

Differentially expressed mRNAs of neural signaling pathway genes in peripheral blood leukocytes as biomarkers for schizophrenia

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Abnormal neurotransmitter regulation plays a role in the pathogenesis of Schizophrenia (SCZ), and the presence of brain tissue-like aberrant expression in the partial transcriptome of peripheral blood leukocytes from patients with SCZ suggests that these aberrantly expressed genes could be potential diagnostic markers. We designed a case-control study to analyze the association between the expression levels of mRNAs and SCZ in peripheral blood leukocytes and to explore their potential value as diagnostic biomarkers for SCZ. Differentially expressed mRNAs associated with neural signaling pathways were screened by RNA sequencing in a small set, comprising 9 patients with SCZ and 20 controls. A case-control study that included 217 cases and 217 controls was further conducted to verify these mRNAs. The differential expression analysis between cases and controls was performed, followed by restricted cubic spline regression analysis. Gene expression score (GES) was constructed for differentially expressed genes to assess their diagnostic value as biomarkers. In SCZ patients, there were higher expression levels of *CREB5*, *PPP3R1* and *PPP1CB* ($P < 0.05$) than in controls. Furthermore, *DUSP1* and *MAPK13* downregulated in undifferentiated SCZ and acute schizophrenia-like psychotic disorder ($P < 0.05$), *PPP3R1* expression upregulated in paranoid SCZ, undifferentiated SCZ and acute schizophrenia-like psychotic disorder ($P < 0.01$), and *CREB5* exclusively upregulated in paranoid SCZ ($P = 0.001$), respectively. The risk of SCZ was nonlinearly correlated with *DUSP1*, *GNG10*, *GNG7*, *PRKACA*, *CREB5*, *PPP3R1* and *PPP1CB* ($P_{overall} < 0.05$, $P_{nonlinear} < 0.05$). Meanwhile, incorporating these 7 genes into the GES improved the model's area under curve to 0.743, significantly enhancing the diagnostic discriminatory ability for SCZ.

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INTRODUCTION

Schizophrenia (SCZ) is a neurological disorder that frequently manifests as both positive and negative symptoms in addition to aberrant cognitive functioning. SCZ usually develops in late adolescence or early adulthood, and males are more likely to develop SCZ than females^{1–3}. For patients with SCZ, early diagnosis and treatment can help improve clinical symptoms, reduce illness suffering, improve quality of life, and extend life expectancy⁴. However, the current diagnosis of SCZ relies primarily on clinical assessment, which is subjective and may lead to misdiagnosis or delayed intervention. Given these limitations, there is an urgent need to identify objective biomarkers to improve diagnostic accuracy and facilitate treatment.

Although the precise cause of SCZ remains unknown, abnormalities in neural signaling pathways, such as abnormal dopamine system, have been accepted as a major contribution to SCZ symptoms^{5–7}. Mood, cognition, sleep and other physiological and behavioral features are influenced by the 5-hydroxytryptamine (5-HT) regulation in the central nervous system^{8–10}. The glutamate hypothesis proposes that SCZ may be linked to abnormal function of the glutamate system, a key neurotransmitter involved in learning, emotion regulation and cognitive processes^{11–13}.

SCZ is a central nervous system disorder and brain tissue samples are not easily available. Therefore, peripheral blood mononuclear cells (PBMCs) can be a useful research vehicle for SCZ because they are easily available and can reveal some

molecular changes in the central nervous system^{14,15}. Approximately 19–22% of the transcriptome was coexpressed between peripheral blood and brain tissue¹⁶. Another study also found that some genes showed differential expression in SCZ patients' peripheral blood cells and brain tissue¹⁷. The expression patterns of pathways linked to phospholipid metabolism, ribosomal signaling and energy metabolism are similar in SCZ patients' brain tissue and PBMCs¹⁸. In addition, study in Parkinson's disease has demonstrated that transcriptomic profiles in postmortem brains can also be detected in peripheral blood, with significant correlations to clinical symptoms¹⁹. These findings suggest that molecular alterations in peripheral blood may partially reflect central nervous system changes, and that identifying the association between peripheral gene expression profiles and SCZ progression could help elucidate underlying mechanisms and potential clinical applications.

