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ORIGINAL RESEARCH

Aberrantly expressed long noncoding RNAs and genes in Parkinson's disease

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Purpose: Parkinson's disease (PD) is a common neurodegenerative movement disorder, but the pathogenesis remains elusive. This study was aimed to explore key genes and long noncoding RNAs (lncRNAs) associated with PD.

Materials and methods: Three patients with PD and three normal controls were enrolled in the present study from July 12, 2017, to August 29, 2017. RNA sequencing and bioinformatics analysis were performed to obtain differentially expressed micro RNAs (DEmRNAs) and lncRNAs (DElncRNAs) between patients with PD and normal controls. PD-specific protein–protein interaction networks were constructed. DEmRNAs transcribed within a 100 kb window upstream or downstream of DElncRNAs were searched, which were defined as *cis* nearby targeted DEmRNAs of DElncRNAs. Datasets GSE57475 and GSE68719 were downloaded from the Gene Expression Omnibus database, which were used to validate the expression of selected DEmRNAs.

Results: A total of 857 DEmRNAs and 77 DElncRNAs were obtained between PD and normal controls. Natural killer cell-mediated cytotoxicity was a significantly enriched pathway in PD. ERBB2, HSPB1, and MYC were three hub proteins of PD-specific protein–protein interaction network. LOC105378701-*TAL1*, LOC102724104-*CX3CR1*, LOC105375056-*TREML1*/*TREML4*, LOC105379392-*ANK1*, and LOC101928100-*KLRK1*/*KLRD1* interactions were identified DElncRNA nearby targeted DEmRNA pairs in PD. Gene expression results validated by GSE57475 and GSE68719 were consistent with our RNA-sequencing results, generally.

Conclusion: This present study identified key genes and lncRNAs associated with PD, which will provide new clues for exploring the pathogenesis and developing potential biomarkers of PD.

Keywords: RNA-sequencing, mRNA, bioinformatics analysis, protein–protein interaction network

Introduction

As a common neurodegenerative movement disorder, Parkinson's disease (PD) is characterized by slowness of movement, rigidity, postural instability, and resting tremor.[1] These clinical manifestations were resulted from progressive loss of dopamine producing neurons in the substantia nigra pars compacta and widespread intracellular aggregation of the protein alpha-synuclein, the principal component of the pathological hallmark of PD, Lewy bodies.[2] Despite the increasing efforts for exploring the etiology of PD, the exact pathology of PD was not fully defined.

Long noncoding RNAs (lncRNAs) are non-protein-coding transcripts over 200 nucleotides bases long. Recently, accumulated evidences have emphasized the importance of lncRNAs in brain function and central nervous system (CNS) disorders.[2–5] The functions of lncRNAs span from regulating brain evolution and neural development[3] to mediating behavioral and cognitive processes.[4,5] Moreover, lncRNAs were reported to regulate the transcription of nearby genes with *cis*-regulatory effects. Up to date,

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several lncRNAs such as NEAT1, SNHG1, MAPT-AS1, and HOTAIR have been demonstrated to play roles in PD.[6–9] Yanxia Fan et al reported differentially expressed genes, *ZFAND4*, *SRMS*, *UBL4B*, *PVALB*, *DIRAS1*, *PDP2*, *LRCH1*, and *MYL4* were potential biomarkers associated with progression rate of PD.[10] The study of Jieshan Chi et al suggested that five significantly down-regulated mRNAs (*MAPK8*, *CDC42*, *NDUFS1*, *COX4I1*, and *SDHC*) and three significantly down-regulated miRNAs (miR-126-5p, miR-19-3p, and miR-29a-3p), were potentially useful diagnostic markers in clinic.[11]

In this present study, we identified the differentially expressed lncRNAs (DElncRNAs) and mRNAs (DEmRNAs) between PD and normal controls by RNA sequencing and bioinformatics analysis. Identification of *cis* nearby targeted DEmRNAs of DElncRNAs and functional annotation of DEmRNAs would facilitate the exploration of the biological functions of DElncRNAs in PD. This study will provide new clues for understanding the pathogenesis and developing potential biomarkers of PD.

Materials and methods

Patients and samples

Three patients with PD and three normal controls were enrolled in the present study from July 12, 2017, to August 29, 2017. PD was diagnosed based on MDS clinical diagnostic criteria.[12] Patients with other long-term chronic disease and serious disease were excluded. The details of these patients were as follows: a 54-year-old male with 3 years of slow movement and jitter of left upper limb at Hoehn-Yahr stage 1.5; a 60-year-old male with >4 years of slow movement and progressive hand shaking at Hoehn-Yahr stage 2.5, and a 52-year-old male with >2 years of slow movement and hand shaking at Hoehn-Yahr stage 2.0, respectively. All these patients have no family history of PD. Three normal controls were 65-, 54-, and 53-year old healthy males. All individuals provided signed informed consent for use of their samples in this present study. The present study has been approved by the Ethics Committee of the First People's Hospital of Nantong.

From each participant, a 2.5 mL peripheral whole blood was collected in PAXgene® RNA blood tubes (PreAnalytiX GmbH, Hombrechtikon, Switzerland) and stored at −80°C prior to processing.

RNA isolation and sequencing

With PAXgene blood RNA kit (PreAnalytiX GmbH, Hombrechtikon, Switzerland), RNA isolation was conducted on the manufacturer's protocol. By using Nanodrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA), the concentration and purity of RNA were assessed. The integrity of RNA was assessed via a 2% agarose gel. Agilent 2100 bioanalyzer was used to obtain the RIN values. The criteria for cDNA library construction were as follows: 1) Total RNA >5 μg; 2) concentration of RNA ≥200 ng/mL; 3) OD 260/280 value 1.8–2.2.

