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Genome-Wide Integrative Analysis Reveals Common Molecular Signatures in Blood and Brain of Alzheimer's Disease

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Abstract: The currently utilized neuroimaging and cerebrospinal fluid-based detection of Alzheimer's disease (AD) suffer several limitations, including sensitivity, specificity, and cost. Therefore, the identification of AD by analyzing blood gene expression may ameliorate the early diagnosis of the AD. We aimed to identify common genes commonly deregulated in blood and brain in AD. Comprehensive analysis of blood and brain gene expression datasets of AD, eQTL, and epigenetics data was analyzed by the integrative bioinformatics approach. The integrative analysis showed nine differentially expressed genes common to blood cells and brain (CNBD1, SUCLG2-AS1, CCDC65, PDE4D, MTMR1, C3, SLC6A15, LINC01806, and FRG1JP). Analysis of SNP and cis-eQTL data showed 18 genes; namely, HSD17B1, GAS5, RPS5, VKORC1, GLE1, WDR1, RPL12, MORN1, RAD52, SDR39U1, NPHP4, MT1E, SORD, LINC00638, MCM3AP-AS1, GSDMD, RPS9, and GNL2 were observed deregulated AD blood and brain tissues. Functional gene set enrichment analysis demonstrated a significant association of these genes in neurodegeneration-associated molecular pathways. Integrative biomolecular networks revealed dysregulation of several hub transcription factors and microRNAs in AD. Moreover, hub genes were observed associated with significant histone modification. This study detected common molecular biomarkers and pathways in blood and brain tissues in AD that may be potential biomarkers and therapeutic targets in AD.

Keywords: Alzheimer's disease; molecular signature; blood-brain common gene; differentially expressed genes; protein-protein interactions; epigenetics.

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease common among elderly individuals that results in progressively severe cognitive impairment. In the USA, 5.7 million people are currently living with Alzheimer's, and this is expected to rise to 14 million by 2050 [1-3]. AD is diagnosed by the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles in the brain and reacts the pathobiological processes that underlie the disease [3-4]. Although the pathogenesis of AD is multifactorial in nature, the application of molecular methods to improve diagnosis and assessment of AD has yet to provide substantiated results, and hence the quest for early AD biomarkers in peripheral blood has received increased attention [3,5]. Successful identification of such blood molecular biomarkers will have a high impact on AD diagnosis, care, and treatment [3].

Positron emission tomography (PET) based neuroimaging techniques, and cerebrospinal fluids are both used in clinical practice to diagnose Alzheimer's [6-7]. However, these procedures suffer serious limitations, including the invasiveness of collecting CSF as well as the sensitivity, specificity, cost, and limited access to neuroimaging [8]. Considering the shortcomings of available resources for the detection of neurodegenerative diseases, many studies have attempted to explore biomarkers in the blood of AD patients [1,3,6,9]. Circulating cells and proteins are easily accessible from fresh blood samples; the collection procedure is less invasive. Since central mechanisms underlying the progression of the disease is still not clear, much attention has been drawn to systems biology approaches as a new avenue to elucidate the possible roles of biomolecules in complex diseases such as AD [1,3,9-11]. For example, evidence of involvement of miRNA deregulation in the development of neurodegenerative diseases [10]. Consequently, biomolecules such as mRNAs, transcription factors (TFs), miRNAs (and mRNA gene transcripts targeted by such TFs and miRNAs) are increasingly being scrutinized for use as new biomarkers for AD. In addition, the role of epigenetic modifications is also a focus of much interest, with evidence for their importance in the development and progression of AD [12]. DNA methylation and histone modifications are common mechanisms for epigenetic regulation of gene expression [12]. It is well understood that factors such as lifestyle, age, environment, and co-morbid states effect epigenetic changes as well as the risk of AD and that gene methylation and histone modification may be implicated as mediators [12].

We employed an integrative approach to identify molecular biomarker signatures that are expressed under similar genetic control in blood cells and the brain in AD using transcriptome and expressed quantitative loci (cis-eQTL). Gene over-representation analysis was performed on core DEGs followed by gene ontology (GO) analysis. Pathway analysis was then used to enrich the DEGs. Core DEGs were further analyzed to identify regulatory factors (TFs, miRNAs) that may affect the DEG in AD-affected tissues, as well as analysis to identify histone modification sites within the identified DEGs. This study specifically focused on biomarker signatures at both transcriptional (mRNAs and miRNAs), and translational levels (hub proteins and TFs) as our intention was to present valuable information that would clarify mechanisms in AD that may provide e_cacious potential biomarkers for early diagnosis and systems medicines (Figure 1).

