

The Effect of miR-1273d Between Chronic Childhood Stress and Positive Symptoms in Schizophrenia: A Preliminary Mediation Analysis

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Purpose: High disability and recurrence rates have driven researchers to explore the etiology and pathological processes of schizophrenia (SCZ). This study aimed to investigate the relationship between chronic childhood stress and positive symptoms in SCZ patients from the perspective of microRNA (miRNA) expression levels in peripheral blood.

Patients and Methods: 80 SCZ patients and 80 sex- and age-matched healthy individuals were enrolled and assessed using Positive and Negative Syndrome Scale (PANSS) and Childhood Chronic Stress Questionnaire (CCSQ). The miRNA expression level were detected by Quantitative Real-Time PCR (qRT-PCR).

Results: The area under the curve of combined receiver operating characteristic (ROC) curve for aberrantly expressed miRNAs was 0.958, with sensitivity and specificity of 0.838 and 0.963, respectively ($P < 0.001$). Score of peer bullying (PB), abuse and neglect (AN), adverse life events (ALE), and CCSQ in SCZ group were higher ($P < 0.05$ or 0.01). Outside miRNA-3064-5p, the Δ CT values of miRNA-1273d, miRNA-1303, miRNA-21, miRNA-3131, miRNA-3687, miRNA-4428, miRNA-4725-3p, and miRNA-5096 negatively correlated with positive symptom subscale (PSS), while PB, AN, ALE, CCSQ positively correlated with PSS and negative symptom scale (NSS) ($P < 0.05$ or 0.01). Multivariate regression analysis suggested miRNA-1273d and AN significantly associated with PSS and accounted for 25.33% of its variation in SCZ patients ($P < 0.05$ or 0.01). The predictive effect of AN on PSS in the SCZ group was not significant in the mediation model ($P = 0.229$), while AN was significantly associated with PSS, miRNA-1273d in the direct model ($P < 0.05$ or 0.001).

Conclusion: Differentially expressed miRNAs as predictors of psychopathological symptoms in SCZ patients may be potential biomarkers for SCZ diagnosis. Chronic childhood stress may regulate susceptibility gene expression and pathological processes of SCZ by affecting miRNA-1273d expression.

Keywords: schizophrenia, microRNA, biomarker, chronic stress, childhood

Introduction

Schizophrenia (SCZ), one of the most severe mental disorders, is characterized mainly by positive symptoms such as hallucinations, delusions, and psychomotor excitement, or negative symptoms such as avolition, anhedonia, social withdrawal, alogia, and blunted affect.¹⁻⁴ Serious damage to cognitive function, affect, and behavior caused by SCZ may lead to a high disability rate worldwide. The improvement of SCZ prevention and prognosis is of great value in reducing medical expenses and care burden.

Based on existing basic research and clinical observations, genetic predisposition was implicated in SCZ onset and development, and genetic epidemiological studies have revealed a strong heritable basis about 60–80% in SCZ.^{5,6} Previous studies argued that genetic, epigenetic, and environmental stress were jointly involved in SCZ pathogenesis through DNA methylation, 5-Methylcytosine (5mC), 5-hydroxycytosine (5hmC), and non-coding RNA which were regarded as key epigenetic regulators in brain development and aging.⁷⁻⁹ MicroRNA (miRNA), a family of non-coding

small molecules and single-stranded RNA with a length of 21–24 nucleotides, were first reported in the 1990s. It was an endogenous small molecule RNA which was considered as a fine-tuner of cellular protein expression by modifying mRNA translation and regulating gene expression at the post-transcriptional level.^{10,11} miRNAs were confirmed to be involved in a variety of physiological processes, including cell proliferation, differentiation, and apoptosis, which played pivotal regulatory roles in development and maturation of the central nervous system.^{12–14} Aberrant expression of miRNAs may perturb the neural development process that was strongly related to various neurological diseases,^{15–18} one previous study using quantitative real-time polymerase chain reaction (qRT-PCR) to verify miRNA microarray expression profiling, which found that expression levels of miRNA-1273d, miRNA-1303, miRNA-21, miRNA-3064-5p, miRNA-3131, miRNA-3687, miRNA-4428, miRNA-4725-3p, and miRNA-5096 in peripheral blood were upregulated and associated with psychotic symptoms and therapeutic efficacy in SCZ patients, the authors believed these miRNAs has potential value as biomarkers for SCZ diagnosis may regulate pathological process.¹⁹ Nevertheless, the diagnostic sensitivity and specificity of these differentially expressed miRNAs for SCZ have not been confirmed.

