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MiR-574-5P, miR-1827, and miR-4429 as Potential Biomarkers for Schizophrenia

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Abstract

Schizophrenia is a severe chronic debilitating disorder with millions of affected individuals. Diagnosis is based on clinical presentations, which are made when the progressive disease has appeared. Early diagnosis may help improve the clinical outcomes and response to treatments. Lack of a reliable molecular diagnostic invokes the identification of novel biomarkers. To elucidate the molecular basis of the disease, in this study we used two mRNA expression arrays, including GSE93987 and GSE38485, and one miRNA array, GSE54914, and meta-analysis was conducted for evaluation of mRNA expression arrays via metaDE package. Using WGCNA package, we performed network analysis for both mRNA expression arrays separately. Then, we constructed protein–protein interaction network for significant modules. Limma package was employed to analyze the miRNA array for identification of dysregulated miRNAs (DEMs). Using genes of significant modules and DEMs, a mRNA-miRNA network was constructed and hub genes and miRNAs were identified. To confirm the dysregulated genes, expression values were evaluated through available datasets including GSE62333, GSE93987, and GSE38485. The ability of the detected hub miRNAs to discriminate schizophrenia from healthy controls was evaluated by assessing the receiver-operating curve. Finally, the expression levels of genes and miRNAs were evaluated in 40 schizophrenia patients compared with healthy controls via Real-Time PCR. The results confirmed dysregulation of hsa-miR-574-5P, hsa-miR-1827, hsa-miR-4429, CREBRF, ARPP19, TGFBR2, and YWHAZ in blood samples of schizophrenia patients. In conclusion, three miRNAs including hsa-miR-574-5P, hsa-miR-1827, and hsa-miR-4429 are suggested as potential biomarkers for diagnosis of schizophrenia.

Keywords Schizophrenia · metaDE · WGCNA · microRNA · Real-Time PCR

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Introduction

Schizophrenia (SCZ) is a chronic debilitating mental disease with a complex identity considered as one of the most mysterious human disorders (van Os and Kapur 2009a). It mainly appears during adulthood primary years affecting both genders, any race, and genetic backgrounds. According to the global estimations, about 20 million people are living with SCZ around the world (James et al. 2018). The affected patients are expected to have a reduced lifespan of 12–15 years compared to healthy individuals which show higher mortality compared to physical diseases (van Os and Kapur 2009b). SCZ diagnosis is based on psychopathological features including psychosis, hallucination or delusion, and impaired emotions and volition in absence of any medical disorder (Taylor et al. 1974). Thus, disease diagnosis is made when the clinical manifestations have appeared,

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and thus, any diagnostic approach to screen the individuals at risk suggests special importance. The impairment of neurotransmitters like dopamine and gamma aminobutyric acid (GABA) has been the center of the main therapeutical strategies in SCZ; however, the results of current treatments are not satisfying indicating requirement for new concepts. Although the etiology of SCZ is still unknown likely to be multifactorial including genetic and environmental risk factors (Richetto and Meyer 2021), we already have known that individuals who have a history of SCZ in their first-grade family members have a higher risk of developing the disorder indicating high degrees of heritability (Lichtenstein et al. 2009). Furthermore, accumulating evidence suggests familial predisposition as the main risk factor for the disease in addition to other provoking conditions like pregnancy and simultaneous infections, impairments in neurological development, and cannabis abuse (Mäki et al. 2005). Different epigenetic mechanisms including DNA methylation, histone modifications, and regulation by non-coding RNAs (ncRNAs) also have been found to be involved in the SCZ pathogeny considered as molecular scars of environmental exposures (Richetto and Meyer 2021; Kuehner et al. 2019). They are particularly known to play role in neural development, and transcriptome studies show methylation changes and dysregulation of several epigenetic factors such as histone deacetylases (HDACs) and microRNAs (miRNAs) in brain samples of SCZ patients (Richetto and Meyer 2021; Sharma et al. 2008; Du et al. 2019). Additionally, recently a brilliant study has showed that transplantation of exosomes from SCZ patients mimics SCZ behaviors in mice (Du et al. 2021). Recent developments have suggested further consideration of epigenetic regulation in pathogeny and diagnostic and therapeutical potentials for SCZ in the future (Richetto and Meyer 2021).

MiRNAs are a novel group of ncRNAs with an average length of 22 nucleotides not encoding any protein unlike the known class of protein-coding messenger RNAs (mRNAs). Their functions have not been yet completely elucidated; however, numerous key regulatory roles in the eukaryotic gene expression have been described for an increasing number of identified miRNAs. In a variety of biological processes such as stress responses (Leung and Sharp 2010), cell proliferation, differentiation, and death (Bushati and Cohen 2007), embryogenesis, organ development, and function (Kloosterman and Plasterk 2006; Maes et al. 2008), miRNAs have been identified with regulatory functions in multicellular organisms. They mainly act at the post-transcriptional level in interaction with protein complexes via binding to complementary regions mostly located on the 3′ untranslated region (UTR) of the target sponged mRNA and thus, drive their degradation by RNA-induced silencing complex (RISC) leading to repressing the expression of critical proteins which play role in essential cellular processes. As expected, biogenesis, processing, nucleolar export, and stability of miRNAs are faced with precise regulation (Treiber et al. 2012) which also suggests their substantial roles. Constantly growing evidence has demonstrated the association of aberrant expression of miRNAs and numerous human diseases such as diabetes (Tang et al. 2008), osteoporosis (Van Wijnen et al. 2013), neurodegenerative diseases (Nelson et al. 2008), viral infections (Islam et al. 2019), and various types of cancer (Seven et al. 2014). Several miRNAs also have been shown to be aberrantly expressed in serum samples (Shi et al. 2012), cortex tissues (Perkins et al. 2007), and gene analyses of SCZ patients (Hauberg et al. 2016). As a result, differentially expressed miRNAs potentially can be considered as SCZ biomarkers (He et al. 2017). This approach can be beneficial particularly since the miRNAs are easily detected in blood samples of the patients and eventually can be employed in early diagnosis.

Through the employment of bioinformatics tools like Weighted Gene Co-Expression Network Analysis (WGCNA) in several studies, interactions between risk co-expressed genes have been identified in patients with SCZ (Torkamani et al. 2010; Kim et al. 2018) in addition to other mental diseases and healthy condition (Oldham et al. 2008). For instance, Wen et al. (Wen et al. 2020) identified 134 SCZ-specific key genes using WGCNA and Radulescu et al. (Radulescu et al. 2020) found 12 gene co-expression network modules in postmortem brain samples of SCZ patients. A number of other modules have been discovered using the construction of WGCNA in other studies (Liu et al. 2020; Zhang et al. 2020; Feltrin et al. 2019). To date, a number of susceptibility genes and characteristic genetic changes have been identified in association with increased risk of SCZ development. The affected genes have been found to mainly play role in the natural neurodevelopment which is believed to be affected in SCZ and other psychiatric disorders. These genes include immune function-related gene loci on chromosome 6p22.1 (Purcell et al. 2009), chromosome 22q11.2 deletion (Bassett et al. 2002), alleles of 5-hydroxytryptamine (5-HT) receptor gene, and dopamine D3 receptor gene (O'Donovan and Owen 1999). Studies have shown that thousands of single-nucleotide polymorphisms (SNPs) and copy number variants (CNVs) can explain parts of the genetic susceptibility to SCZ (Maric and Svrakic 2012). Currently known genetic mechanisms, however, have not been convincing in the independent elucidation of the SCZ pathophysiology (Pries et al. 2017). Same with many other diseases, epigenetic regulations along with genetic alterations including methylation, acetylation, phosphorylation, and SUMOylation on DNA bases or histone residues have been found to be involved in molecular mechanisms associated with SCZ.

