**Bioinformatic Identification and Validation of Key Genes Associated with Schizophrenia**

**Jiao wang1, Fushuai Wang1, Haicong Zhou1, Hu Feng1, Junyi Zhuang1, Yinping Zhou1, Kai Wang2, Haotian Bai3, Yanyan Kong4\*, Jiang Xie3\***

1Laboratory of Molecular Neural Biology, School of Life Sciences, Shanghai University, Shanghai, China, 2Central Laboratory, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai, China, 3School of Computer Engineering and Science, Shanghai University, Shanghai, China, 4 Position Emission Computed Tomography Center, Huashan Hospital, Fudan University, Shanghai, China

**\* Correspondence:**

Yanyan Kong

Email: ykong11@fudan.edu.cn

Jiang Xie

Email: jiangx@shu.edu.cn

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**Abstract**

Genetic factors contribute substantially to the pathology of schizophrenia, a severe mental disorder. However, the molecular basis of schizophrenia has not been comprehensively characterized, thereby limiting treatment strategies. In particular, previous studies of schizophrenia have generally focused on the single-gene level. In our study, we used a bioinformatics approach to systematically analyze genome-wide differences in gene expression between patients with schizophrenia and healthy controls. We identified 62 differentially expressed genes (DEGs) in patients with schizophrenia. These genes were enriched for various pathways, such as ion transmembrane transport, axon guidance, and cell surface pathways. A competing endogenous RNA network analysis showed that miRNAs regulate the expression of these DEGs, including miRNAs with established roles in schizophrenia. PET results revealed key brain regions in the pathology of schizophrenia, suggesting that some DEGs function in these brain regions, such as the prefrontal lobe. Finally, a qPCR analysis of the prefrontal lobe of mice with schizophrenia confirmed the differential expression of some genes, supporting the general role of these DEGs in schizophrenia. In conclusion, these results indicate that the miRNA-mediated regulation of target genes in key brain regions is an important molecular pathway for schizophrenia, providing a theoretical foundation for further studies of the molecular basis of schizophrenia.

**Introduction**

Schizophrenia is a serious mental illness involving multiple psychological disorders, with positive (fantasy, speech, and behavioral disorders) and negative (excessive or weakened mood) symptoms [[1](#_ENREF_1)] and a core feature of cognitive deficits [[2](#_ENREF_2)]. Its cause is currently unknown. About 1% of the general population suffer from schizophrenia worldwide, with a high frequency in adolescence or early adulthood [[3](#_ENREF_3)], seriously affecting quality of life. Schizophrenia currently lacks a clear etiology. Studies have pointed out that genetic factors play an important role in the pathophysiology of schizophrenia, accounting for about 80% [[4](#_ENREF_4)].

The Psychiatric Genomics Association conducted a genome-wide association study (GWAS) of 36,989 patients with schizophrenia and 113,075 healthy controls; several genetic variants associated with schizophrenia were identified and the overall genetic composition of patients was described [[4](#_ENREF_4)]. More than 180 loci are strongly associated with schizophrenia [[5](#_ENREF_5)], including microRNA 137 (MIR137), zinc finger protein 804A (ZNF804A), vaccinia-associated kinase 2 (VRK2), arsenite methyltransferase (AS3MT), and susceptibility genes in major histocompatibility complexes in European and East Asian populations [[6](#_ENREF_6)].

Studies of epigenetic mechanisms have focused on DNA methylation, histone modifications, and non-coding miRNAs [[7](#_ENREF_7)]. MicroRNAs (miRNAs) are short non-coding RNAs (19–23 nucleotides) that regulate expression at the transcriptional and post-transcriptional levels via interactions with the 3′ untranslated region (UTR) of the target mRNA [[8](#_ENREF_8)]. At the post-transcriptional level, miRNAs play important roles in brain development and the pathophysiology of many mental illnesses. A study of miRNA expression in the dorsal lateral prefrontal cortex of patients with schizophrenia at different physiological periods has revealed the temporal dynamics of miRNAs in this region, suggesting that schizophrenia and dysregulated miRNAs enriched in infancy and adolescence have possible connections [[9](#_ENREF_9)].