Brain development is a dynamic process that constantly exchanges energy and matter with the environment and receives information stimuli through the sensory system for promoting development of brain function and human sociality. Early life stress (ELS), such as public violence, parental conflict, physical abuse, emotional abuse, sexual abuse, and physical and/or emotional neglect in the family, can seriously damage normal neuronal growth and brain development with long-term psychological and physiological effects in adulthood. Some studies found ELS led to difficulties in emotion regulation by reducing hippocampal volume and heightening amygdala activation. Individuals who experienced ELS in childhood may form life-long personalized stress responsiveness patterns characterized by sensitivity to fear or anxiety stimuli. In addition, ELS brought about disabilities in decision-making, planning, impulse control, empathy, understanding and describing one's own emotions as well as differentiating feelings from bodily signals of arousal by disrupting general development of the prefrontal cortex (PFC), thereby causing difficulties in building solid and appropriate interpersonal relationships. The ELS has also been shown to induce dysfunction of serotonergic and dopaminergic neurons, which can impair emotional and motivational processes,^{20–23} these disorders in brain structure and function were believed to be strongly associated with pathological process of SCZ. It was confirmed that miRNA-mediated epigenetic mechanisms may exert an impact on interactions of environmental stress, susceptibility gene expression, and pathological changes in the brain. A previous study reported exposure to stress and psychoactive substances dysregulated miRNA-mediated gene expression involved in the neural development process, such as proliferation, differentiation, synaptogenesis, neural plasticity, and apoptosis, which may further induce pathological processes associated with SCZ.^{24–26}

In summary, this study aimed to analyze the diagnostic value of differentially expressed miRNAs in peripheral blood for SCZ using the receiver operating characteristic (ROC) curve and explored the relationship between chronic childhood stress, miRNA expression, and SCZ for providing a scientific basis of SCZ prevention. This study proposed hypotheses that specifically expressed miRNAs may exhibit ideal diagnostic value for SCZ due to distinct spatial, temporal, and cell type-specific patterns associated with pathological condition,²⁷ and a mediating effect of miRNA between chronic childhood stress and psychotic symptoms may be observed in SCZ patients. A flow diagram for mediating effect of miRNA is shown in [Figure 1](#).

Materials and Methods

Participants

Study Group

Eighty patients with SCZ, including 38 males and 42 females aged 18–50 years old were admitted to the No. 904th Hospital between January 2022 and December 2024. All enrolled patients met the diagnostic criteria for schizophrenia in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) and were either first-visit patients (53 in total) or have not taken antipsychotic drugs within 3 months prior to enrollment (27 in total). SCZ patients with other severe mental diseases, craniocerebral injury, cerebrovascular disease, alcohol or drug abuse history, blood transfusion within 1 month before enrollment in this study, and those who had received modified electroconvulsive therapy (MECT) within 3 months were excluded.