To our knowledge based on the literature, protein networks and protein-miRNA networks have not been yet

identified in SCZ. In this study, we aimed to identify aberrantly expressed genes and miRNAs and associated cellular mechanisms among Iranian SCZ patients using gene co-expression network analyses. Retrieved hub miRNAs and genes were confirmed using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and their diagnostic values were assessed through receiver operating characteristic (ROC) curve. The retrieved candidate miRNAs and genes suggested as potential biomarkers for SCZ patients.

Materials and Methods

Data Collection and Processing

Three expression arrays including Gene Expression Omnibus (GEO) series numbers GSE93987, GSE38485, and GSE54914 were obtained from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). According to the datasets, in GSE93987 individual pyramidal cells in dorsolateral prefrontal cortex layers 3 or 5 were captured by laser microdissection from 36 subjects diagnosed with SCZ or schizoaffective disorder and matched healthy controls. In GSE38485, the gene co-expression network in whole blood samples retrieved from 106 SCZ patients was compared to those from 96 healthy controls. In GSE54914 through a processing method, the probes were converted to the corresponding genes using their platforms, then the raw data were normalized by the quantile normalization function in the Limma package (Smyth 2005).

Meta-analysis

Using the metaDE package (James et al. 2018), meta-analysis was performed for the two mentioned mRNA expression datasets, and differentially expressed genes (DEGs) between SCZ and healthy samples were identified. A false discovery rate (FDR) < 0.05 was selected as the significance threshold for screening of the DEGs.

Weighted Gene Co-expression Network Construction and Module Detection

After identification of DEGs, the corresponding gene co-expression networks were constructed using the WGCNA package (Langfelder and Horvath 2008). For network construction, first a similarity matrix was made by calculating the correlations of all gene pairs. By applying the pickSoftThreshold function, the appropriate soft-thresholding power B was selected to assess the scale-free topology. Afterward, to reduce the noise and spurious association impact, adjacency was transformed to the topological overlap matrix and the corresponding dissimilarity was calculated. Hierarchical clustering was utilized to produce a dendrogram of genes. The modules with significant association with the measured clinical traits were selected according to two parameters including correlation and *p*-value in the next step. Then, we quantified the association of individual genes with our trait of interest (status) by defining Gene Significance (GS) as the absolute value of the correlation between the gene and the corresponding trait. For each module, we also defined a quantitative measure of module membership (MM) as the correlation between the module eigengene and the gene expression profile. This allows us to quantify the similarity of all array genes to each module. In the final step of network analysis, using the GS and MM measures we identified the genes with high significance for the status as well as high MM in the selected modules.

Protein–Protein Interaction (PPI) Network Analysis and Pathway Enrichment Analysis

To identify the protein–protein interaction (PPI) information, the proteins of the selected modules were matched to the search tool for the retrieval of interacting genes (STRING) database (https://string-db.org/cgi/input.pl). The retrieved data was imported to the Cytoscape software (Shannon et al. 2003), and the corresponding PPI was visualized based on the degree and betweenness centrality. To elucidate the potential dysregulated signaling pathways, the ClueGO was utilized, and accordingly, the most related signaling pathways were identified (Bindea et al. 2009).

Identification of Differentially Expressed miRNAs (DEMs)

By searching the GEO database, GSE54914 miRNA array was used for the identification of differentially expressed miRNAs (DEMs) between the SCZ patients and healthy controls. Significant miRNAs were identified based on *p*-value threshold of < 0.05 and logFC > 1.

MiRNA-Protein Interaction Network Construction and Identification of Hub miRNAs

DEMs and the most important genes retrieved from PPI were used for the construction of miRNA-protein interaction. For this purpose, the CyTargetLinker plugin (Kutmon et al. 2013) based on the Cytoscape software was employed, and a regulatory network between the retrieved miRNAs and genes was made using the miRBase (Griffiths-Jones et al. 2007), TargetScan (Agarwal et al. 2015), and TransmiR (Wang et al. 2010) databases. By consideration of the miRNA-protein network, three miRNAs and three genes were identified for further analyses.

Validation of Detected miRNAs by the ROC Curve

The receiver operating characteristic (ROC) curve was plotted to confirm the diagnostic performance of the core miRNAs based on the GSE54914, and accordingly, the area under the curve (AUC) was estimated. The miRNAs with the p-value < 0.05 and AUC > 0.8 were considered as the strong potential diagnostic biomarkers.

Validation of the Detected Genes and miRNAs Based on the Clinical Samples

To further confirmation of the results of bioinformatics data, qRT-PCR was conducted to evaluate the expression level of the selected genes and miRNAs in blood samples of the SCZ patients ($n = 40$) recruited to Imam Khomeini, Mohammad Kermanshahi, and Farabi Hospitals, Kermanshah University of Medical Sciences, Kermanshah, Iran. Written informed consent was obtained from all patients. The demographic and clinical characteristics of the patients were provided in Supplementary Table 1.

RNA Extraction and cDNA Production

Whole blood RNA purification kit (Qiagen Cat. No. 52304) was used for RNA extraction. Total RNA was extracted from 2-ml whole blood collected from each subject. Using reverse transcriptase enzyme, complementary DNA (cDNA) was synthesized, and then treated with DNase I enzyme to remove the genomic DNA. The purity of the extracted RNA was measured by calculation of the ratio A_{260}/A_{280} using UV spectrophotometry.

Primer Design

The online miRNA designing tool (http://genomics.dote.hu:8080/mirnadesigntool/) was employed for the primer designing. To analyze the expression of the selected genes, primer design was performed using the GenScript online tool (https://www.genscript.com/tools/real-time-pcr-tagman-primer-design-tool)

qRT-PCR

Primers arrived in the lyophilized form (Cinaclone, Iran). For preparation, sterile distilled water was added to each tube containing the lyophilized primer (based on the information provided for each primer), and the solution was placed in stoke at −20 °C. Applied Biosystems Real-Time PCR instrument and Takara SYBR green kit were used for the analysis of miRNA and mRNA expression alterations. We also set up for each gene at each time, a negative control to examine the presence of contamination in each reaction.