In this study, we validated the association of differentially expressed mRNAs of neural signaling pathways in peripheral blood leukocytes screened by RNA-Sequencing analysis and evaluated the diagnostic values as biomarkers for SCZ.

METHODS

Subjects

This case-control study for validation included a total of 217 cases with SCZ and 217 controls. All patients with SCZ were recruited

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from the Huai'an No. 3 People's Hospital, Jiangsu Province. The controls were selected from the cohort which employed a multistage cluster random sampling method to select residents aged ≥ 18 years old in Siyang county adjacent to Huai'an city, and matched by age (difference ≤ 2 years) and gender. All SCZ patients were experiencing an acute episode and were not receiving antipsychotic medication. Two independent clinicians used the International Classification of Disease, Tenth Revision (*ICD-10*) as the reference for the SCZ's diagnostic criteria.

The study complies completely with the Declaration of Helsinki. The Institutional Review Board of Nanjing Medical University in China gave its approval to the protocols and consent forms (NJMUER201600334, NJMUER2019929). The informed consent form was signed by each participant or their legal guardians.

Data collection

The data collected from the study population included general information: age, gender, diagnosis type, smoking and drinking status. SCZ family history data were collected through interviews with participants or their first-degree relatives.

Trained psychiatrists assessed the patients based on the Positive and Negative Syndrome Scale (PANSS), including its three subscales: the positive subscale, the negative subscale and the general psychopathology subscale, after admission and before treatment.

RNA sequencing and mRNAs selection

There were 9 SCZ patients and 20 controls in the discovery set. We performed whole transcriptome RNA sequencing by Illumina Novaseq6000 platform. Differentially expressed mRNAs at $|\log_2\text{FC}| > 1$ and $P < 0.05$ were used as the basis for an enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG). The primers were amplified by primer gradient experiments and verified using Sanger sequencing. The specific primer validation process is shown in Supplemental Materials.

RNA extraction and mRNAs expression detection

Over 8 hours of fasting of participants, venous blood was collected using an anticoagulant tube with ethylene diamine tetraacetic acid (EDTA)-k2, and white blood cells were rapidly separated and mixed with human peripheral blood RNA preservation solution (Eaglink, EGEN2026, China). The experimental procedures and the $2^{-\Delta\Delta\text{CT}}$ calculation method are provided in Supplemental Materials. Relative expression levels of genes were determined using this method.

Statistical analysis

All variables had descriptive statistics generated. The data were reported using the median and interquartile range for continuous variables that did not fit the normal distribution requirements and the mean \pm standard deviation for those that did. Continuous variables were compared between cases and controls using the Mann-Whitney U test for non-normally distributed data and the t-test for normally distributed data. The Chi-square (χ^2) test was used to compare differences between cases and controls for categorical variables. Fold change (FC) is the ratio of gene expression in the cases to gene expression in the controls, and is used to measure the difference in gene expression between cases and controls. Stratified analyses were performed for age and gender, and heterogeneity tests were performed using generalized linear models. Subgroups were analyzed by diagnosis type and the median PANSS total score. Spearman rank correlation was used to examine the relationships between gene expression levels and the PANSS total score, the positive scale score (P Score), the negative scale score (N Score) and the general psychopathology scale score (G Score).

The dose-response relationship between gene expression levels and the risk of SCZ was investigated using restricted cubic spline (RCS) regression, corrected for extremes by median ± 3 -fold interquartile range, adjusted for age and gender, and using the median of gene expression as the reference point, with the number of nodes at four. Genes that were differentially expressed between cases and controls or had a non-linear association with the SCZ were included in the gene expression score (GES). Gene expression was grouped into quartiles (Q1, Q2, Q3, Q4) and included in logistic regression models, and odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for gene expression and SCZ. The GES was calculated for each subject, its formula is: $\text{GES} = \sum_{i=1}^n (\beta_i * x_i)$, β_i is the logistic regression coefficient for each gene and x_i is the expression of each gene. Receiver operating characteristic (ROC) curve were constructed to assess the area under curve (AUC) of gene expression. Traditional risk factors (TRF) include age, gender and SCZ family history. Gene expression level enhancement was assessed by the Delong test, net reclassification index (NRI) and integrated discrimination improvement (IDI).