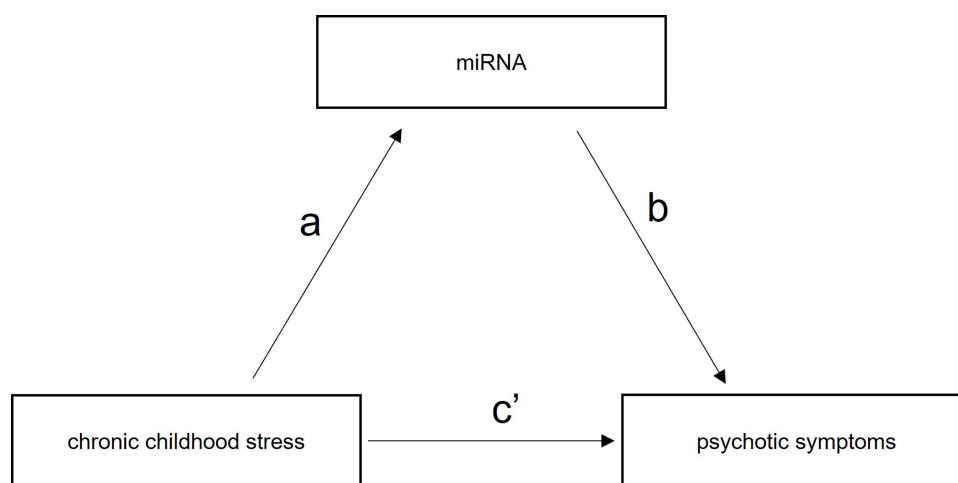


Figure 1 Schematic plot for mediating effect of miRNA between chronic childhood stress and psychotic symptoms in SCZ patients (a: Effect of chronic childhood stress on miRNA; b: Effect of miRNA on psychotic symptoms; c': Direct effect of chronic childhood stress on psychotic symptoms in mediation model).

Control Group

Eighty hospital staff members and health examinees in the No. 904th Hospital, including 36 males and 44 females aged 19–48 years, were recruited as the control group in this study. The inclusion criteria were as follows: no family history of serious mental disorders, including schizophrenia, depressive disorder, anxiety disorder, obsessive-compulsive disorder, and bipolar disorder; and no major traumatic event exposure in the past 6 months. Individuals with a history of blood transfusions in the past 1 month and those with difficulties in basic reading, comprehension, and writing were excluded.

All the participants in the study group were Han Chinese with basic abilities in reading, writing, comprehension and verbal communication assessed by two experienced clinicians. There were no between-group differences in age ($t=0.448$) and sex ($\chi^2=0.101$) ($P>0.05$).

Mental Assessment Scale

Positive and Negative Syndrome Scales (PANSS)

PANSS, consisting of 30 items, including 7 items for the positive symptom subscale (PSS), 7 items for the negative symptom scale (NSS), and 16 items for the general psychopathology scale (GPS), was used to assess the symptom severity in different SCZ types.

Childhood Chronic Stress Questionnaire (CCSQ)

Sixty items of the CCSQ rated on a scale of 1–5 were assigned to three dimensions: peer bullying (PB), abuse and neglect (AN), and adverse life events (ALE). Higher scores indicated more severe chronic stress during childhood. CCSQ was firstly developed by Hu in Chinese version with Cronbach's α of 0.946 and subscale reliabilities of 0.868, 0.835, and 0.716, respectively.²⁸

Quantitative Real-Time PCR (qRT-PCR) for Testing of Circulating miRNA

According to the results in a previous study,¹⁹ miRNA-1273d, miRNA-1303, miRNA-21, miRNA-3064-5p, miRNA-3131, miRNA-3687, miRNA-4428, miRNA-4725-3p, and miRNA-5096 were investigated using qRT-PCR. Total RNAs were extracted from purified plasma using TRIzol reagent for quantitative detection of miRNAs. Complementary DNA was synthesized using the TaqMan microRNA reverse transcription kit and miRNA-specific stem loop primers according to the manufacturer's instructions. The 5 × RT primers (miRNA-specific stem-loop primers) and 20 × miRNA-specific PCR primer/probe mix were supplied by TaqMan MicroRNA Assays based on the miRNA sequences obtained from the miRBase database. Data were collected using SDS 2.3 software. After normalization to RNU48, the expression levels of miRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method.^{19,29}

