Common molecular biomarker signatures in blood and brain of Alzheimer's disease

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Abstract

Background: The Alzheimer's is a progressive neurodegenerative disease of elderly peoples characterized by dementia and the fatality is increased due to lack of early stage detection. The neuroimaging and cerebrospinal fluid based detection is limited with sensitivity and specificity and cost. Therefore, detecting AD from blood transcriptsthat mirror the expression of brain transcripts in the AD would be one way to improve the diagnosis and treatment of AD. The present study aimed to identify blood transcript that mirror the expression in brain of the AD. *Materials and Methods:* We used blood (GSE18309) and brain (GSE4757) based microarray gene expression datasets from NCBI-GEO. *Result:* We analyzed each datasets, and identified 9 common (CNBD1, SUCLG2-AS1, CCDC65, PDE4D, MTMR1, C3, SLC6A15, LINC01806, and FRG1JP) DEGs from blood and brain. Then we identified 18 significant genes (HSD17B1, GAS5, RPS5, VKORC1, GLE1, WDR1, RPL12, MORN1, RAD52, SDR39U1, NPHP4, MT1E, SORD, LINC00638, MCM3AP-AS1, GSDMD, RPS9, and GNL2**)** from eQTL data of blood and brain. The significant neurodegeneration associated molecular signaling pathways (Ribosome and complement pathways) were identified. We integrated the different omics data and revealed transcription factor (TFs) (SREBF2, NR1H2, NR1H3, PRDM1, XBP1), microRNAs (miRNAs) (miR-518e, miR-518a-3p, miR-518b, miR-518c, miR-518d-3p, miR-518f). Several histone modifications sites of the DEGs were identified. *Conclusion:* In sum, we identified new putative links between pathological processes in brain and blood cells. Thus, our formulated methodologies demonstrate the power of gene and gene expression analysis for brain-related pathologies transcriptomics, cis-eQTL, and epigentics data from brain and blood cells.

Keywords: Alzheimer's disease; biomarkers; blood-brain common gene; differentially expressed genes; protein-protein interaction; epigenetics.

1. Introduction

The Alzheimer's disease (AD) is the progressive neurodegenerative disease of elderly with cognitive impairment function. Now, 5.5 million peoples are affected with AD and will rise to 13.8 million by 2050 in USA [1]. The presence of extracellular amyloid plaques and intracellular neurofibrillary tangles in brain is characterized as the hallmarks of pathobiology of the AD [1]. Despite many factors are implicated in the pathogenesis of AD, the application of molecular mechanisms in the diagnosis of the AD is still unclear. Thus quest of peripheral biomarkers in the AD is an unmet challenge [2,3]. Therefore, studies focused on the molecular biomarkers should have high impact on the AD diagnosis and treatment [4].

The positron emission tomography (PET) based neuroimaging techniques and cerebrospinal fluids (CSF) are used in everyday clinical practice to diagnose dementia [5–7], but these procedures suffer with limitation as invasiveness of collecting CSF, sensitivity, specificity, and cost and limited access of neuroimaging studies [8]. Considering these shortcomings of the available clinical practice of detection of neurodegeneration, many studies attempted to explore biomarkers in blood of the AD patients [9,10]; blood tissue is easily accessible and less invasive process to collect and suitable for frequent measures throughout the disease course. Despite the significant findings from these studies about Alzheimer's disease, central mechanism of the disease is still not clear. Therefore, much attention is drawn to systems biology analyses in recent years to elucidate the possible roles of biomolecules in different complex diseases [11–15].

Evidence showed that miRNAs are involved in deregulation of neurodegenerative diseases [16]. Consequently, the biomolecules i.e., mRNAs, TFs, miRNAs are increasingly being studied in the exploration of biomarkers in the AD [19-20].

The role of epigenetic modifications is evident in development and progression of the neurodegenerative disease including the AD [17,18]. DNA methylation, histone modifications are considered mechanism of epigenetic regulation [19]. Various external factors such as life style, age, and environment and disease state are contributor in epigenetic changes [19]. Recent studies identified the most consistent methylation genes and histone modification with AD [19].

