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Alteration of microRNA expression in lymphocytes in patients with first-episode schizophrenia

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Abstract

Background The development of schizophrenia is related to a combination of genetic and epigenomic factors. MicroRNAs (miRNAs) play a crucial role in epigenetic processes and are relevant to the onset and progression of schizophrenia. They can regulate target genes during the growth and development of neurons and can be affected by genetic and environmental factors associated with schizophrenia. Although prior studies have found abnormal miRNA expression in schizophrenia, few studies have examined the miRNA level in first-episode schizophrenia (FES). The present study aimed to examine the expression of lymphocyte microRNA (miR-107, miR-181a, miR-181b, miR-223, miR-219, miR-137, miR-125b) in patients with first-episode schizophrenia who had never been treated.

Method We investigated the expression of miRNAs using the real-time polymerase chain reaction (RT–PCR) technology. The severity of clinical symptoms was assessed using Positive and Negative Syndrome Scale (PANSS). The prognostic value of biomarkers was analyzed using receiver operating characteristic (ROC) curves, and the predictive value of these biomarkers was also compared. Logistic regression analysis was used to assess the relative risk related to microRNA alteration in schizophrenia. Logistic regression analyses were then performed to identify the most significant and sensitive miRNA biomarkers.

Results Compared with the control group, the patient group exhibited significantly higher levels of expression for six miRNAs (miR-181a, miR-137, miR-223, miR-107, miR-181b, and miR-125b) ($P < 0.05$). The ROCs indicated that miR-223 exhibited the highest diagnostic value, with an area under the curve being 0.916.

Conclusions The present study provided some insights into the alteration of miRNA expression, which might improve our understanding of the complex global changes in gene expression in the pathophysiology of schizophrenia. This study identified six miRNAs (miR-223, miR-181a, miR-181b, miR-125b, miR-219, and miR-107) that might facilitate the diagnosis of schizophrenia.

Keywords MicroRNA, Schizophrenia, Biomarker

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Background

Schizophrenia (SCZ) is one of the most debilitating and genetically complex neurodevelopmental disorders with a lifetime prevalence of 0.7% in the general population worldwide [1]. The clinical presentation of schizophrenia includes positive symptoms such as hallucinations, delusions, disorganized thinking, poor planning, disorganized or abnormal motor behaviors, and negative symptoms such as affective flattening and reduced speech

[2]. SCZ imposes a huge burden on individuals, families and the whole society [3]. This disease usually occurs in late adolescence or early adulthood, with the prodromal period coinciding with the critical period of growth and development of adolescence. First-episode SCZ is often diagnosed between the ages of 15 (adolescence) and 25 years (early adulthood) [4]. Even with proper treatment, approximately two-thirds of the affected individuals have persisting or fluctuating symptoms [5]. Despite the concerns, only a small number of recent studies have provided insight into the early stage of SCZ; thus, the etiology of SCZ remains largely unknown. The interaction between susceptibility genes and environmental factors has been proposed as a possible mechanism of SCZ [6]. Prior findings have shown that genetic and environmental factors may impact the neurodevelopmental process, which leads to abnormalities in the plasticity and connectivity of the brain, thereby resulting in SCZ [7]. Notably, key epigenetic reprogramming processes may take place in the presence of major environmental risk factors for schizophrenia during the vital period of neurodevelopment in adolescence [8]. The environment may continue to shape epigenetics through early adulthood and across lifespans, with epigenetic mechanisms acting as downstream effectors of environmental signals [9]. Epigenetic marks may also record altered gene expression [10]. DNA methylation, post-translational histone modification, and RNA interference, particularly through microRNAs (miRNAs), are three major epigenetic mechanisms [9].