However, the specific mechanism underlying schizophrenia is still unclear, and this limits the development of novel treatments of schizophrenia. Furthermore, little is known about the roles of competing endogenous RNAs (ceRNAs) in the pathogenesis of schizophrenia. Therefore, we used bioinformatics methods to establish a ceRNA network in order to evaluate the relationships between miRNAs and target genes, exploring the molecular mechanism of schizophrenia.

**Materials and Methods**

**Animals**

C57LB6 (wild-type) mice were reared at a constant temperature (22 ± 1°C) and maintained on a 12/12 h light/dark cycle, with food and water provided ad libitum. All animals were treated in accordance with the International Guidelines for Animal Research. The study design was approved by the Animal Ethics Committee of Shanghai University.

**Positron emission tomography (PET)**

According to normal operating procedures, PET was performed on mice using the Siemens Inveon PET/CT System (Siemens Medical Solutions, Knoxville, KY, USA) by the PET CENTER HUASHAN HOSPITAL FUDAN UNVIERSITY. Briefly, 500μmci 18F-DTBZ was injected through the tail vein. The detection interval was 50 min after injection, and the PET detection time was 10 min. Mice were anesthetized using isoflurane.

**Identification of DEGs and functional annotation**

To identify DEGs between patients with schizophrenia and healthy individuals, previously reported raw RNA-Seq data were obtained [[10](#_ENREF_10)] (NCBI accession number GES121376). A locally developed Perl script was used to delete low-quality bases at the 5′ and 3′ ends (Q20 ≤ 20). HISAT2 (version 2.0.5) [[11](#_ENREF_11)] was used to map reads to the human transcriptome (GRCm38.p5). Read counts were generated using BEDTools [[12](#_ENREF_12)], and expression values were calculated using the RPKM (read reads per million bases) method. DEGs were obtained using a fold change threshold of ≥1.50 or ≤0.67 and p-values obtained by *t-*tests of ≤0.05. DAVID 6.8 was used for a functional enrichment analysis of schizophrenia-related genes [[13](#_ENREF_13)].

**Construction of a ceRNA network**

miRNAs were collected from miRbase [[14](#_ENREF_14)]. mRNA and lncRNA sequences were obtained from NCBI (GRCm38.p5). All differentially expressed mRNAs and lncRNAs were used for target identification. The miRNA–mRNA and miRNA–lncRNA regulatory relationships were determined using RNAhybrid (setting the seed region from coordinates 1 to 6, 2 to 7, 3 to 8, 4 to 9, and 5 to 10; mfe ≤ -25 kcal/mol, p ≤ 0.01) and BLASTN (word size of 10 and e-value of 1000, removing the miRNA on the forward alignment and miRNA (position of the alignment greater than 6), and the intersection was obtained. The ceRNA network was visualized using Cytoscape 3.5.1 [[15](#_ENREF_15)].

**Brain imaging**

A cohort of 44 individuals (including 18 with schizophrenia and 26 healthy controls) was collected from HuaShan Hospital, Shanghai, China. All Digital Imaging and Communications in Medicine (DICOM) data were converted into NIfTI-formatted files using DCM2NII (http://people.cas.sc.edu/rorden/mricron/index.html).

Two steps were used to establish the boundaries of the brain for analyses. In the preprocessing stage, statistical parametric mapping (SPM12, www.fil.ion.ucl.ac.uk/spm) was implemented in MATLAB R2016a; every image was normalized into a standard brain space by the ICBM East Asian template and the low-frequency background noise was simultaneously removed. Next, an isotropic Gaussian smoothing kernel with a FWHM value of 10 × 10 × 10 mm³ was applied. Regions of interest (ROIs) were compared between groups using a two-sample Student’s *t*-test based on 18 patients with schizophrenia and 26 healthy controls. By setting the peak threshold to p < 0.01 and applying an error correction with a threshold of 20 voxels, the regions were localized. Using Talairach Client [21], individual and batch labels of the ROI created were discovered.