We employed integrative approach to identify systems molecular biomarker signatures in that are expressed under similar genetic control in blood and brain in Alzheimer's disease from transcriptome and expressed quantitative loci (cis-eQTL) considering the integrative cellular and signaling process in cell. The gene overrepresentation analysis was performed on core DEGs identified Gene Ontology and significant pathways enriched by the common DEGs. The core DEGs was further analyzed to identify regulatory (TFs, miRNAs) of the DEG. Further analysis was performed to identify histone modification sites of DEGs. This study is specifically focused on to reveal biomarker signatures at RNA level (mRNAs and miRNAs) and protein levels (hub proteins and TFs) intended to present valuable information to clarify the mechanisms of molecular biomarkers in the AD that may provide efficacious potential biomarkers for early diagnosis and systems medicines (Figure 1).

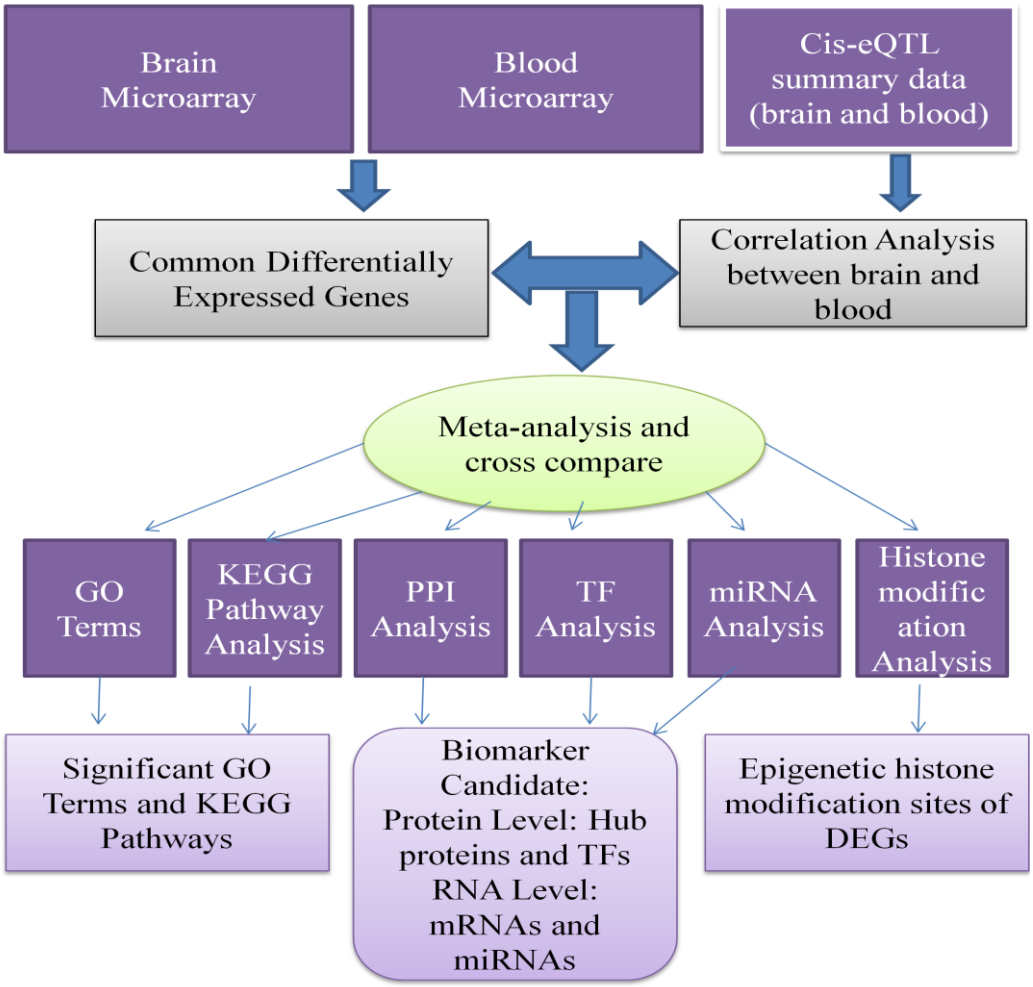

Figure 1: An integrative analysis methodology employed in this study. Gene expression transcriptomics datasets of blood and brain of Alzheimer's disease (AD) were obtained from the NCBI-GEO. The eQTL data of human blood and brain were obtained from GTEx. The datasets were analyzed using limma in R to identify common differentially expressed genes (DEGs) between blood and brain. Then, a meta-analysis network based approach was used to identify common genes from eQTL. Functional enrichment analyses of DEGs from microarray and eQTL were performed to identify significantly enriched pathways and Gene Ontology terms. Protein-protein interaction networks were reconstructed around DEGs. The significant TFs and miRNAs were obtained from Enrichr. The histone modification sites of the hub genes were also obtained from Human Histone Modification database.

