel with best classification performance

To identify a smaller set of putative lipid biomarkers for SCZ, 12 lipid metabolites with AUROCs >0.850 (Table 2) were selected to perform backward stepwise logistic regression models in the training set samples. According to the criterion of the smallest AIC, a set of seven biomarkers was selected: LPC(0:0/18:0), LPC(18:0), LPC(20:0), PC(18:2/18:2), PC(O-16:0/18:2), LPE(20:4), and PE(P-18:0/18:2). Since LPC(0:0/18:0) and LPC(18:0) are isomers, we selected LPC(18:0), which had VIP and AUROC values that were both larger than those of LPC(0:0/18:0) to simplify the potential biomarker panel. Thus, the six lipid metabolites LPC(18:0), LPC(20:0), PC(18:2/18:2), PC(O-16:0/18:2), LPE(20:4), and PE(P-18:0/18:2) were used to build a new PLS-DA model in the training set. This PLS-DA model constructed by the biomarker panel for discrimination between SCZ patients and healthy controls showed good classification performance with AUROC = 0.991 (95% CI: 0.980–1.000; sensitivity = 0.976, specificity = 0.925) for the training set (Fig. 5A), and AUROC = 0.980 (95% CI: 0.951–1.000; sensitivity = 0.971, specificity = 0.969) for the test set (Fig. 5B).

4. Discussion

Lipid profiling is an effective approach for the identification of metabolites in various biochemical pathways that are altered in response to disease (Stephenson et al., 2017). The present study utilized LC-MS-based metabolic profiling to gain a deeper understanding of global perturbations in phospholipid metabolism in SCZ, and to capture potential markers that may facilitate its diagnosis.

In this study, we found a significant decrease in the serum levels of most differential PCs, LPCs, and all PEs, as well as significant increases in most SMs levels in SCZ patients compared with healthy controls. We also detected significant alterations of three LPEs, with increased levels of 16:0 and 20:4, and decreased levels of 20:0. The total levels of LPC and PE classes were significantly reduced while the total levels of LPE

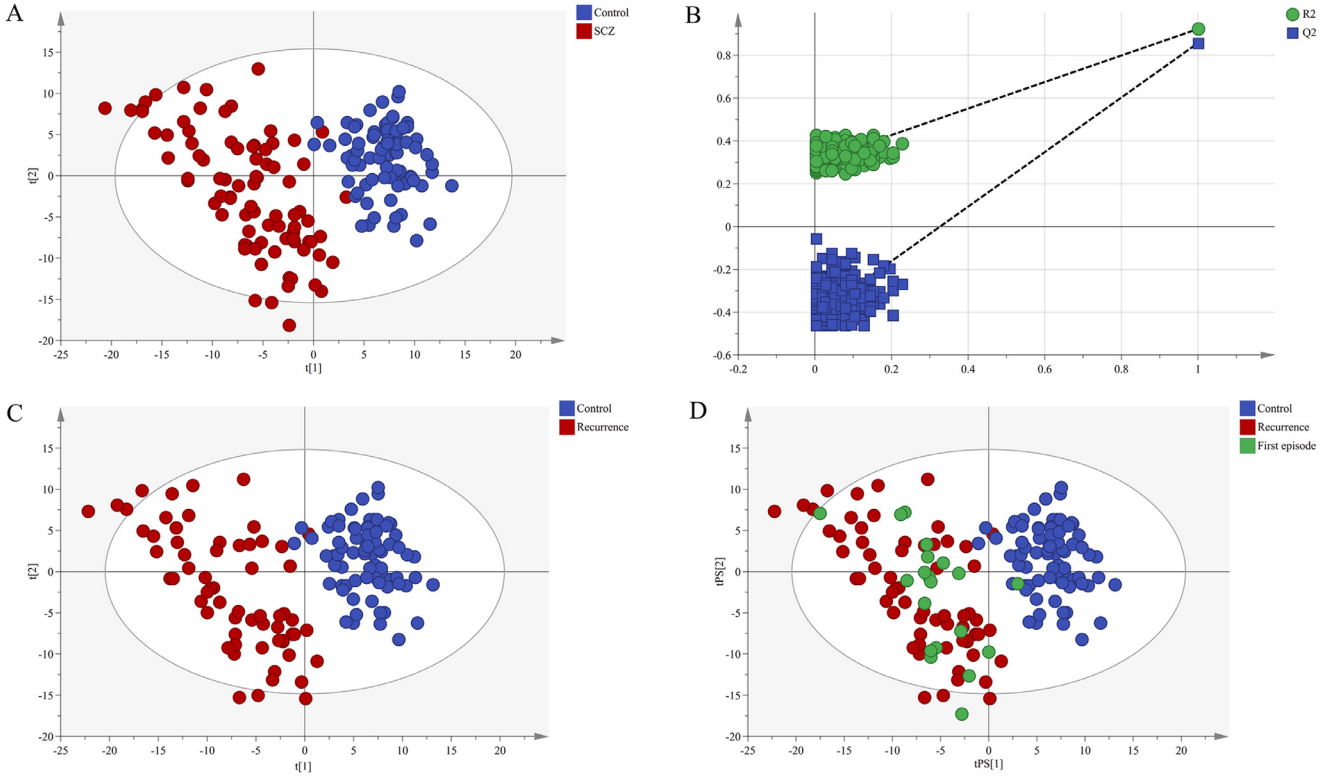

Fig. 1. Metabolomics analysis of serum samples from SCZ patients and healthy controls. (A) Score plot of the PLS-DA model showing a clear discrimination between 84 patients and 77 healthy controls in the training set. The axis t[1] and t[2] represent the scores of first and second PLS-component, respectively. (B) Statistical validation of the PLS-DA model. A 300-iteration permutation test showed that the values of R^2 (green dots) and Q^2 (blue dots) from the permuted analysis (bottom left) was significantly lower than corresponding original values (top right), suggesting this PLS-DA model was valid. (C) The PLS-DA model was reconstructed with data from 64 recurrent patients and 77 healthy controls in the training set. (D) This regenerated PLS-DA model in (C) was used to classify 20 first episode and drug-naïve patients in the training set; 19 of the 20 patients were accurately classified. The axis tPS[1] and tPS[2] represent predicted scores.

