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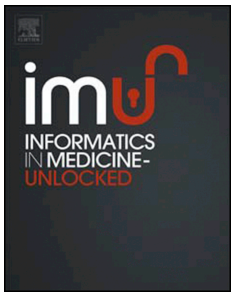

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Abstract

Bipolar disorder (BD) is a severe psychiatric disorder and among the leading causes of disability worldwide that has a significant genetic component with high heritability. The prognosis for BD patients remains poor because of the lack of reliable biomarkers and also pathogenesis and diagnostic methods remain limited. The purpose of this study was the elucidation potential candidate hub miRNAs related to BD. We searched the gene and miRNA expression patterns of BD from five expression arrays (GSE74358, GSE69486, GSE12654, GSE46416 for gene expression, and GSE152267 for miRNA expression) from the Gene Expression Omnibus (GEO) database to recognize differentially expressed genes (DEGs) and differentially expressed miRNAs (DEMs) between normal and BD samples. Using limma package, 87 DEGs were identified and Enrichment analysis for identified genes was performed. Due to more validation of DEGs, their expression was evaluated by 8 independent expression arrays including GSE12649, GSE23848, GSE39653, GSE46449, GSE5388, GSE5389, GSE53987, and GSE62191. By merging DEMs and DEGs, miRNA-mRNA network was constructed. After elucidation of hub miRNAs, the ability of detected miRNAs to discriminate BD from healthy controls was assessed by area under the curve (AUC) using the ROC curve analysis. Furthermore, RT-qPCR on 50 BD samples and 50 healthy controls was performed to validate the in silico results. Our results showed that hsa-miR-19b-3p and hsa-miR-23b-3P could be potential biomarkers of BD.

Keywords: bipolar disease, microRNA, biomarker, real time PCR.

Introduction

Non-coding RNAs comprise a large proportion of human transcriptome mainly with no known encoded protein; however, they have been found to play essential roles in regulation of cellular functions such as cell cycle and proliferation (1). Moreover, microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) are the main classes of ncRNAs with have been associated with several human diseases particularly various types of cancer and therefore, suggested as potential biomarkers with applications in diagnosis, prediction of prognosis, and treatment (2). MiRNAs have shown expression dysregulation with potential applications in psychiatric disorders like schizophrenia (3). Also, studies in postmortem brain tissues and in the periphery of BD patients also have shown changed expression of miRNAs such as miR-34a and miR-137 in addition to possibility of miRNAs-encoding genes in susceptibility to BD (4). In another study, Maffioletti *et al*. (5) assessed the expression level of more than 1700 miRNAs in blood samples of patients with mood disorders through a microarray technique. They showed aberrant expression of 5 miRNAs in blood of 20 BD patients in addition to alterted expression of 5 others in manic depressive patients. Furthermore, Camkurt *et al*. (6) demonstrated upregulation of 4 miRNAs in blood samples of BD patients, 7 others with higher upregulation in manic BD patients, and a couple in manic patients. They suggested miR-106a-5p and miR-107 as selective putative biomarkers for manic BD patients.

Bipolar disorder (BD), also known as manic-depressive illness, is an affective disorder among the most distinct psychiatric illnesses characterized by manic-depressive fluctuations in mood state and responsible for a considerable proportion of disabilities ranking 17th among all diseases globally (7, 8). This disorder tends to a severe and chronic illness with recurrent episodes, with

significant morbidity, poor outcomes, and also higher mortality compared to the general population (9). The prevalence of BD among the population is yearly 2%, and suicide and comorbidities mainly lead to the premature mortality and reduced life expectancy among the affected patients (10-12). The distinguishing manifestation of BD is relapsing episodes of mania/hypomania occasionally replaced by episodes of depression, which along with other clinical symptoms including grandiosity, overconfidence, talkativeness, and changes in eating and sleeping habits constitute the basics of disorder diagnosis (10). Since diagnosis of the disorder, although not easy, is based on clinical presentations according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-TR) guidelines (13), which means that BD is diagnosed when the manifestations of the disease have appeared, and so clinical management requires early diagnosis prior to disease. The current DSM has different major weakness such as “Cultural Bias”. DSMs are mainly published by anglo-Americans and most of the behaviour that is considered as “normal” in the DSM is actually what is considered normal by the anglo-Americans. That is, some of the behaviour that is considered as abnormal in the DSM might be considered as normal in other cultures. Another weakness would be that DSM promotes a mechanical approach to mental disorder assessment. The clinicians may focus excessively on the signs and symptoms of mental disorders and they might not put much emphasis on a more in-depth understanding of the clients/patients problems. Another weakness and debate about the DSM is that it is an unscientific system and it is the opinion of a few powerful psychiatrists. There are many other weaknesses with specific disorders of DSM especially about the diagnosis criteria and some other issues such as whether 'Paraphilias' should be included or not (14). Currently, no neuroimaging method or laboratory test is available for helping early diagnosis of BD patients (15). Family and molecular genetic studies provide convincing evidence that BD is a multifactorial disorder, with genetic and

environmental factors contributing to its development. On the basis of twin and family studies, the heritability of BD is estimated at 60–85%. Genome-wide association studies (GWASs) have led to valuable insights into the genetic etiology of BD. The largest such study has been conducted by the Psychiatric Genomics Consortium (PGC), in which genome-wide SNP data from 29,764 patients with BD and 169,118 controls were analyzed and 30 genome-wide significant loci were identified (PGC2) (16), Thus, precise medicine is expected to help development of biomarkers with potentials of differentiating BD patients or susceptible individuals from healthy people, which accordingly can benefit the early diagnosis of the disorder (15).

Although previous studies have searched the role of miRNAs on BD, almost none of them considered high-throughput data and also useful packages like limma for the identification of miRNA biomarkers. Bioinformatic tools have facilitated estimation of selective miRNAs, while extensively used high-throughput RNA sequencing has helped identification of a huge number of miRNAs with putative biological importance and potential applications in diagnosis and management of human diseases (17). In this study, we aimed to evaluate the expression levels and diagnostic values of miRNAs using *in situ* tools and validation via experimental analyses in blood samples of BD patients. Accordingly, the results can be used in development of biomarkers with applications for easy and quick detection of BD-susceptible individuals.

Materials and methods

Acquisition of high throughput sequencing data and pre-processing

The Gene expression omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) was used for downloading raw data of bipolar and healthy controls. By searching keywords, bipolar and healthy controls, four gene expression arrays including GSE74358, GSE69486, GSE12654, GSE46416

and one miRNA array, GSE152267, were identified. We also extracted raw data of eight expression arrays including GSE12649, GSE23848, GSE39653, GSE46449, GSE5388, GSE5389, GSE53987, and GSE62191 for more validation of results. In GSE74358, 28 samples, 14 BP and 14 healthy controls expression arrays were analyzed. In GSE69486, total RNA obtained from skin fibroblast cells of 5 healthy control samples that were pooled into 2 samples and from 10 bipolar disorder patients, in order to compare their gene expression profiles. 15 healthy controls and 11 BP samples of GSE12654 from postmortem brains obtained from the Stanley Medical Research Institute were used for DNA microarray analysis. GSE46416 compare the gene expression values of 10 healthy controls and 22 BP samples. In GSE152267, Peripheral blood samples of 7 BD patients and 6 healthy individuals were collected for the miRNA extraction and hybridization on Affymetrix microarrays. The information of eight validation data is collected in Table 1. In general DEGs between 240 control subjects and 260 BP samples were used for identification of molecular mechanism during BP.