2. Materials and Methods

2.1 Identification of Differentially Expressed Genes from High-throughput Microarray Datasets

We obtained two gene expression microarray datasets GSE18309 (peripheral blood tissues) and GSE4757 (brain tissue) of AD patients from National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database [20]. We first directly applied the logarithmic transformation on both blood and brain microarray datasets to reach the datasets near normality and to mitigate the effect of outliers. Then we applied linear Models for Microarray (limma) which is implemented in bioconductor platform in R to identify the DEGs from each datasets. The overlapping DEGs between two datasets were considered for further analysis. We screen out the statistically significant DEGs if they satisfy adjusted p-value <0.05 and absolute values of log2 fold control >=1.0. The Benjamini-Hochberg (BH) method was used to adjust the p-values.

2.2 Geneset Enrichment Analyses to Identify Gene Ontology and Molecular Pathways

We performed gene set enrichment analysis via Enrichr [21] to find out the Gene Ontology (GO) terminology and KEGG pathway of the detected overlapping DEGs. The ontology comprises three categories: biological process, molecular function and cellular component. The p-value<0.05 was considered as the cut-off criterion for all the enrichment analyses.

2.3 Protein-protein Interaction Network Analysis

Considering the experimentally verified physical interaction of the proteins, we reconstructed the protein-protein interaction (PPI) network around the proteins encoded by the core common DEGs in blood and brain using STRING database [22]. The confidence score 400 was selected in STRING Interactome. Network visualization and topological

analyses were performed through NetworkAnalyst [23]. Using topological parameters degree and betweenness parameters were used to designate hub (i.e., central) proteins from PPI analysis.

2.4 Identitification of Histone Modification Sites

Histone modification data for the hub genes were retrieved from human histone modification database [24].

2.5 Identification of Transcriptional and Post-transcriptional Regulator of the Differentially Expressed Genes

We used experimentally verified TF-target gene interactions from TRANSFAC [25] and JASPAR database [26] to identify the TFs. The miRNAs-target gene interactions were obtained from miRTarBase [27] to identify miRNAs . We identified significant miRNAs ($p<0.01$) and TFs ($p<0.05$) via Enrcihr [21].

2.6 Association of eQTL Effects Between Blood and Brain Tissues

We used eQTL data of both blood and brain from the GTEx Portal which is a database for the Genetic Association data (https://gtexportal.org/home/). The eQTL databases link gene SNPs to gene expression. We used these from the GTEx database to find genes with similar genetic control of expression in the two tissues using meta-analysis approaches.

2.7 Cross Validation of the Candidate Biomarkers

We cross checked the identified common DEGs using AlzGene database which is a collection of published AD genetic association studies [28]. We also cross compared the identified miRNAs with a blood based miRNAs signatures which detected 12 diagnostic miRNAs (brain-miR-112,brain-miR-161, hsa-let-7d-3p, hsa-miR-5010-3p, hsa-miR-26a-5p,

hsa-miR-1285-5p, hsa-miR-151a-3p, hsa-miR-103a-3p, hsa-miR-107, hsa-miR-532-5p, hsa-miR-26b-5p and hsa-let-7f-5p) [29].

3. Results

3.1 Identification of Differentially Expressed Genes

We used microarray gene expression datasets of brain (GSE4757) and blood (GSE18309). The datasets were analyzed and identified 9 (nine) common DEGs (CNBD1, SUCLG2-AS1, CCDC65, PDE4D, MTMR1, C3, SLC6A15, LINC01806, and FRG1JP) in blood and brain.

3.2 Identification of AD-associated Genes in Blood that Mirror Those in Brain from eQTL

We used a meta-analysis approach to identify genes from GTEx database that show similar expression pattern in both blood and brain tissues using eQTL database that link gene SNPs to gene expression. Thus, we identified 673 blood-brain co-expressed genes (BBCG) using the correlation and meta-analysis approach as explained in the method section. We identified 18 significant genes (HSD17B1, GAS5, RPS5, VKORC1, GLE1, WDR1, RPL12, MORN1, RAD52, SDR39U1, NPHP4, MT1E, SORD, LINC00638, MCM3AP-AS1, GSDMD, RPS9, and GNL2**)** was commonly dysregulated between blood and brain for AD using SNP and cis-eQTL data of blood and brain using curated gold-benchmark databases OMIM and GWAS catalogues.