and SM classes were elevated in patients. The PC class did not show an obvious change compared to controls. These findings suggest that SCZ involves a number of lipid abnormalities.

Previous metabolomics studies for lipid metabolites in human-based studies and animal models of schizophrenia are summarized in Table 3. Certain findings of the current study are echoed by previous literature, but most of the differential lipids in Table 2 have not been reported in previous studies on schizophrenia. Kaddurah-Daouk et al. found significantly decreased level of plasma LPC(18:0) in SCZ patients (Kaddurah-Daouk et al., 2007), which was also observed in the current study. Some differential lipids in this study were also reported in other metabolomics studies, although they had no significant differences between SCZ patients and controls, such as LPC(14:0), LPC(15:0), LPC(20:0), PC(16:0/16:0), PC(16:0/18:1), SM(d18:1/16:0) (Hamazaki et al., 2010; Kaddurah-Daouk et al., 2007;. Oresic et al., 2011).

As is well known, glycerophospholipids are the main components of the cell membranes and play a major role in cell signaling, membrane anchoring, and substrate transport. PC is the major glycerophospholipid of mammalian cell membranes and is also abundant in circulation (Vance and Tasseva, 2013). The reduction of PC levels has previously been reported in different regions of brains of SCZ patients (Hamazaki et al., 2010; Schmitt et al., 2004; Schwarz et al., 2008), and plasma PC was observed to be slightly downregulated in SCZ patients (Kaddurah-Daouk et al., 2007). In our study, the total PC class was not different from controls, but there were highly significant changes in individual PC levels, with 6 PCs increased and 15 decreased. Phosphatidylcholine is synthesized from its precursor phosphocholine (Sastry, 1985). It is one of the phosphomonoesters that has consistently been shown to be decreased in the brain of SCZ patients (Fukuzako et al., 1999; Pettegrew et al., 1991; Stanley et al., 1995). Thus, our finding of decreased PC in serum might be related to reduced PC synthesis.

Our study also indicated that most LPC levels were decreased, which is consistent with a recent serum lipidomics study (Oresic et al., 2012). Among these identified LPCs, the reduced species all contained unsaturated or monounsaturated fatty acid chains, while only elevated LPC consisted of a polyunsaturated fatty acid chain 22:6. The peripheral circulating LPCs are derived from several metabolic pathways (Reichel et al., 2015). Blood LPCs can originate from hepatic secretion following the hydrolysis of cellular membrane PC but the major fraction is directly synthesized in the blood stream from lipoprotein-PC (Reichel et al., 2015). The responsible enzymes are liver secreted lecithin-cholesterol acyltransferase (LCAT) or one of several phospholipase As (PLAs). Depending on the enzymes' specificity for *sn*-1 or *sn*-2 ester bonds and the molecular PC species consumed, the enzymatic reactions result in saturated or unsaturated LPC species. The majority of plasma LPC species carry saturated fatty acids, because most of the blood LPCs are derived from the LCAT reaction that preferentially transfers linoleate from the *sn*-2 position of PC to cholesterol (Schmitz and Ruebsaamen, 2010). In contrast, highly unsaturated plasma LPC may preferentially arise from hepatic secretion (Sekas et al., 1985). The decreased saturated LPCs in our study might suggest the inhibition of LPC-generating enzymes, as well as their regulation via the decreased availability of the precursor molecule PCs. Note that PC 20:4/14:0, 22:6/14:0, 18:2/17:0, and 18:2/17:1 were also decreased. The increased LPC (22:6) might indicate that increased breakdown of membrane PC might be involved in SCZ. In addition, LPC has proinflammatory properties, and affects the function of immunoregulatory cells to modulate inflammatory processes and immune responses (Hasegawa et al., 2011). Inflammation in the central nervous system is closely related to neurodegeneration (Na et al., 2014). Our

Table 2
Differential lipids for the discrimination between SCZ patients and healthy controls.