miRNAs, single-stranded non-coding RNAs involved in the post-transcriptional suppression or instability of the mRNAs of numerous target genes, are classified in the epigenetic category [11]. Overexpression of miRNAs is prone to causing decreased expression of target gene [12]; however, miRNAs may upregulate gene expression in rare cases [13]. The miRNAs are highly enriched in the brain [14]. In some cases, individual miRNAs can target multiple mRNAs, effectively controlling expression of a series of genes. Thus, the alteration of a single miRNA with regard to genomic sequence, copy number, and/or expression has broad implications for the development and cellular function throughout the lifetime [15, 16]. Although the origin of circulating miRNAs is unclear, it is believed that they play a role in cell-to-cell signaling [17]. Compared with healthy controls, the expression of miRNAs was found altered in postmortem studies on the brain tissue [18, 19], plasma [20], serum [21], exosomes [22] and lymphocytes [14, 23]. Du et al. [24] discovered that the exosomes of schizophrenia patients had the highest level of upregulated miR-206 expression. Twenty key genes associated with schizophrenia, including NRG1, may be regulated by the differently expressed miRNA [22]. The aberrant levels of exosome metabolites are linked to the beginning of schizophrenia, and they can be utilized as a differential diagnostic in schizophrenia [25]. miRNAs regulate biological processes related to the aberrant neurodevelopment in patients with schizophrenia [26]. Previous literature revealed a large number of abnormal miRNA changes in the peripheral blood of patients with SCZ [27, 28], as well as in the exosomes and cadaveric brain [29, 30]. The main component of peripheral blood mononuclear cells (PBMCs) is leukocytes, which are easily obtainable [31, 32]. In several neuropsychiatric disorders, alteration of metabolism and cellular functions in the central nervous system, as well as disturbances in neurotransmitters and hormones, is concomitant with altered function and metabolism of lymphocytes in the blood [33].

It has been found that several miRNAs (miR-107 [34], miR-181a [35, 36], miR-181b [37], miR-223 [27], miR-219 [38, 39], miR-137 [40], and miR-125b [41] are involved in the regulation of the glutamate pathway, which can inhibit the expression of glutamate receptors and are related to the dysfunction of glutamate receptors. miR-137 is capable of regulating synaptic function and is linked to synapse development in schizophrenia [42]. miR-219 is involved in the regulation of neural stem cells proliferation [43]. Antipsychotic medications may have an impact on miRNA levels [20]. The study of early onset of schizophrenia, after eliminating the interference of drugs on the body, is of great significance to explore the pathogenesis of schizophrenia. There is little research on first-episode, drug-naive schizophrenia, despite the large number of miRNA studies in schizophrenia. For SCZ, the time from onset to diagnosis may last for years, and there is a lack of specific laboratory indicators. The diagnosis of SCZ is primarily based on clinical manifestations, as there are no laboratory or physical tests or biological markers with sufficient diagnostic value at the moment [44]. Furthermore, Considering the extent of microRNA alterations and their broad range of impact, this phenomenon may be a significant factor in the etiology of schizophrenia. [45]. The detection of biomarkers in the blood have become a promising field of research on SCZ. Recently, studies on SCZ suggested the great importance of miRNAs in the pathogenesis of SCZ [46, 47]. In the present study, we used Quantitative real-time RT-PCR analysis to detect the expression of miRNAs (miR-107, miR-181a, miR-181b, miR-223, miR-219, miR-137, and miR-125b) in patients with first-episode SCZ and healthy controls, looking to identify potential biomarkers for the diagnosis of SCZ.

Materials and methods

Participants

Twenty-six people with schizophrenia were recruited for the study. All the enrolled patients were treatment naïve. *The inclusion criteria were as follows*: primary school education or above; aged between 18 and 65; no antipsychotics, mood stabilizers, or antidepressants use; no acute or chronic infectious diseases, and no immunosuppressants, antibiotics or hormone drugs use. *The exclusion criteria were as follows*: other primary mental illnesses; other substance use disorders (not excluding nicotine); organic brain disease, epilepsy, or craniocerebral injury; history of blood transfusion; major physical illness.In the present study, all the participants were Han Chinese diagnosed with schizophrenia according to DSM-V by at least two trained psychiatrists.

Twenty-six sex- and age-matched healthy controls were also enrolled in the study. *The inclusion criteria were as follows*: primary school education or above; aged between 18 and 65; no acute or chronic infection, no immunosuppressants, antibiotics, or hormone drugs use. *The exclusion criteria were as follows*: a history of mental illness; a history of substance dependence other than nicotine; Organic brain disease, epilepsy, or craniocerebral injury; psychotropic drugs use. This study was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University, and informed consent was obtained from all participants. The study was conducted in accordance with the regulations and guidelines established by this committee.

Selection of miRNA

We aimed to find miRNAs that are both implicated in NMDA receptor dysfunction and exhibit aberrant expression in schizophrenia, based on the hypothesis that NMDA receptor dysfunction occurs in schizophrenia. The seven miRNAs tested in this study were selected through extensive literature search of the PubMed database and Web of science. All published studies related to miRNA in SCZ from January 2000 to May 2022 were searched and analyzed. Based on the retrieved publications, seven miRNAs associated with SCZ were selected for this study.