3.3 Gene Ontology and Pathways Analysis

To clarify the biological significance of the identified DEGs, we performed the geneset enrichment analysis. The significant GO terms were enriched in the biological process, cellular component, and molecular functions (Table 1). The significant pathways

analysis revealed Ribosome, Alternative Complement Pathway, Classical Complement Pathway, Lectin Induced Complement Pathway, and Cytoplasmic Ribosomal Proteins (Table 2).

Table 1: Gene Ontology (biological process, Cellular component and molecular functions) of common differentially expressed genes between blood and brain tissue of Alzheimer's disease.

Category	GO ID	Term	Adjusted P-value	Z-score	Combined Score	Genes
Biological process	GO:0045047	protein targeting to ER	0.014	-2.085	16.872	RPS9;RPL12;RPS5
	GO:0006614	SRP-dependent cotranslational protein targeting to membrane	0.014	-1.84	15.352	RPS9;RPL12;RPS5
	GO:0000184	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	0.014	-1.997	15.323	RPS9;RPL12;RPS5
	GO:0019083	viral transcription	0.014	-1.784	13.649	RPS9;RPL12;RPS5
	GO:0019080	viral gene expression	0.014	-1.579	12.203	RPS9;RPL12;RPS5
Cellular component	GO:0005840	ribosome	0.004	-2.246	19.781	RPS9;RPL12;RPS5
	GO:0022626	cytosolic ribosome	0.01	-1.333	9.84	RPS9;RPL12;RPS5
	GO:0044445	cytosolic part	0.014	-1.613	10.753	RPS9;RPL12;RPS5
	GO:0022627	cytosolic small ribosomal subunit	0.015	-1.507	9.321	RPS9;RPS5
	GO:0015935	small ribosomal subunit	0.015	-1.492	9.0023	RPS9;RPS5
Molecular function	GO:0019843	rRNA binding	0.0013	-2.098	22.001	RPS9;RPL12;RPS5

Table 2: The significant molecular pathways of common differentially expressed genes between blood and brain tissue of Alzheimer's disease.

Category	Pathways	Adjusted P-value	Z-score	Combined Score	Genes
KEGG	Ribosome	0.021	-1.746	12.418	RPS9;RPL12;RPS5
BioCarta	Alternative Complement Pathway	0.02	-1.322	5.702	C3
	Classical Complement Pathway	0.02	-1.452	5.678	C3
	Lectin Induced Complement Pathway	0.02	-1.352	5.478	C3
WikiPathways	Cytoplasmic Ribosomal Proteins	0.005	-2.011	16.844	RPS9;RPL12;RPS5

3.5 Protein-protein Interaction Analysis to Identify Hub Proteins

A protein-protein interaction network was constructed encoded by the DEGs to reveal the central protein the so called hub proteins considering the degree measures (Figure 2). RPS5, RPS9, RAD52, PDE4D, RPL12, GNL2, WDR1, GLE1, C3, and SORD were identified as the hub proteins. They might be potential biomarker and therapeutic targets in the AD.

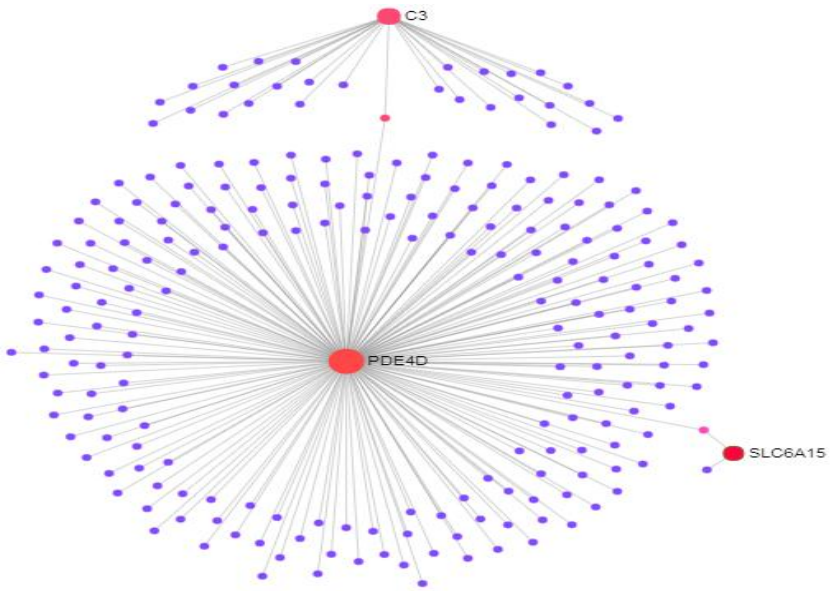

Figure 3: Protein-protein interaction network of the differentially expressed genes (DEGs) in Alzheimer's disease. The nodes indicate the DEGs and the edges indicate the interactions between two genes.