Possible lipid metabolites	Analysis mode	Molecular formula	Accurate *m/z*	Theoretical *m/z*	Error (ppm)	RT (min)	*q*[a]	FC[b]	VIP[c]	AUROC	95% CI
LysoPC(14:0)[d]	Positive	C22H46NO7P	468.3086	468.3090	−0.854	7.07	5.38×10^{-6}	0.65	1.10	0.690	0.608–0.773
LysoPC(15:0)	Positive	C23H48NO7P	482.3237	482.3247	−2.073	7.32	1.11×10^{-5}	0.73	1.16	0.722	0.642–0.803
LysoPC(17:0)[d]	Positive	C25H52NO7P	510.3557	510.3560	−0.588	7.75	7.34×10^{-11}	0.67	1.51	0.832	0.770–0.895
LysoPC(17:1)[d]	Positive	C25H50NO7P	508.3395	508.3403	−1.574	7.45	2.74×10^{-4}	0.71	1.00	0.686	0.604–0.768
LysoPC(18:0)[d]	Positive	C26H54NO7P	524.3709	524.3716	−1.335	7.94	2.40×10^{-16}	0.71	1.71	0.887	0.838–0.936
LysoPC(0:0/18:0)[d]	Positive	C26H54NO7P	524.3712	524.3716	−0.763	7.83	3.64×10^{-15}	0.66	1.66	0.867	0.813–0.920
LysoPC(19:0)[d]	Positive	C27H56NO7P	538.3867	538.3873	−1.114	8.14	2.61×10^{-14}	0.50	1.72	0.903	0.857–0.949
LysoPC(20:0)[d]	Positive	C28H58NO7P	552.4027	552.4029	−0.362	8.33	4.56×10^{-18}	0.43	1.84	0.924	0.884–0.964
LysoPC(20:1)	Positive	C28H56NO7P	550.3870	550.3873	−0.545	8.03	6.12×10^{-11}	0.67	1.54	0.846	0.788–0.904
LysoPC(22:6)[d]	Negative	C30H50NO7P	612.3304	612.3301	0.490	7.38	2.98×10^{-13}	1.53	1.44	0.816	0.750–0.882
LysoPE(16:0)[d]	Negative	C21H44NO7P	452.2780	452.2777	0.663	7.56	1.50×10^{-9}	1.44	1.33	0.786	0.715–0.857
LysoPE(20:0)	Negative	C25H52NO7P	508.3403	508.3403	0.000	7.94	3.41×10^{-12}	0.78	1.54	0.834	0.771–0.897
LysoPE(20:4)[d]	Negative	C25H44NO7P	500.2781	500.2777	0.800	7.41	6.02×10^{-17}	1.49	1.78	0.899	0.852–0.946
PC(15:0/18:1)	Positive	C41H80NO8P	746.5683	746.5700	−2.277	10.29	3.39×10^{-7}	1.57	1.28	0.760	0.686–0.834
PC(16:0/16:0)[d]	Positive	C40H80NO8P	734.5689	734.5700	−1.497	10.49	1.88×10^{-9}	1.19	1.21	0.752	0.676–0.829
PC(16:0/18:1)[d]	Positive	C42H82NO8P	760.5837	760.5856	−2.498	10.65	1.65×10^{-17}	1.22	1.83	0.904	0.858–0.950
PC(16:0/22:5)	Positive	C46H82NO8P	808.5832	808.5856	−2.968	10.25	1.10×10^{-6}	1.15	1.16	0.751	0.677–0.825
PC(16:1/16:0)[d]	Positive	C40H78NO8P	732.5527	732.5543	−2.184	9.96	8.77×10^{-10}	1.65	1.45	0.799	0.729–0.869
PC(18:2/17:0)[d]	Positive	C43H82NO8P	772.5836	772.5856	−2.589	10.53	3.00×10^{-7}	0.76	1.32	0.795	0.725–0.865
PC(18:2/17:1)	Positive	C43H80NO8P	770.5674	770.5700	−3.374	10.00	3.44×10^{-6}	0.53	1.22	0.735	0.657–0.814
PC(18:2/18:2)[d]	Positive	C44H80NO8P	782.5675	782.5700	−3.195	9.87	2.41×10^{-12}	0.59	1.59	0.870	0.816–0.923
PC(20:3/16:0)[d]	Positive	C44H82NO8P	784.5833	784.5856	−2.931	10.32	2.15×10^{-4}	0.89	1.03	0.659	0.573–0.744
PC(20:4/14:0)[d]	Negative	C42H76NO8P	798.5297	798.5285	1.503	9.51	5.30×10^{-6}	0.75	1.09	0.719	0.641–0.797
PC(20:4/18:0)[d]	Positive	C46H84NO8P	810.5983	810.6013	−3.701	10.86	1.56×10^{-5}	1.12	1.12	0.740	0.663–0.817
PC(20:4/18:2)[d]	Positive	C46H80NO8P	806.5675	806.5700	−3.100	9.83	2.34×10^{-9}	0.77	1.37	0.802	0.735–0.870
PC(22:6/14:0)[d]	Positive	C44H76NO8P	778.5375	778.5387	−1.541	9.45	8.10×10^{-7}	0.59	1.24	0.757	0.683–0.830
PC(O-16:0/18:2)[d]	Positive	C42H82NO7P	744.5893	744.5907	−1.880	10.65	2.91×10^{-15}	0.52	1.74	0.886	0.832–0.939
PC(O-16:0/20:3)	Positive	C44H84NO7P	770.6048	770.6064	−2.076	10.81	9.29×10^{-12}	0.58	1.56	0.846	0.787–0.906
PC(O-16:0/20:4)[d]	Positive	C44H82NO7P	768.5891	768.5907	−2.082	10.60	5.57×10^{-6}	0.81	1.27	0.741	0.665–0.817
PC(O-18:0/20:4)[d]	Positive	C46H86NO7P	796.6201	796.6220	−2.385	11.46	2.01×10^{-4}	0.82	1.09	0.691	0.609–0.774
PC(O-18:0/22:6)	Positive	C48H86NO7P	820.6207	820.6220	−1.584	11.31	4.24×10^{-7}	0.65	1.38	0.779	0.706–0.851
PC(P-16:0/18:2)[d]	Positive	C42H80NO7P	742.5738	742.5751	−1.751	10.51	3.51×10^{-7}	0.75	1.28	0.753	0.679–0.827
PC(P-18:1/18:1)	Positive	C44H84NO7P	770.6056	770.6064	−1.038	11.33	3.99×10^{-9}	0.63	1.45	0.803	0.735–0.871
PC(20:4/P-18:1)	Positive	C46H82NO7P	792.5874	792.5907	−4.164	10.27	6.93×10^{-7}	0.60	1.33	0.778	0.707–0.848
PE(O-16:0/20:4)	Negative	C41H76NO7P	724.5289	724.5281	1.104	10.71	5.61×10^{-13}	0.45	1.74	0.876	0.823–0.929
PE(O-16:0/22:5)	Negative	C43H78NO7P	750.5439	750.5438	0.133	10.79	1.47×10^{-8}	0.50	1.48	0.815	0.749–0.881
PE(O-16:0/22:6)	Negative	C43H76NO7P	748.5286	748.5281	0.668	10.64	2.36×10^{-2}	0.86	1.16	0.648	0.563–0.733
PE(O-18:0/20:4)	Negative	C43H80NO7P	752.5604	752.5594	1.329	11.51	1.29×10^{-5}	0.48	1.29	0.771	0.699–0.844
PE(P-16:0/18:2)	Negative	C39H74NO7P	698.5132	698.5125	1.002	10.56	2.65×10^{-14}	0.52	1.73	0.878	0.825–0.931
PE(P-16:0/20:4)[d]	Negative	C41H74NO7P	722.5126	722.5125	0.138	10.49	9.18×10^{-7}	0.72	1.32	0.743	0.667–0.820
PE(P-16:0/22:6)	Negative	C43H74NO7P	746.5131	746.5125	0.804	10.38	2.95×10^{-3}	0.79	1.14	0.673	0.591–0.755
PE(P-18:0/18:2)[d]	Negative	C41H78NO7P	726.5447	726.5438	1.239	11.39	5.05×10^{-15}	0.48	1.73	0.878	0.825–0.930
PE(P-18:0/20:4)[d]	Negative	C43H78NO7P	750.5445	750.5438	0.933	11.31	1.09×10^{-6}	0.68	1.28	0.746	0.670–0.822
PE(P-18:0/22:6)	Negative	C45H78NO7P	774.5445	774.5438	0.904	11.17	1.44×10^{-5}	0.66	1.30	0.740	0.665–0.816
PE(P-18:1/22:6)	Negative	C45H76NO7P	772.5288	772.5281	0.906	10.54	1.68×10^{-5}	0.72	1.36	0.749	0.674–0.824
SM(d18:0/18:1)	Positive	C41H83N2O6P	731.6055	731.6067	−1.640	10.42	5.76×10^{-11}	1.47	1.48	0.817	0.752–0.883
SM(d18:0/18:2)	Positive	C41H81N2O6P	729.5898	729.5911	−1.782	9.91	1.08×10^{-14}	1.50	1.67	0.885	0.834–0.935
SM(d18:1/16:0)[d]	Positive	C39H79N2O6P	703.5734	703.5754	−2.843	9.73	1.07×10^{-7}	1.09	1.18	0.724	0.646–0.802
SM(d18:1/24:1)[d]	Negative	C47H93N2O6P	857.6755	857.6748	0.816	12.37	8.12×10^{-5}	1.33	1.02	0.713	0.630–0.795
SM(d18:2/22:1)	Negative	C45H87N2O6P	827.6288	827.6278	1.208	10.76	1.90×10^{-12}	1.68	1.55	0.842	0.784–0.901
SM(d18:2/24:1)[d]	Positive	C47H91N2O6P	811.6681	811.6693	−1.478	11.62	3.66×10^{-8}	1.40	1.27	0.772	0.700–0.844