RNA isolation and reverse transcription

Whole blood (10 ml) was collected from all the patients with SCZ and healthy controls using an EDTA anticoagulant tube. The plasma was separated by centrifugation and transferred into a fresh RNase/DNase-free microcentrifuge tube, and the mononuclear leukocytes were separated using a lymphocyte separation tube (Dakewe Biotech Co, Ltd., Shenzhen, China). The harvested cells were then rinsed and centrifuged to remove platelets, plasma, and any Ficoll-Paque PLUS. These mononuclear leukocyte samples were stored at −80℃ for later use. According to the manufacturer's instructions, total RNA was extracted from PBMCs using the Trizol reagent (Invitrogen, CA, USA) and stored at −80 °C until use. The reverse transcription reactions were performed at 37℃ for 60 min and then at 85℃ for 5 min according to the manufacturer's protocol in a PE 2400 PCR system (Xiamen Jinyu Industry Co., Ltd.); the product was held at 4℃.

Quantitative real-time RT-PCR

Total RNA (1 ug) was reverse-transcribed and amplified by fluorescent dye-based real-time quantitative PCR using the All-in-One™ miRNA qPCR Kit and the All-in-One™ miRNA qPCR Primer(GeneGopoeia), with relative expressions determined using the $2^{-\triangle\triangle Ct}$ method. SYBR Green I (LightCycler 480 SYBR Green I Masters, Roche) is a highly sensitive DNA fluorescent dye. By measuring the strength of the fluorescence signal, the number of PCR products can be determined. The relative expression level of each miRNA was normalized to the level of miRNA U6. U6(HmiRQP9001), miR-107(HmiRQP0030), miR-181a-5p(HmiRQP0232), miR-181b-5p(HmiRQP0234), miR-137-3p(HmiRQP0175), miR-223-3p(HmiRQP0342),miR125b-5p(HmiRQP0096),miR-219–2-3p(HmiRQP0331)were purchased from GeneGopoeia. The real-time PCR reaction was performed in 5 uL of reagent mixture containing 2.5 ul of TaqMan Universal PCR Master Mix II, 0.25 uL of miRNA-specific primer/probe mix (Applied Biosystems), and 2.25 uL of diluted RT cDNA product. The PCR reaction was run in a 7900HT fast real-time PCR system (Applied Biosystems) with following cycling parameters: 95℃ for 10 min, followed by 40 cycles of reaction at 95℃ for 10 s, 60℃ for 20 s and 72℃ for 10 s. All the reactions were performed in triplicate, with snRNA U6 used as the endogenous control. All reactions including negative controls were duplicated. Melting curve analysis was then performed to ensure that only a single product was amplified at a time.

Statistical analysis

The expression of each miRNA was quantified using its normalized threshold cycle number $\triangle$Ct, where $\triangle$Ct = [Ct miRNA] – [Ct snRNAU6] and the relative expression level was calculated as $2^{-\triangle\triangle Ct}$, which is generally applied in genome-wide profiling research of miRNAs. Samples with Ct values greater than 35 were excluded from the analysis. All data were processed using the Statistical Package for the Social Sciences version 17.0 (SPSS, Chicago, IL, USA). Wilcoxon rank sum test was used to compare the expression level of miRNAs between the patient group and the control group. Receiver operating characteristics (ROC) curves(GraphPad Prism 8) were generated to evaluate the diagnostic performance

of miRNAs. Binomial Logistic regression analyses (SPSS, Chicago, IL, USA) were then performed to identify the most significant and sensitive miRNA biomarkers. For all the analyses, $P<0.05$ was considered statistically significant.

Results

Demographic characteristics

The majority of patients were male for both groups, and no significant difference was noticed in terms of age and sex between the patient group and the control group (Table 1). Among the 26 participants in the patient group, 6 participants had a family history of SCZ.