3.6 Epigenetic Regulation of the Differentially Expressed Genes

In order to identify the probable epigenetic regulation of the hub genes, histone modification data for eight hub genes (Table 3) were retrieved from HHMD. Table 3 shows that all the hub genes were associated with several histone modification sites.

Table3: Histone modification patterns (obtained from HHMD) of hub genes with respect to the already known histone modification sites in neurodegenerative diseases.

Official Symbol of DEGs and Hub Genes	**RefSeq ID**	**Histone modification sites already known in neurodegenerative diseases**				
		H3K27	**H3K4**	**H3K9**	**H3K9/H4K20**	**H4R3**
RPS5	NM_001009	✓	✓	✓	✓	✓
RAD52	NM_134424	✓	✓	✓	✓	✓
PDE4D	NM_001104631	✓	✓	✓	✓	✓
RPL12	NM_000976	✓	✓	✓	✓	✓
RPS9	NM_001013	✓	✓	✓	✓	✓
GNL2	NM_013285	✓	✓	✓	✓	✓
WDR1	NM_017491	✓	✓	✓	✓	✓
GLE1	NM_001499	✓	✓	✓	✓	✓
C3	NM_000064	✓	✓	✓	✓	✓
SORD	NM_003104	✓	✓	✓	✓	✓

3.7 Identification of Regulatory Biomolecules

We identified TFs and miRNAs and their targeted DEGs to reveal the regulatory biomolecules that regulate the expression of DEGs at transcriptional and post transcriptional level. The analysis revealed significant TF (SREBF2, NR1H2, NR1H3, PRDM1, and XBP1) and miRNAs (miR-518e, miR-518a-3p, miR-518b, miR-518c, miR-518d-3p, and miR-518f) (Table 4 and Table 5).

Table 4: The significant transcription factors (TFs) regulating common differentially expressed genes between blood and brain tissue of Alzheimer's disease.

Term	**Genes**	**P-value**
SREBF2	GSDMD;C3;WDR1;HSD17B1;RPL12;NPHP4	0.008
NR1H2	GLE1;GSDMD;SORD	0.02
NR1H3	GSDMD;VKORC1;GLE1;SORD;GNL2	0.03
PRDM1	MTMR1;WDR1;PDE4D;SLC6A15;MT1E	0.04
XBP1	RPS9;PDE4D;SORD	0.04

Table 5: The significant microRNAs (miRNAs) regulating common differentially expressed genes between blood and brain tissue of Alzheimer's disease.

Term	Genes	P-value
miR-518e	RPS9;WDR1;HSD17B1	0.01
miR-518a-3p	RPS9;WDR1;HSD17B1	0.02
miR-518b	RPS9;WDR1;HSD17B1	0.02
miR-518c	RPS9;WDR1;HSD17B1	0.02
miR-518d-3p	RPS9;WDR1;HSD17B1	0.02
miR-518f	RPS9;WDR1;HSD17B1	0.02

3.8 Cross Validation of the Candidate Biomarker Biomolecules

The DEGs and hub proteinswere compared with the 618 AD-related biomarkers depositedin AlzGene database. The result revealed no overlapped of the DEGs with the AD-related biomarkers in AlzGene database. We compared the identified miRNAs with the blood aberrant miRNAs were collected from a study which detected 12 diagnostic miRNAs of AD patients of peripheral blood samples. No overlapping miRNA found with this study.

4. Discussion

The unavailability of the peripheral blood biomarkers leads to much study to elucidate the molecular mechanism of the AD, since biomarkers are needed to diagnosis, monitor and treatment of the disease. In biomedical research, microarray is widely used and considered as main resources for candidate biomarkers; consequently, we analyzed two gene expressions datasets from peripheral blood and brain of the AD patients in order to identify potential biomarkers candidate. The analysis revealed 9 common DEGs in two transcriptomics datasets of blood and brain tissues. The geneset enrichment analyses revealed AD associated molecular signaling pathways i.e., ribosome [30], complement [31,32] .