Statistical Analysis

All data were statistically analyzed using DataAssist v3.0, and SPSS v17.0. The relative expression level of miRNA was represented by the difference in the cycle threshold (Ct) between miRNA and the internal reference RNU48 ($\Delta Ct = Ct_{miRNA} - Ct_{RNU48}$). A lowered ΔCt value suggested that miRNA expression was upregulated.¹⁹ The ROC curve was used to determine the sensitivity and specificity of differentially expressed miRNAs in differentiating patients with SCZ from healthy controls, and Logistic regression was used to acquire predicted probability for the following combined ROC with Hosmer-Lemeshow goodness-of fit test for internal validation ($\chi^2=8.099$, $P=0.424>0.05$), the training-testing split was a ratio of 7:3. Spearman correlation was used for association analysis of miRNA expression level and psychopathological symptoms and Pearson's were employed for psychopathological symptoms and chronic childhood stress in SCZ patients. Collinearity diagnostics for regression analysis showed all tolerances were more than 0.1, and Kolmogorov–Smirnov test verified the data of dependent variables conformed to normal distribution ($P>0.05$). All statistical analyses were two-sided significance tests with a P value of < 0.05 , which was considered statistically significant.

Results

ROC Curve of Differentially Expressed miRNAs for Differentiating SCZ from Health Conditions

The ROC curve was constructed using health conditions (1 = patients with SCZ, 2 = healthy controls) as state variables and differentially expressed miRNAs (miRNA-1273d, miRNA-1303, miRNA-21, miRNA-3064-5p, miRNA-3131, miRNA-3687, miRNA-4428, miRNA-4725-3p, and miRNA-5096) as test variables. The results showed that the area under the curve (AUCs) of the nine differentially expressed miRNAs ranged from 0.678 to 0.878 ($P < 0.001$), and the AUC of the combined ROC curves of the differentially expressed miRNAs was 0.958 with a sensitivity and specificity of 0.838 and 0.963, respectively ($P < 0.001$), Youden index of combined ROC curves was calculated as 0.801, see Figure 2A, B and Table 1.