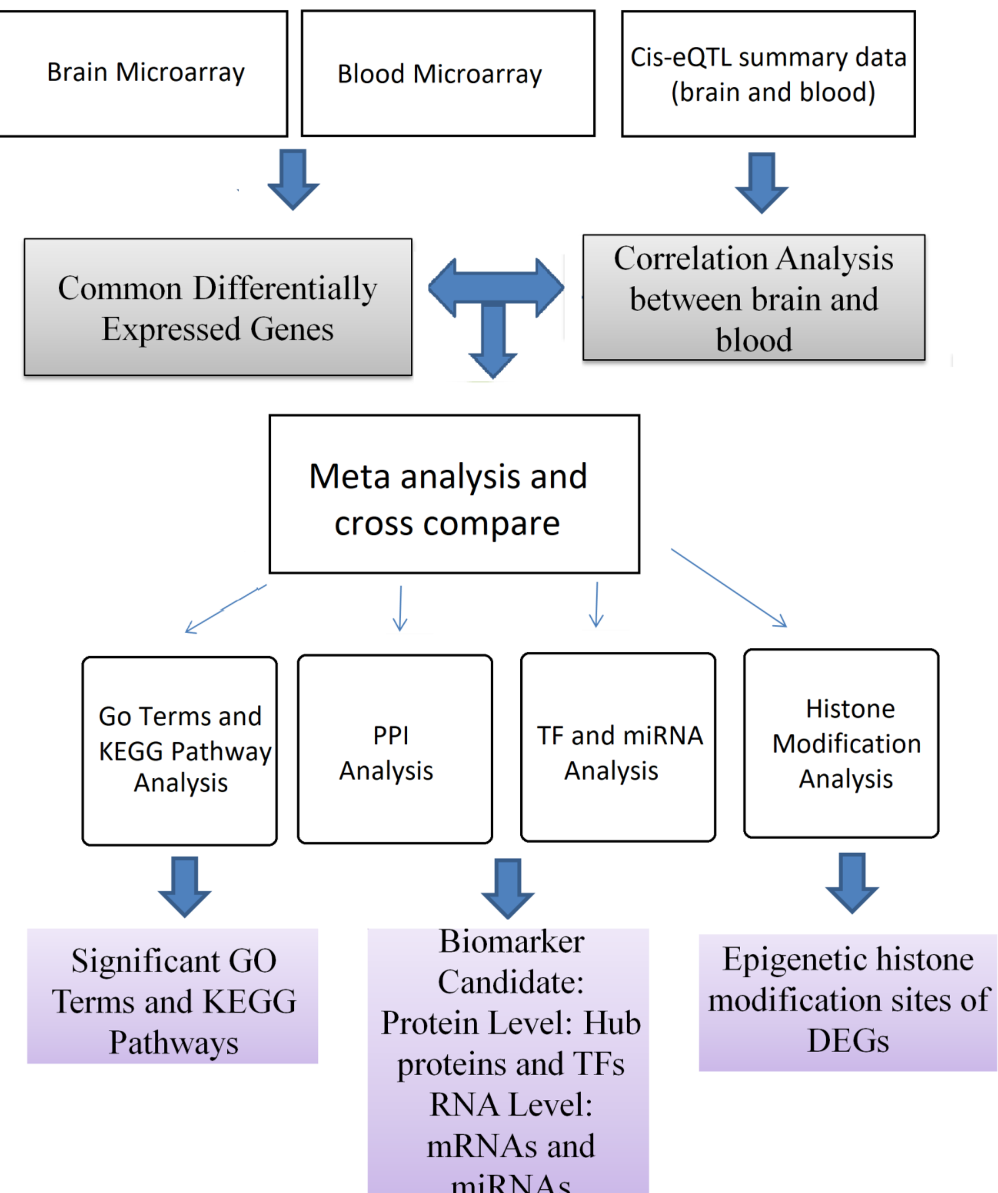

Figure 1. The systems biology pipeline employed in this study. Gene expression datasets of the blood of AD were obtained from the GEO and GTEx portal. The datasets were analyzed using in Bioconductor environment in R to identify common DEGs between brain and blood tissue. The significantly enriched pathways, GO terms were identified through functional enrichment analyses. PPI network was constructed to identify hub proteins. TF-target gene interactions and miRNA-target genes interactions were studied to identify regulatory biomolecules.

2. Materials and Methods

2.1 Identification of differentially expressed genes from microarray datasets.

We obtained two gene expression microarray datasets GSE18309 (peripheral blood mononuclear cells (PBMCs) expression dataset) and GSE4757 (brain tissue) of AD patients from NCBI-GEO database [13]. The peripheral blood tissue datasets (PBMCs) contained 6 samples where 3 samples were AD blood tissues of AD while 3 were matched control (excluded 3 mild cognitive impairment samples from analysis). The brain tissues dataset

GSE4757 had 20 samples where matched pair samples of AD from neurons were obtained. The gene expression profile of 10 mid-stage AD of the entorhinal cortex containing neurofibrillary tangles and 10 paired normal neurons (without neurofibrillary tangles). We applied a logarithmic transformation to both blood and brain microarray datasets to approximate the datasets to normality and to mitigate the effect of outliers. Following this, we applied linear Models for Microarray (limma) through the bioconductor platform in R in order to identify the DEGs from each dataset. The overlapping DEGs between the two datasets were considered for further analysis. We then screened for statistically significant DEGs that satisfied an adjusted p-value < 0:05 and absolute values of log2 fold for control>= 1:0. The Benjamini-Hochberg (BH) method was used to adjust p-values.