The expression fold change of genes examined in this study was evaluated using the Threshold Cycle (CT) method by the formulas below. 5 s rRNA and GAPDH were used as the reference genes for this study.

$$R = 2^{-(\Delta\Delta CT)}$$

$$\Delta\Delta CT = (CT_{target} - CT_{reference})_{healthy} - (CT_{target} - CT_{reference})_{patient}$$

Results

Meta-analysis

Two datasets with available SCZ mRNA expression data, including GSE93987 and GSE38485, and also 1 miRNA expression data, GSE54914, were downloaded from the GEO database. In the meta-analysis using the metaDE package, we identified 3840 DEGs, which were selected for further investigations (Fig. 1).

Construction of Weighted Gene Co-expression Network

The WGCNA package was used in each mRNA expression datasets separately to construct the co-expressed networks and identify the co-expression modules retrieved from the results of the meta-analysis. Clustering dendrogram of the samples for the DEGs retrieved from each dataset was conducted based on their Euclidean distance (Figs. 2a and 3a). We then quantified the association between the individual genes and the trait of interest (SCZ and healthy) by defining the GS and MM values (Figs. 2b and 3b). A total number of 43 modules were identified for the DEGs retrieved from the GSE38485, and only genes of the lightcyan module were selected for further investigation. Seven modules were also identified for the GSE93987 and the blue module was selected for further analysis (Fig. 3b). According to the results, it is obvious that the GS and MM measures are highly correlated, illustrating that the genes with significant association with a trait are also the central elements of the modules associated with the trait (Figs. 2c and 3c). Based on Fig. 2c, genes of the light cyan module show correlation and p-value of 0.63 and 3.5e −24, respectively, and according to Fig. 3c, genes of the blue module demonstrate the corresponding values of 0.32 and 4.6e − 10, respectively.

Protein–Protein Interaction Network and Pathway Enrichment Analysis

All genes of the favorite modules (light cyan and blue) were used for the network construction. For the light cyan module,

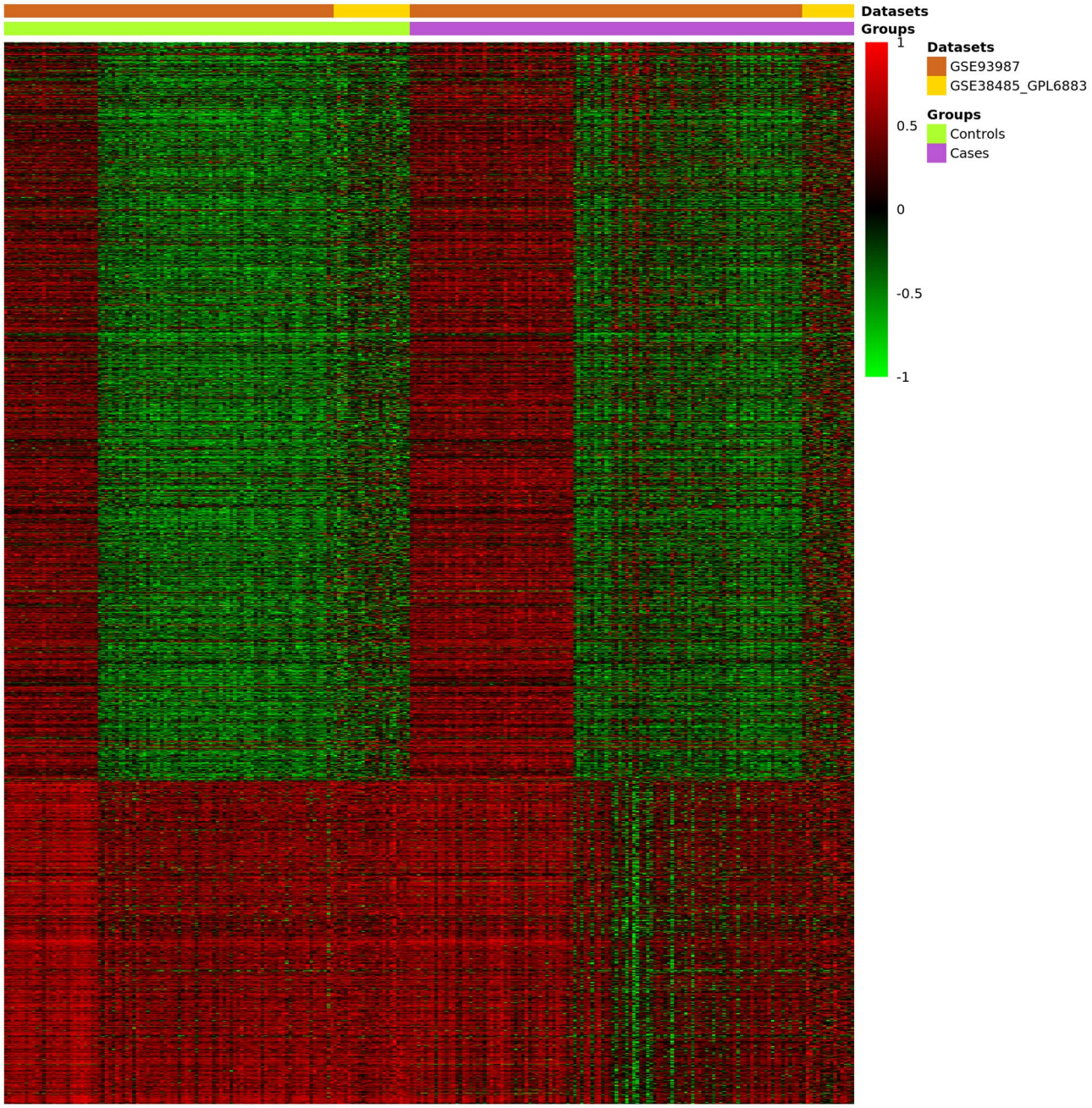


Fig. 1 Heatmap for DEGs between schizophrenia and healthy controls for two datasets including GSE93987 and GSE38485. Red colors represent upregulated genes and green colors represent downregulated genes

242 genes were selected, and using both STRING database and the Cytoscape software, the PPI network was made (Supplementary Fig. 1a). The color and size of each protein were set based on the degree and betweenness centrality. For the blue module, 362 proteins were selected, and according to the same protocol, the corresponding protein network was constructed (Supplementary Fig. 2a). To analyze the pathway enrichment, the CluGO plugin based on the Cytoscape software was used to illustrate the results of KEGG path analysis for each of the gene groups separately (Supplementary Figs. 1b and 2b). For the genes of light cyan module, some cellular processes and signaling pathways were identified to be dysregulated. These included vitamin B6 binding, fucosyltransferase activity, single-stranded DNA binding, polyubiquitin modification-dependent protein binding, DNA replication origin binding, and double-stranded RNA binding. Also, dysregulated signaling pathways for the blue module included the epithelial cell signaling in