To verify the robustness of the findings, two sensitivity analyses were performed in this study: (1) excluding acute schizophrenia-like psychotic disorder, and (2) excluding both acute schizophrenia-like psychotic disorder and their matched controls. The stability of the diagnostic value of SCZ was assessed by comparing the changes in differentially expressed genes in the two sensitivity analyses.

All statistical analyses were performed in R software (version 4.3.2). A two-tailed $P < 0.05$ was considered significant.

Ethics approval and consent to participate

The study complies completely with the Declaration of Helsinki. The Institutional Review Board of Nanjing Medical University in China gave its approval to the protocol and consent forms (NJMUER201600334, NJMUER2019929).

RESULTS

Bioinformatics analysis

A total of 1205 mRNAs showed differential expression ($P < 0.05$, $|\log_2\text{FC}| > 1$), of which 623 genes showed up-regulation and 582 genes showed down-regulation. Based on the results of differentially expressed mRNAs, KEGG enrichment analysis screened 20 mRNAs in three neural signaling pathways, including glutamatergic synapses, dopaminergic synapses and 5-hydroxytryptaminergic synapses. A total of 8 genes, *DUSP1*, *GNG10*, *GNG7*, *PRKACA*, *CREB5*, *PPP3R1*, *PPP1CB* and *MAPK13*, were validated by primer gradient experiments and Sanger sequencing. Primer sequences are shown in Table S1.

Characteristics of study population

The characteristics of the study participants are summarized in Table 1. Briefly, cases and controls were well-matched on age and gender. The SCZ case group had lower proportions of smoking and drinking. Four clinical diagnosis types including paranoid SCZ (59.5%), undifferentiated SCZ (22.1%), acute schizophrenia-like psychotic disorder (17.5%) and simple SCZ (0.9%) were enrolled in this study.

Comparison of gene expression levels between SCZ cases and controls

Compared with the controls, SCZ cases displayed elevated expression levels of *CREB5* ($\text{FC} = 1.260$, $P = 0.001$), *PPP3R1* ($\text{FC} = 1.434$, $P < 0.001$) and *PPP1CB* ($\text{FC} = 1.319$, $P < 0.001$) (Table 2), whereas the expression levels of *DUSP1*, *GNG10*, *GNG7*, *PRKACA*

Table 1. Demographic and clinical characteristics between SCZ cases and controls.

Characteristic	Group/subtype	Case (n = 217)	Control (n = 217)	Z/χ ²	P
Age		36.0 (30.0, 47.0)	37.0 (29.0, 47.0)	0.219 ^a	0.827
Gender (%)	Male	95 (43.8)	95 (43.8)	0.980 ^b	1.000
	Female	122 (56.2)	122 (56.2)		
Smoking (%)	No	205 (94.5)	179 (82.5)	15.280 ^b	<0.001
	Yes	12 (5.5)	38 (17.5)		
Drinking (%)	No	212 (97.7)	160 (73.7)	50.882 ^b	<0.001
	Yes	5 (2.3)	57 (26.3)		
Diagnosis type (%)	Paranoid SCZ	129 (59.5)			
	Undifferentiated SCZ	48 (22.1)			
	Acute schizophrenia-like psychotic disorder	38 (17.5)			
	Simple SCZ	2 (0.9)			
PANSS total scores		85.00 (74.00, 100.00)			
P Scores		23 (19, 27)			
N Scores		21 (15, 26)			
G Scores		44 (37, 49)			
Family History (%)		40 (18.43)			

^aMann-Whitney U test.^bχ² test.**Table 2.** Comparison of mRNAs expression level between SCZ cases and controls.

mRNA	SCZ case (n = 217)	Control (n = 217)	Z	FC	P ^a
<i>DUSP1</i>	1.158 (0.363, 2.175)	1.043 (0.550, 2.081)	0.281	1.110	0.778
<i>GNG10</i>	0.884 (0.396, 1.591)	1.033 (0.632, 1.739)	1.884	0.856	0.060
<i>GNG7</i>	0.981 (0.608, 1.681)	1.034 (0.684, 1.509)	0.265	0.949	0.791
<i>PRKACA</i>	1.063 (0.672, 1.616)	1.040 (0.778, 1.389)	0.432	1.022	0.666
<i>CREB5</i>	1.240 (0.721, 2.106)	0.984 (0.640, 1.462)	3.453	1.260	0.001
<i>PPP3R1</i>	1.434 (0.862, 2.071)	1.000 (0.747, 1.334)	4.813	1.434	<0.001
<i>PPP1CB</i>	1.266 (0.809, 1.735)	0.960 (0.768, 1.354)	3.868	1.319	<0.001
<i>MAPK13</i>	0.891 (0.604, 1.341)	1.049 (0.682, 1.516)	1.888	0.849	0.059

^aMann-Whitney U test.

and *MAPK13* did not differ between SCZ cases and controls ($P > 0.05$; Table 2).