Ribosomal RNA was removed with Ribo-Zero Magnetic kit (EpiCentre, Madison, WI, USA). Then, RNA was purified and fragmented into fragments with 140–160 nt. The first cDNA strand was synthesized via RNA fragments primed with random hexamer primers. The second cDNA strand was synthesized with dUTP instead of dTTP. End repair was conducted by using End Repair Enzyme mix (NEB, Ipswich, MA, USA). Subsequently, 3′ end adenylation and adapter ligation were performed. After digesting the second cDNA strand with UNG enzyme (Illumina, Inc., San Diego, CA, USA), PCR was performed for 15 cycles to amplify the libraries. Purification and recovery of libraries were performed by using commercial magnetic beads. Sequencing was performed on the Illumina Hiseq X-ten platform (Illumina, Inc.).

Quality control of raw sequence and mapping of clean reads

By using Base Calling version 0.11.4 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/),[35] the FASTQ sequence data were obtained from the RNA-sequencing data. Reads with low quality (adaptor sequences, sequences with a quality score <20, and sequences with an N base rate of raw reads >10%) were removed with Cutadapt version 1.9.1 (https://cutadapt.readthedocs.io/en/stable/)[36] to obtain the clean reads.

Identification of DEmRNAs and DElncRNAs in PD compared with normal controls

TopHat release 2.2.1 (http://tophat.cbcb.umd.edu/)[37] was used to align the clean reads with the human reference genome, Ensemble GRCh38.p7 (ftp://ftp.ncbi.nlm.nih.gov/genomes/Homo_sapiens).[38] With Cuffquant version 2.2.1 (http://cufflinks.cbcb.umd.edu/),[39] expressions of mRNAs and lncRNAs were normalized and outputted. Fragments per Kilobase of exon per million fragments mapped (FPKM) was used to determine the transcription abundance of lncRNAs and mRNAs. With Cuffdiff version 2.2.1 (http://cufflinks.cbcb.umd.edu/),[39] FPKM of lncRNAs and mRNAs were

calculated. Both DEmRNAs and DElncRNAs were obtained with DESeq2 (http://bioconductor.org/packages/DESeq2/)[40] in R version 3.3.3 with *P*-value <0.05. Hierarchical clustering analyses of DElncRNAs and DEmRNAs were conducted by using R package "pheatmap".

Functional annotation of DEmRNAs between PD and normal control

Functional annotation, including Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of the DEmRNAs between PD and normal control, was performed using the GeneCoDis3 tool (http://genecodis.cnb.csic.es/analysis).[41] False discovery rate (FDR) <0.05 was set as the cutoff for significance.

Protein–protein interaction (PPI) network construction

With the Biological General Repository for Interaction Datasets (BioGrid, http://www.uniprot.org/database/DB-0184),[42] top 100 up- and downregulated DEmRNAs were scanned. PPI network was then constructed using Cytoscape software (version 3.3.0, http://www.cytoscape.org)[43] in order to further explore the biological functions of the DEmRNAs.

Cis nearby targeted DEmRNAs of the DElncRNAs

To obtain the targeted DEmRNAs of DElncRNAs with *cis*-regulatory effects, DEmRNAs transcribed within a 100 kb window upstream or downstream of DElncRNAs were searched, which were defined as *cis* nearby targeted DEmRNAs of DElncRNAs.

Validation in the GEO dataset

GSE57475 and GSE68719 datasets were downloaded from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/),[44] which consisted of 49 patients with PD and 93 normal controls, and 29 patients with PD and 44 normal controls, respectively. The dataset GSE57475 examined the blood sample and GSE68719 examined brain tissue, which were all from USA. The expression patterns of selected DEmRNAs were validated with GSE57475 and GSE68719 datasets.

Results

RNA-sequencing data

Total RNA isolated from each blood sample met the criteria for cDNA library construction and RNA sequencing. After trimming of the raw reads, 6.7×10^7 clean reads were obtained from each blood sample from patients with PD and normal controls. The mapping ratio was calculated following clean reads of each sample aligned to the human reference genome (GRCh38.p7). Mapping ratio of each sample was >84%.

DEmRNAs and DElncRNAs between PD and normal controls

A total of 857 DEmRNAs (304 upregulated and 553 downregulated DEmRNAs) and 77 DElncRNAs (38 upregulated and 39 downregulated DElncRNAs) between PD and normal controls were identified. The top ten up- and downregulated DElncRNAs and DEmRNAs between PD and normal controls are summarized in Tables 1 and 2, respectively. Hierarchical clustering analysis of DElncRNAs and top 100 DEmRNAs is displayed in Figure 1A and B, respectively. Furthermore, all these DElncRNAs were distributed in all chromosomes (chr.), with the exception of chr.15 and chr.16, and DEmRNAs were widely distributed in all chromosomes (Figure 1C).

Functional annotation

Blood coagulation (FDR =4.11E–11), platelet activation (FDR =2.55E–09), plasma membrane (FDR =1.48E–11),

Table 1 Top ten up- and downregulated DElncRNAs between PD and normal controls

DElncRNAs	Log2 fold change	*P*-value	Regulation
TM4SF19-TCTEX1D2	1.91E+00	7.41E–07	Up
LOC101927369	1.21E+00	1.37E–04	Up
LOC102724104	1.04E+00	1.60E–04	Up
LINC01871	1.11E+00	1.79E–04	Up
LOC105373420	1.25E+00	9.26E–04	Up
LOC105371464	7.73E–01	1.94E–03	Up
LINC00943	9.86E–01	3.50E–03	Up
LOC105370060	1.09E+00	4.41E–03	Up
LOC101927012	9.74E–01	5.26E–03	Up
LOC105372055	9.94E–01	5.88E–03	Up
LOC102724765	−1.07E+00	1.38E–04	Down
LOC105369772	−1.34E+00	4.76E–04	Down
KRT73-AS1	−1.25E+00	5.00E–04	Down
LOC105379392	−9.44E–01	6.04E–04	Down
JHDM1D-AS1	−8.08E–01	1.96E–03	Down
LOC105372185	−1.03E+00	2.02E–03	Down
LOC105377225	−7.01E–01	2.02E–03	Down
LOC105378701	−1.18E+00	2.15E–03	Down
LOC105375056	−1.12E+00	2.34E–03	Down
LOC105373204	−7.02E–01	3.74E–03	Down

Abbreviations: DElncRNAs, differentially expressed long noncoding RNAs; PD, Parkinson's disease.