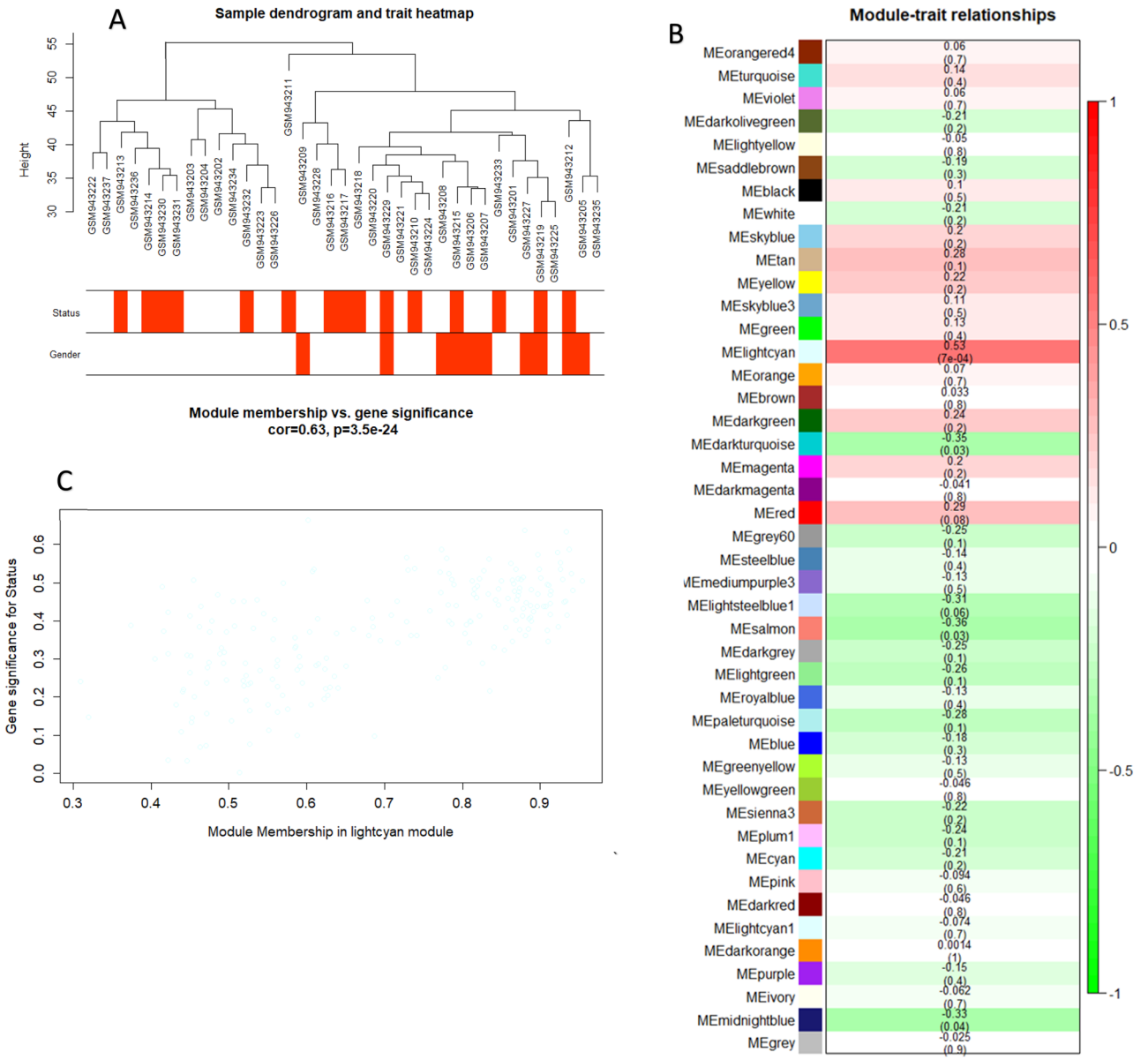


Fig. 2 Network construction for GSE38485 using WGCNA. (**A**) Clustering dendrogram of samples based on their Euclidean distance. (**B**) Module-trait associations. Each row corresponds to a module eigengene, column to a trait. Each cell contains the corresponding correlation and *p*-value. The table is color-coded by correlation according to the color legend. (**C**) A scatterplot of Gene Significance (GS) for weight vs. Module Membership (MM) for the light cyan module

Helicobacter pylori infection, vasopressin-regulated water reabsorption, dopaminergic synapse, proteasome, and *Salmonella* infection.

Identification of the Differentially Expressed miRNAs

In the GSE54914 dataset, the miRNA expression profiles of 18 SCZ patients and 12 healthy controls were compared. Raw data were normalized via log2 transformation and also quantile normalization function in the Limma package. The results demonstrated 191 upregulated and 2 downregulated miRNAs (Fig. 4).

MRNA-miRNA Network Construction

Based on two initial mRNA arrays, two miRNA-mRNA networks were made (Fig. 5A, B). According to the DEGs extracted from the GSE38485 and the DEMs identified by the GSE54914, a network was constructed (Fig. 6). As obvious, the hsa-miR-4429 shows direct effect on several DEGs including SORCS1, ZNF652, CHEK1, FAM63B,