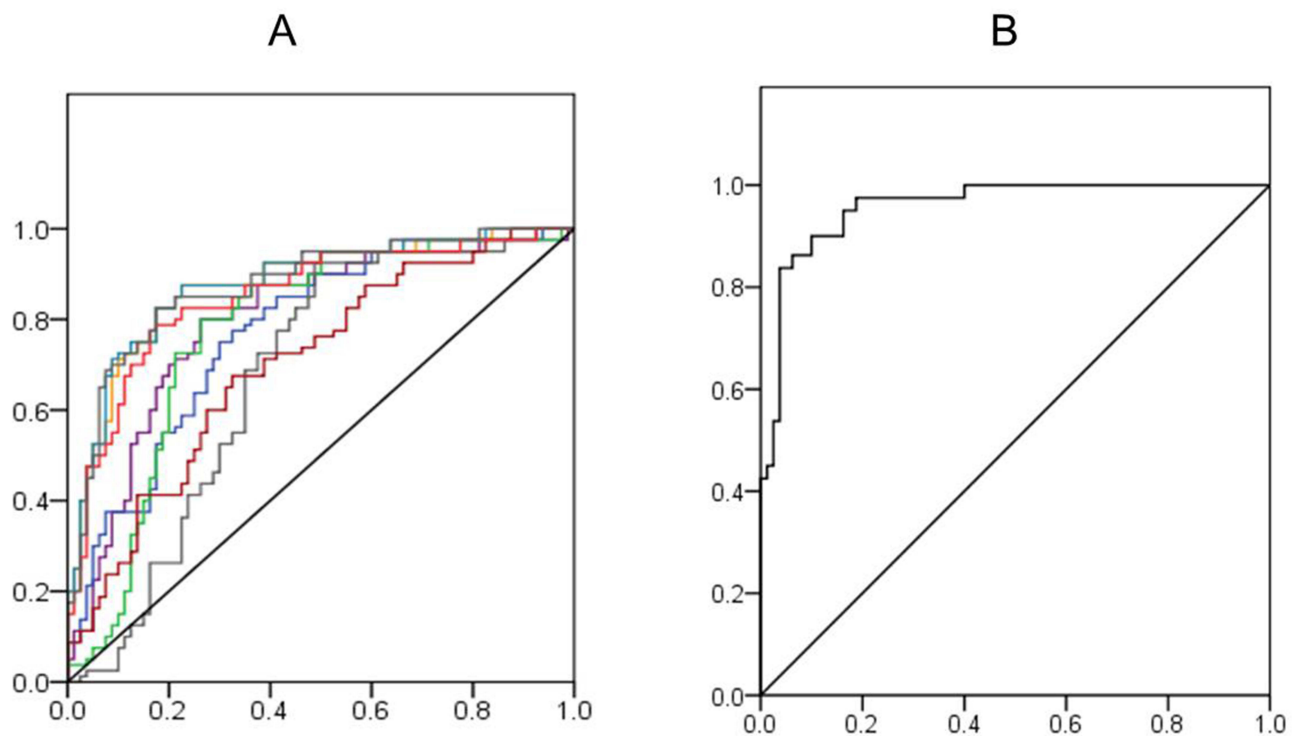


Figure 2 ROC curves for differentially expressed miRNAs between patients with SCZ and healthy controls ((**A**) ROC curves for single miRNA; (**B**) Combined ROC curve of differentially expressed miRNA). Vertical Axis (HA), Horizontal Axis (HS) are sensitivity and 1-specificity, respectively.

Table 1 Results of the ROC Curve About Aberrant Expression of miRNAs in the SCZ Group and Healthy Controls

Test Variable	AUC	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval		Sensitivity	Specificity
			Lower Bound	Upper Bound		
miRNA-1273d	0.773	<0.001	0.700	0.845	0.775	0.675
miRNA-1303	0.773	<0.001	0.696	0.850	0.800	0.738
miR-21	0.678	<0.001	0.591	0.764	0.925	0.512
miRNA-3064-5p	0.702	<0.001	0.621	0.782	0.675	0.675
miRNA-3131	0.875	<0.001	0.820	0.930	0.825	0.825
miRNA-3687	0.851	<0.001	0.791	0.912	0.775	0.838
miRNA-4428	0.878	<0.001	0.824	0.932	0.875	0.875
miRNA-4725-3p	0.875	<0.001	0.820	0.929	0.688	0.925
miRNA-5096	0.796	<0.001	0.725	0.868	0.800	0.738

Notes: AUC was area under the curve; Sig.^b is statistical significance of the regression coefficient.

Comparison for Chronic Stress in Childhood Between SCZ Patients and Healthy Controls

The independent sample *t*-test suggested the scores of PB, AN, ALE, and CCSQ were significantly higher in SCZ patients than the control group ($P < 0.05$ or 0.01), see [Table 2](#).

Correlation Analysis of miRNAs, Chronic Stress in Childhood and Psychopathological Symptoms in SCZ Group

As shown in [Table 3](#), Spearman correlation showed Δ CT values of miRNA-1273d, miRNA-1303, miRNA-21, miRNA-3131, miRNA-3687, miRNA-4428, miRNA-4725-3p, and miRNA-5096 except miRNA-3064-5p negatively correlated with PSS, and Pearson correlation suggested scores of PB, AN, ALE, CCSQ positively correlated with PSS and NSS ($P < 0.05$ or 0.01).

Table 2 Between-Group Comparisons for Chronic Stress in Childhood ($\bar{X} \pm SD$)

Dimension	SCZ Group	Control Group	<i>t</i>	<i>P</i>	Cohen's <i>d</i>
PB	42.31 ± 10.50	33.26 ± 16.11	4.209	<0.001	0.633
AN	65.54 ± 17.01	53.41 ± 33.74	2.870	0.005	0.444
ALE	38.41 ± 10.00	31.27 ± 15.53	3.462	0.001	0.528
CCSQ	146.26 ± 35.37	117.94 ± 56.21	3.864	<0.001	0.586

Notes: PB was peer bullying; AN was abuse and neglect, ALE was adverse life events; CCSQ was Childhood Chronic Stress Questionnaire.

Table 3 Correlation Analysis of miRNAs, Chronic Stress in Childhood and Psychopathological Symptoms in the SCZ Group (*r*)

Item	PSS	NSS	GPS	PANSS
miRNA-1273d	-0.335**	-0.030	-0.143	-0.004
miRNA-1303	-0.343**	-0.036	-0.144	-0.005
miRNA-21	-0.309**	0.047	-0.061	0.087
miRNA-3064-5p	-0.209	-0.050	-0.206	-0.114

(Continued)

Table 3 (Continued).