Expression of miRNAs in PBMCs

It can be seen from Fig. 1 and Table 2, the results of Wilcoxon rank sum test showed that the level of expression of miR-181a ($Z=-2.324$, $P=0.020$) miR-125b ($Z=-4.374$,$P<0.0010.000$), miR-137 ($Z=-2.434$, $P=0.015$), miR-223 ($Z=-5.152$, $P<0.001$), miR-181b ($Z=-2.956$, $P=0.003$) miR-107 ($Z=-2.690$, $P=0.007$), and miR-125b ($Z=-4.374$, $P<0.001$) in patients with first-episode SCZ were significantly higher than that in the healthy controls ($P<0.05$). No significant differences were found in the expression of miR-219 between the two groups (Fig. 1b).

Table 1 The demographic characteristics of the participants

Variable	Patients with schizophrenia ($n=26$)	Healthy controls ($n=26$)	Comparison
			Statistics *P* value
Age, years			
Mean (SD)	23.54 (0.94)	23.88 (0.44)	*t* = -0.33 0.74
Range	18–65	18–65	
Gender			
Female	12 (44.4%)	6 (20.0%)	0.08
Male	14 (55.6%)	20 (80%)	
Education			
< 9 years	6 (22.2%)		
> 9 years	20 (77.8%)		
Ethnicity			
Han	26	26	
Ethnic minorities	0	0	
Marital status			
Married	0	0	
Unmarried	26	26	
Family history			
Present	6	-	
Absent	20	-	

The ROC curve analysis

ROC curved analysis was performed to assess the diagnostic value of circulating miRNAs in schizophrenia. Comparing patients with SCZ and healthy controls, the AUC of miR-181a was 0.688 (95%CI: 0.545–0.8312), with a sensitivity of 50.00% and a specificity of 80.77% (Fig. 2A); the AUC of miR-137 was 0.697 (95%CI: 0.554–0.840), with a sensitivity of 61.92% and a specificity of 76.92% (Fig. 2B); the AUC of miR-223 was 0.916 (95%CI: 0.842–0.990), with a sensitivity of 76.2% and a specificity of 100% (Fig. 2C); the AUC of miR-181b was 0.739 (95%CI: 0.603–0.875), with a sensitivity of 80.77% and a specificity of 61.54% (Fig. 2D); the AUC of miR-107 was 0.718 (95%CI: 0.567–0.868), with a sensitivity of 73.08% and a specificity of 76.93% (Fig. 2E); the AUC of miR-125b was 0.854 (95%CI: 0.748–0.959), with a sensitivity of 73.08% and a specificity of 92.31% (Fig. 2F).

Logistic regression analysis

A logistic regression model of the level of miRNA expression was built to identify first-episode SCZ. The result showed that four miRNAs, namely miR-181b, miR-137, miR-223, and miR-125b, exhibited greater diagnostic value for SCZ (Table 3).

Discussion

Our findings showed that the expression of miRNA-181a, miRNA-181b, miRNA-223, miRNA-137, miRNA-125b, and miRNA-107 was considerably upregulated in first-episode SCZ, indicating that these six miRNAs are linked to the early onset of schizophrenia. There are many previous findings in the literature that support the results of this study. MiR-137 is highly conserved in mammals. miR-137 regulates the maturation of synapses and the formation of neural cells [28]. A previous study found that miR-137 was upregulated in schizophrenia [40]. Prior studies suggested an association between miRNAs and psychiatric symptoms. Song et al. [20] found significantly elevated plasma levels of miRNA-181b, miRNA-30e, miRNA-34a, and miRNA-7 in patients with first-episode SCZ, and the level of miRNA-181b declined with improvement of psychotic symptoms after six weeks of treatment with antipsychotics. Prior findings also indicated a positive correlation between the improvement of negative symptoms and a decrease level of miR-181b (which was previously elevated). The level of miR-219–2-3p was found significantly correlated with the severity of psychiatric symptoms in schizophrenia [48]. The interaction of hsa-miR-219, CAKM2G, GRIN2B and GRIN3A polymorphisms may predispose Chinese to schizophrenia [49]. MiR-107 has been found significantly upregulated in the prefrontal cortex of patients with SCZ [34]. A