Table 2 Top ten up- and downregulated DEmRNAs between PD and normal controls

DEmRNAs	Log2 fold change	*P*-value	Regulation
NINL	1.67E+00	6.23E–13	Up
GZMB	1.28E+00	6.70E–11	Up
COL6A2	1.23E+00	1.48E–09	Up
GZMH	1.19E+00	4.64E–09	Up
ERBB2	1.12E+00	5.98E–09	Up
FGFBP2	1.13E+00	1.71E–08	Up
GNLY	1.10E+00	6.61E–08	Up
C1orf21	9.67E–01	7.48E–08	Up
APOBEC3B	1.23E+00	9.63E–08	Up
PRSS23	1.09E+00	1.07E–07	Up
LRRN3	–1.49E+00	4.27E–14	Down
KRT73	–1.72E+00	1.11E–13	Down
MYL4	–1.29E+00	3.20E–13	Down
FKBP8	–1.05E+00	5.03E–11	Down
KIAA1324	–1.43E+00	3.18E–10	Down
XK	–1.08E+00	2.81E–08	Down
GNG11	–1.13E+00	3.12E–08	Down
TMEM158	–1.19E+00	4.10E–07	Down
IL6ST	–6.84E–01	5.64E–07	Down
LOC102723750	–9.81E–01	8.30E–07	Down

Abbreviations: DEmRNAs, differentially expressed micro RNAs; PD, Parkinson's disease.

protein binding (FDR =2.40E–30), and cytoplasm (FDR =2.54E–36) are significantly enriched GO terms in PD (Figure 2A–C). Natural killer cell-mediated cytotoxicity (FDR =2.36E–08), pathways in cancer (FDR =6.82E–06), and protein processing in endoplasmic reticulum (FDR =1.54E–05) are three significantly enriched KEGG pathways in PD (Figure 2D).

PPI network

The PD-specific PPI network was consisted of 186 nodes and 194 edges. ERBB2 (degree =13), HSPB1 (degree =13), and MYC (degree =11) were three hub proteins of PD-specific PPI network (Figure 3).

Cis nearby targeted DEmRNAs of DElncRNAs

A total of 39 DElncRNAs nearby targeted DEmRNA pairs were obtained which was consisted of 28 DElncRNAs and 36 DEmRNAs. LOC105378701-*TAL1*, LOC102724104-*CX3CR1*, LOC105375056-*TREML1*/*TREML4*, LOC105379392-*ANK1*, and LOC101928100-*KLRK1*/*KLRD1* interactions were identified DElncRNAs nearby targeted DEmRNA pairs in PD (Table 3).

Validation in the GEO dataset

The expression patterns of six DEmRNAs including TAL bHLH transcription factor 1 (*TAL1*), triggering receptor expressed on myeloid cells like 1 (*TREML1*), triggering receptor expressed on myeloid cells like 4 (*TREML4*), erb-b2 receptor tyrosine kinase 2 (*ERBB2*), chemokine (C-X3-C) receptor 1 (*CX3CR1*) and ankyrin 1 (*ANK1*) were verified using GSE57475 dataset. As shown in Figure 4, *TAL1*, *TREML1*, *TREML4*, and *ANK1* were downregulated while *ERBB2* and *CX3CR1* were upregulated in PD compared with normal controls, which were consistent with our RNA-sequencing results (Figure 4).

In GSE68719 dataset, the expression patterns of *ERBB2*, *CX3CR1*, and *ANK1* were similar to that in GSE57475, while for *TAL1* and *TREML1*, it displayed the opposite. *TREML4* was not found in GSE68719. The result may be due to the difference of the tissue types between GSE57475 and GSE68719. We further determined the expression of killer cell lectin like receptor D1 (*KLRD1*), a DEmRNA between PD and normal controls, and found that its expression status was consistent with our RNA-sequencing results (Figure 5).

Discussion

Increasing evidences have indicated that lncRNAs play important roles in the pathogenesis of PD.[6–9] This present study identified abundant lncRNAs that were differentially expressed between PD and normal controls.

JHDM1D antisense 1 (JHDM1D-AS1) was an lncRNA that arises from the antisense strand of JHDM1D and was downregulated in PD in this present study. In in vitro and in vivo experiments, JHDM1D-AS1 was found to be upregulated in cancer cells and tumor tissues under nutrient starvation, which promotes tumorigenesis by upregulating angiogenesis and triggering inflammation.[13] Accumulated evidence indicated that neuroinflammation plays key roles in the pathogenesis of neurodegenerative diseases.[14,15] Angiogenesis has been found in various neurodegenerative diseases such as Alzheimer's disease (AD)[16] and PD[1] which was speculated to make a contribution for neuroinflammation by failing to protect the parenchyma from peripheral immune cells and inflammatory or toxic factors in the peripheral circulation.[1] Hence, we made a hypothesis that JHDM1D-AS1 might be involved with the process of PD by regulating angiogenesis and neuroinflammation. Further experiments are needed to explore the precise role of JHDM1D-AS1 in PD.

However, the biological functions of most identified DElncRNAs between PD and normal control remain unclear.