[a] *q* values were FDR corrections for *p*-values which were calculated from GLM with adjustment for age, gender, BMI, current smoking, current drinking and psychiatric family history.

[b] Fold changes (FC) were calculated as the ratios of the geometric means of metabolite levels between two groups. FC >1 indicated a relatively higher concentration of metabolites while FC <1 indicated a relatively lower concentration in SCZ patients as compared to healthy controls.

[c] Variable importance to projection (VIP) values >1.

[d] Metabolites were validated with pure chemical standards in-house to confirm the MS spectra and retention time. The other metabolites were identified according to MS and MS/MS data using public databases. *m/z*, mass-to-charge ratio. RT, retention time.

study showed that most LPCs were diminished in SCZ patients compared to controls, which suggested that SCZ patients may be more susceptible to inflammatory disorders. One previous study on the SCZ-rheumatoid arthritis connection also suggested that prolonged low levels of LPC in early life increases susceptibility to infection (Takatera et al., 2007).

Previous studies on central and peripheral membrane lipid abnormalities of SCZ showed a substantial decrease in PE content (Kaddurah-Daouk et al., 2007; Ryazantseva et al., 2002). Similarly, a decrease in PE lipids was also observed in serum in our study. Potential mechanisms underlying these abnormalities may involve modified membrane phospholipid transporters or degradative enzymes, which result in the alterations in phospholipid distribution between the external and internal membrane leaflets (Nuss et al., 2009). The combination of diminished PE and elevated LPE levels suggested a hyperactive state of the enzyme phospholipase A2 (PLA2) (Dennis et al., 2011). Also the PLA2 activation is related to increased inflammation. In a genetically induced model of neurodegeneration, it indicated that some specific lysophospholipids were responsible for microglial activation and that cytosolic PLA2 (cPLA2) inhibition protected neurons from associated local inflammation and glutamate-mediated neurotoxicity (Sundaram et al., 2012).