Data pre-processing, quality control and identification of differentially expressed genes

To normalize the selected datasets, the quantile normalization method in the limma package (18) and also the log2 transformation scale were used. Since each gene may have several probes, for better results, the aggregate function of the S4Vectors package was used to measure the average expression of all probes related to each gene. There were significant differences in gene expression levels between patients with mental illness and those with other diseases like tumors. For example, if we chose the screening criteria for DEGs of tumor diseases as |log fold change (log FC)|>1 or 2, even 0.2 to 0.9, there were only approximately 10 DEGs that could be screened out for mental diseases. To avoid missing more valuable DEGs, we artificially defined $P<0.05$ and |log FC|>0.5

as inclusion criteria for DEGs. The differentially expressed genes were identified using Limma package (19).

Protein–protein interactions (PPI) network analysis

Search Tool for the Retrieval of Interacting Genes (STRING v.11) online tool was used to evaluate the functional interactions of DEGs (https://string-db.org/). The DEGs were uploaded in the STRING tool. The identified network was imported to Cytoscape (version 3.8.0) for further analysis and PPI network visualization.

GO enrichment analysis and KEGG pathway analysis

In the next step, DEGs were studied using Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway *via* ClueGO (20). The p-value < 0.05 was defined as a meaningful enrichment analysis result. Potential functions were predicted using GO and KEGG pathway analysis.

Validation of DEGs by independent datasets

Previously published data sets including GSE12649, GSE23848, GSE39653, GSE46449, GSE5388, GSE5389, GSE53987, and GSE62191 were downloaded and normalized and the expression values of DEGs was evaluated by these datasets.

Screening the differentially expressed miRNAs

The limma package was used for differential expression analysis for both BD patients and healthy individuals' samples. MiRNAs with the FDR < 0.05 and $|\text{LogFC}| \geq 1$ were considered differentially expressed miRNAs (DEMs).

Construction of miRNA-mRNA network

The identified DEMs in addition to highly significant genes achieved in previous section were employed for constructing the miRNA-mRNA network. Accordingly, we applied the CyTargetLinker app of Cytoscape (21), and then constructed a miRNA-gene regulatory network *via* employing three miRNA-based web servers including miRBase (22), TargetScan (23), and TransmiR (24). Based on the results retrieved from the network, 2 miRNAs were screened as the hub miRNAs.

Validation of the hub miRNAs

For further validation of the identified DEMs, we aimed demonstrate the diagnostic power of the hub miRNAs through plotting the receiver operating characteristic (ROC) curve and estimation of the area under the curve (AUC) using GSE152267.

Hub miRNAs qRT-PCR validation

To validate the *in situ* results, we performed quantitative reverse-transcription PCR (qRT-PCR) to analyze the genes and miRNAs expression in whole blood collected from the BD patients (n = 50) enrolled in Tabriz University of Medical Sciences, Tabriz, Iran. All patients were requested to fill the informed consent regarding the study.

RNA extraction and cDNA production

For each patient, total RNA was extracted from a 2ml venous blood specimen using purification kit (Qiagen Cat. No. 52304). Then, synthesizing complementary DNA (cDNA) was performed, followed by removing the genomic DNA by treating the generated cDNA with DNase I. The

extracted cDNA was purity-checked using UV-spectrophotometry and handled for carrying out qRT-PCR.

Primer design

To design the forward and reverse primers, we used the web-based miRNA primer designing tool (25).

qRT-PCR

The primers, which were received as lyophilized powder (Cinaclone, Iran), were prepared by adding sterile distilled water to the tubes according to the data provided by the company and then stored at −20 °C. To analyze the expression changes in both miRNAs and mRNAs, real-time PCR thermocycler (Applied Biosystems) and SYBR green qRT-PCR kit (Takara, China) were employed. Negative controls were applied in all reactions to reveal possible contaminations. Changes in the threshold cycle (CT) (as the formula below) was used to evaluate the expression fold alterations of the miRNAs and genes, while 5s rRNA considered the reference to whom the expression levels of the examined genes were compared.

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

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

Results

Differentially expressed genes (DEGs)

Employing the limma package with an P.value < 0.05 and |log fold change (log FC)|>0.5, upregulated and downregulated genes for each of GSE74358, GSE69486, GSE12654, and GSE46416 datasets were identified and volcano plot of each dataset can be seen in figure 1 (Figure 1A, 1B, 1C ,1D). A total of 87 common DEGs between GSE74358, GSE69486, GSE12654, and GSE46416 were identified (including 48 up-regulated genes and 39 down-regulated genes) (Figure 1E, 1F), The list of DEGs can be seen in Table 2.

PPI network analysis and Pathway enrichment analysis

All 87 DEGs were used for the network construction. Using STRING database and the Cytoscape software, the PPI network was made (Figure 2). The color and size of each protein was set based on the expression and degree respectively. Upregulated genes were red while downregulated ones were green and the size of each protein represents the degree or the number of connections with other proteins, these genes and their PPI parameters is visible in table 2. To analyze the pathway enrichment, the CluGO plugin based on the Cytoscape software was used to illustrate the results of KEGG path analysis for DEGs (Figure 3). Some cellular processes and signaling pathways were identified to be dysregulated. These included G-protein mediated events, MET activates PTK2 signaling, Collagen biosynthesis and modifying enzymes, nuclear body organization, regulation of insulin receptor signaling pathway, and Yersinia infection.

Validation of DEGs by independent datasets

The expression status of all DEGs were evaluated by other separate datasets. The expression status of these genes were evaluated using GSE12649, GSE23848, GSE39653, GSE46449, GSE5388, GSE5389, GSE53987, and GSE62191and can be seen as heatmap (Figure 4). The expression evaluation validated 48 upregulated genes including NRK, KLHL11, LAT, BAIAP2L1, RFX4,

FLCN, LHX9, EML2, CSMD2, EXD3, PRICKLE3, SLC44A4, CXCL14, C1orf87, PPFIBP1, NR6A1, TSTD2, MPP4, GDF15, ZNF24, POLN, POPDC2, COL11A2, HSD17B14, GYPE, B3GNT2, EFCAB6, ENPP1, PLCB4, GABPB1, EIF1AY, ARSB, OAS1, MAP2, CNNM2, GM2A, TFEC, DIRAS3, FARP1, ACSM3, SAA1, PKP2, MERTK, EZR, ELP4, MYO6, PRDM2, and SLC4A4 and 39 downregulated genes including SEMA3A, ARHGAP20, IL18R1, HOXB8, FAM20A, COL5A3, SEMA3A, A2M, CTSC, LIMCH1, SLC9A9, PLA2R1, PML, IFT81, NPL, RAB3IP,PDK4, ROR2, FBXW8, COL16A1, NBEA, LRRC8B, PIK3R1, MAPK9, ATP2B2, SF1, SRPK2, ENPP2, RFC5, CACNA2D3, ATP2B1, CADPS2, TPR, PREPL, PRKAR1A, WSB1, ITGA4, FYB, DEPDC5, LAMB1, FCGR2A, UCP3, IFNAR2, HLA-DMA, KRT7, BAZ1A, NLRP3, ABCC9, ADH1A, SCAMP1, SCAI.