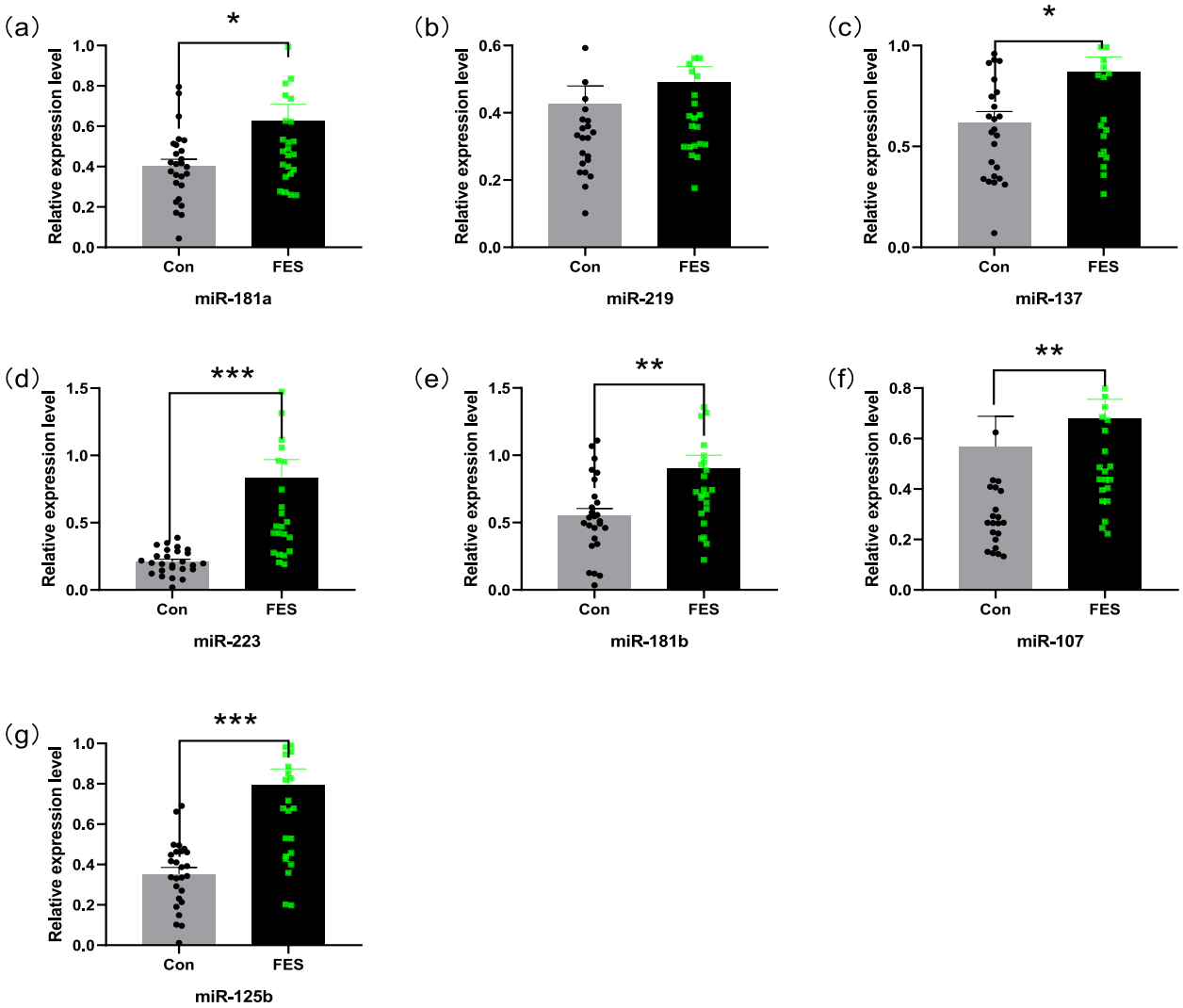

Fig. 1 **a**-**g** Compared with healthy controls ($n=26$), the expression of miR-107, miR-181a, miR-181b, miR-223, miR-137, and miR-125b was significantly upregulated in patients with first-episode SCZ($n=26$). The horizontal bar in the figures indicate the standard error. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 2 Level of microRNA expression in PBMCs of the patient group and the control group (ΔCt)

miRNA	SCZ ($n=26$)	HCs ($n=26$)	Z	*P* value
miR-181a*	0.63 ± 0.41	0.40 ± 0.18	−2.324	0.020
miR-219	0.49 ± 0.23	0.43 ± 0.23	−1.519	0.129
miR-137*	0.87 ± 0.37	0.62 ± 0.28	−2.434	0.015
miR-223***	0.83 ± 0.69	0.21 ± 0.09	−5.152	< 0.001
miR-181b**	0.90 ± 0.69	0.55 ± 0.29	−2.956	0.003
miR-107**	0.68 ± 0.39	0.57 ± 0.62	−2.690	0.007
miR-125b***	0.79 ± 0.41	0.35 ± 0.17	−4.374	< 0.001