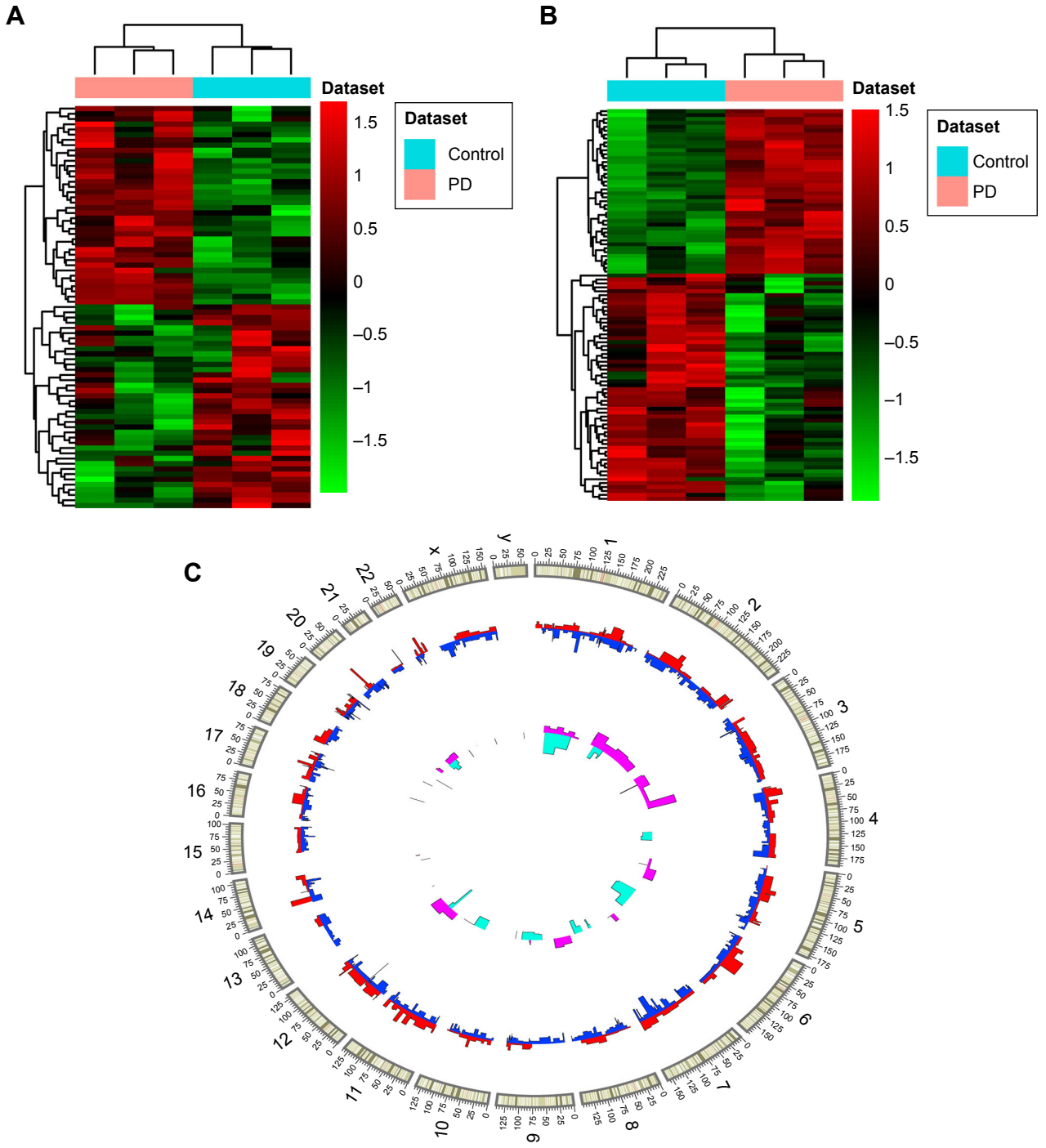

Figure 1 DElncRNAs and DEmRNAs between PD and normal controls.

Notes: (**A**, **B**) Hierarchical clustering results of DElncRNAs and top 100 DEmRNAs between PD and normal controls, respectively. Row and column represent DElncRNAs/DEmRNAs and tissue samples, respectively. The color scale represents the expression levels. (**C**) Distribution of DElncRNAs and DEmRNAs on chromosomes. The outer layer cycle was the chromosome map of the human genome hg19 (GRCh37). The red and blue inner layer represents the distribution of up- and downregulated DEmRNAs on different chromosomes, respectively. The pink and light blue inner layer represents the distribution of up- and downregulated DElncRNAs on different chromosome, respectively.

Abbreviations: DElncRNAs, differentially expressed long noncoding RNAs; DEmRNAs, differentially expressed micro RNAs; PD, Parkinson's disease.

Previous studies indicated that lncRNAs might regulate the expression of their nearby genes by *cis*-regulatory effects.[17] To further research the functions of DElncRNAs in PD, we searched the DEmRNAs transcribed within a 100 kb window upstream or downstream of DElncRNAs which served as *cis* nearby targeted DEmRNAs of DElncRNAs.

In this present study, downregulated *TAL1* was found in PD compared with normal controls, and interacted with