DEMs Identification

In the GSE152267 dataset, the miRNA expression profile of 7 BD patients and 6 healthy controls were compared. Raw data were normalized *via* log2 transformation and also the quantile normalization function in the Limma package. The results demonstrated 11 upregulated miRNAs, hsa-miR-3921, hsa-miR-23b-3p, hsa-miR-1180-3p, hsa-miR-297, hsa-miR-378h, hsa-miR-337-3p, hsa-miR-423-5p, hsa-miR-548x-3p, hsa-miR-6776, hsa-miR-486-3p, and hsa-miR-151a-5p and 5 downregulated miRNAs, hsa-miR-22-3p, hsa-miR-19b-3p, hsa-miR-4734, hsa-miR-378a-3p, hsa-miR-4745-5p (Figure 5).

MRNA-miRNA network construction

Relying on the identified DEMs and the DEGs, construction of a miRNA-mRNA network was conducted (Figure 6). As obvious, the hsa-miR-19b-3p and hsa-miR-23b-3P show direct effect on highest number of DEGs and are considered hub miRNAs.

Validation of the hub miRNAs by the ROC curve

Plotting the ROC curves was conducted according to the GSE152267 raw data and through a single-gene test. This was carried out by assigning the sensitivity and specificity values estimated for differentiating the BD patients' samples from healthy controls to each side of the curve for two hub miRNAs. The Hanley and McNeil test (26) was employed for comparing the AUC in ROC curves (Figure 7). The results demonstrated significant values for both miRNAs as AUC of 1, and *p-value* of 0.0027 for hsa-miR-19b-3p, and AUC of 0.8, and *p-value* of 0.0433 for hsa-miR-23b-3P.

qRT-PCR

Expression of the selected miRNAs was evaluated *via* qRT-PCR in a total of 50 BD patients in addition to healthy controls (Figure 8). Both hub miRNAs demonstrated aberrant expression, of them Hsa-miR-19b-3p showed downregulation, while hsa-miR-23b-3P was upregulated in BD patients compared to healthy individuals.

Discussion

As one of the most significant mental conditions, bipolar disorder, is a highly heritable psychiatric disorder(27). Investigations on the blood and post-mortem brains of people with this disorder have found aberrant gene expression(28). Gene Ontology Analysis of the genes has revealed molecular mechanisms that appear to be crucial in the pathogenesis of the psychological disorders. In the past few years, changes in miRNA expression have been recognized to have a role in pathophysiology of mental illnesses and have the potential to behave as peripheral biomarkers of disease and therapy response. So, investigating common miRNA biomarkers of BD may assist comprehend its connection pathology and developing treatments to decrease the symptoms in BD

patients. Numerous miRNAs showed the ability to significantly differentiate between patients with BD and healthy, normal ones. In the present study, we formed a regulatory pattern implicating the BD-specific mRNAs-miRNAs by merging all the regulatory details received from our results. According to the bioinformatic databases, we identified 148 DEGs and 16 crucial DEMs (including 11 upregulated and 5 downregulated) from bipolar samples and adjacent normal individuals. In a subsequent analysis, we selected hsa-miR-23b-3p and hsa-miR-19a-3p based on p-value, fold change, and their known role as regulators of gene expression in patients with BD. Our findings demonstrated that compared with controls, miR-23b-3p was noted to be considerably more upregulated and miR-19b-3p was downregulated. These two miRNAs have great potential to be a useful tool for the diagnosis of BD and directly regulates the expression of several DEGs (miR-19b-3p shows the direct impact on several DEGs such as ACP1, NPTN, NBEA, NDEL1, TCEAL1, and RNF1. However, the hsa-miR-23b-3P directly regulates the expression of several other DEGs including CASD1, DSTN, ADAM23, and IPO5). All of these target genes were demonstrated to be involved in the NOS process, regulation of organic acid transport, regulation of cellular response to insulin stimulus, neuroinflammatory response, and etc. Several studies have reported aberrant expression of miR-19b-3p associated with other brain-derived disorders(29-32) Additionally, the study done by Lee et al. determined over-expression of miR-23b-3p in bipolar patients compared with healthy individuals(33). In another study, serum expression levels of miR-23b-3p significantly increased in bipolar disorder patients(34). However, many researches confirmed the role of specific pathways in initiation and development of BD. Modified NOS signaling has been associated with the pathophysiology of BD (35-37). In a recent study conducted by Loeb et al. NOS activity revealed as a promising biomarker for major depressive disorders(38). In addition to the NOS pathway, in multiple studies, the importance of the regulation of the organic

acid transport pathway has also been described in BD(39). Based on experiments, patients with BD had a more increased level of Uric Acid, particularly in mania seizures. Uric Acid may be a potential biomarker to indicate BD(40).

Besides all these investigations and in silico analytic results, we also conducted a series of experiments on 50 BD patients compared to normal individuals, which had the same results from statistical studies. According to our qPCR results, Hsa-miR-19b-3p was shown to be downregulated (*p-value:* 0.0027), while hsa-miR-23b-3P was shown to be upregulated (*p-value:* 0.0433).

Conclusion

In the current analysis, we examined circulating miRNAs in plasma samples of patients with BD in comparison to those of normal healthy controls among illness episodes like mania and depression. 87 DEGs and 16 DEMs showed significant alterations in patients with BD in comparison to healthy individuals; among these genes and miRNAs, hsa-miR-23b-3p and hsa-miR-19a-3p, and their related DEGs such as ACP1, NPTN, NBEA, NDEL1, TCEAL1, RNF1, CASD1, DSTN, ADAM23, and IPO5 expressed significantly. Moreover, the ROC analysis and qPCR were confirmed that miR-23b-3p (AUC: 0.8 and *p-value*: 0.0433) was noted to be considerably more upregulated and miR-19b-3p (AUC: 1 and *p-value*: 0.0027) was downregulated in patients with BD. Our findings imply that these two miRNAs may be biomarker candidates for BD.

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Competing interests

Authors have no conflict of interest

Authors' contributions

Conceptualization: Yazdan Rahmati and Omran Davarinejad, Computational analyses and Experiments: Payam Mohammadi, Omran Davarinejad, Yazdan Rahmati, Zahra Foruzandeh, Farzaneh Golmohammadi,, Writing manuscript: Farzaneh Golmohammadi, Davood Ghavi, MohammadReza Alivand, Supervision and Finalization: Yazdan Rahmati

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Data availability statement

The datasets analyzed during the current study are available in the Gene expression omnibus https: //www.ncbi.nlm.nih.gov/geo/' repository and also are available in supplementary data.

Consent for publication

The participant and authors have consented to the submission of this report to the journal.

Ethics Approval and Consent to Participate

All patients gave their signed written informed consent letters. Medical Research and Ethical Committee of Tabriz university of Medical Sciences, approved the study performed under ethical principles contained in the 7th and current (2013) editions of Helsinki Declaration.