Item	PSS	NSS	GPS	PANSS
miRNA-3131	-0.271*	0.044	-0.126	0.007
miRNA-3687	-0.273*	-0.011	-0.167	-0.035
miRNA-4428	-0.257*	0.020	-0.141	-0.016
miRNA-4725-3p	-0.264*	0.046	-0.100	0.028
miRNA-5096	-0.274*	-0.015	-0.113	0.001
PB	0.285*	-0.186	-0.203	0.270*
AN	0.258*	-0.203	-0.188	0.296**
ALE	-0.138	0.227*	-0.162	0.230*
CCSQ	0.258*	0.238*	-0.207	0.305**

Notes: *was $P < 0.05$, **was $P < 0.01$. PB was peer bullying; AN was abuse and neglect, ALE was adverse life events; CCSQ was childhood chronic stress questionnaire. PSS was positive symptom subscale; NSS was negative symptom scale; GPS was general psychopathology scale; PANSS was positive and negative syndrome scales. ΔCt was the difference in the cycle threshold (Ct) between miRNA and the internal reference RNU48 ($\Delta Ct = Ct_{miRNA} - Ct_{RNU48}$). A lowered ΔCt value suggested that miRNA expression was upregulated.

Regression Analysis for Association Between Positive Symptoms in Patients with SCZ and miRNA Expression Levels, Chronic Stress in Childhood

Multiple regression analysis was conducted with PSS as the dependent variable (DV) and ΔCt values of miRNAs, PB, AN, and ALE as independent variables (IVs). The results suggest only miRNA-1273d and AN were entered into the regression equation of PSS and accounted for 25.33% of PSS variance ($P < 0.05, 0.01$), see Table 4.

The Mediating Effect Analysis for miR-1273d Between Chronic Stress in Childhood and Positive Symptoms in SCZ Patients

As has been shown in multiple regression analysis, only miRNA-1273d and AN significantly predicted positive symptom severity of SCZ patients, hierarchical regression analysis was conducted according to the test procedure of mediating

Table 4 Regression Analysis for Associations Between Positive Symptoms in Patients with SCZ and miRNA Expression Levels, Chronic Stress in Childhood

DV	IV	Beta	SE	95% CI		t	P	R ²
				LB	UB			
PSS	miRNA-1273d	-0.298	0.876	-0.550	-0.045	-2.624	0.011	0.253
	miRNA-1303	-0.110	0.596	-0.278	0.499	-1.296	0.115	
	miRNA-21	-0.106	0.506	-0.435	0.583	-1.243	0.127	
	miRNA-3064-5p	-0.098	0.682	-0.360	0.364	-1.163	0.148	
	miRNA-3131	0.023	0.578	-0.827	0.472	0.204	0.839	
	miRNA-3687	-0.022	0.731	-0.280	0.637	-0.124	0.865	
	miRNA-4428	0.122	0.427	0.228	0.672	1.181	0.142	
	miRNA-4725-3p	-0.192	0.075	-0.838	0.453	-1.644	0.162	
	miRNA-5096	-0.144	0.900	-0.941	0.653	-1.571	0.208	
	PB	-0.070	0.118	-0.307	0.166	-0.593	0.555	
	AN	0.231	0.091	0.150	0.513	3.346	0.002	
	ALE	-0.073	0.123	-0.319	0.172	-0.595	0.554	

Notes: DV and IV indicate the dependent variable, independent variable, respectively. R² represents the coefficient of determination.

Abbreviations: SE, standard error; LB, lower bound; UB, upper bound; CI, confidence interval; PSS, positive symptom subscale.