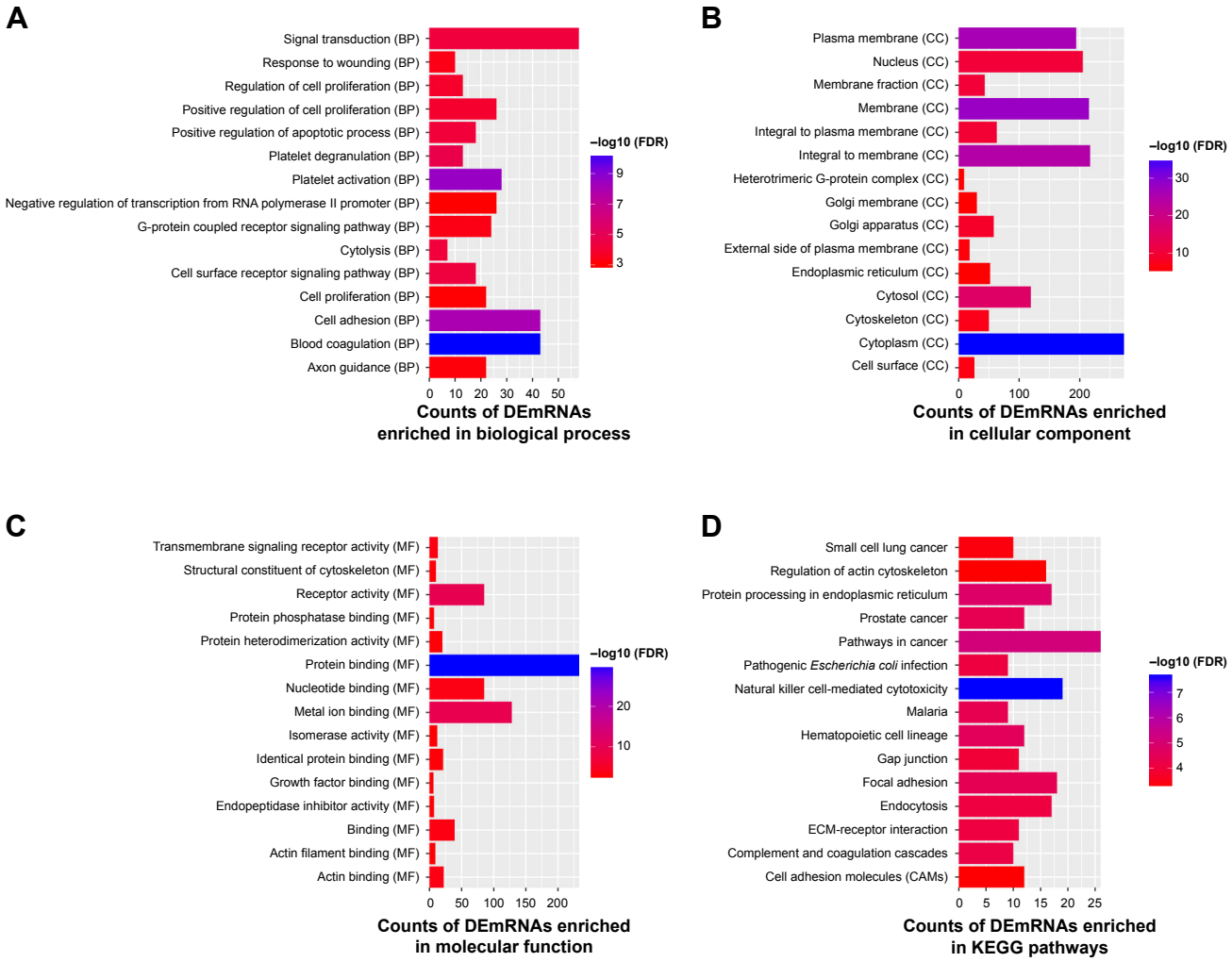

Figure 2 Significantly enriched GO terms and KEGG pathways of DEmRNAs between PD and normal controls.
Notes: (**A**) BP, (**B**) CC, (**C**) MF, and (**D**) KEGG pathways. The x-axis shows counts of DEmRNAs enriched in GO terms or KEGG pathways and the y-axis shows GO terms or KEGG pathways. The color scale represented –log FDR.
Abbreviations: BP, biological process; CC, cellular component; DEmRNAs, differentially expressed mRNAs; FDR, false discovery rate; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular function.

seven DEmRNAs of PD. Microglia is essential for innate neuroimmune function and CNS homeostasis, and plays crucial roles in neurodegeneration and brain aging.[18] *TAL1* is a transcription factor that involves with microglial aging.[19] Hence, *TAL1* was speculated to play key roles in initiation of PD by regulating many key DEmRNAs between PD and normal controls.[20] Additionally, TAL1 was identified to be a nearby targeted DEmRNA of LOC105378701, which suggested that LOC105378701 might involve with PD by regulating the expression of *TAL1* with *cis* effect.

CX3CR1 is a specific receptor of fractalkine (also called *CX3CL1*) that is exclusively expressed in microglia in the CNS.[21] Fractalkine/*CX3CR1* signaling plays an inhibitory role in control of microglial inflammatory response.[22] Knockout of *CX3CR1* was found to exacerbate inflammation and neurodegeneration in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxin model of PD.[23] Sun et al demonstrated for the first time that *CX3CR1* is involved in the neuroinflammatory process in 1-methyl-4-phenylpyridiniumion (MPP+) rat model of PD.[24] Both the RNA-sequencing results and our gene expression validation results found that *CX3CR1* was upregulated in PD compared with normal control, which emphasized the importance of CX3CR1 in PD. Further research is needed to explore its precise role in PD. Moreover, *CX3CR1* was a nearby targeted gene of LOC102724104, which suggested that LOC102724104 might be a potential regulator of PD by regulating *CX3CR1*.

ERBB2, also known as human epidermal growth factor receptor 2 (*HER2*), a receptor tyrosine kinase, was originally identified based on its role in cancer research. Wang et al first