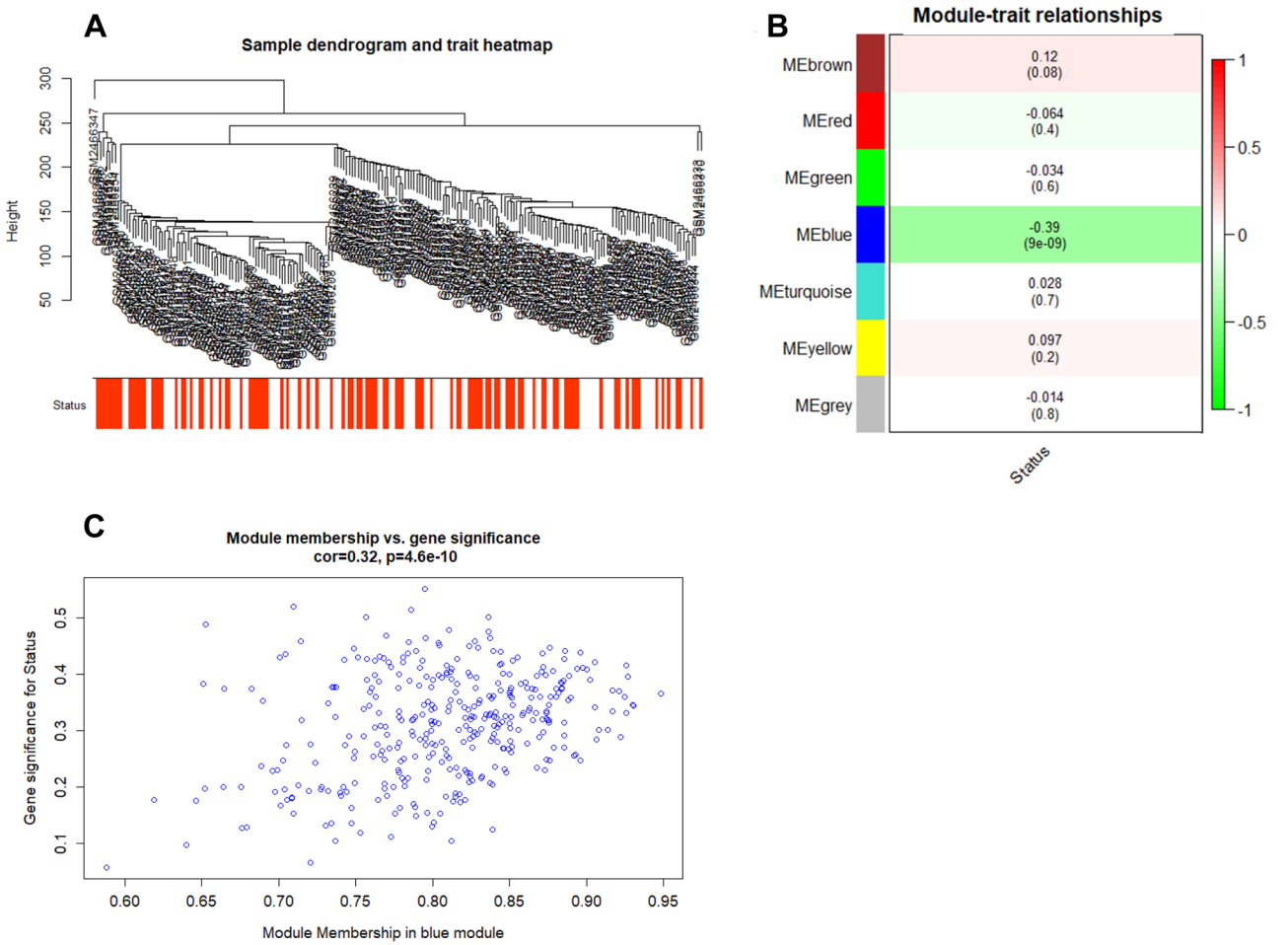


Fig. 3 Network construction for GSE93987 using WGCNA. (**A**) Clustering dendrogram of samples based on their Euclidean distance. (**B**) Module-trait associations. Each row corresponds to a module eigengene, column to a trait. Each cell contains the corresponding correlation and *p*-value. The table is color-coded by correlation according to the color legend. (**C**) A scatterplot of Gene Significance (GS) for weight vs. Module Membership (MM) in the blue module

PDCD4, and FOXM1. Also, hsa-miR-574-5P directly regulates the expression of several other DEGs including MCM8, SLC35E1, HS6ST3, and ZRANB. The third miRNA hsa-miR-1827 was shown to regulate the expressions of some DEGs like ACBD7, XPNPEP3, PPM1K, and CENPM.

Identification of Hub Genes

Using the DEGs retrieved from the GSE93987 and GSE38485 and DEMs identified by the GSE54914, two miRNA-mRNA networks were constructed (Fig. 5A, B). According to these networks, three miRNAs including hsa-miR-4429, hsa-miR-1827, and hsa-miR-574-5P play a central role. Therefore, we considered them as the hub miRNAs for further analyses. The common target genes of these miRNAs were identified using the Cytargetlinker plugin based on the Cytoscape software (Fig. 6A, B). Several genes like CREBRF, ARPP19, TGFBR2, YWHAZ, SRSF7, MAPK1IP1L, FAM117B, and BVES were demonstrated to be regulated by the hub miRNAs. We also evaluated the expression value of the detected genes in three independent datasets including the GSE62333, GSE93987, and GSE38485 (Fig. 6C).

Validation of the Selected miRNAs by the ROC Curve

The ROC curves were plotted for the raw data retrieved from the results of Real-Time PCR and GSE54914 using a single-gene test in SCZ versus normal tissues for three miRNAs by plotting the sensitivity versus specificity. The results demonstrated significant values for the hub miRNAs. These included for hsa-miR-4429 (AUC: 0.88 and *p*-value: 0.0001, AUC: 0.9 and *p*-value: 0.0001), hsa-miR-1827 (AUC: 0.83 and *p*-value: 0.0001, AUC: 0.84 and *p*-value: 0.0013), and hsa-miR-574-5P (AUC: 0.76 and *p*-value: 0.0001, AUC: 0.9

and *p*-value: 0.0002). The AUC was analyzed by the Hanley and McNeil method (Supplementary Fig. 3).

qRT-PCR

To evaluate the expression levels of the hub genes and miRNAs, qRT-PCR was carried out in 40 SCZ samples and matched healthy individuals (Fig. 7). Among the selected genes, CREBRF, TGFBR2, YWHAZ, and MAPK1IP1L were shown to be downregulated in the SCZ samples compared to the controls, while the hub miRNAs were shown to be upregulated.

Discussion

SCZ is one of the most debilitating mental disorders and a complex disease with an unknown etiology. Diagnosis is made based on the clinical manifestations on appearance, which makes the disease hardly manageable. Real-time diagnosis may help improve or slow down the progression of the consequences and response to treatment, which eventually promotes the patient's quality of life and social activities. Molecular diagnostics are potential nominates for on-time detection of susceptible or affected individuals. In accordance to the advancements in the past decades, numerous genetic studies have shown correlation between several genes and susceptibility to SCZ development in the people with familial history. Transcriptome studies also have demonstrated dysregulation of a growing number of miRNAs in tissues and plasma of SCZ patients. MiRNAs as a novel group of ncRNAs act as the key regulators of gene expression playing substantial roles in several critical biological processes. Accordingly, dysregulation in their biogenesis has been associated with various human diseases including SCZ, for which a number of miRNAs are identified with aberrant expression in patient tissues compared with healthy individuals. Importantly, miRNAs are easily detected in human body fluids suggesting them as potential biomarkers for a wide variety of human disorders particularly in conditions for those no diagnostic approaches have been developed or diagnosed via invasive methods. Since accessibility to the brain biopsies is not possible for screening the SCZ susceptible individuals, development of a diagnostic approach based on the differentially expressed miRNAs potentially can benefit this society.

In the current study, we identified 3 hub miRNAs including hsa-miR-574-5P, hsa-miR-1827, and hsa-miR-4429 to be aberrantly expressed in blood sample of SCZ patients via network construction. The results were confirmed by the qRT-PCR. These miRNAs demonstrated acceptable diagnostic values in the ROC curve. The biological significance of these mRNAs and miRNAs has not been fully elucidated; however, they have been found to be dysregulated in several human disorders. Hsa-miR-574-5P, also known

Fig. 5 mRNA-miRNA regulatory network between the DEMs and DEGs related to the (**A**) light cyan and (**B**) blue module genes, respectively. Every node represents a gene or a miRNA, and each edge demonstrates the interaction between genes and miRNAs