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Figure 1. DEGs in expression microarrays of four datasets of bipolar and healthy controls with cutoff, P-value <0.01, logFC >0.5 and common DEGs between four mentioned expression arrays. Red dots are upregulated while green dots are downregulated. (A) GSE69486. (B) GSE12654. (C) GSE46416. (D) GSE74358. (E) Venn diagram of upregulated genes. (F) Venn diagram of downregulated genes.

Figure 2. The PPI network of DEGs in BP. The colors represent the expression value of genes. The grades of the colors represent the expression levels. Red gradient shows upregulated genes and green gradient represent downregulated genes. Number of nodes: 175, number of edges: 406, average node degree: 6.29, avg. local clustering coefficient: 0.401, PPI enrichment p-value: < 1.0e-16.

Figure 3. Dysregulated signaling pathways and interacted genes (nodes with red mark) after functional enrichment analysis using ClueGO, a Cytoscape plug-in.

Figure 4. Validation of DEGs by independent expression arrays. Red color represents upregulated genes and green color represents downregulated genes.

Figure 5. Volcano plot showing the differential miRNAs in the expression microarray (GSE152267) (P-value <0.05, logFC >1). Red points indicate upregulated miRNAs, green points indicate downregulated miRNAs.

Figure 6. MRNA-miRNA regulatory network between DEMs and DEGs. Every node represents a gene or a miRNA, and each edge represents the interaction between genes and miRNAs. The color blue represents genes, and red represents upregulated miRNAs and green color represents downregulated miRNAs.

Figure 7. ROC curve analysis of two core miRNAs based on GSE152267 dataset. Receiver operating characteristic (ROC) curve and area under the curve (AUC) statistics are used to evaluate the capacity to discriminate BD from healthy controls with excellent specificity and sensitivity.

Figure 8. Real time PCR analysis for miRNA expression of BD in comparison with healthy controls in serum samples. Bar diagram shows fold change in expression of core miRNAs. Statistical comparisons were made with respective 5 s rRNA, $P < 0.05$ for all miRNAs.

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Table 1. The list of expression arrays that were used.

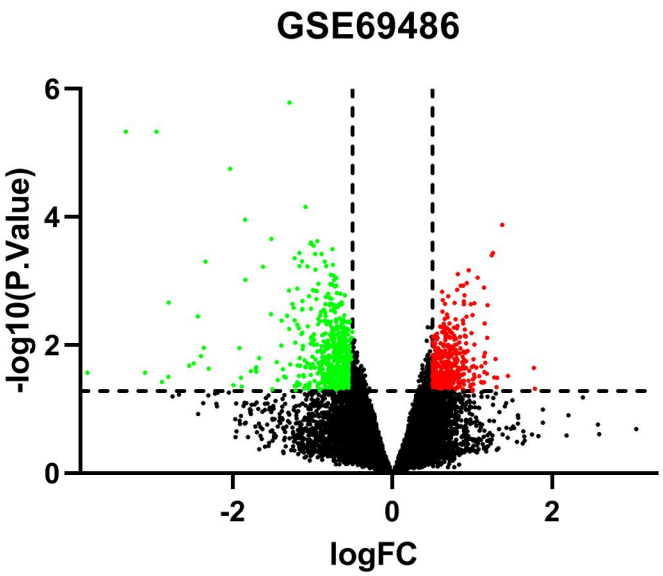
Datasets	Platform	comparison	
GSE74358	Affymetrix Human Genome U133 Plus 2.0 Array	Healthy controls	Bipolar disorder
GSE69486	Illumina HumanHT-12 V4.0 expression beadchip	Healthy controls	Bipolar disorder
GSE12654	Affymetrix Human Genome U95 Version 2 Array	Healthy controls	Bipolar disorder
GSE46416	Affymetrix Human Exon 1.0 ST Array	Healthy controls	Bipolar disorder
GSE12649	Affymetrix Human Genome U133A Array	Healthy controls	Bipolar disorder
GSE23848	Sentrix Human-6 v2 Expression BeadChip	Healthy controls	Bipolar disorder
GSE39653	Illumina HumanHT-12 V4.0 expression beadchip	Healthy controls	Bipolar disorder
GSE46449	Affymetrix Human Genome U133 Plus 2.0 Array	Healthy controls	Bipolar disorder
GSE5388	Affymetrix Human Genome U133A Array	Healthy controls	Bipolar disorder
GSE5389	Affymetrix Human Genome U133A Array	Healthy controls	Bipolar disorder
GSE53987	Affymetrix Human Genome U133 Plus 2.0 Array	Healthy controls	Bipolar disorder
GSE62191	Agilent-014850 Whole Human Genome Microarray	Healthy controls	Bipolar disorder
GSE152267	Affymetrix Multispecies miRNA-4 Array	Healthy controls	Bipolar disorder

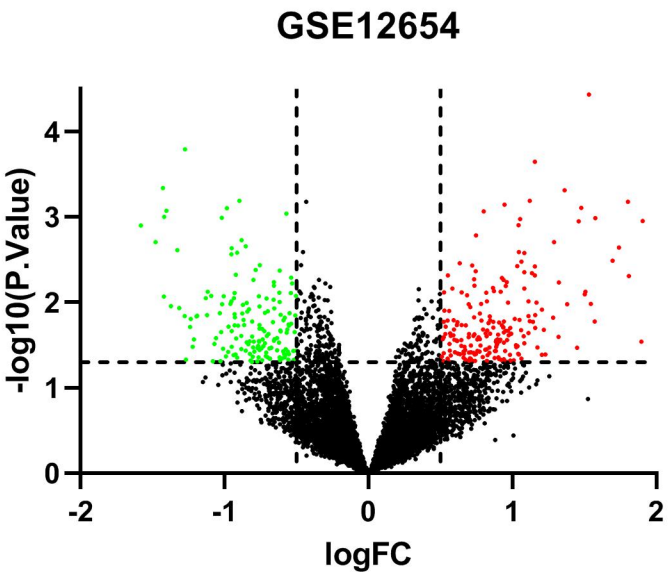
Table 2. The list of DEGs and their PPI parameters.