**RNA extraction and real-time quantitative PCR (qPCR)**

The prefrontal lobe of mice was dissected quickly in 0°C PBS and placed in RNA extraction solution (Promega, Madison, WI, USA). RNA extraction was performed according to the manufacturer's protocol. The final reaction volume contained 2 µg of total RNA, 4 µl of 5× RT Master Mix (TaKaRa, Kusatsu, Japan), and RNA-free water (up to 20 µl). cDNA was synthesized at 25°C for 5 min, 37°C for 30 min, 85°C for 10 s, and 12°C for 10 min. To quantify the target genes, real-time PCR was performed using qPCR SYBR Green Master Mix (Yeasen, Shanghai, China). Each reaction contained 1 µl of cDNA sample (100–200 ng/µl), 10 µl of qPCR SYBR Green Master Mix, 0.8 µl (10 µM) of designated primers, and RNA-free water (up to 20 µl). The PCR conditions were as follows: 95°C for 30 s, 55°C for 20 s, 72°C for 30 s, with 40 thermal cycles. The mRNA expression levels were normalized to *GAPDH* levels.

**Construction of a mouse model of schizophrenia**

The *N*-methyl-D-aspartate (NMDA)-type glutamate-receptor antagonist MK-801 (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in physiological saline and injected into the abdominal cavity of mice for 7 days (0.6 mg/kg, once daily) [[16](#_ENREF_16)]. The same mice were injected with the same amount of normal saline as a control. PET and qPCR experiments were performed within 1 week after the injection.

**Statistical analysis**

All data were analyzed using GraphPad Prism and results are presented as means ± SEM. Levels of mRNA expression were compared between schizophrenic mice and the control group using *t*-tests. Significance was set to p < 0.05.

**Results**

**DEGs between patients with schizophrenia and normal controls**

To determine molecular differences between patients with schizophrenia and normal controls, we performed an RNA sequencing analysis (bulk) of interneurons in the two groups. Raw RNA-Seq reads from two independent datasets, each based on 14 independent iPSC lines, were downloaded from GEO (https://www.ncbi.nlm.nih.gov/geo/; accession number GSE121376) [[17](#_ENREF_17)]. We identified 62 DEGs between 28 patients with schizophrenia and 28 normal people. Among these 62 genes, 22 genes were up-regulated in most patients with schizophrenia compared with levels in healthy individuals and 40 genes were down-regulated in schizophrenia (Figure 1). Some of these genes are associated with schizophrenia based on previous studies. For example, *HLA-B* is a risk factor for CIAG (clozapine-induced agranulocytosis (CIA)/clozapine-induced granulocytopenia (CIG)), which is a life-threatening event for individuals with schizophrenia [[18](#_ENREF_18)]. Mutations in *HLA-B* in the prefrontal lobe may affect central DA signals and indirectly influence the development of schizophrenia [[19](#_ENREF_19)]. Dysfunction of the brain glutamate system may be involved in the pathophysiology of schizophrenia, and *MYO5B* is involved in glutamate receptor 1 (GluR1) recycling, suggesting that it is linked to schizophrenia [[20](#_ENREF_20)]. The close relationship between C1orf54 and schizophrenia has been validated by a GWAS [[21](#_ENREF_21)]. SET domain containing 7 (*SETD7*) is a methyltransferase that can methylate non-histone substrates, such as forkhead box O3 (*FOXO3*). *SETD7* was downregulated in patients with schizophrenia in our study, consistent with previous results showing that *FOXO3* transcriptional activity is reduced by methylation. Thus, we can infer that *SETD7* may be crucial for schizophrenia via the methylation of FOXO3, which promotes neuronal apoptosis [[22](#_ENREF_22)]. A previous study has shown that *DGKD* is vital for the development and function of the central nervous system, and a lack of *DGKD* can result in schizophrenia, consistent with our results showing that *DGKD* expression is low in patients, further supporting the role of *DGKD* in schizophrenia [[23](#_ENREF_23)]. Potassium voltage-gated channel subfamily A member 2 (*KCNA2*) had low expression in patients with schizophrenia in our study, similar to the results of a previous study showing that mutations in the voltage-gated K+ channel protein may lead to the loss of K+ channels, causing neurotransmitter release disorders and nerve meta-excitability changes, while its overexpression could rescue coordinated motor control [[24](#_ENREF_24)]. Therefore, the DEGs identified in our analysis are specifically related to schizophrenia.