Disturbed SM metabolism has been reported in other neurodevelopmental disorders like Alzheimer's disease, but it is less studied in

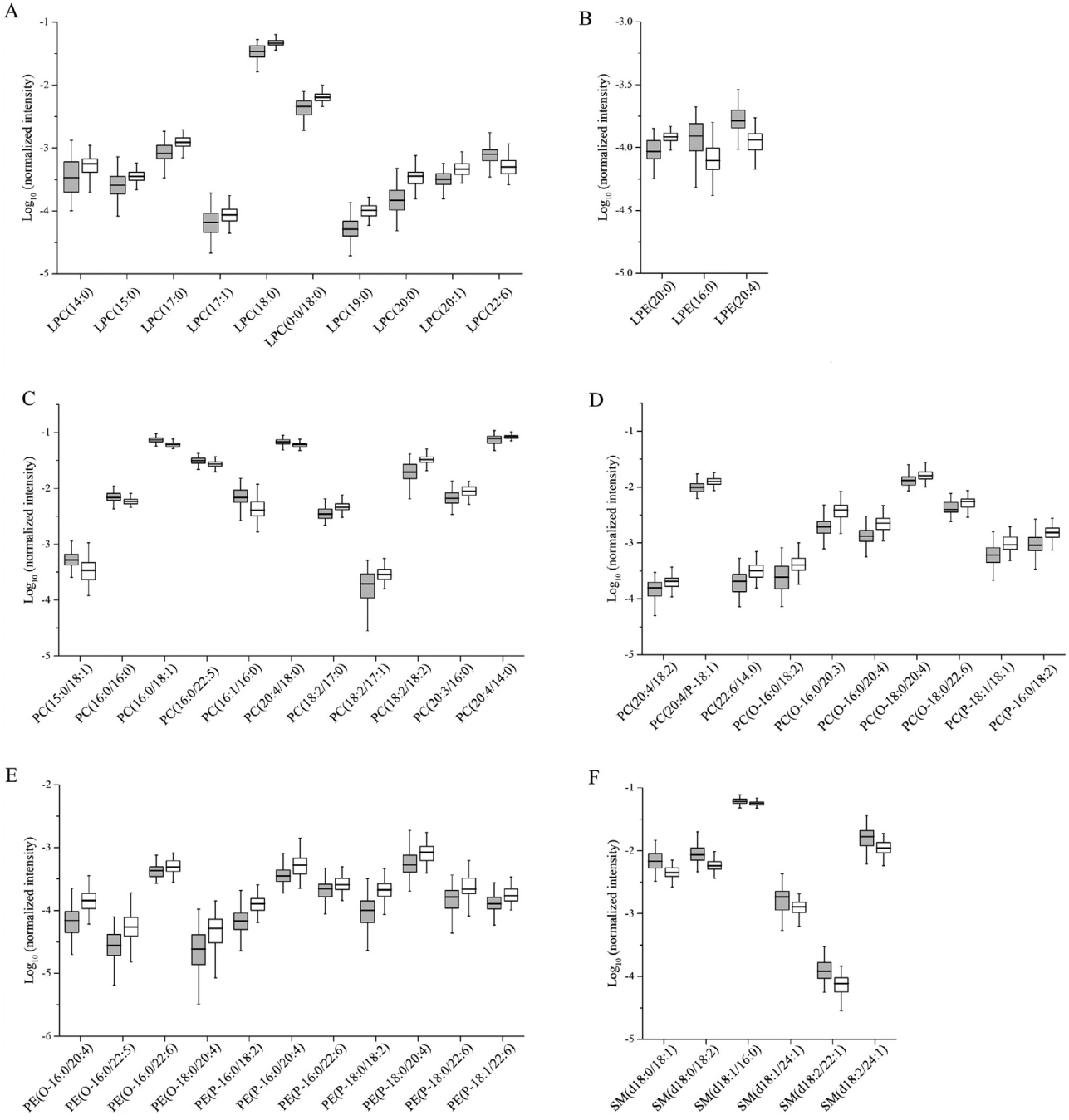

Fig. 2. Box plots of 51 differential lipid metabolites for the comparison between SCZ patients and controls. The vertical axis represents the $\log_{10}$-transformed value of the normalized peak intensity which is calculated by dividing the original peak intensity by MS total useful signal. Grey and white boxes represent SCZ patients and healthy controls, respectively.

SCZ. In the present study, all individual SMs were increased in the serum of SCZ patients, which fits well with earlier studies showing an increased level of SM in erythrocyte membranes (Keshavan et al., 1993; Ponizovsky et al., 2001). Myelin sheaths are the membrane extensions of oligodendrocytes, of which 70%–75% are composed of lipid components (Sastry, 1985). SM is one of the major components of myelin membranes. A decrease in SM levels in the central nervous system, indicating apoptosis and oligodendrocyte dysfunction in SCZ with impaired myelination, was observed by imaging and post-mortem studies (Davis et al., 2003; Flynn et al., 2003). The increased individual SMs observed in our serum-based study of SCZ suggests that there might be a different mechanism in the systematic regulation of lipid balance among different physiological compartments with SM abnormalities. In line with our findings, increased SMs had association with diminished PC levels (Schwarz et al., 2008), since PC is the choline donor to SM in neurons and oligodendrocytes (Schmitt et al., 2004). Partial correlation analysis indicated that some LPCs and SMs were highly inversely correlated in our study. SM in the blood was shown to inhibit the activities of LCAT (Subbaiah et al., 2012) and, thus, to be an important determinant of blood PC and especially LPC content (Reichel et al., 2015).