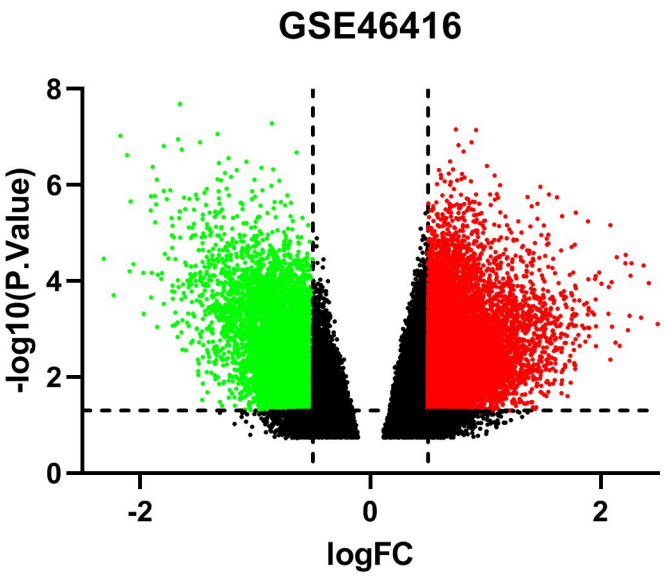
Gene name	AverageShortestPath	ClosenessCentrality	Degree	Eccentricity	DirectedEdges	Radiality
A2M	2.90410959	0.34433962	14	6	14	0.78843227
ABCC9	4.56621005	0.219	1	8	1	0.60375444
PKP2	3.57077626	0.28005115	4	7	4	0.71435819
ACSL4	1	1	1	1	1	1
ACSM3	5	0.2	1	8	1	0.55555556
FTCD	4.00456621	0.24971494	2	7	2	0.66615931
ADH1A	5.84018265	0.17122752	1	9	1	0.46220193
GABRA6	4.84474886	0.20640905	3	8	3	0.57280568
CETP	3.05479452	0.32735426	5	6	5	0.7716895
HMOX1	2.84018265	0.35209003	13	6	13	0.79553526
HGF	2.49771689	0.40036563	25	6	25	0.83358701
LTF	3.03652968	0.32932331	6	6	6	0.77371892
GDF15	3.03652968	0.32932331	3	6	3	0.77371892
ENPP2	2.96347032	0.33744222	4	6	4	0.78183663
FCGR2A	2.75799087	0.36258278	14	6	14	0.80466768
PLA2R1	3.10502283	0.32205882	1	6	1	0.76610857
SCAI	3.10502283	0.32205882	1	6	1	0.76610857
TCF20	2.86757991	0.34872611	3	6	3	0.79249112
DKK1	2.76712329	0.36138614	7	6	7	0.80365297
PAX7	2.84931507	0.35096154	8	6	8	0.79452055
CR1	3.00456621	0.33282675	3	6	3	0.77727042
NLRP3	2.71232877	0.36868687	11	6	11	0.80974125
EZR	2.88127854	0.34706815	7	6	7	0.79096905
MAP2	2.96803653	0.33692308	4	6	4	0.78132927
KRT7	2.78082192	0.35960591	7	6	7	0.8021309
ATP2B2	3.86757991	0.25855962	3	7	3	0.68138001
SLC4A4	3.91780822	0.25524476	2	7	2	0.67579909
PLA2G7	3.44292237	0.29045093	6	7	6	0.72856418
DDAH1	3.83105023	0.26102503	1	7	1	0.68543886
TPR	3.16438356	0.31601732	7	6	7	0.75951294
TSTD2	4.73059361	0.21138996	1	8	1	0.5854896
ATP2B1	1	1	1	1	1	1
CNNM2	1	1	1	1	1	1
MYO6	3.50228311	0.28552803	4	7	4	0.72196854
RFC5	3.3652968	0.29715061	11	6	11	0.73718924
SRPK2	3.96347032	0.25230415	2	7	2	0.67072552
B3GNT2	3.62557078	0.27581864	3	7	3	0.70826991
SCAMP1	4.62100457	0.21640316	1	8	1	0.59766616
IFT81	3.92237443	0.25494761	2	7	2	0.67529173
PRICKLE3	5.47031963	0.18280467	1	8	1	0.50329782

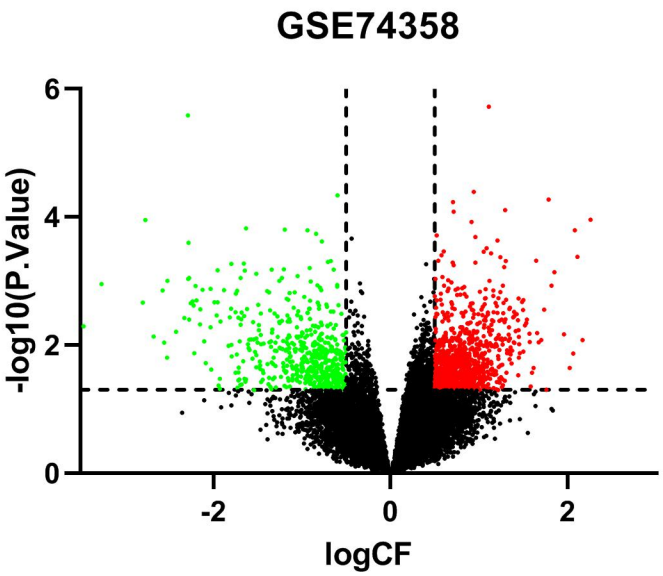
FARP1	3.60273973	0.27756654	3	7	3	0.7108067
BAZ1A	3.70319635	0.27003699	3	7	3	0.69964485
POLN	3.96347032	0.25230415	2	7	2	0.67072552
CADPS2	1	1	1	1	1	1
PDK4	3.89497717	0.25674091	3	7	3	0.67833587
UCP3	3.89954338	0.25644028	2	7	2	0.67782851
MERTK	3.56164384	0.28076923	3	7	3	0.71537291
ITGA4	2.89954338	0.34488189	14	6	14	0.78893962
FYB	3.49315068	0.28627451	3	7	3	0.72298326
COL11A2	3.26027397	0.30672269	7	6	7	0.74885845
COL5A3	3.67579909	0.27204969	4	7	4	0.70268899
COL16A1	3.62557078	0.27581864	5	7	5	0.70826991
HLA-DMA	4.20091324	0.23804348	2	7	2	0.64434297
LAMB1	3.02283105	0.33081571	9	6	9	0.77524099
PIK3R1	2.60730594	0.38353765	20	6	20	0.82141045
CTSC	3.80365297	0.26290516	3	7	3	0.688483
IFNAR2	3.34703196	0.29877217	7	7	7	0.73921867
CXCL14	3.44292237	0.29045093	2	7	2	0.72856418
DEPDC5	4.60730594	0.21704658	1	7	1	0.59918823
FLCN	3.61187215	0.27686473	2	6	2	0.70979198
PRKAR1A	3.38812785	0.29514825	4	7	4	0.73465246
ENPP1	3.77625571	0.26481258	5	7	5	0.69152714
SEMA3A	3.00456621	0.33282675	8	6	8	0.77727042
OAS1	4.18721461	0.23882225	3	8	3	0.64586504
PRDM2	3.23287671	0.30932203	1	7	1	0.75190259
RFX4	3.21917808	0.3106383	2	7	2	0.75342466
MAPK9	3.03196347	0.32981928	5	7	5	0.77422628
WSB1	4.7260274	0.2115942	2	8	2	0.58599696
LAT	3.42009132	0.29238985	3	7	3	0.73110096
RAB3IP	5.60730594	0.17833876	1	9	1	0.48807712
GABPB1	4.08675799	0.24469274	1	8	1	0.65702689
GM2A	5.37899543	0.18590832	1	8	1	0.51344495
NPL	4.82191781	0.20738636	1	8	1	0.57534247
PML	3.61187215	0.27686473	6	7	6	0.70979198
TFEC	3.83561644	0.26071429	1	7	1	0.68493151
HSD17B14	4.21461187	0.23726977	1	8	1	0.6428209
IL18R1	3.33789954	0.2995896	3	7	3	0.74023338
PLCB4	3.28310502	0.30458971	5	7	5	0.74632166
ROR2	3.9086758	0.25584112	3	8	3	0.6768138
TBC1D22B	1	1	1	1	1	1
SF1	4.15068493	0.24092409	2	7	2	0.6499239