**Functional enrichment analysis of DEGs**

To further explore the functions of the DEGs, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses for functional classification using DAVID (https://david.ncifcrf.gov/). The GO analysis showed that 59 DEGs were enriched for 15 GO terms, including terms in the three classes, biological processes, cellular components, and molecular functions. In particular, 17 genes were enriched for biological processes, including protein homooligomerization, K+ transport ion transmembrane transport regulation, axon guidance, regulation of protein kinase activity, and retinal homeostasis. Twenty-nine genes were enriched for cellular components, including cell junctions, cell surface, extracellular exosomes, and Golgi. Thirteen genes were enriched for molecular functions, including voltage-gated potassium channel activity, polysaccharide binding, RNA polymerase II sequence-specific DNA binding, delayed rectifier potassium channel activity, and potassium channel activity (Figure 2A, B, and C). Previous studies have confirmed that these processes are connected to schizophrenia, including Kv3 channels in which K+ promotes excitability and neurotransmitter release [[25](#_ENREF_25)], axon guidance [[26](#_ENREF_26)], regulation of protein kinase activity [[27](#_ENREF_27)], retinal homeostasis [[28](#_ENREF_28)], cell surface antibodies [[29](#_ENREF_29)], and voltage-gated potassium channel activity [[30](#_ENREF_30)]. Similar results were obtained by a KEGG analysis. Ten DEGs were components of five important pathways: graft-versus-host disease, allograft rejection, aldosterone-mediated sodium reabsorption, nicotine addiction, and type I diabetes (Figure 2D). Potassium voltage-gated channel subfamily D member 3 (*KCND3*) is a voltage-gated potassium channel protein that affects axon guidance. *KCND3* encodes a voltage-gated potassium channel-Kv4.3, which mediates the rapid activation and inactivation of membrane depolarization and is vital for transient A-type potassium currents in the brain [[31](#_ENREF_31)]. Therefore, *KCND3* is likely to alter neuronal excitability, resulting in cell death or loss of function and thereby promoting the development of schizophrenia. Additionally, glutamate ionotropic receptor NMDA type subunit 2B (GRIN2B), a subunit of the NMDA receptor glutamate-gated ion channel, is essential for synaptic plasticity. Thus, a deficiency will damage cognitive function and hinder the formation of higher-order cognitive abilities. These cognitive disorders usually occur in patients with schizophrenia [[32](#_ENREF_32)]. Based on these findings, the functions of these genes are related to the promotion of schizophrenia.