Table 5 Hierarchical Regression Analysis for Mediating Effect of miR-1273d Between Chronic Stress in Childhood and Positive Symptoms in Patients with SCZ

Step	DV	IV	Beta	SE	95% CI		t	P
					LB	UB		
Step1	PSS	AN	0.105	0.045	0.015	0.195	2.325	0.023
Step2	miRNA-273d	AN	-0.195	0.022	-0.239	-0.051	-4.278	0.000
Step3	PSS	AN	-0.060	0.049	-0.158	0.038	-1.213	0.229
		miRNA-273d	-0.482	0.227	-0.933	0.030	-2.126	0.037

Abbreviations: DV, dependent variable; IV, independent variable; SE, standard error; CI, confidence interval; LB, lower bound; UB, upper bound; PSS, positive symptom subscale; AN, abuse and neglect.

effect analysis:^{30,31} (1) Taking AN as IV and PSS as DV; (2) taking miR-1273d as DV and AN as IV; and (3) taking PSS as DV and AN miRNA-1273d as IVs. It was verified AN did not significantly predict PSS score in the mediating model ($P = 0.229$), while significantly associated with scores of PSS and ΔCt value of miRNA-1273d in the direct model ($P < 0.05$ or 0.001), see Table 5. The mediating effect value in the model was $0.482 \times 0.195 = 0.094$, and ratio of mediating effect in total effect was $0.482 \times 0.195 / 0.105 = 89.51\%$.

Discussion

The gene–environment interactions aim to describe how genetic and environmental factors jointly influence the risk of developing SCZ. Previous twin studies for SCZ yielded proband wise concordance rates of 41–65% in monozygotic (MZ) pairs and 0–28% in dizygotic (DZ) pairs, and heritability estimates of approximately 80–85%.³² These results highlight the contradiction between clinical observation and genetic evidence in SCZ, which suggests that there are other factors involved in the pathological process of SCZ.

Firstly, this study found AUCs of 9 upregulated miRNAs ranging from 0.678 to 0.878, and AUC of combined ROC curve was 0.958 with sensitivity and specificity of 0.838 and 0.963, respectively. Except for miRNA-3064-5p, ΔCt values of 8 other aberrant expressed miRNAs was negatively associated with positive symptoms. These results primarily indicated combined ROC curve of the 9 upregulated miRNAs had a high discriminative validity for SCZ and the higher the expression level of miRNAs, the more severe the positive symptoms in SCZ patients. miRNAs are a class of RNA molecules that do not encode proteins but play pivotal roles in fine-tuning gene regulation in neurons and cellular processes greatly relating to synaptic transmission, synaptic plasticity, neuronal survival, and immune cell activation.^{33–36} These biological processes were believed to be involved in neuropathogenesis, such as cognitive impairment, hallucinations, and inflammatory responses, which were strongly associated with SCZ.^{37–39} Furthermore, dysfunctional patterns of cortical connections disrupted by neuronal migration or differentiation disorders associated with miRNA dysregulation during brain development, may cause region-specific abnormalities in neuronal morphology and cytoarchitectural organization or metabolic alterations in the hippocampus, frontal cortex, cingulate cortex, and entorhinal cortex in adulthood,^{40–43} these physiological alterations were deemed to be greatly related to SCZ pathogenesis. Therefore, it may be hypothesized a SCZ-specific miRNA expression profile may regulate the pathological process and disease onset via a temporal-spatial mechanism.

Epigenetics, serving as a bridge between environmental exposure and genetic background, mainly focused on exploring heritable changes in gene expression without altering DNA sequences, which ultimately drove phenotypic changes in offsprings.^{44,45} Epigenetics well interpreted the physiological process for acquired changes when genetic background was consistent. This present study found higher scores of PB, AN, ALE, and CCSQ in SCZ patients compared with healthy controls positively correlated with PSS, NSS. Hierarchical regression analysis further verified miRNA-1273d fully mediated the relationship between chronic childhood stress and positive symptoms.^{30,31} These results could highlight chronic childhood stress as a predictor of SCZ onset may induce positive symptoms by regulating miRNA-1273d expression in SCZ patients. Compared with PB and ALE, AN perpetrators were often parents or significant others characterized by unique, dependent, long-term, unavoidable relationships with victimized children;