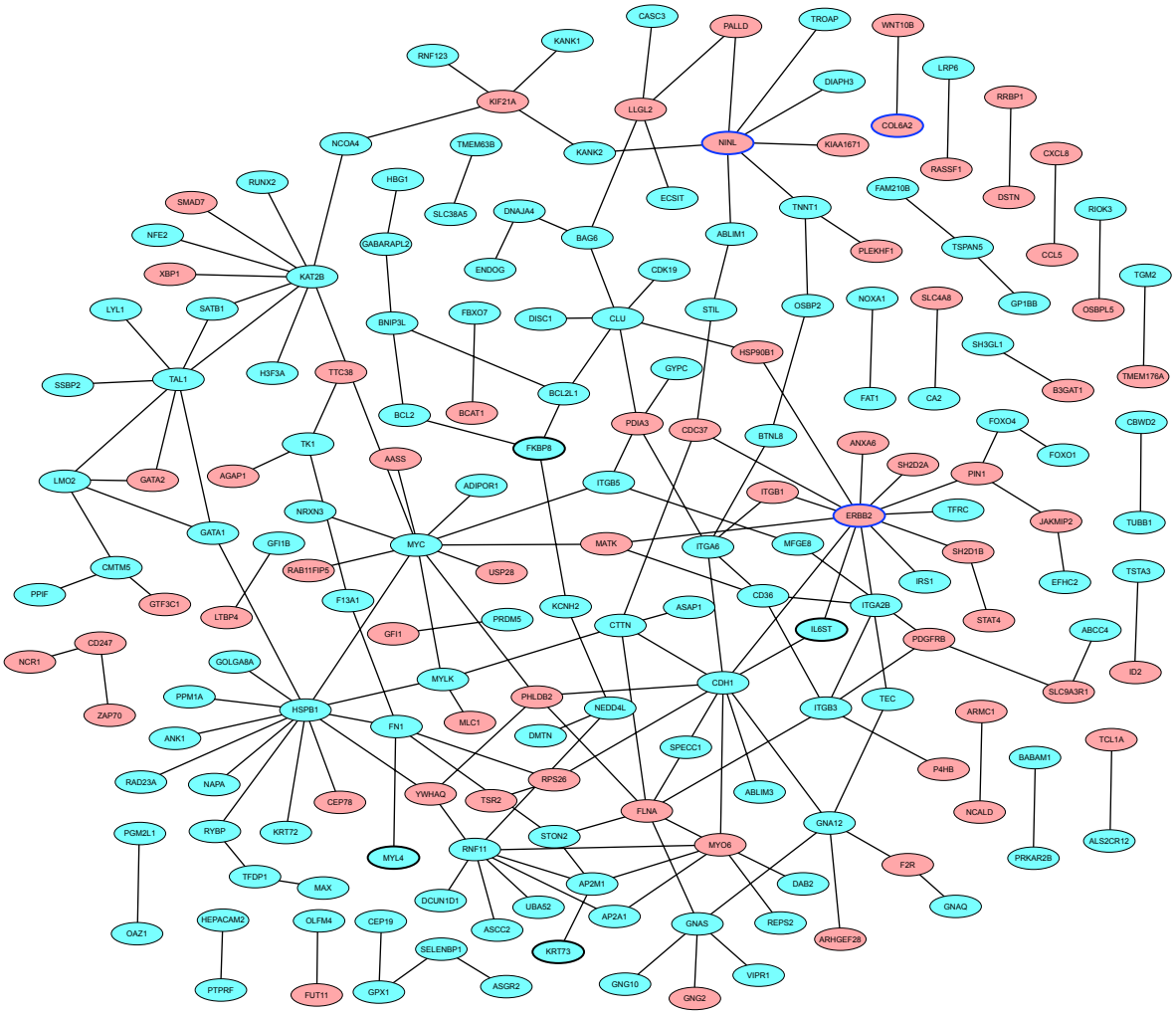

Figure 3 PD-specific PPI network.
Notes: The red and blue ellipses represent proteins encoded by up- and downregulated DEmRNAs between PD and normal control. Ellipses with black and blue border are DEmRNAs derived from top ten down- and upregulated DEmRNAs between PD and normal control.
Abbreviations: DEmRNAs, differentially expressed mRNAs; PD, Parkinson's disease; PPI, protein–protein interaction.

suggested a link between PD and *HER2* polymorphism, and they indicated that different signals or potency of the kinase activities resulting from the Ala1170Pro allele of *HER2* may be associated with vulnerability to stress on dopaminergic neurons in PD.[25] In this present study, *ERBB2* was a hub protein of PD-specific PPI network. Moreover, *ERBB2* was a nearby targeted gene of LOC105372578, which suggested that LOC105372578 might be a potential regulator of PD by regulating *ERBB2*.

AD is also a type of neurodegenerative disease. We identified three AD-regulated genes that were differentially expressed between PD and normal control as well.

ANK1 is a known susceptibility gene for type 2 diabetes which was recognized to own some similarities with AD.[26] Aberrant methylation and expression of *ANK1* were found in AD.[27,28] Cortical-specific hypermethylation of *ANK1* was robustly associated with AD-related neuropathology.[28] Lunnon et al speculated that the brain-expressed *ANK1* protein could be associated with pathology of AD by its function on compartmentalization of the plasma membrane.[28]

The triggering receptors expressed on myeloid (TREM) family is known to play a key role in modulating inflammation in the innate immune response.[29] Both *TREML1* and *TREML4* were reported to be plausible risk genes of AD.[30] Reliable expression of *TREML1* was found in both cerebellum and temporal cortex of brain.[30] Variant of *TREM1* (rs6910730) was associated with increased AD pathology burden and increased rate of cognitive decline, independently.[31] Moreover, *TREML1* was reported to be involved with promoting vascular homeostasis and neuroinflammation that was speculated to be a potential mediator

Table 3 Nearby targeted DEmRNAs of DElncRNAs between PD and normal controls

DElncRNAs			Nearby targeted DEmRNAs		
Symbol	**Start-100 kb**	**End +100 kb**	**Symbol**	**Start**	**End**
CTD-2201118.1	79891295	80183665	SERINC5	80111226	80256082
EPHA1-AS1	143253399	143623449	EPHA1	143390813	143408892
EPHA1-AS1	143253399	143623449	FAM131B	143353399	143382304
HCG11	26386648	26627393	BTN3A1	26402237	26415216
HCG11	26386648	26627393	BTN3A2	26365158	26453415
KRT73-AS1	52485588	52720133	KRT1	52674735	52680407
KRT73-AS1	52485588	52720133	KRT72	52585588	52602900
KRT73-AS1	52485588	52720133	KRT73	52585588	52620133
LEF1-AS1	107947544	108276430	LEF1	108047544	108176430
LOC101927012	147459993	147882848	JAKMIP2	147559993	147782848
LOC101927369	35997885	36256994	CCL4	36097885	36156994
LOC101928100	10263768	10510146	KLRD1	10238384	10329607
LOC101928100	10263768	10510146	KLRC4	10407384	10409757
LOC101928100	10263768	10510146	KLRK1	10372353	10390054
LOC101929866	45078476	45291638	PI3	45174898	45176544
LOC101929866	45078476	45291638	SLPI	45230820	45290352
LOC102724104	39132519	39363406	CX3CR1	39263493	39281735
LOC105369772	52525675	52730770	KRT1	52674735	52680407
LOC105369772	52525675	52730770	KRT72	52585588	52602900
LOC105369772	52525675	52730770	KRT73	52585588	52620133
LOC105370556	70420816	70664742	TTC9	70641786	70675360
LOC105371464	159700480	159916257	FCRL6	159800480	159816257
LOC105372491	560703	796189	SRXN1	646614	658840
LOC105372578	24819978	25032985	CST7	24949229	24959928
LOC105372716	62683185	62894281	COL9A3	62817061	62937952
LOC105372881	207265821	207473252	CD55	207321471	207360966
LOC105373943	235394088	236231800	AGAP1	235494088	236131800
LOC105374771	64290955	64525399	LGALSL	64454192	64461383
LOC105375056	41049096	41278568	TREML4	41228291	41239386
LOC105375056	41049096	41278568	TREML1	41149096	41178568
LOC105375796	143451962	143663062	TSTA3	143612617	143618043
LINC02084	27612180	27814006	EOMES	27715948	27722715
LOC105377110	58950175	61351474	FHIT	59050175	61251474
LOC105378678	41328211	41564911	HIVEP3	41506364	42035925
LOC105378701	47072216	47277080	STIL	47250138	47314787
LOC105378701	47072216	47277080	TAL1	47216289	47232389
LOC105379392	41553224	41996762	ANK1	41653224	41896762
SIRPG-AS1	1529151	1786516	SIRPG	1629151	1686516
TMEM9B-AS1	8847200	9076283	AKIP1	8911116	8933006