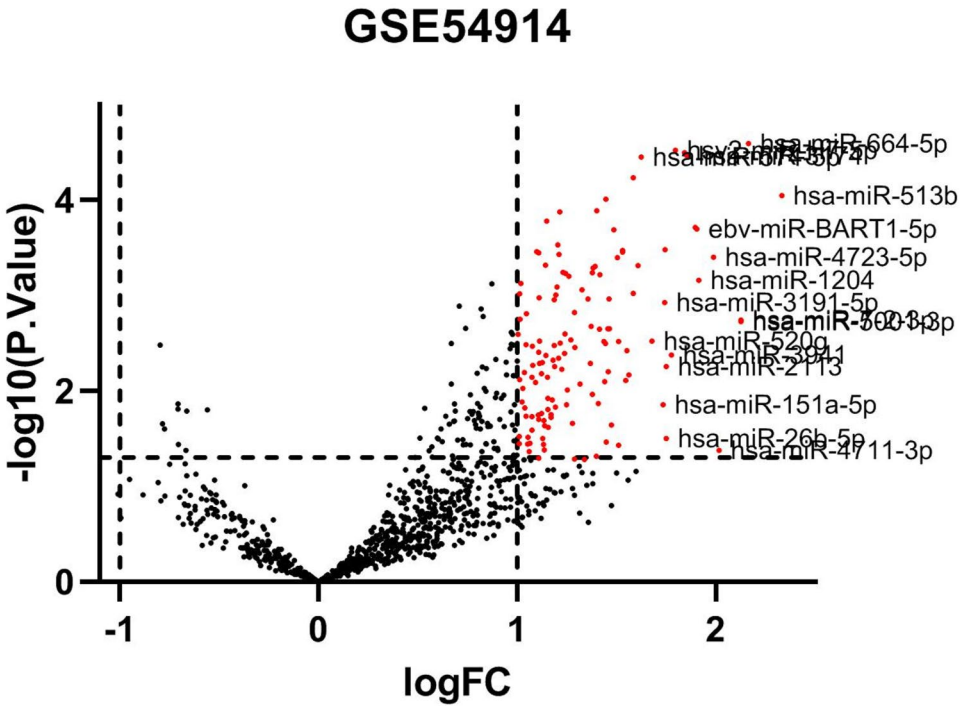
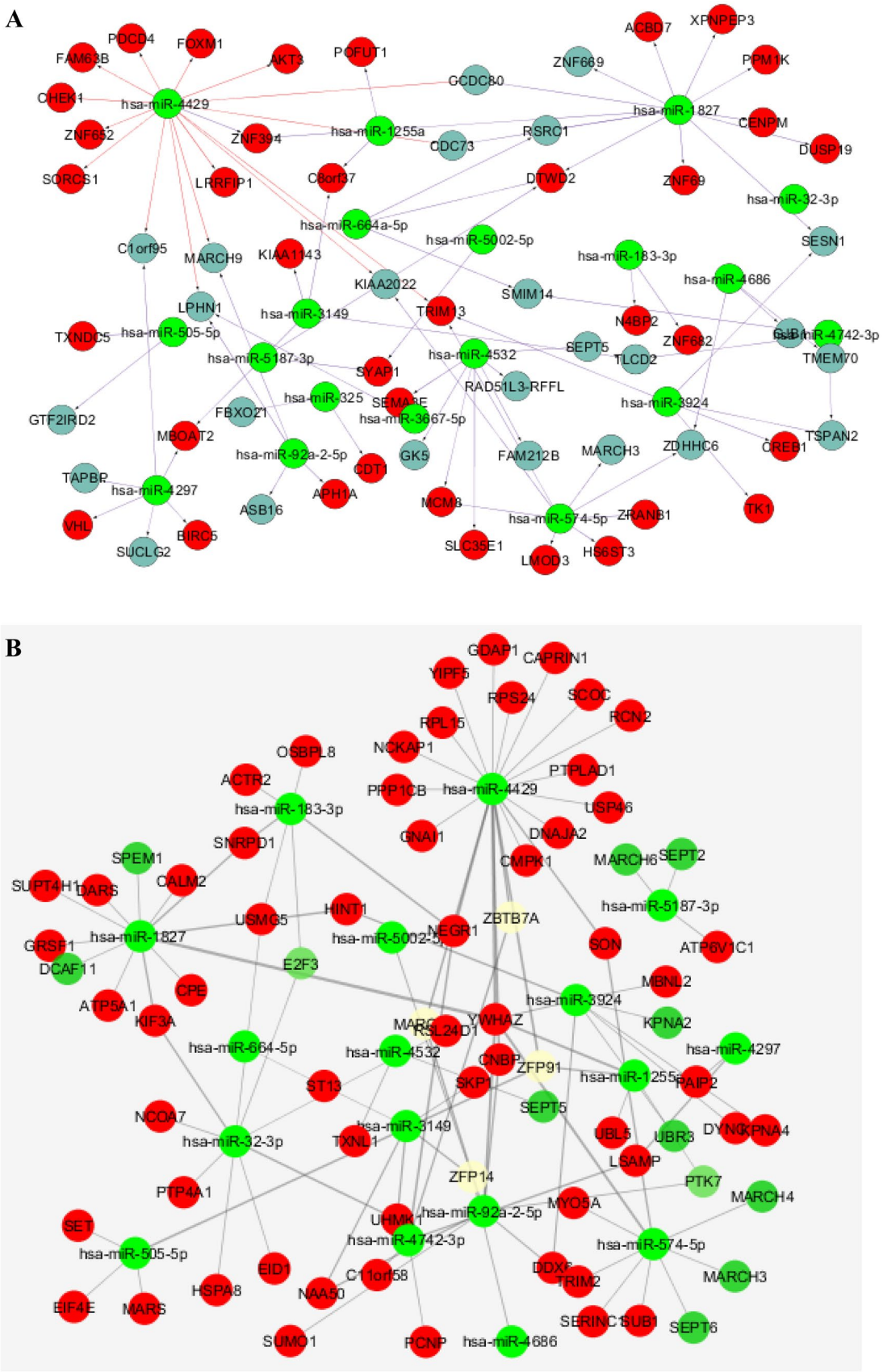


Fig. 4 Volcano plot display of differentially expressed miRNAs between schizophrenia and healthy controls. Every point in the plot represents a miRNA. Red points indicate miRNAs that are upregulated, and blue points indicate miRNAs that are downregulated