**ceRNA regulatory network analysis of miRNAs that regulate DEGs**

RNA-seq data for patients with schizophrenia and normal controls [[17](#_ENREF_17)] were used to construct ceRNA networks, including miRNA–mRNA and miRNA–lncRNA interactions (Figure 3). As shown in Figure 3, miRNAs, the key nodes in the network, regulate the expression of the DEGs and related lncRNAs. This result indicates that the abnormal expression of these DEGs in schizophrenia is regulated by miRNA. Additionally, miRNAs are crucial factors in the pathogenesis and development of schizophrenia [[33](#_ENREF_33)]. Furthermore, a previous study demonstrated that miRNAs interact with related genes in neural development and other aspects, and miRNA alterations are important factors in the complex pathophysiology of schizophrenia [[34](#_ENREF_34)]. As shown in Figure 3, hsa-miR-619-5p (light blue nodes) regulates many lncRNAs and mRNAs. tRNA-yW synthesizing protein 5 (*TYW5*) is a schizophrenia-related risk gene and is a target gene of hsa-mir-619-5p, which is regulated by hsa-mir-619-5p [[35](#_ENREF_35), [36](#_ENREF_36)]. In addition, RASD family member 2 (*RASD2*; dark blue nodes in Figure 3) is regulated by a variety of miRNAs, including has-miRNA-4763-3p, has-miRNA-4695-5p, and has-miRNA-6769b-5p, indicating that *RASD2* is regulated by multiple miRNAs and may be a key target gene for schizophrenia. *RASD2* gene expression is decreased in the postmortem prefrontal lobe of patients with schizophrenia [[37](#_ENREF_37)]. *RASD2* can regulate dopaminergic neurotransmission [[19](#_ENREF_19)]. It can also regulate the signal cascades of AKT and mTOR [[38](#_ENREF_38), [39](#_ENREF_39)], which are related to mental disorders [[40](#_ENREF_40)]. In conclusion, miRNAs are key regulatory factors with roles in schizophrenia related to the regulation of DEGs, such as *RASD2*.

**Validation of interactions between miRNAs and DEGs**

As shown in Figure 3, we detected 43 interactions between miRNAs and mRNAs in the ceRNA network. To further demonstrate the interactions between miRNAs and target genes, we constructed images using RNAhybrid and BLASTN (Figure 4a,b). Based on the minimum free energy (MFE) values (Figure 4a), there were clear interactions between miRNAs and target genes. Some single target genes were regulated by multiple miRNAs, as shown in Figure 4a. CLEC18B was regulated by several miRNAs, including hsa-miR-328-3p, hsa-miR-5006-5p, hsa-miR-6804-3p, and hsa-miR-7113-3p. Among these miRNAs, has-miR-328-3p is involved in the regulation of Glutamate metabotropic receptor 4 (GRM4), which is coupled to Go/i proteins and expressed in brain circuits important in schizophrenia [[41](#_ENREF_41), [42](#_ENREF_42)]. In addition, hsa-miR-6845-3p interacted with *SPDYE2* (Figure 4a); decreased *SPDYE2* expression promotes axonal outgrowth [[43](#_ENREF_43)]. Genes enriched in the axon guidance pathway are associated with the risk of schizophrenia [[44](#_ENREF_44)]; therefore, we predicted that has-miR-6845-3p may participate in the regulation of schizophrenia. Overall, accumulating evidence suggests that these miRNAs play a crucial regulatory role in schizophrenia. To further describe how miRNAs interact with target genes, hybridization between *RASD2* and miRNAs was evaluated using RNAhybrid (Figure 4b), establishing that *RASD2* can be regulated by miRNAs. To verify the interactions between miRNAs and target genes, the RPKM method was used (Figure 4c). Based on the degree of association with miRNAs (Figure 4a), six highly correlated target genes were chosen, including *RASD2, CLEC18B, CLEC18C, NCR3LG1, SPDYE2,* and *SPDYE2B*. Compared with levels in the healthy control group, the RPKM values in the schizophrenia group were significantly altered for all six target genes. Moreover, a GO analysis showed that *RASD2* was enriched for synaptic transmission, dopaminergic, and small GTPase-mediated signal transduction, indicating that *RASD2* expression changes were induced by miRNAs. Therefore, the 43 miRNAs regulate corresponding mRNAs to function in schizophrenia.