Abbreviations: DElncRNAs, differentially expressed long noncoding RNAs; DEmRNAs, differentially expressed micro RNAs; PD, Parkinson's disease.

of neuronal protection and injury in AD and possibly other CNS diseases.[30]

To the best of our knowledge, we first found the down-regulation of *ANK1*, *TREML1*, and *TREML4* in PD compared with normal controls, which might involve with PD as well. Both *TREML1* and *TREML4* were nearby targeted genes of LOC105375056, and ANK1 was a nearby targeted gene of LOC105379392, which suggested that LOC105375056 and LOC105379392 and their interactions with *TREML1*/*TREML4* or *ANK1* might implicate in PD.

In addition, several studies of the familial PD genes had emphasized the importance of RNA metabolism, particularly mRNA translation, in the disease process. Bingwei Lu et al had indicated that several familial PD genes, including *LRRK2*, *PINK1*, *Parkin*, and *eIF4G1*, have been shown to interact with components of the translation initiation machinery or interact with modulators of the translation initiation process, such as miRNAs and the mTORC1 signaling pathway.[32–34] In the future, the research on RNA metabolism in PD needs to be carried more.

According to the KEGG enrichment analysis, natural killer cell-mediated cytotoxicity was a significantly enriched pathway in PD. *KLRD1* and killer cell lectin like receptor K1 (*KLRK1*) were the two DEmRNAs enriched in the pathway of natural

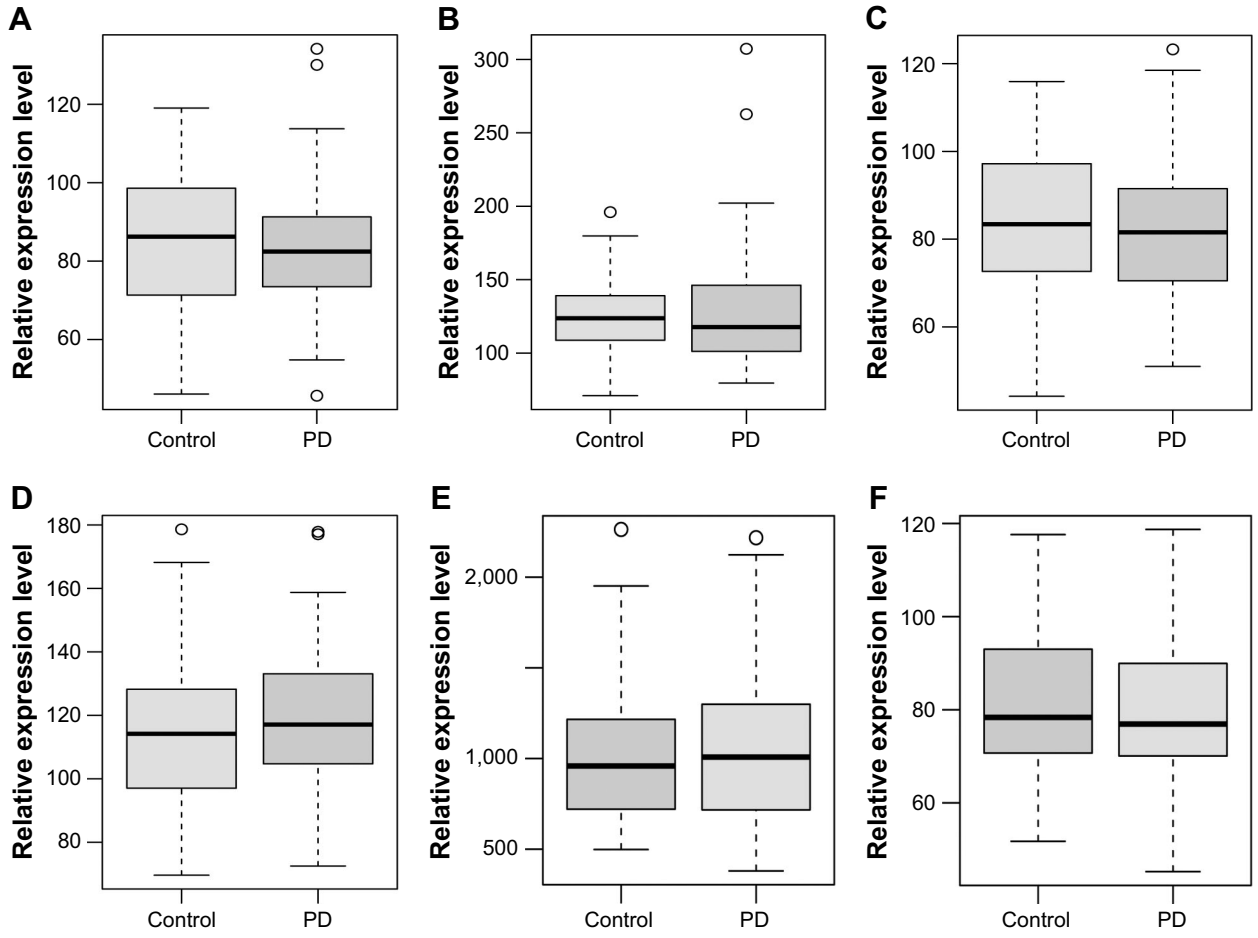

Figure 4 Validation of selected DEmRNAs in GSE57475.
Notes: The x-axis shows PD and normal control groups and the y-axis shows relative expression levels. (**A**) TAL1; (**B**) TREML1; (**C**) TREML4; (**D**) ERBB2; (**E**) CX3CR1; (**F**) ANK1. The circles represent outliers.
Abbreviations: DEmRNAs, differentially expressed micro RNAs; PD, Parkinson's disease.