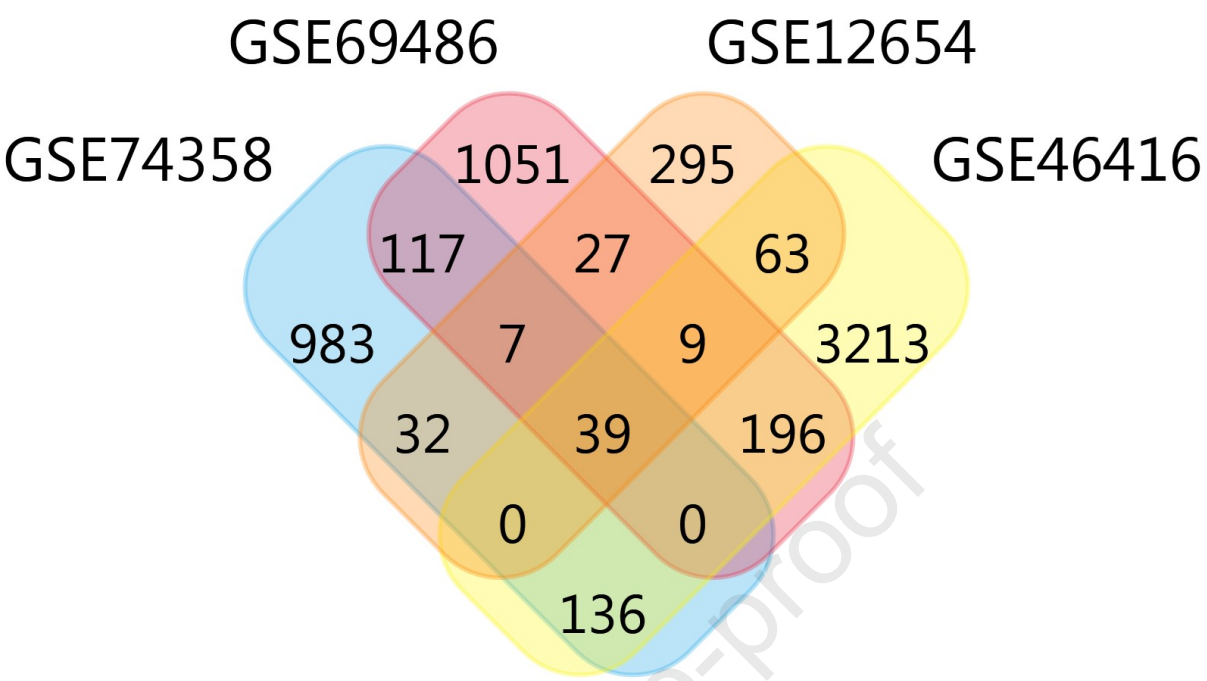
ZNF24	1	1	1	1	1	1
CADPS2	1	1	1	1	1	1
CSMD2	1	1	1	1	1	1
CAPSL	4.08219178	0.24496644	1	7	1	0.65753425
IBSP	3.08675799	0.3239645	9	6	9	0.768138
CASP4	3.70776256	0.26970443	1	7	1	0.69913749
GAK	4.10045662	0.24387528	3	7	3	0.65550482
E2F2	3.06849315	0.32589286	7	7	7	0.77016743
PDK4	3.89497717	0.25674091	3	7	3	0.67833587
COMP	3.10502283	0.32205882	9	6	9	0.76610857
UCP3	3.89954338	0.25644028	2	7	2	0.67782851
MERTK	3.56164384	0.28076923	3	7	3	0.71537291
ITGA4	2.89954338	0.34488189	14	6	14	0.78893962
FYB	3.49315068	0.28627451	3	7	3	0.72298326