hence, greater stress caused by AN was regarded as being associated with the pathological process, onset, and symptoms of SCZ.^{46–48} How environmental stress leads to psychopathological symptoms by altering the epigenetic map has long been an outstanding issue in the mental health field; recent studies argued environmental stress can rapidly reshape the brain epigenetic map through glucocorticoid-mediated histone phosphorylation, which caused abnormal miRNA expression patterns and H3K9me2 loss in the promoter region of the BDNF gene. Aberrant miRNA and H3K9me2 also regulate the gene expression of glutamatergic and dopaminergic signaling pathways associated with the pathological process of SCZ.^{49–52} In summary, glucocorticoid-mediated histone phosphorylation maybe one key pathway in which chronic childhood stress regulated miRNA-1273d expression in SCZ patients.

This article is conducive to promoting the progress of precise SCZ diagnosis and elucidating pathophysiological processes in the following aspects. First, the identification of accessible biomarkers in bodily systems such as blood may lead to a more accurate diagnosis, more effective treatments, and could assist in monitoring disease evolution.⁵³ Based on existing research,¹⁹ this paper further verified the diagnostic value of differentially expressed miRNAs in peripheral blood for SCZ using an ROC curve, provided preliminary scientific evidence for miRNA as a biomarker of SCZ diagnosis. Second, as a basic consensus in the academic community, SCZ is a severe mental illness with an intricate pathophysiology involving genetics, environmental factors, and their interactions.⁵⁴ However, how environmental and genetic factors interact with each other is still poorly understood. Guided by the basic theoretical orientation of epigenetics, this study explored the relationship between chronic stress in childhood, miRNA expression levels, and psychopathological symptoms, and clarified the regulatory role of miR-1273d in chronic stress in childhood and positive symptoms in SCZ patients.

Limitations and future directions: (1) The sample size was relatively small, and it may be necessary to conduct multi-center, double-blind, randomized controlled and prospective studies in the future for further confirming the diagnostic accuracy of differentially expressed miRNAs in SCZ. Based on these external validations, peripheral blood miRNA detection kits could be developed for the clinical diagnosis and treatment efficacy evaluation of SCZ. Furthermore, selection bias for participants enrollment needs to be prevented in the future exploration due to confounding effects of some possible variables (eg smoking, BMI, and inflammation markers); (2) The exploration of the relationship between chronic stress in childhood, miRNA expression levels, and psychopathological symptoms of schizophrenia in this study is still preliminary, and many inferences based on this study still need further verification, such as the relationship between miRNA expression levels, especially miR-1273d, and BDNF, Glutamate, and dopamine, which could further clarify the etiology and pathological process of SCZ; (3) As a cross-sectional study, the observation of chronic stress in childhood was conducted through a mental scale assessment, which may limit the reliability of the conclusions due to recall bias in this study. In the future, animal models and longitudinal studies on the association between chronic stress, miRNAs, and SCZ may provide new evidence for existing theories.⁵⁵ Moreover, intervention measures for adverse childhood experiences in susceptible individuals could be carried out to longitudinally observe the relationship between intervention strategies and mental health status.

Conclusion

The results of this study show aberrant expressed miRNAs with potential diagnostic value for SCZ and chronic childhood stress are predictors of psychopathological symptoms, especially positive symptoms, in SCZ patients. Chronic stress in childhood may regulate susceptibility gene expression and pathological processes in SCZ by affecting the expression level of miRNA-1273d.

Abbreviations

SCZ, schizophrenia; miRNA, microRNA; qRT-PCR, quantitative real-time PCR; PANSS, Positive and Negative Syndrome Scale; CCSQ, Childhood Chronic Stress Questionnaire; ROC, receiver operating characteristic curve; AUC, area under curve; PB, peer bullying; AN, abuse and neglect; ALE, adverse life events; PSS, positive symptom subscale; NSS, negative symptom scale; GPS, general psychopathology scale.

Data Sharing Statement

The dataset used in the current study is available from the corresponding author upon request.

Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki, and approved by the Medical Ethics Committee of the No. 904th Hospital (Permit numbers, 2018-340-1). All the participants and their legal guardians signed an informed consent form.

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Disclosure

The authors declare no conflicts of interest in this work.

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