The data is presented as mean ± standard deviation. The variable △Ct was used to compare the level of miRNA expression, and the conversion factor $2^{-\triangle\triangle CT}$ was used to calculate relative miRNA expression levels. *miR* microRNA, *SCZ* schizophrenia

* $P<0.05$. ** $P<0.01$. *** $P<0.001$

previous study found that miR-181a was upregulated in brain post-mortem tissues of schizophrenia [50]. Schizophrenia patients had considerably higher serum levels of miR-223. And in miR-223 overexpression cells, they discovered that the mRNA levels of INPP5B, RHOB, SKIL, and SYNE1 associated with the cytoskeleton or cell movement were drastically downregulated [27].

The hyperactive dopaminergic function related to SCZ may also be caused by dysregulated n-Methyl-d-Aspartic Acid (NMDA) receptors neurotransmission [51]. It has been hypothesized that the dysfunction of NMDA receptors is a contributing factor for SCZ [52, 53]. Studies have shown that miRNA regulates the expression of the G protein-coupled receptor gene and influences the activity of the NMDA receptor [54].

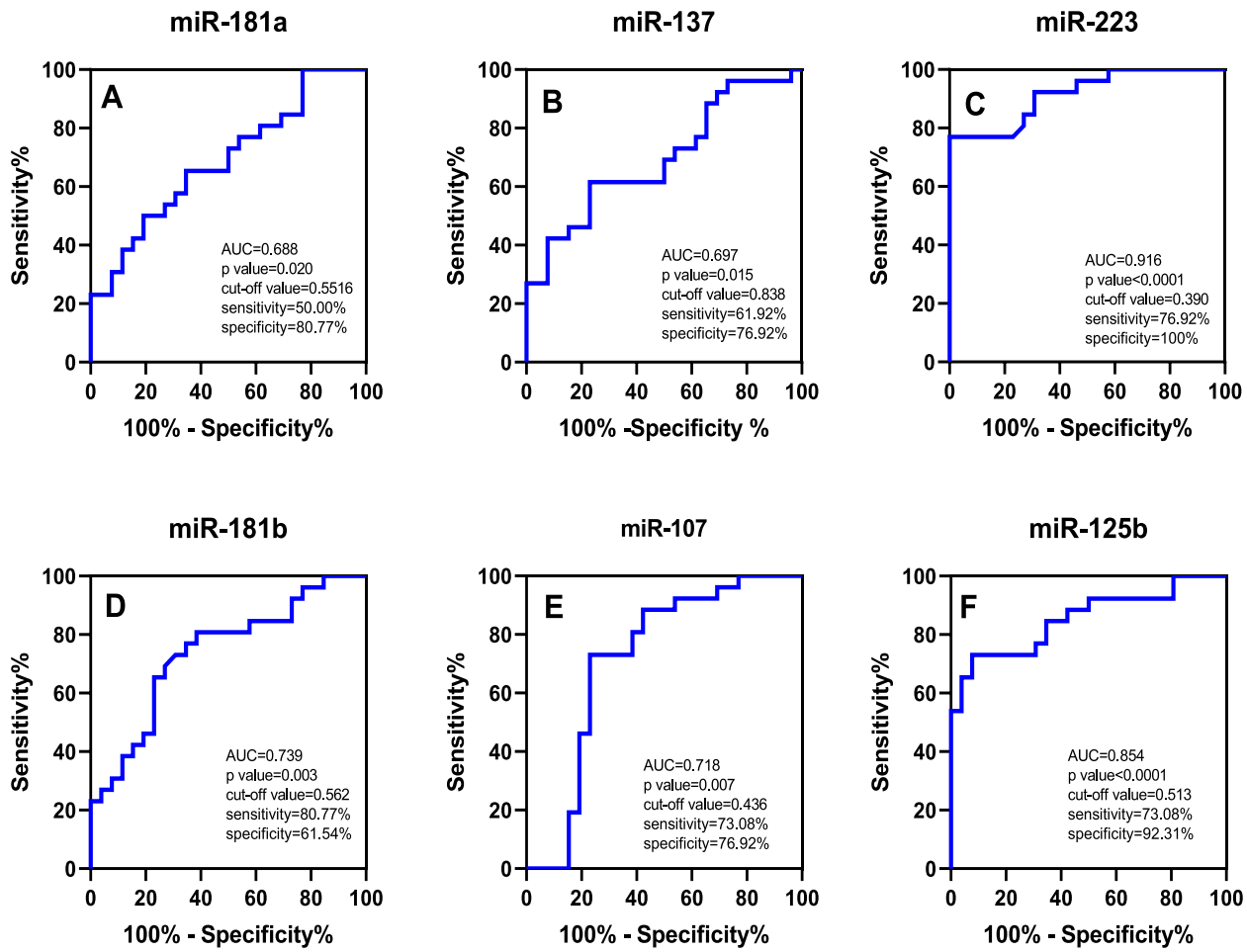

Fig. 2 ROC curves for miR-181a, miR-181b, miR-137, miR-223, miR-125b, and miR-107, the expression of which were upregulated in patients with first-episode schizophrenia. The sensitivity, specificity and AUC value of each miRNA in the diagnosis of schizophrenia are presented in the figure

Table 3 Logistic regression analysis on the relative risk ratio of microRNA expression for patients with schizophrenia

Item	B	SE	Wald	*P* value	Exp (B)
miR-181b	2.680	1.023	6.862	0.009	14.590
miR-137	2.362	0.955	6.111	0.013	10.611
miR-223	1.439	0.451	10.171	0.001	4.216
miR-125b	0.666	0.196	11.519	0.001	1.946

B, regression coefficient, Exp (B), relative risk, *miR* microRNA, *SE* standard error, *Wald* chi-squared value

Hauberg et al. discovered that miRNA plays a significant role in the regulation of schizophrenia risk genes [55].All the seven miRNAs with aberrant expression were involved in the glutamate pathway. Through highly conserved binding to mouse, rat, and human 3 'UTR complementary mRNA sites, miR-223 [56] can directly inhibit the expression of the AMPAR subunit GluR2 and the NMDAR subunit NR2B in the central nervous system, thereby specifically regulating neuronal excitability in response to glutamate. Produced by lymphocytes, miR-223 can cross the blood–brain barrier to affect the expression of genes in the brain. In patients with SCZ, the upregulation of miR-223 may lead to dysfunction of NMDA receptors and decreased synaptic activity, which might be related to the development of SCZ [57]. Beveridge et al. [45] investigated the expression of miRNAs in the cerebral cortex of individuals with schizophrenia, which showed that miR-181b