killer cell-mediated cytotoxicity, and *KLRD1* and *KLRK1* were also the nearby targeted DEmRNAs of LOC101928100. These findings suggested the potential roles of LOC101928100 and its interactions with *KLRK1/KLRD1* in PD.

Conclusion

Our data showed that an abundant of novel DElncRNAs may be associated with the pathology of PD. lncRNA interaction pairs of JHDM1D-AS1, LOC105378701-*TAL1*,

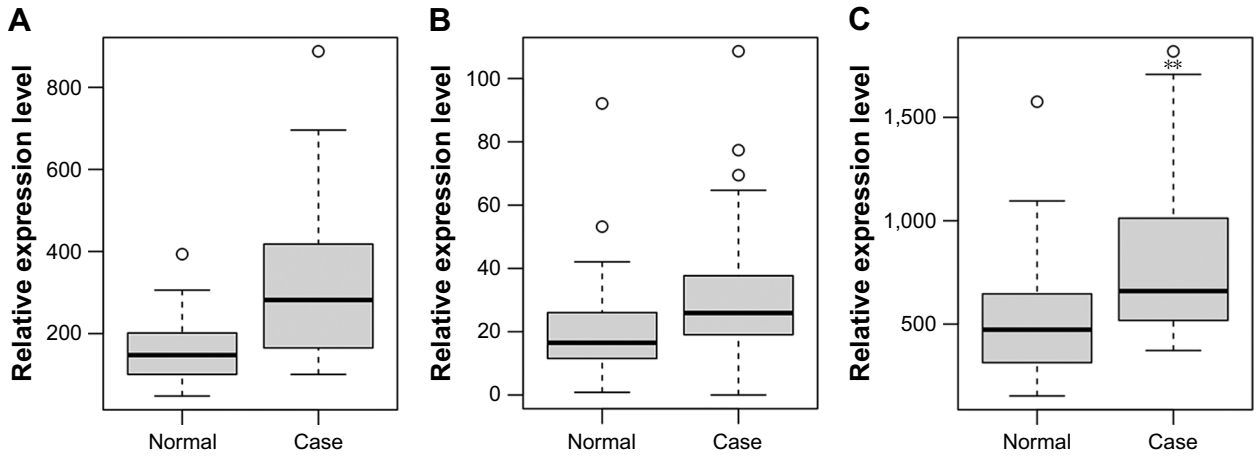

Figure 5 (*Continued*)

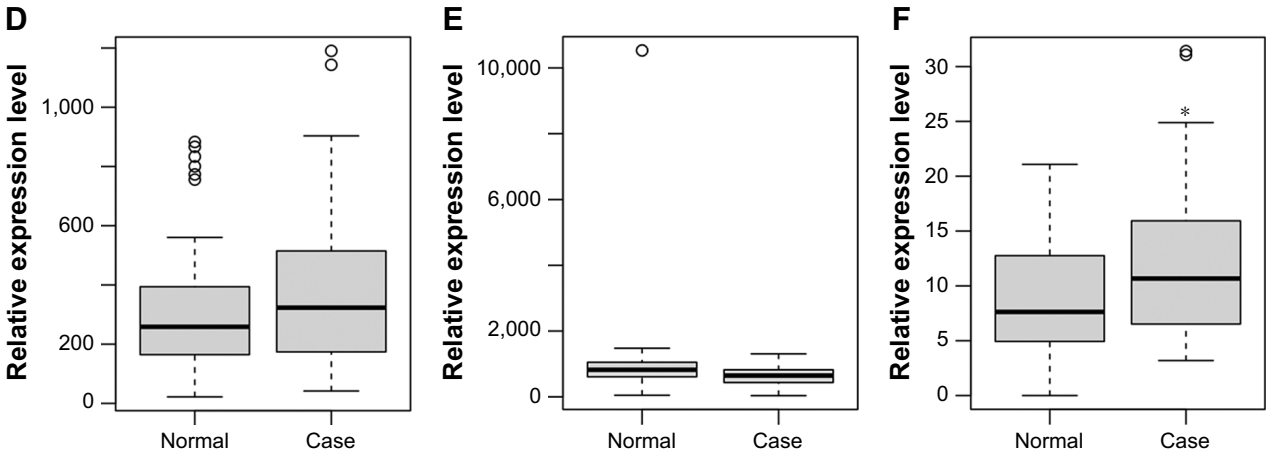

Figure 5 Validation of selected DEmRNAs in GSE68719.
Notes: The x-axis shows PD and normal control groups and the y-axis shows relative expression levels. (**A**) TAL1; (**B**) TREML1; (**C**) ERBB2; (**D**) CX3CR1; (**E**) ANK1; (**F**) KLRD1. The circles represent outliers. *$P < 0.05$ and **$P < 0.01$.
Abbreviations: DEmRNAs, differentially expressed micro RNAs; PD, Parkinson's disease.

LOC102724104-*CX3CR1*, LOC105375056-*TREML1*/*TREML4*, LOC105379392-*ANK1*, and LOC101928100-*KLRK1*/*KLRD1* might involve with PD. Their detailed roles in PD need to be clarified in our future work.

Limitations

The sample size for RNA sequencing in this study was small. Although the validation based on GSE57475 and GSE68719 suggested that our RNA-sequencing results were generally convincing, studies with larger sample size are needed to confirm this conclusion.

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Disclosure

The authors report no conflicts of interest in this work.

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