Subgroups analysis of comparing mRNAs expression level between SCZ cases and controls by age, gender, diagnosis type and PANSS score

PPP3R1 expression level of SCZ cases was higher than that of controls both in subgroups aged ≤ 35 years ($FC = 1.456, P = 0.001$) and aged > 35 years ($FC = 1.404, P < 0.001$). *CREB5* ($FC = 1.289, P = 0.001$) and *PPP1CB* ($FC = 1.344, P < 0.001$) expression levels were higher only in SCZ cases aged > 35 years, while *MAPK13* expression level of SCZ cases was lower than controls ($FC = 0.776, P = 0.020$) in the subgroup aged ≤ 35 years (Table S2).

Both in male and female SCZ cases had higher expression levels of *PPP3R1* and *PPP1CB* ($FC > 1, P < 0.05$; Table S3) than the controls, while in male SCZ cases had higher levels of *CREB5* expression level than controls ($FC = 1.335, P = 0.002$). There is no difference in other mRNAs expression levels between SCZ cases and controls, regardless of gender ($P > 0.05$).

Compared with the controls, paranoid SCZ cases displayed increased expression levels of *PPP3R1* ($FC = 1.503, P < 0.001$), *PPP1CB* ($FC = 1.457, P < 0.001$) and *CREB5* ($FC = 1.287, P = 0.001$), undifferentiated SCZ cases displayed increased expression levels

of *PPP3R1* ($FC = 1.275, P < 0.01$) and *PPP1CB* ($FC = 1.321, P < 0.01$) but decreased expression level of *DUSP1* ($FC = 0.702, P < 0.05$), and acute schizophrenia-like psychotic disorder cases displayed a increased *PPP3R1* expression level ($FC = 1.431, P < 0.01$) but decreased expression level of *MAPK13* ($FC = 0.748, P < 0.05$), respectively (Table 3).

Both in the PANSS < 85 and the PANSS ≥ 85 subgroups, *PPP3R1* and *PPP1CB* expression levels were higher than controls ($FC > 1, P < 0.01$; Table S4). SCZ cases displayed increased expression levels of *PRKACA* and *CREB5* but decreased expression level of *GNG10* than controls only in the PANSS ≥ 85 group (*PRKACA*: $FC = 1.140, P = 0.018$; *CREB5*: $FC = 1.755, P < 0.001$; *GNG10*: $FC = 0.682, P = 0.041$; Table S4).

None of the above differences in gene expression was heterogeneous between subgroups of age and gender ($P > 0.05$, Table S2, S3).

Association of gene expression with SCZ risk

The expression levels of seven genes *DUSP1*, *GNG10*, *GNG7*, *PRKACA*, *CREB5*, *PPP3R1* and *PPP1CB* showed "U" shaped dose-response relationships with SCZ ($P_{overall} < 0.05$, $P_{nonlinear} < 0.05$; Fig. 1).

Table 3. Comparison of mRNAs expression level between SCZ cases of different diagnosis type and controls.