**ceRNA network analysis of schizophrenia-related genes and miRNAs**

To further investigate the roles of miRNAs in schizophrenia, we collected data for schizophrenia-related genes from the UniProt database for comparisons with the DEGs we obtained from patients with schizophrenia (Figure 5a). These schizophrenia-related genes did not overlap with DEGs. Therefore, to further investigate the regulation of miRNAs, we constructed a ceRNA network of miRNAs and schizophrenia-related genes. Based on this analysis, the expression of schizophrenia-related genes was regulated by multiple miRNAs. Interestingly, it is worth noting that some miRNAs can regulate both DEGs identified in our patients and the expression of schizophrenia-related genes, further demonstrating that miRNAs have key regulatory roles in schizophrenia. As shown in Figure 3, has-miRNA-4763-3p regulates the expression of *RASD2*. As shown in Figure 5b, has-miRNA-4763-3p regulates the expression of the schizophrenia-related gene Catechol-*O*-methyltransferase (*COMT*). *COMT* is widely expressed throughout the brain and strongly regulates dopamine (DA) levels in the prefrontal cortex. DA is an important factor affecting cognitive function in schizophrenia [[45](#_ENREF_45)]. Similarly, hsa-miR-619-5p regulates the expression of the DEG *NCR3LG1* and the schizophrenia-related gene *MTHFR* (Figure 3 and Figure 5b). Therefore, the construction of the ceRNA network of miRNAs and schizophrenia-related genes further confirmed the key regulatory roles of miRNAs in schizophrenia.

**PET scans of the pathological brain area**

To detect the key brain regions of schizophrenia development, PET technology was used to specifically detect 5-HT receptors [[46](#_ENREF_46)] in patients with schizophrenia by injecting 18F-MPPF, a highly selective 5-HT antagonist [[47](#_ENREF_47)]. PET data were collected from 44 samples (including 18 patients with schizophrenia and 26 healthy controls) from Huashan Hospital in Shanghai, China, and analyzed using MATLAB R2016a. By comparing data from patients with schizophrenia and healthy individuals, we obtained the key brain regions (Figure 6). As shown in Figure 6, enhanced brain regions (marked in red) mainly included the frontal lobe, temporal lobe, and sub-lobar region. Reduced brain regions (blue) mainly included the occipital lobe, posterior lobe, and left temporal lobe. Among these, the prefrontal lobe is a key brain region in schizophrenia [[48](#_ENREF_48)]. Interestingly, among the DEGs identified in our study, *RASD2* levels are reduced in the prefrontal lobe of patients with schizophrenia, causing cognitive impairment [[19](#_ENREF_19)]. Therefore, we speculated that a decrease in *RASD2* in the prefrontal lobe affects the expression of neurotransmitter receptors and neurotransmitter release disorders, which may cause schizophrenia. In conclusion, changes in DEGs in corresponding brain regions may cause schizophrenia by regulating neural signaling.

**Validation of DEGs in mice with schizophrenia**

To further verify the role of DEGs, we constructed a mouse model of schizophrenia by intraperitoneal injection of the NMDA receptor antagonist MK-801 [[16](#_ENREF_16)]. After injection with MK-801, mice showed intense schizophrenia behavior, a decrease in normal conscious behavior, and an increase in abnormal conscious behavior, such as walking instability, violent jumping, and constant turning (data not shown) [[16](#_ENREF_16)], revealing that MK-801 successfully induced schizophrenia. Studies have shown that dopamine receptors play an important role in mediating dopamine function and may be related to the occurrence of schizophrenia [[49](#_ENREF_49)]. After model construction, we injected 18F-DTBZ into the mice by the tail vein and detected dopamine receptor expression by PET. We detected reduced dopamine receptor expression (Figure 7a), further confirming the occurrence of schizophrenia in mice. The occurrence of schizophrenia is related to a reduction in dopamine receptor levels [[49](#_ENREF_49)]. To verify the DEGs (Figure 1), the expression of *RASD2, GRIN2B, KCTD12, FOXO3*, and *SETD7* were evaluated in schizophrenic and normal mice by qPCR (Figure 7b). The DEGs, such as *RASD2,* were abnormally expressed in schizophrenic mice and showed the same trends in expression detected in the DEG analysis. These results indicate that the differential expression patterns of *RASD2* and other genes in mice and humans are the same, indicating that the functions of these genes in schizophrenia are conserved among humans and mice. Therefore, the abnormal expression of the DEGs identified in our study, such as *RASD2,* provides a feasible theoretical basis for revealing the molecular mechanism of schizophrenia.