Importantly, our study revealed highly differential lipid metabolites which could discriminate SCZ patients from healthy controls. On the basis of the untargeted metabolomics data in training set, six metabolites, decreased LPC (18:0), LPC (20:2), PC (18:2/18:2), PC (O-16:0/18:2), PE (P-18:0/18:2), and increased LPE (20:4) were selected as a combinational biomarker panel for SCZ discrimination, which achieved

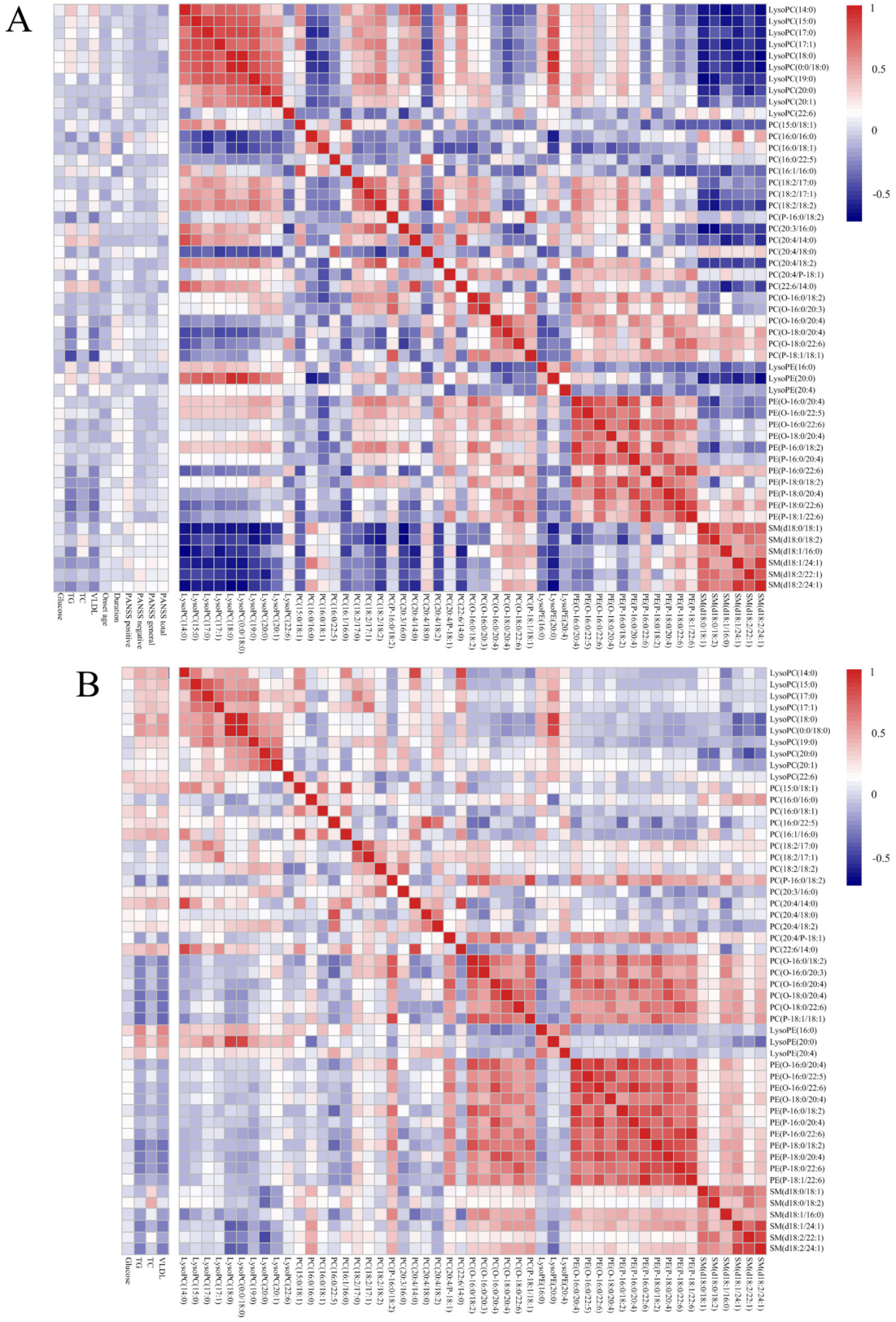

Fig. 3. Partial correlation coefficients among different lipid metabolites, and among lipid metabolites and clinical characteristics with adjustment for age, gender, BMI, smoking, drinking and psychiatric family history in (A) SCZ patients, and (B) healthy controls. Blue and red represent negative and positive correlations, respectively.