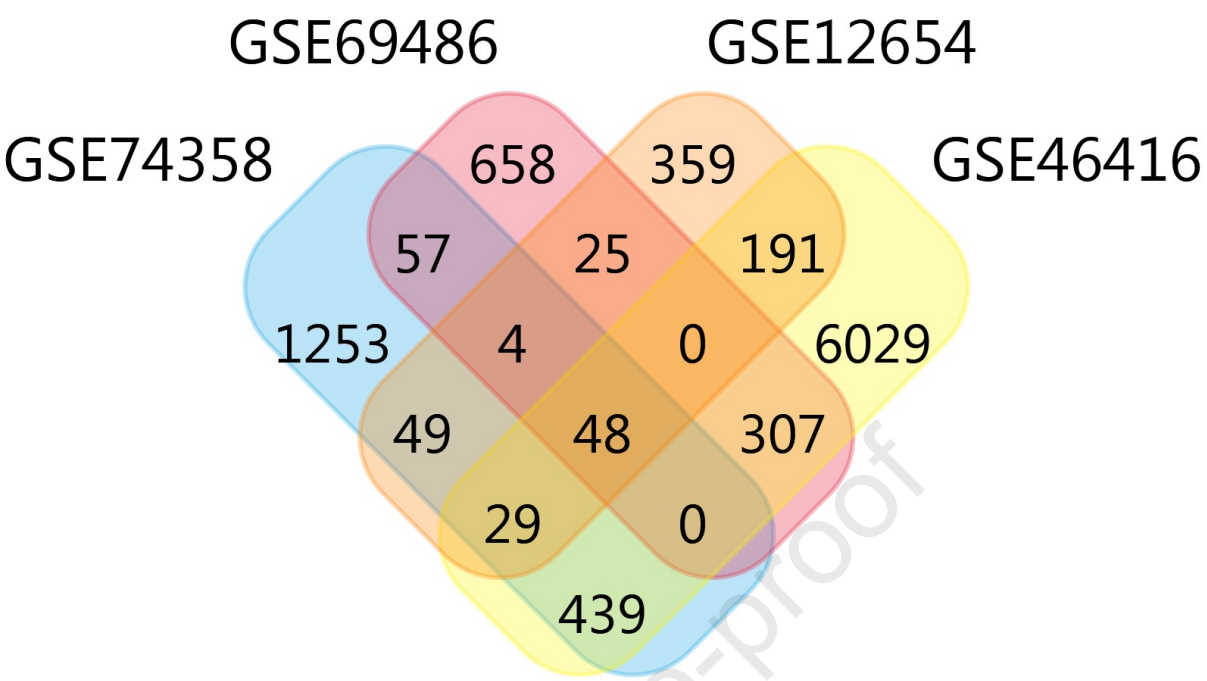


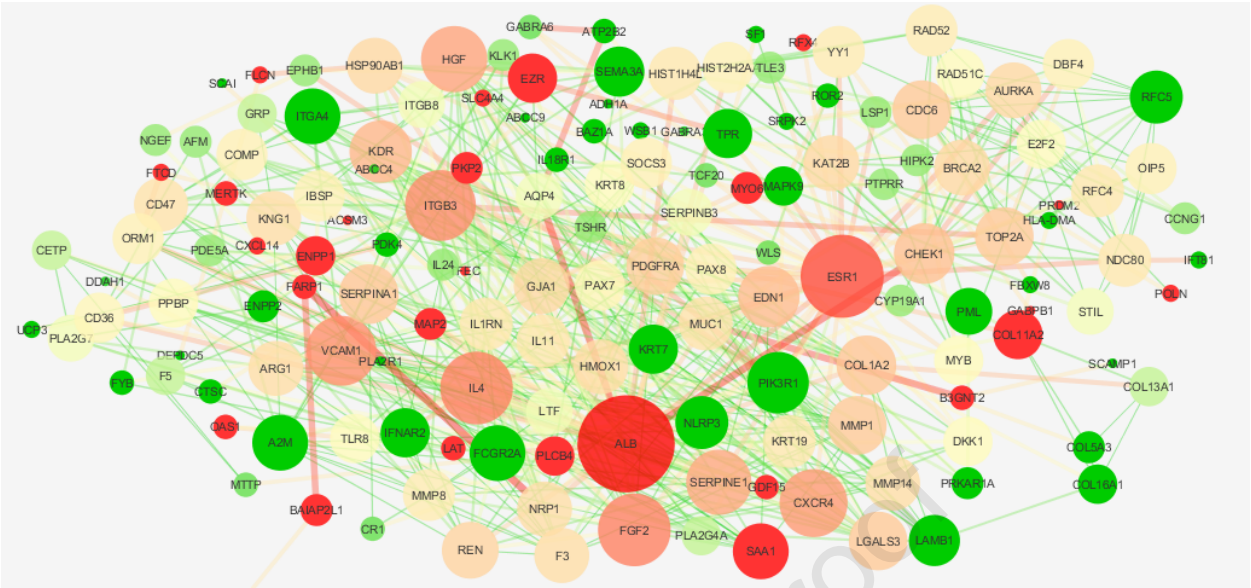


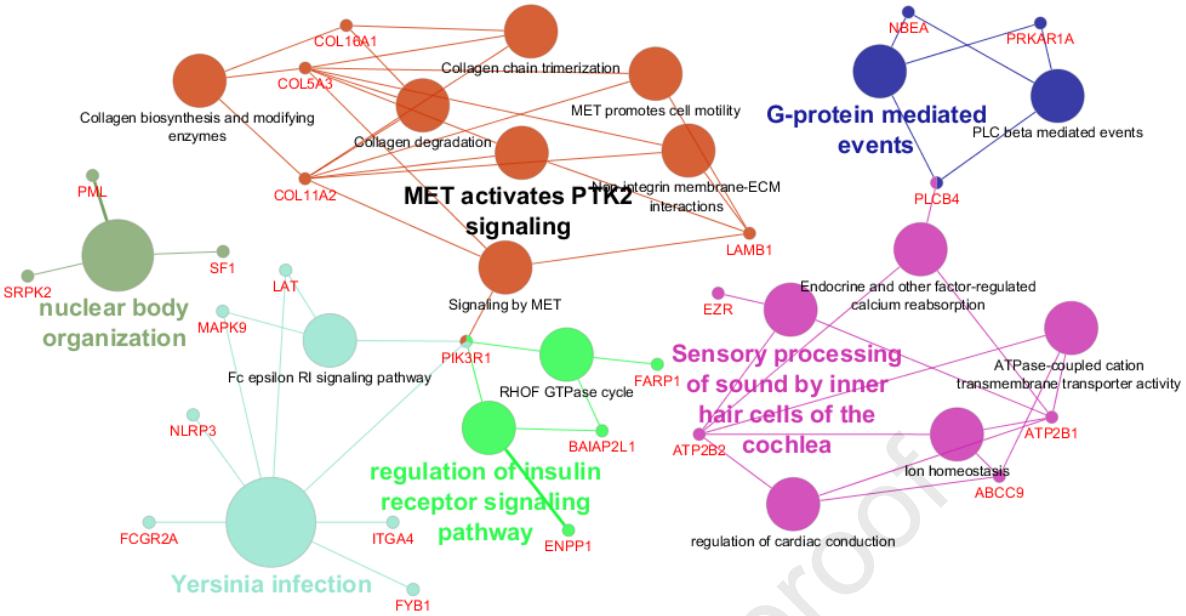


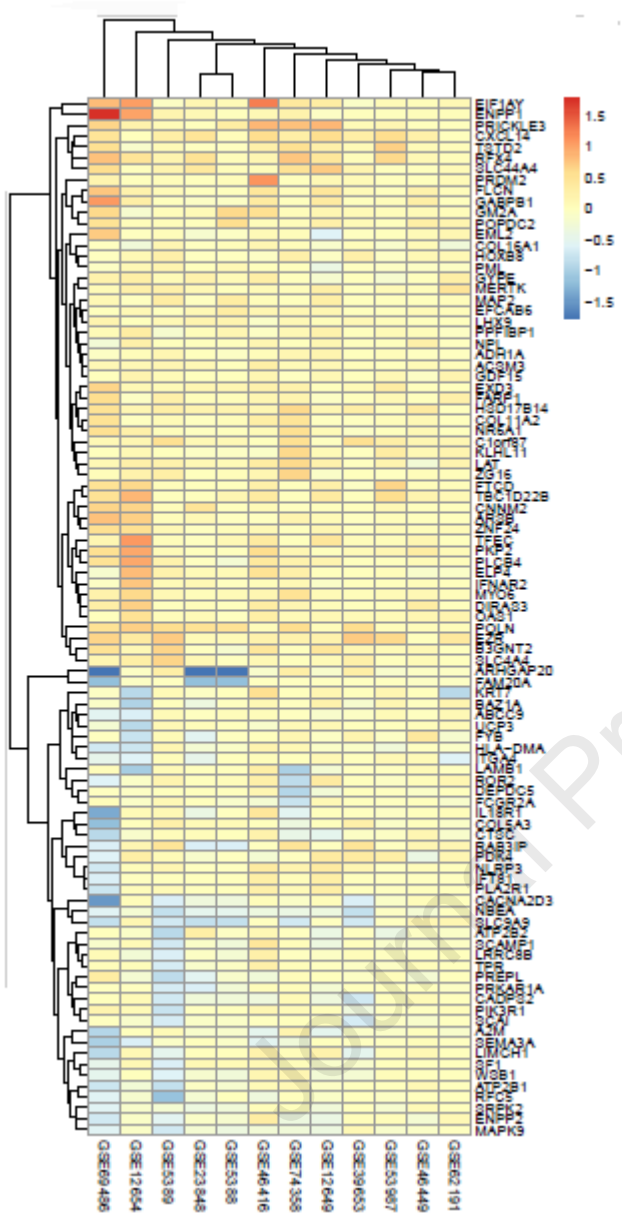


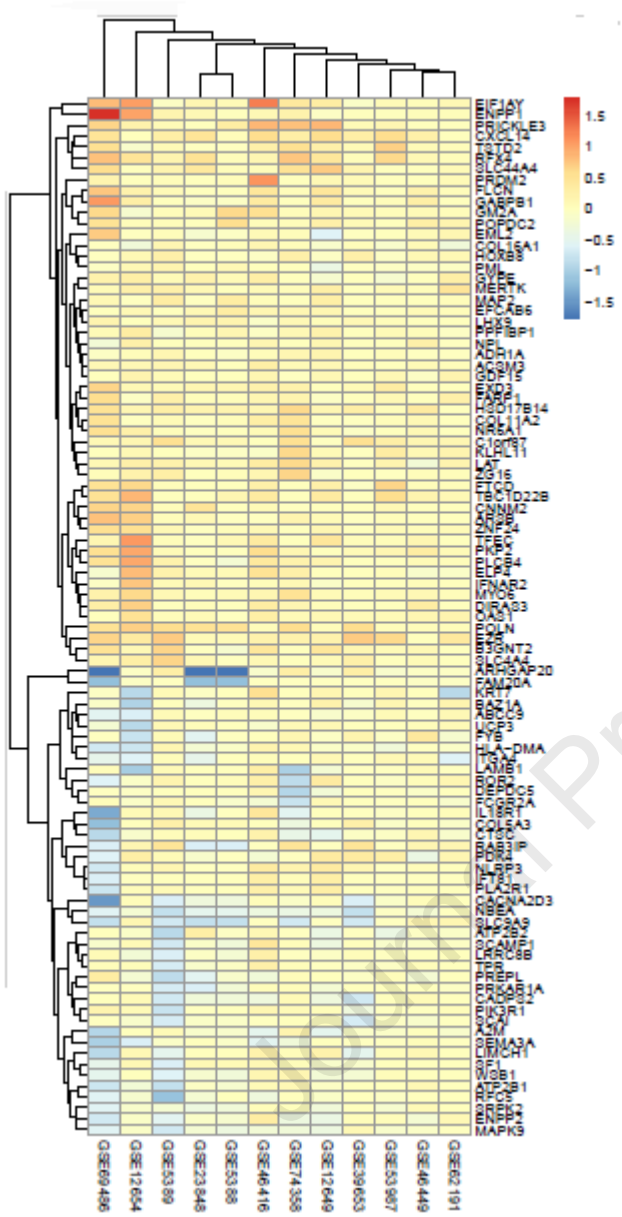