mRNA	Control (n = 217)		Paranoid SCZ (n = 129)		Undifferentiated SCZ (n = 48)			Acute Schizophrenia-like Psychotic Disorder(n = 38)		
	M(Q1,Q3)	M(Q1,Q3)	FC	P ^a	M(Q1,Q3)	FC	P ^b	M(Q1,Q3)	FC	P ^c
<i>DUSP1</i>	1.043 (0.550,2.081)	1.274 (0.394,2.757)	1.221	0.580	0.732 (0.254,1.425)	0.702	0.014	1.342 (0.739,2.369)	1.287	0.296
<i>GNG10</i>	1.033 (0.632,1.739)	0.839 (0.378,1.591)	0.812	0.071	0.885 (0.406,1.813)	0.857	0.413	0.886 (0.452,1.467)	0.858	0.210
<i>GNG7</i>	1.034 (0.684,1.509)	0.981 (0.613,1.596)	0.949	0.693	1.064 (0.640,1.789)	1.029	0.510	0.883 (0.528,1.519)	0.854	0.397
<i>PRKACA</i>	1.040 (0.778,1.389)	1.043 (0.626,1.438)	1.003	0.867	1.222 (0.868,1.668)	1.175	0.131	1.036 (0.675,1.674)	0.996	0.953
<i>CREB5</i>	0.984 (0.640,1.462)	1.266 (0.745,2.262)	1.287	0.001	1.261 (0.730,1.981)	1.282	0.060	1.176 (0.646,2.227)	1.195	0.147
<i>PPP3R1</i>	1.000 (0.747,1.334)	1.503 (0.850,2.200)	1.503	<0.001	1.275 (0.864,1.923)	1.275	0.006	1.431 (1.002,2.055)	1.431	0.001
<i>PPP1CB</i>	0.960 (0.768,1.354)	1.399 (0.775,1.910)	1.457	<0.001	1.268 (0.979,1.623)	1.321	0.001	1.032 (0.762,1.331)	1.075	0.591
<i>MAPK13</i>	1.049 (0.682,1.516)	0.877 (0.604,1.362)	0.836	0.139	1.029 (0.616,1.338)	0.981	0.497	0.785 (0.527,1.200)	0.748	0.030

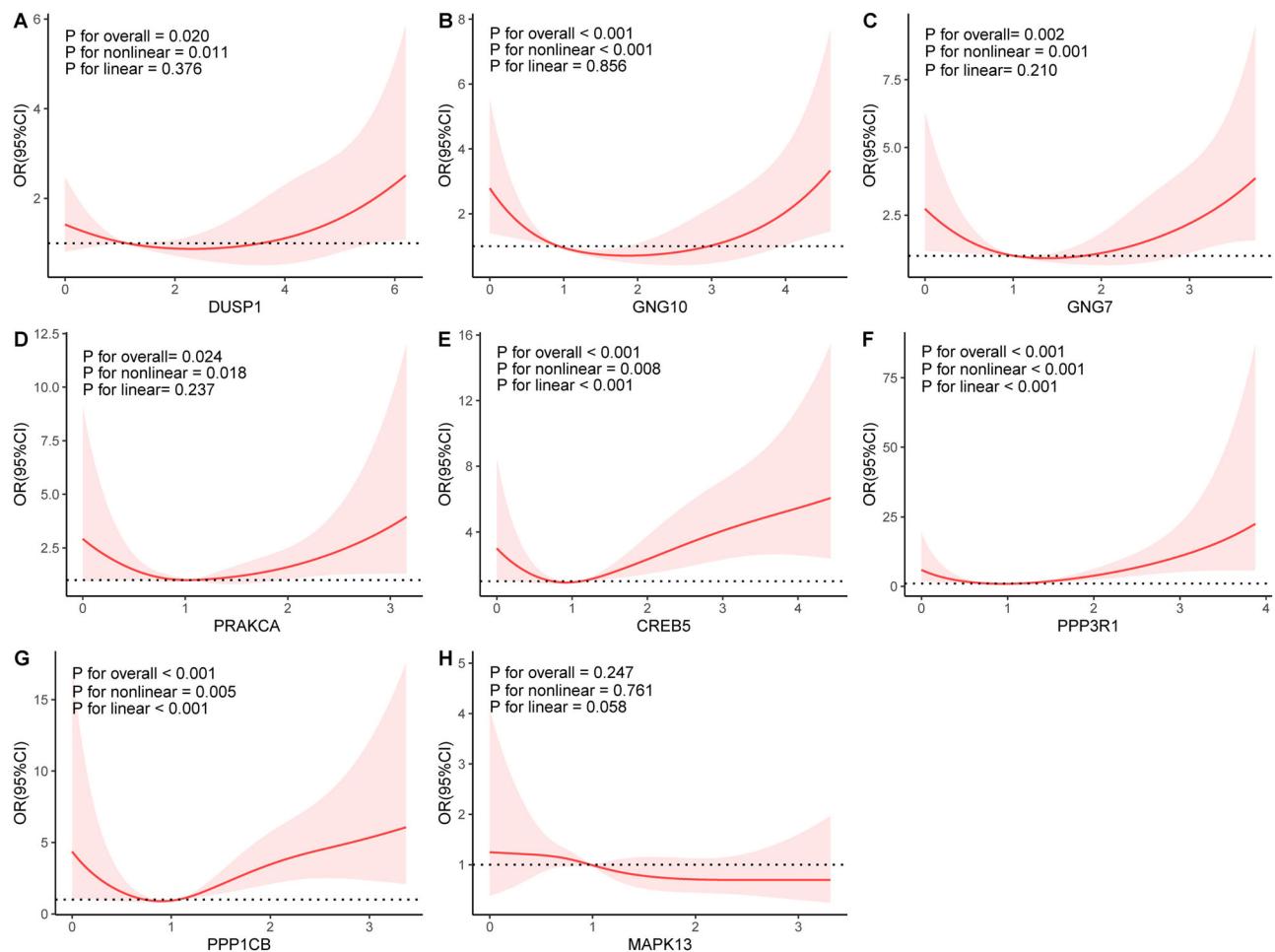
^aMann-Whitney U test.^aparanoid SCZ vs control.^bundifferentiated SCZ vs control.^cacute schizophrenia-like psychotic disorder vs control.