was overexpressed in the superior temporal gyrus. MiR-181b, predominantly expressed in lymphoid cells and known to regulate the inotropic glutamate receptor, is encoded by two genetic loci located on chromosomes 1 and 9. Upregulation of miR-181b has been linked to the downregulation of genes associated with schizophrenia (SCZ), including VSNL1 and GRIA2 [58]. According to recent studies, overexpression of miR-181a

could significantly and negatively regulate the expression of GRIA2 and inhibit the GRIA2 protein activity [59]. Camkurt et.al [60] investigated the expression of miRNAs in the peripheral whole blood of individuals with schizophrenia, which showed that miR-125b was overexpressed. miR-125b can directly and specifically regulate the mRNA expression of glutamate NMDA receptor subunit NR2A, thereby modulating NMDA receptor function [41]. As the miRNAs inhibit the expression of glutamate receptors, the abnormally expressed miRNAs might be associated with hypofunction of NMDA receptors. MiR-107 inhibits the expression of GRIN3A and has been found significantly upregulated in the prefrontal cortex of patients with SCZ [34]. In vivo, Increased miR-137 expression in vivo can lead to changes in the distribution of synaptic vesicles, nerve fiber damage, dementia, hippocampus-dependent learning, and memory deficits. The dysregulation of miR-137 may impair synaptic plasticity in the hippocampus [61]. MiR-137 modulates the glutamatergic synaptic transmission, and its effect on glutamate signaling may lead to the pathogenesis of SCZ. A variety of glutamatergic receptors, including AMPA and the NMDA receptor subunits GluA1 and GluN2A, as well as a number of presynaptic targets involved in neurotransmitter release, are regulated by miR-137 [62, 63]. Thus, overexpression of miR-137 may impact synaptogenesis, pre-synaptic micro-structure, and function of synapses, thereby affecting the density and function of synapses, which might also be a potential central disruption related to schizophrenia [28]. Studies indicated that the disruption of NMDA receptor signaling by dizocilpine could reduce the level of miR-219 (a miRNA specifically in the brain) in the mouse prefrontal cortex [48]. miR-219 [48] inhibits the expression of glutamate receptor ionotropic NMDA 1 (GRIN1). It was found that, in the hippocampus and amygdala of patients with epilepsy, the expression level of miR-219 was negatively associated with the expression of GRIN1, suggesting that miR-219 might regulate NMDA activity in this patient population [38]. Based on the above findings, it could be inferred that the abnormal elevation of the above miRNAs may affect the glutamatergic pathway by inhibiting the expression of NMDA receptors, which is associated with psychotic symptoms in SCZ.