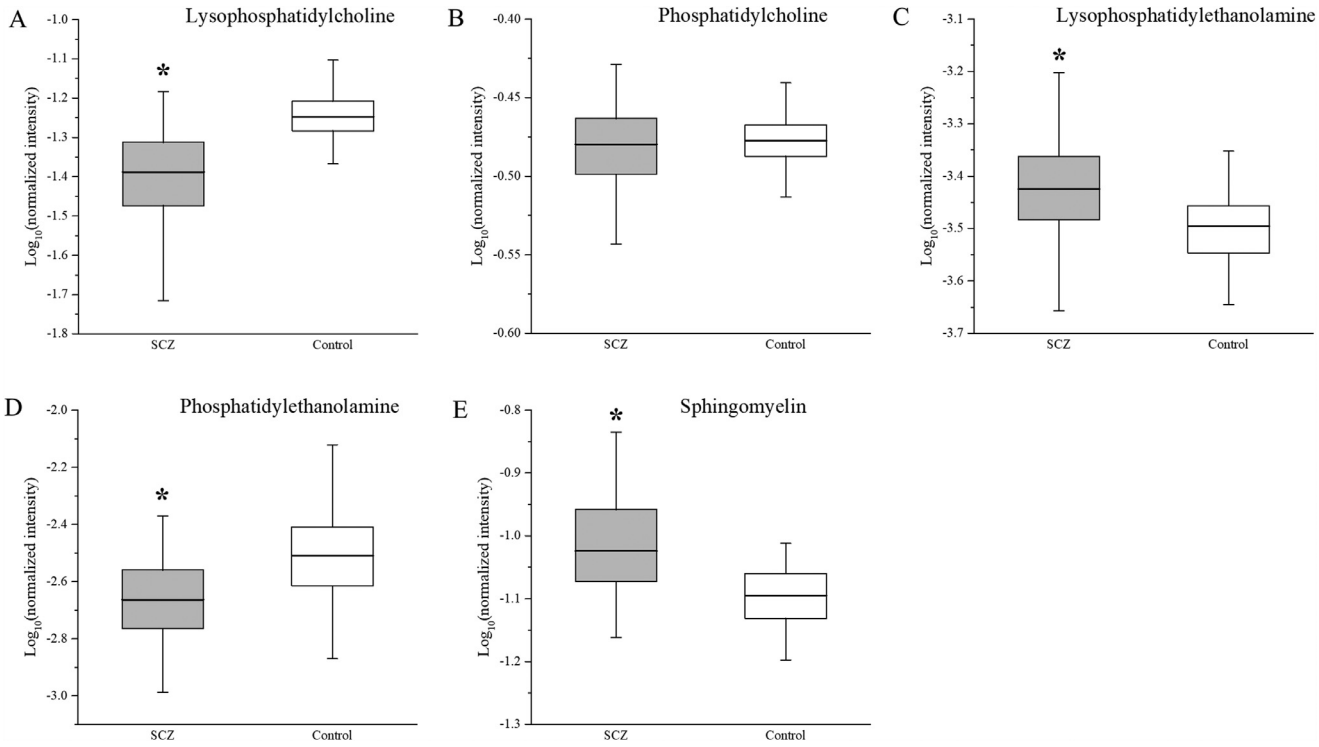

Fig. 4. Box plots of five major lipid classes. (A) lysophosphatidylcholine, (B) lysophosphatidylethanolamine, (C) and (D) phosphatidylcholine, (E) phosphatidylethanolamine, and (F) sphingomyelin in SCZ patients and controls. The vertical axis represents the $\log_{10}$-transformed value of the normalized peak intensity which is calculated by dividing the original peak intensity by MS total useful signal. Grey and white boxes represent SCZ patients and healthy controls, respectively. * $p < 0.05$, comparison between patients and controls.

good classification performances in both training and test set. Despite having measured phospholipids from serum, the observed variation may have direct implications for the lipid metabolism in the brain (Reichel et al., 2015). For instance, LPCs act as transporters of glycerophospholipid components between tissues, and are preferred carriers of polyunsaturated fatty acids across the blood-brain barrier (Lagarde et al., 2001; Nguyen et al., 2014). Thus, these phospholipid species are potentially interesting biomarkers to monitor the presence of neuropsychiatric disorders. Given the limited sample size and the heterogeneity of the disease, further studies are necessary to clarify the accuracy and specificity of these potential markers.

There are some limitations that should be noted in the present study. Firstly, the putative lipid biomarkers identified in this study should be further validated using targeted metabolomics methods and verified in larger prospective studies. Secondly, all controls in this study were healthy. Further evidence will need to be collected to indicate the specificity of these markers for SCZ. Therefore, future work recruiting extra controls, such as patients with other psychiatric disorder(s) is needed to validate the clinical value of these potential biomarkers on the classification between different types of psychiatric disorders. Moreover, only 23.5% of enrolled patients were drug-naïve first-episode patients in our study. The previous treatment effects might