Fig. 1 Expression level of mRNAs and the risk of SCZ. Restrict cubic spline regression analysis of the association of mRNAs expression level and the risk of SCZ with odds ratio (OR) and 95% confidence interval (CI) after adjustment for age and gender. **A** *DUSP1* expression showed nonlinear relationships with SCZ, P for overall = 0.020, P for nonlinear = 0.011. **B** *GNG10* expression showed nonlinear relationships with SCZ, P for overall < 0.001, P for nonlinear < 0.001. **C** *GNG7* expression showed nonlinear relationships with SCZ, P for overall = 0.002, P for nonlinear = 0.001. **D** *PRKACA* expression showed nonlinear relationships with SCZ, P for overall = 0.024, P for nonlinear = 0.018. **E** *CREB5* expression showed nonlinear relationships with SCZ, P for overall < 0.001, P for nonlinear = 0.008. **F** *PPP3R1* expression showed nonlinear relationships with SCZ, P for overall < 0.001, P for nonlinear < 0.001. **G** *PPP1CB* expression showed nonlinear relationships with SCZ, P for overall < 0.001, P for nonlinear = 0.005. **H** *MAPK13* expression didn't show linear or nonlinear relationships with SCZ, P for overall = 0.247, P for nonlinear = 0.761.

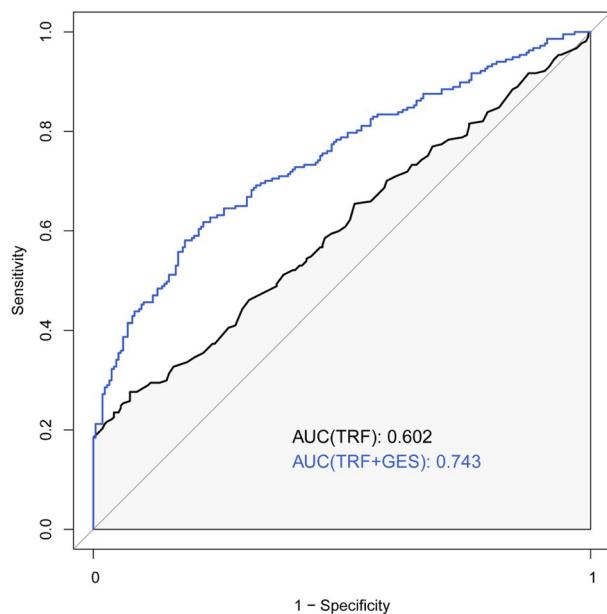


Fig. 2 The ROC curve of GES and TRF for SCZ diagnosis. Traditional risk factor (TRF) model including age, gender and family history of SCZ. Gene expression score (GES) model including the expression levels of *DUSP1*, *GNG10*, *GNG7*, *PRKACA*, *CREB5*, *PPP3R1* and *PPP1CB*. The area under curve (AUC) of TRF was 0.602. The AUC of TRF+GES was 0.743.