The central hub proteins regulate the most of the cellular processes; consequently protein-protein interaction network is extensively used to identify the hub proteins. These hub

proteins are considered as the key drivers in the mechanisms behind the disease [33]. Therefore, we reconstructed the protein interaction network around DEGs to identify key hub proteins. The identified hub proteins have the potential to contribute to the formation and progression of neurodegenerative diseases including the AD. The hub protein RPS5 is implicated in neurodegenerative disease [34]. The dysregulation of RAD52 is implicated in AD [35]. The hub protein PDE4D came into prominence since recent studies suggested that phosphosdiesterases as promising therapeutic drug target in AD [36,37]. WDR1 is associated with cardiovascular diseases [38]. SORD is associated with cataract and cardiovascular disease according to GeneCards database. GLE1 is associated with amyotrophic lateral sclerosis [39]. The role of ribosomal proteins (RPS9 and RPL12) in the neurodegenerative disease is obscure. GNL2 is implicated in neurogenesis of retina [40]. Another hub C3, may play important role in the pathogenesis of the AD [41,42].

Epigenetic alterations are present in different tissues along the aging process in neurodegenerative disorders such as Alzheimer's disease (AD). Epigenetics affect life span and longevity. AD-related genes exhibit epigenetic changes, indicating that epigenetics might exert a pathogenic role in dementia. Epigenetic modifications are reversible and can potentially be targeted by pharmacological intervention. Epigenetic drugs may be useful for the treatment of major problems of health (e.g., cancer, cardiovascular disorders, brain disorders) [43]. The epigenetic regulations of hub genes are shown in Tables 3. We have studied the histone modification patterns of DEGs and hub genes. Histone modification refers to the posttranslational modifications of the amino-terminal tails of histone proteins which upon modification affect the downstream molecular interactions, hence regulates the gene expression. Interestingly, we found several histone modification sites those are already

known to be associated with several neurodegenerative diseases [44] present within these DEGs and hub genes (Table 3).

Significant relevant regulatory TFs and miRNAs were also identified in the present study since they regulate the gene expression at the level of transcriptional and post-transcriptional (Table 4). The dysregulation of SREBF2 implicated in AD [45]; NR1H3 is associated with the progression of AD [46]; NR1H2 may associated with AD [47]. PRDM1 regulate the DEGs (MTMR1, PDE4D, and SLC6A15) associated with systemic lupus erythematosus [48]. The miRNAs play role in gene regulation and emerging evidences showed its potential as biomarkers for the diagnosis and prognosis of the AD; consequently miRNAs may play significant role in the pathogenesis of AD [49,50]. The identified miR-518e and miR-518a-3p plays role in AD [51]; miR-518c is a biomarker of Parkinson disease [52]; miR-518b is dysregulated in esophageal carcinoma [53]. The role of miR-518d-3p and miR-518f in AD is not reported yet.

5. Conclusion

In the present study, we analyzed blood and brain transcriptomics, eQTL data to identify the commonly expressed DEGs between the two tissues. We integrated these common DEGs into pathways analysis protein-protein interaction and TFs and miRNAs. The 9 significant DEGs (CNBD1, SUCLG2-AS1, CCDC65, PDE4D, MTMR1, C3, SLC6A15, LINC01806, and FRG1JP) were identified from microarray data of blood and brain. Then we identified 18 significant eQTL genes (HSD17B1, GAS5, RPS5, VKORC1, GLE1, WDR1, RPL12, MORN1, RAD52, SDR39U1, NPHP4, MT1E, SORD, LINC00638, MCM3AP-AS1, GSDMD, RPS9, and GNL2**)** common in blood and brain. The neurodegeneration associated molecular signaling pathways (ribosome, complement systems) were identified. The significant TF (SREBF2, NR1H2, NR1H3, PRDM1, and XBP1) and miRNAs (miR-518e, miR-518a-3p, miR-518b, miR-518c, miR-518d-3p, and miR-518f) were identified as

transcriptional and post-transcriptional regulator of the DEGs. Several histone modifications sites of the hub proteins were identified. In this study, we have identified potential biomarkers that are commonly dysregulated both blood and brain tissues. The biomarkers can be detected as transcripts in blood cells. The biomarkers candidate identified using blood cell transcripts and brain cell transcript that are commonly dysregulated. This novel approach to find markers that are can be employed in a tissue (blood) that is easily accessible in the clinic and does not require specialized methods of evaluation beyond gene expression analysis. Since these biomarkers are evident in cells outside the central nervous system, and so reflect responses to systemic factors. But these results will need to establish through further experimental studies.

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