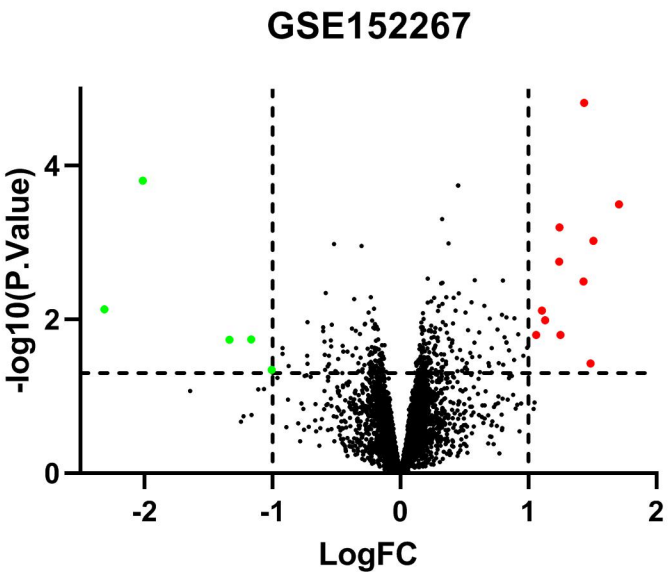


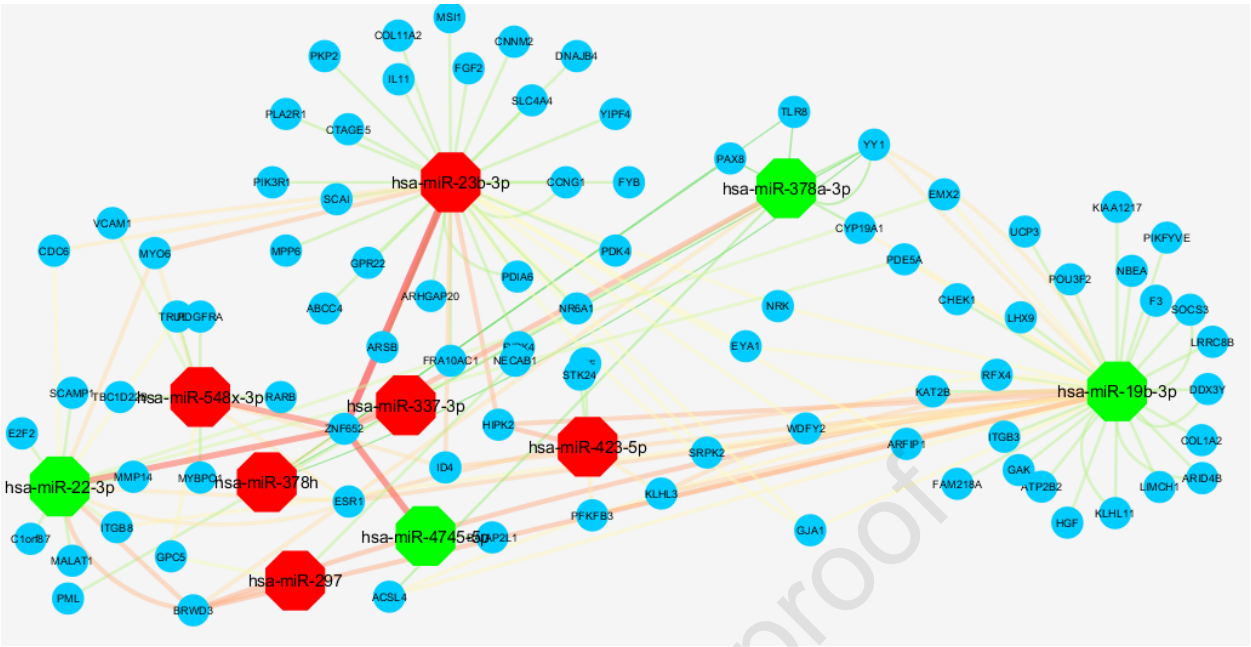


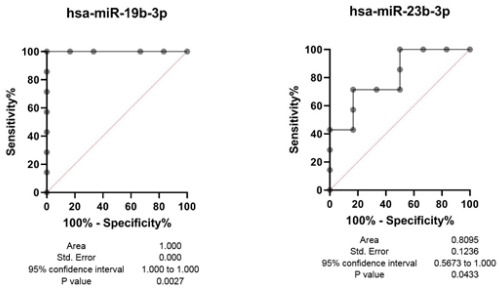


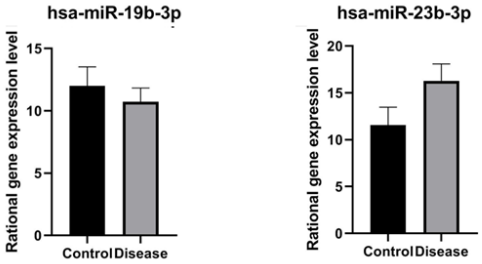












Conflict of interest

Authors have no conflict of interest