Diagnostic value of mRNAs for SCZ

The AUC (95% CI) was 0.602 (0.549–0.655) for the TRF model (Fig. 2 and Table S5). Incorporating GES including *DUSP1*, *GNG10*, *GNG7*, *PRKACA*, *CREB5*, *PPP3R1* and *PPP1CB* to the TRF, TRF + GES model significantly improved the AUC to 0.743 (95% CI: 0.697–0.789; Table S5) with P less than 0.001 by Delong test. The NRI was 0.212 (95% CI: 0.116–0.309; P < 0.001) and the IDI was 0.083 (95% CI: 0.058–0.107; P < 0.001).

Correlation between gene expression and PANSS score

GNG10 expression showed weak negative correlations with P score ($r = -0.219$, $P = 0.009$), G score ($r = -0.188$, $P = 0.027$) and PANSS total score ($r = -0.200$, $P = 0.018$), and *PPP1CB* expression showed weak positive correlations with P score ($r = 0.269$, $P = 0.001$) and PANSS total score ($r = 0.167$, $P = 0.048$) (Table S6).

Sensitivity analysis

The expression levels of *CREB5*, *PPP3R1*, and *PPP1CB* were still higher in SCZ cases than in controls ($FC > 1$, $P < 0.05$), after excluding acute schizophrenia-like psychotic disorder cases (Table S7) and further excluding the paired controls (Table S8).

The nonlinear associations of mRNAs expression levels of *DUSP1*, *GNG10*, *GNG7*, *CREB5*, *PPP3R1* and *PPP1CB* except *PRKACA* persisted ($P_{overall} < 0.05$, $P_{nonlinear} < 0.05$) whether excluding acute schizophrenia-like psychotic disorder cases alone and further excluding their paired controls (Figures S1 and S2).

Furthermore, the TRF + GES model maintained stable discriminatory power ($AUC > 0.7$, $P < 0.001$) for SCZ after excluding acute schizophrenia-like psychotic disorder cases and further excluding the corresponding paired controls (Tables S9 and S10).

DISCUSSION

Recently, altered mRNAs expression levels in peripheral blood were observed in patients with SCZ^{20,21}, while the role of neural signaling pathways in the pathophysiological mechanisms of SCZ is

still being explored. This study investigated eight differentially expressed mRNAs in neural signaling pathways identified by RNA-sequencing screening and validated *CREB5*, *PPP3R1* and *PPP1CB* for SCZ and particularly, higher *DUSP1* and *MAPK13* expression levels in patients with undifferentiated SCZ and acute schizophrenia-like psychotic disorder respectively. In addition, *CREB5*, *PPP3R1*, *PPP1CB*, *DUSP1*, *GNG10*, *GNG7* and *PRKACA*, showed "U" shaped dose-response relationships with SCZ.

The cAMP-response element-binding (CREB) protein family, which includes *CREB5*, is crucial for learning memory, neuronal cell growth, synaptic plasticity, and gene expression downstream of cAMP signaling^{22,23}. Numerous investigations have demonstrated that SCZ patients exhibit reduced CREB protein and mRNAs expression level^{24,25}. Even though *CREB5* expression differed significantly between cases and controls, our research revealed a more complex nonlinear relationship between *CREB5* expression level and SCZ rather than a straight forward linear one, which may indicate a more intricate mechanism between this gene and SCZ.

PPP3R1, a signaling pathway member of calmodulin phosphatase. The lack of calmodulin phosphatase may cause changes in synapse function, attention problems and cognitive change^{26,27}, as well as a range of behavioral impairments linked to SCZ, including cognitive dysfunction, working memory loss, and decreased social interaction with evidence of animal study²⁸.

PPP1CB encodes protein as one of the three catalytic subunits of protein phosphatase 1 (PP1), which is involved in the regulation of synaptic plasticity and neurological disorders^{29,30}. PP1 deficiency is associated with the development of learning and memory impairments, and thus, *PPP1CB* may be associated with the onset of SCZ by regulating PP1.