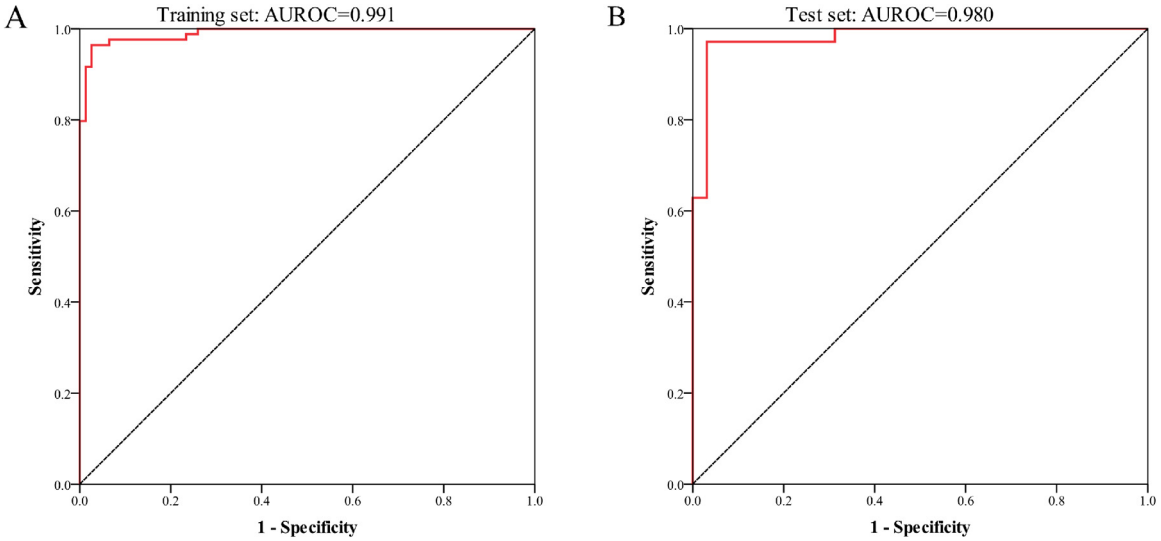

Fig. 5. ROC curves illustrate the classification performance of the combinational biomarker panel for distinguishing between (A) SCZ patients and healthy controls in the training set, (B) SCZ patients and healthy controls in the test set.

Table 3
Application of metabolomics for identification of lipid metabolites in human-based studies and animal models of schizophrenia.

Model/ Subject	Samples	Sample size (cases/ controls)	Analytical techniques	Metabolites	References
Human	Post-mortem brain tissue (thalamus)	18/23	TLC	PC (↓), SM (↓), LPC and PE (-)	Schmitt et al. (2004))
Human	Plasma	50/16	TLC, HPLC-ELSD, GC-FID	PCs (↓), LPCs (↓), PE (↓), total PC and total LPC (-), total PE (↓)	Kaddurah-Daouk et al. (2007))
Human	Post-mortem brain tissue (White matter, grey matter)	15/15	UPLC-MS	White matter PCs (↓), grey matter PCs (↑)	Schwarz et al. (2008))
Human	Post-mortem brain tissue (hippocampus)	35/35	HPLC-ESI-MS	Total PC and PE (-) PEs (-), most PCs (-)	Hamazaki et al. (2010))
Human	Serum	45/45	UPLC-QTOF-MS	Major PCs, LPCs, SMs (-)	Oresic et al. (2011)
Human	Serum	19/19	UPLC-QTOF-MS	LPCs (↓)	Oresic et al. (2012))
Human	Plasma	265/216	Commercially available Absolute*IDQ* p150 kit	PC(38:6) (↓), PC(34:3), (36:5), (42:1), (38:5), (38:0) (-)	He et al. (2012)
Human	Plasma	20/29	TLC-GC	Total PC, total LPC, and total PE (-)	McEvoy et al. (2013))
Human	Brain tissue	11/15	31P MRS	Total PC and PE within specific regions (↓)	Weber-Fahr et al. (2013))
Human	Post-mortem brain tissue (Frontal cortex)	10/10	ESI-MS/MS	Choline plasmalogens lacking PUFAs (↑), ethanolamine plasmalogens lacking PUFA (↑), ethanolamine plasmalogens possessing PUFA (↓)	Wood (2014))
Animal	Hippocampus	11/11	ESI-MS/MS	Choline plasmalogens lacking PUFA (↑), ethanolamine plasmalogens (-)	Wood (2014))
Human	Plasma	23/27	ESI-MS/MS	Choline plasmalogens and ethanolamine plasmalogens (↓)	Wood et al., 2015)
Human	Platelets	23/27	ESI-MS/MS	Choline plasmalogens (↑), ethanolamine plasmalogens (↓)	Wood et al. (2015))
Human	Post-mortem brain tissue (Frontal cortex)	10/10	Orbitrap-MS	Choline plasmalogens and ethanolamine plasmalogens (↑)	Wood and Holderman (2015))
Human	Red blood cell	74/40	LC-MS/MS	Total SM (↓), total PC and PE (-)	Tessier et al. (2016)
Human	Fibroblasts	10/10	HPLC-Exactive Orbitrap MS	PCs (↓), C40:7 PC plasmalogen and C34:1 PC plasmalogen-B (↓)	Huang et al. (2017)

The symbol "↑" and "↓" represent significantly increased and decreased levels of metabolites in SCZ patients as compared to healthy controls, respectively ($p < 0.05$). The symbol "-" represents statistically non-significant values ($p < 0.05$).

influence our findings, although our study observed similar profiles between treatment naïve patients and recurrent patients after at least 1 month without antipsychotics. The reported results in this study may encourage the exploration of lipid metabolites in drug-naïve first-episode patients with schizophrenia and large sample cohorts with longer longitudinal durations.

5. Conclusion

In this study, using a LC-MS-based metabolic profiling approach, we provided evidence that extensive disturbances of phospholipids, including PCs, LPCs, PEs LPEs and SMs were involved in the development of SCZ. Differential lipid biomarkers identified from this metabolomics analysis enabled discrimination of SCZ patients from healthy controls with high performance. These findings contribute to reveal the underlying molecular mechanisms of SCZ, and lay the foundation for the development of objective biomarkers for the diagnosis of SCZ.

Contributors

Wang JY, Wang DF and Zhang CB conceived and designed the study; Wang DF, Cao B, Sun XY and Yan JJ collected the data; Wang DF, Gu HW, and Cao B performed the statistical analysis; Wang DF, Cheng LH, Fei Q, Raftery D, Yan LL, and Wang JY contributed to the discussion; Gu HW, Raftery D, and Wang JY revised the paper. All authors have read and approved the final version of this article.

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Conflict of interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2018.12.008.

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