A
PDCD4
FOXM1
FAM63B
AKT3
POFUT1
CCDC80
CHEK1
hsa-miR-4429
ZNF652
ZNF394
hsa-miR-1255a
CDC73
RSRC1
SORCS1
LRRFIP1
C8orf37
DTWD2
ZNF669
ACBD7
XPNPEP3
PPM1K
hsa-miR-1827
CENPM
DUSP19
ZNF69
hsa-miR-32-3p
SESN1
hsa-miR-664a-5p
hsa-miR-5002-5p
C1orf95
MARCH9
KIAA1143
KIAA2022
hsa-miR-183-3p
hsa-miR-4686
SMIM14
LPHN1
hsa-miR-3149
TRIM13
N4BP2
TXNDC5
hsa-miR-505-5p
SEPT5
ZNF682
hsa-miR-4742-3p
hsa-miR-5187-3p
hsa-miR-4532
TLCD2
TMEM70
SYAP1
RAD51L3-RFFL
hsa-miR-325
hsa-miR-3924
GTF2IRD2
FBXO21
hsa-miR-3667-5p
MBOAT2
TSPAN2
hsa-miR-92a-2-5p
GK5
FAM212B
MARCH3
ZDHHC6
CDT1
TAPBP
hsa-miR-4297
APH1A
ASB16
MCM8
TK1
VHL
hsa-miR-574-5p
ZRANB1
BIRC5
SLC35E1
HS6ST3
SUCLG2
LMOD3
B
GDAP1
CAPRIN1
YIPF5
RPS24
SCOC
RCN2
RPL15
NCKAP1
OSBPL8
PTPLAD1
ACTR2
PPP1CB
hsa-miR-4429
hsa-miR-183-3p
USP46
GNAI1
DNAJA2
SNRPD1
CMPK1
MARCH6
SEPT2
SPEM1
SUPT4H1
DARS
CALM2
hsa-miR-5187-3p
USMG5
HINT1
ZBTB7A
GRSF1
hsa-miR-1827
hsa-miR-5002-5p
NEGR1
SON
ATP6V1C1
DCAF11
E2F3
MBNL2
CPE
hsa-miR-3924
ATP5A1
KIF3A
MARC
YWHAZ
KPNA2
RSL24D1
hsa-miR-664-5p
hsa-miR-4532
hsa-miR-4297
CNBP
ZFP91
ST13
SKP1
hsa-miR-1255a
PAIP2
SEPT5
NCOA7
hsa-miR-3149
UBL5
UBR3
DYNC
KPNA4
hsa-miR-32-3p
TXNL1
LSAMP
ZFP14
PTK7
PTP4A1
MARCH4
MYO5A
hsa-miR-92a-2-5p
SET
UHMK1
hsa-miR-4742-3p
hsa-miR-574-5p
hsa-miR-505-5p
EID1
DDX6
TRIM2
MARCH3
C11orf58
HSPA8
NAA50
EIF4E
MARS
SERINC1
SUB1
SEPT6
SUMO1
PCNP
hsa-miR-4686

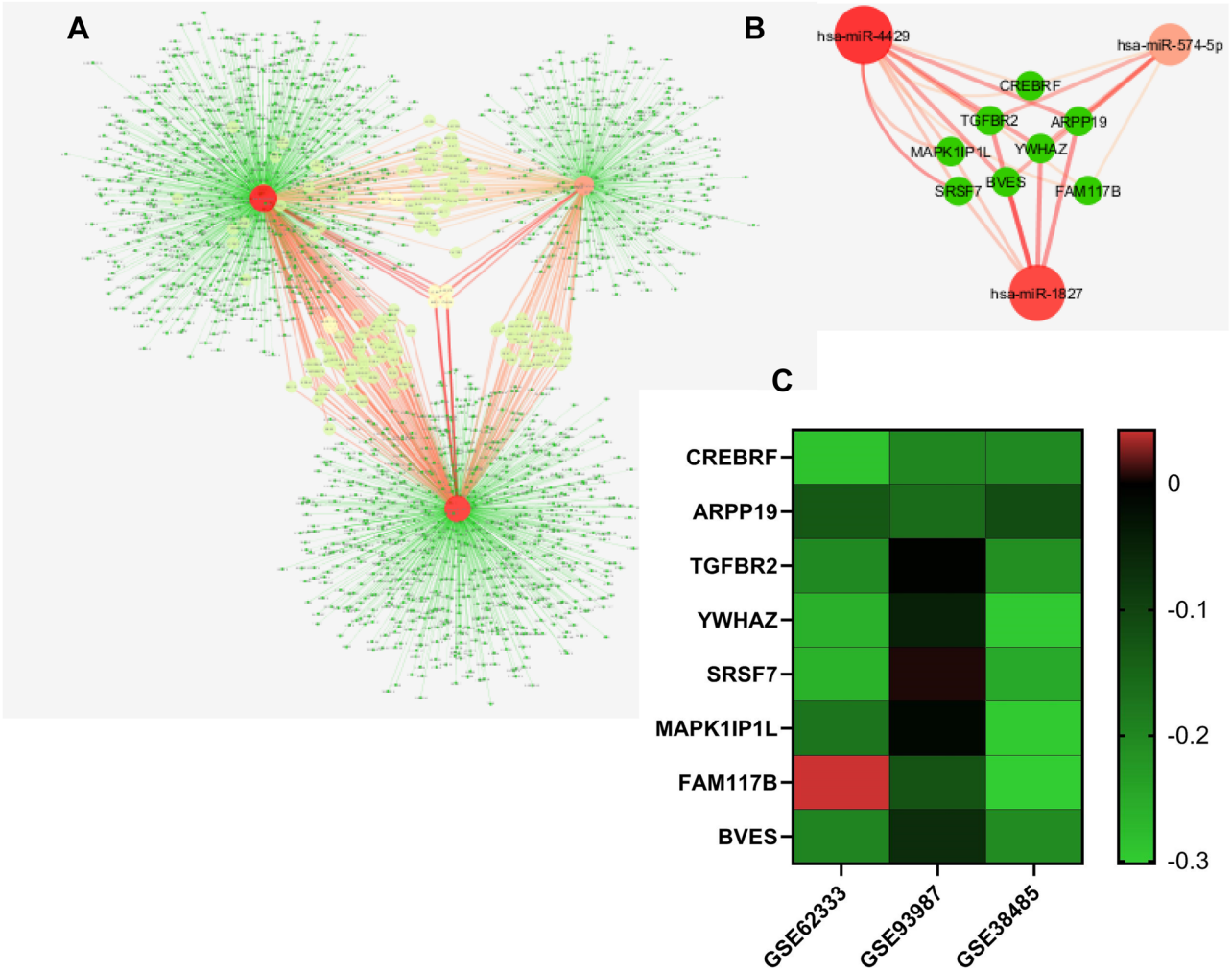


Fig. 6 MiRNA-target interactions of the hub miRNAs including Has-miR-4429, Has-miR-1827, and HasmiR-574-5P. (**A**) Nodes between three big red nodes (Has-miR-4429, Has-miR-1827, and Has-miR-574-5P) are common target genes of them and were shown as yellow nodes. (**B**) Eight genes including CREBRF, ARPP19, TGFBR2, YWHAZ, SRSF7, MAPK1IP1L, FAM117B, and BVES are common target genes of three mentioned hub genes. (**C**) The expression status of these common target genes evaluated in three independent expression arrays (GSE62333, GSE93987, and GSE38485) and the results are shown as heatmap