Our findings suggested that the expression levels of *DUSP1* and *MAPK13* decreased in patients with undifferentiated SCZ and acute schizophrenia-like psychotic disorder. The mitogen-activated protein kinase (MAPK) phosphatase family, of which *DUSP1* is an important member, regulates the MAPK signaling pathway to contribute to learning, memory, neuronal stress response and synaptic plasticity^{31,32}. And *MAPK13* encodes a member of the MAPK family³³. Thus, *DUSP1* and *MAPK13* may be linked to the pathophysiological mechanisms of SCZ by regulating the MAPK signaling pathway.

G protein-coupled receptors (GPCR) are central mediators of neurotransmitter signaling, and *GNG10* may affect neurotransmission, emotional and cognitive functions by modulating the GPCR signaling pathway^{34,35}. A previous study observed that *GNG10* expression level in peripheral blood was up-regulated in patients with first-episode SCZ³⁶. However, our study found a nonlinear association of *GNG10* expression level with SCZ but not a single linear association. Furthermore, the correlation analysis observed a negative correlation between *GNG10* expression level and PANSS total score and its subscale scores, which suggested that a lower expression level of *GNG10* may be associated with more severe positive, general psychopathological and overall symptoms of SCZ.

GNG7 binds to G protein α and β subunits and participates in D₁ dopamine receptor-mediated neuroprotective responses³⁷. Behavioral alterations result from the loss of the G protein gamma (7-) subunit, which is involved in signaling pathways that regulate the stability or formation of heterotrimers of particular G proteins^{38,39}.

The *PRKACA* encodes the protein kinase A (PKA) catalytic subunit Ca, which is considered the predominant isoform, is expressed in most tissues, and is a core kinase of the cyclic adenosine monophosphate (cAMP) pathway^{40,41}. The dysregulation of cAMP signaling pathway in patients with SCZ⁴², and our findings support the association of *PRKACA* and SCZ by affecting the cAMP signaling pathway.

The strengths of this study mainly include, firstly, analyzing the expression changes of specific pathways (glutamatergic synapses, dopaminergic synapses and 5-hydroxytryptaminergic synapses),

which helps to deeply understand the pathogenesis of SCZ. Secondly, the GES can significantly improve the differentiation and prediction ability of the model. Thirdly, peripheral blood samples are easier to obtain than brain tissue samples, which greatly reduces the difficulty and cost of obtaining samples and is less traumatic to the human body. Lastly, we use of RCS regression models to investigate the association between gene expression levels and SCZ risk. This approach sensitively identifies and characterizes potential dose-response relationships, and provides more new evidence for elucidating the biological effects of differentially expressed mRNAs involved in the complex mechanisms of SCZ.

The limitations of this study include, firstly, that some subgroups had too few patients. Secondly, this was a single-center study; future multicenter studies could enhance the generalizability of the findings. Thirdly, fewer covariates were adjusted in this study, and further relevant data could be collected for future analyses. Lastly, the sample size of screening set is constrained by using the current threshold ($P < 0.05$ and $|\log_2\text{FC}| > 1$) to identify differentially expressed mRNA and further study with sufficient conditions would be warranted in the future.

CONCLUSION

The expression levels of *CREB5*, *PPP3R1*, and *PPP1CB* were elevated in the neural signaling pathway of peripheral blood leukocytes of patients with SCZ; there was a significant dose-response relationship between the expression levels of *DUSP1*, *GNG10*, *GNG7*, *PRKACA*, *CREB5*, *PPP3R1*, and *PPP1CB* and the risk of SCZ; the gene expression scores by integrating these genes could significantly improve the diagnostic ability of SCZ.

DATA AVAILABILITY

The datasets analyzed during the current study are available from the corresponding author on reasonable request due to ethical limitations.

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AUTHOR CONTRIBUTIONS

C.S. and Y.X. designed the study. M.Y.Z. contributed to the data collection. Y.Z., F.F.W. and C.S. conducted data analysis and interpretation. Y.Z., Y.F., and C.S. drafted the manuscript. Y.Z., M.Y.Z., F.F.W., S.Q.M., Y.X., and C.S. contributed administrative, technical, or material support. Y.Z. and M.Y.Z. are co-first author. Y.X. and C.S. supervised the draft and revision of the manuscript. The corresponding author attests that all the listed authors meet authorship criteria and that no others meeting the criteria have been omitted. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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