**Discussion**

We identified 62 DEGs between patients with schizophrenia and healthy controls (Figure 1). These genes were mainly enriched for functions in the regulation of ion transmembrane transport, axon guidance, cell surface (Figure 2), indicating roles in ion transport and normal neuronal growth. Furthermore, a ceRNA network analysis showed that miRNAs regulate DEGs and schizophrenia-related genes (Figures 3–5), demonstrating the key regulatory role of miRNAs in schizophrenia. Furthermore, PET confirmed that the brain regions that were altered in patients with schizophrenia include the frontal lobe, temporal lobe, and occipital lobe (Figure 6) and some DEGs were altered in these brain regions. Finally, qPCR further confirmed the expression of DEGs in mice with schizophrenia (Figure 7), and these results were consistent with those for human RNA-seq data.

Intriguingly, the expression patterns of some DEGs identified in schizophrenia patients were consistent with the results of previous studies. Moreover, previous studies have shown that some of the DEGs identified in our analysis are associated with schizophrenia, such as *FOXO3* [[22](#_ENREF_22)] and *DGKD* [[23](#_ENREF_23)]. We also detected some novel genes that have not previously been linked to the formation of schizophrenia. In addition, a functional enrichment analysis indicated that these genes function in various processes, including axon guidance and protein kinase activation, which have been linked to schizophrenia, suggesting that the DEGs may directly or indirectly affect schizophrenia (Figure 2). Our data revealed novel candidate genes linked to schizophrenia and provided theoretical evidence for future studies of the mechanisms underlying the development of schizophrenia.

To further explore the regulatory relationships involving these DEGs, we constructed a ceRNA network (Figure 3). Based on this network, we found that the DEGs are regulated by various miRNAs, including miRNAs associated with schizophrenia, such as has-miR-328-3p and hsa-miR-6845-3p [[42](#_ENREF_42), [50](#_ENREF_50)]. *RASD2* (Figure 3, dark blue nodes) is a typical example; it was regulated by different miRNAs with downregulated expression, consistent with previous results showing that *RASD2* expression is slightly reduced in the postmortem prefrontal lobe of patients with schizophrenia[[19](#_ENREF_19)]. Thus, these miRNAs are candidate biomarkers. However, the comprehensive connections among mRNAs, miRNAs, and lncRNAs remain to be characterized and should be a focus of future research.

The ceRNA networks generated using schizophrenia-related genes from the UniProt database and DEGs obtained in this study did not overlap. There are several explanations for the differences, including (1) our inclusion of mRNAs only (and not miRNAs and lncRNAs) and (2) insufficient studies and, as a consequence, incomplete data for DEGs in the UniProt database. Furthermore, some DEGs were newly discovered schizophrenia-related genes, providing a theoretical basis for further studies of the molecular mechanisms underlying schizophrenia. As shown in Figure 5b and Figure 3, some miRNAs regulate the expression of schizophrenia-related genes, suggesting that miRNAs have key regulatory roles in schizophrenia. Furthermore, as determined by PET, the frontal, temporal, and occipital lobes were key brain regions in schizophrenia. Interestingly, RASD2 is reportedly down-regulated in the prefrontal lobe of patients with schizophrenia [[19](#_ENREF_19)], consistent with our results (Figure 1). Additionally, hsa-miR-4763-3p regulates the expression of *RASD2* and *COMT* (Figure 3 and Figure 5b). Therefore, the regulatory network of miRNAs and target genes provides key insights into the molecular mechanism underlying schizophrenia. However, the specific mechanisms by which RASD2 contributes to the development of schizophrenia require further research. Finally, we performed qPCR experiments using a mouse model of schizophrenia and observed the abnormal expression of DEGs, such as *RASD2* (Figure 7b), identified in the human RNA-seq analyses. Accordingly, these DEGs are conserved and the mechanism underlying schizophrenia is similar in humans and mice, suggesting that the mouse model is effective for related research.