as miR-574-5P, has been studied as a malignancy promoter and cancer biomarker for several human tumors like lung cancer (Foss et al. 2011), nasopharyngeal carcinoma (Lin et al. 2020), colorectal cancer (Cui et al. 2014), and squamous cell carcinoma (Yang et al. 2013) in addition to its acceptable prognostic ability in prediction of prognosis in the patients with incident asthma (Li et al. 2021a). In lung cancer, vesicle-derived miR-574-5P is known to regulate prostaglandin E_2 (PGE_2) expression via Toll-like receptors (TLR) 7/8 (Donzelli et al. 2021). MiR-574-5P/TLR 7/8 axis is also identified to be involved in the pathogenesis of rheumatoid arthritis via induction of osteoclast differentiation (Hegewald et al. 2020). Additionally, it is known to be affected in several other human disorders such as rheumatoid arthritis (Hegewald et al. 2020), kidney injury in sepsis (Liu et al. 2021), and diabetes mellitus (Wang et al. 2021a). Regulatory functions of miR-574-5p also have been found on complement C7 involved in the pathogenesis of diabetic nephropathy (Guo et al. 2021), and serum lipids and blood glucose (Wang et al. 2021a) in addition to its antiviral activity against hepatitis B virus (HBV) (Wu et al. 2021). Another hub miRNA hsa-miR-1827 in addition to playing role in various human malignancies (Zhou et al. 2021a; Shen et al. 2021; Guo et al. 2020; Wang et al. 2020), regulation of osteogenic differentiation (Zhu et al. 2017), and primary immune thrombocytopenia(ITP) (Sun et al. 2021) has been found as a potential biomarker with dysregulation in samples of patients with Alzheimer's disease (Soleimani Zakeri et al. 2020). According to the literature, the third miRNA hsa-miR-4429 have been associated with particularly various types of cancer with tumor suppressor functions (Zhou et al. 2021b; Li et al. 2021b; Wang et al. 2021b), although in a few

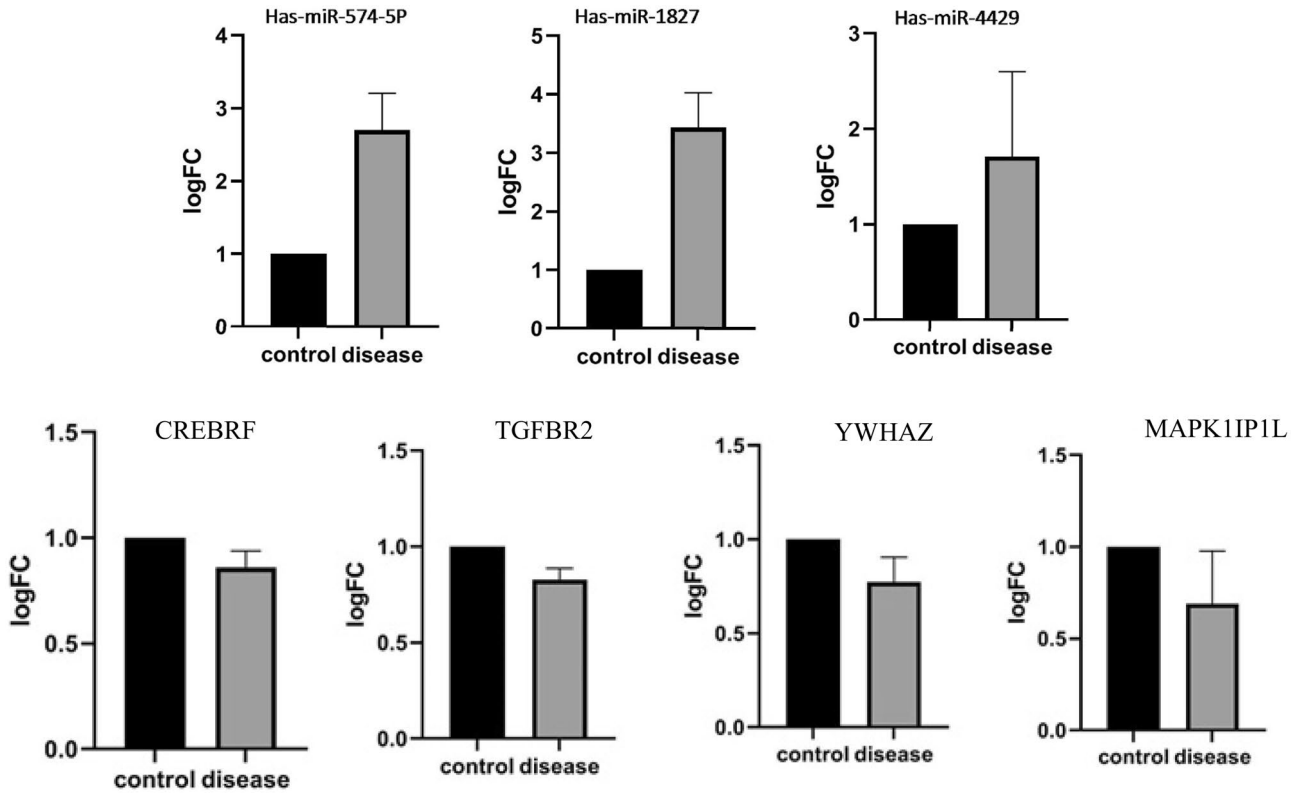


Fig. 7 Real-Time PCR analysis for miRNAs and mRNAs for schizophrenia in comparison with healthy controls in blood samples. Bar diagram shows fold change in expression of core miRNAs. Statistical comparisons were made with respective 5 s rRNA for miRNAs and GAPDH for mRNAs. $p < 0.05$ for all genes and miRNAs

studies is found to be dysregulated in other conditions like acute ischemic stroke and biliary atresia (Jickling et al. 2014; Dong et al. 2016). Among the DEGs, some have been known to play a role in neural health and function with changes contributing to neurological/neurodegenerative diseases like axon degeneration, Alzheimer's disease, and bipolar disease (Soleimani Zakeri et al. 2020; Li et al. 2021c; Reitz et al. 2011; Starnawska et al. 2016; Kim et al. 2001; Maycox et al. 2009). Taken together, the interaction we identified between the hub miRNAs and the hub genes can suggest potential role for the miRNAs in the SCZ pathogenesis. Still not elucidated, the association of these genes with the SCZ pathogenesis can be further explored to find diagnostic and therapeutical targets.

Overall, identification of these miRNAs and genes, already have been associated with several human diseases, in blood samples of SCZ patients may help develop a diagnostic approach via easily detection in the peripheral blood and so, earlier screening and monitoring the SCZ-affected individuals can be achieved. Further investigations are recommended to explore the biological functions and potential clinical application of these miRNAs.

In conclusion, this study is one of the first attempts to construct a miRNA-mRNA network using the metaDE, WGCNA, and limma packages. We identified 4 hub genes and 3 miRNAs in three separate datasets. Then, their expression levels were verified by other independent datasets and real-time PCR, and their prognostic and diagnostic power was validated by the ROC curve.

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Declarations

Ethics Approval and Consent to Participate All patients gave their signed written informed consent letters. Medical Research and Ethical Committee of Kermanshah University of Medical Sciences (Kermanshah,

Iran; registration no. IR.KUMS.REC.1397.490; grant number 97542) approved the study performed under ethical principles contained in the 7th and current (2013) editions of Helsinki Declaration.

Conflict of Interest The authors declare no competing interests.

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