Although the present study is not the first one to examine the expression of miRNA in PBMCs in SCZ, it has a broader scope and yielded findings that the level of circulating miRNAs might be associated with SCZ and other related syndromes. The overexpression of several miRNA targets, indicating a potential deficiency in microRNA-based control of gene transcription in SCZ [64]. Although the cortical miRNA-related pathophysiology of SCZ still remains unclear, it is possible that the alteration of expression is present outside the brain, indicating that peripheral miRNAs might be biomarkers for SCZ. Furthermore, studies on peripheral miRNA suggested that miRNA dysregulation occurs in the early stage of SCZ. Our results indicated that some miRNAs could inhibit the expression of glutamate receptor subunits [48, 56, 62], affect the function of glutamate receptors, and result in the hypofunction of NMDA receptors. Despite the increasing number of studies, biomarkers of SCZ are still underexplored [65], with often inconsistent and even conflicting findings yielded in existing studies. There are also several limitations to this study, with the most important ones being the small sample size and lack of validation using human brain tissues; thus, the present findings are only preliminary and should be interpreted with caution.

There are some limitations to this study. This study was a cross-sectional study and did not examine the longitudinal study of indicator changes. The study lacks an exploration of the possible influence of external factors, such as medications, on miRNA levels. The sample size of this experiment is insufficient. There is a gender imbalance and age difference in the study population of this experiment, which may have an impact on the results of the experiment. Although the majority of the participants in this experimental investigation were from Hunan Province, regional disparities may still exist. The role of miRNA in cell-to-cell signaling is speculative. Given the similarity between neuronal cells and PBL in the mechanisms of receptor expression and transduction, it has been demonstrated that changes in miRNA expression in lymphocytes or leukocytes are correlated, but it has yet to be established whether blood RNA can be used in place of RNA in brain tissue. While the expression levels of specific miRNAs were found altered in SCZ patients, the mechanism by which these miRNAs influence the disorder is not fully elucidated.

In conclusion, the present study provides preliminary evidence that six miRNAs are implicated in the pathogenesis of SCZ and have significant diagnostic value for this mental disorder. These miRNAs regulate the glutamate signaling pathway, which is one of the most important mechanisms for the development of psychotic symptoms and one of the most prominent factors affecting the development of the cerebral cortex in people at a high risk of SCZ. Future studies may focus on miRNAs as epigenetic regulators of expression of genes related to mental disorders. Hopefully, microRNAs may serve as state biomarkers for the diagnosis and personalized management of SCZ.

Abbreviations

miRNAs	MicroRNAs
FES	First-episode schizophrenia
RT–PCR	Real-time polymerase chain reaction
PANSS	Positive and Negative Syndrome Scale
ROC	Receiver operating characteristic
PBMCs	Peripheral blood mononuclear cells
NMDA	N-Methyl-d-Aspartic Acid
GRIN1	Glutamate receptor ionotropic NMDA 1

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Authors' contributions
Jingjing Huang implemented experiments, analyzed data, and wrote the main manuscript text. Xuyi Wang guided our experiment and edited the manuscript.

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Data availability
The datasets generated and analysed during the current study are not publicly available due to our lab confidentiality policy but are available from the corresponding author on reasonable request. The subject's personal information is not publicly available due to their containing information that could compromise the privacy of research participants.

Declarations

Ethics approval and consent to participate
This study was approved by Ethics Committee of National Clinical Medical Research Center, The Second Xiangya Hospital, Central South University, Peoples R China. Informed consent was obtained from all subjects. Declare all of this research method is carried out in accordance with the relevant guidelines and regulations.

Consent for publication
All participants in this study have signed informed consent forms.

Competing interests
The authors declare no competing interests.

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