In this study, a comprehensive analysis of the molecular mechanisms underlying schizophrenia was performed using bioinformatics methods. The large number of DEGs associated with schizophrenia identified in this study provide a basis for further functional studies. In addition, we found that miRNAs regulate the expression of these DEGs, providing a potential molecular pathway for schizophrenia. PET results showed key brain areas where these DEGs may function. Finally, the consistent results obtained using a mouse model of schizophrenia suggest that this is an effective model for future research. In conclusion, our data provide a theoretical basis for further studies of the molecular mechanism underlying schizophrenia.

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**Conflicts of interest**

There are no conflicts of interest to declare.

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**Figure legends**

Figure 1. Heatmap comparing gene expression differences between patients with schizophrenia (n = 28) and healthy controls (n = 28). Red represents up-regulation and blue represents down-regulation. Only protein-coding genes are shown. LncRNAs are not shown. RNA-seq data were downloaded from GEO (https://www.ncbi.nlm.nih.gov/geo/; accession number GSE121376). Thresholds for DEG identification: FC ≥ 1.3 || ≤ 0.77 and p-value ≤ 0.05, schizophrenia/control.

Figure 2. GO and KEGG functional enrichment analyses of DEGs (protein-coding genes). GO functional enrichment analyses were performed for the three classes: (a) biological processes (BP), (b) cellular components (CC), and (c) molecular functions (MF). (d) Summary of KEGG functional enrichment analysis of DEGs.

Figure 3. ceRNA network of DEGs (miRNA–mRNA and miRNA–lncRNA). Regulatory relationships between miRNA–mRNA and miRNA–lncRNA were determined using RNAhybrid and BLASTN, taking the intersection (light blue nodes, has-miRNA). Red nodes represent mRNA up-regulation and dark blue nodes represent mRNA down-regulation. Orange nodes represent lncRNA up-regulated lncRNAs and green nodes represent down-regulated lncRNAs. Gray lines represent interactions between two nodes.

Figure 4. Relationship between miRNAs and mRNAs verified by RNAhybrid and BLASTN. (a) miRNA–mRNA interactions predicted by RNAhybrid and BLASTN, showing values for protein-coding genes regulated by at least four different miRNAs. (b) miRNA–mRNA interactions predicted by RNAhybrid for RASD2. Red (2–7) and green are labeled seed sequences on miRNAs (5–10). (c) RPKM values for six genes in the schizophrenia group and the healthy control group.

Figure 5. ceRNA network of schizophrenia-related genes. (a) Veen diagram of schizophrenia-related genes (left) and DEGs (right). The intersection is empty. schizophrenia-related genes were retrieved from the UniProt database and schizophrenia-related diseases. (b) ceRNA network of schizophrenia-related genes. Purple nodes represent hsa-miRNAs; red nodes represent schizophrenia-related genes; green nodes represent lncRNAs. Gray lines represent interactions between two nodes.

Figure 6. PET image of the brain of a patient with schizophrenia. Red represents enhanced brain areas and blue represents reduced brain areas.

Figure 7. PET images of the mouse brain and qPCR verification. (a) 18F-DTBZ PET images of mice brain; green represents dopamine receptors. (b) qPCR verification of *RASD2, GRIN2B, KCTD12, FOXO3*, and *SETD7*.