2.2. Geneset enrichment analyses to identify gene ontology and molecular pathways.

We performed gene set enrichment analysis via Enrichr [14] to identify GO and pathways of the overlapping DEGs. The ontology comprised of three categories: biological process, molecular function, and cellular component. The p-value< 0:05 was considered as the cut-off criterion for all enrichment analyses.

2.3. Protein-protein interaction network analysis.

We retrieved the PPI networks based on the physical interaction of the proteins of DEGs using STRING database [15]. A confidence score of 900 was selected in the STRING Interactome. Network visualization and topological analyses were performed through NetworkAnalyst [16]. Using topological parameters, the degree (greater than equal 18 degrees) was used to identify highly interacting hub proteins from PPI analysis.

2.4. Identification of histone modification sites.

Histone modification data for the hub genes were retrieved from the human histone modification database (HHMD) [17]. HHMD is a public repository that contains human histone modifications information obtained from experimental studies.

2.5. Identification of transcriptional and post-transcriptional regulators of the differentially expressed genes.

We used TF-target gene interactions from TRANSFAC [18] and JASPAR databases [19] to identify TFs. The miRNA-target gene interactions were obtained from miRTarBase [20]. We have considered statistically signi_cant miRNAs and TFs with (p < 0:05) computed by Fishers' exact test via Enrichr [14].

2.6. 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 Genetic Association data (https: //gtexportal.org/home/). These eQTL databases link gene SNPs to gene expression. We used them to identify genes with similar genetic control of expression in the two tissues using meta-analysis approaches.

If we allow $\tilde{x}$ to be the estimated effect of the top-linked cis-eQTL for a gene, we can calculate $\tilde{x}$ based on the method explained in [21] and as below:

$$\tilde{x} = x + \varrho \qquad (1)$$

where x is the true effect, and ϱ is the estimated error. The covariance of the estimated cis-eQTL effects between tissues i and j across genes can be partitioned into the co-variance of true cis-QTL effects and the co-variance of estimation errors. Thus we can estimate the correlation of true cis-eQTL effect sizes across genes between tissues i and j.

2.7. Cross-validation of the differential expression of differentially expressed genes.

We utilized an independent whole blood gene expression dataset of advanced AD cases and controls (GEO accession, GSE97760). The demographic summary and details of the patients are described in the respective publication (PMID: 25079797). However, the dataset had nine advanced AD cases and 10 age-matched healthy who are all females. The gene expression data were normalized by log-transformation and quantile normalization using the Limma package in the Bioconductor environment in R implemented in the RStudio.

2.8. Statistical analysis.

The data visualization of differential expression was done via GraphPad Prism 5.

3. Results and Discussion

3.1. Identification of common deferentially expressed genes between blood and brain tissues.

We analyzed microarray gene expression datasets of the brain and blood samples of AD patients. The analysis revealed 9 (nine) common DEGs (CNBD1, SUCLG2-AS1, CCDC65, PDE4D, MTMR1, C3, SLC6A15, LINC01806, and FRG1JP) in blood and brain. We also identified AD-associated genes in the blood that mirror those in brain from eQTL. We used a meta-analysis approach to identify genes from GTEx database that display a similar expression pattern in both blood and brain tissues using eQTL database that link gene variants (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 methods section. We identified 18 DEGs (HSD17B1, GAS5, RPS5, VKORC1, GLE1, WDR1, RPL12, MORN1, RAD52, SDR39U1, NPHP4, MT1E, SORD, LINC00638, MCM3AP-AS1, GSDMD, RPS9, and GNL2) that were commonly dysregulated between AD blood and brain compared to control tissues using SNP and cis-eQTL data of curated, gold-benchmarked OMIM and GWAS catalogs. In this way, we have identified 27 DEGs that were commonly dysregulated in blood and brain in AD from microarray and eQTL data analysis. To clarify the biological significance of the identified DEGs, we performed a gene set enrichment analysis. The significant GO terms were enriched in biological processes, molecular functions, and cellular components (Table 1). The pathways analysis revealed significant pathways in the 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 dysregulated genes common to blood cells and brain tissue of Alzheimer's disease.

Category	GO ID	Term	Adjusted P-value	Genes
Biological process	GO:0045047	protein targeting to ER	0.014	RPS9;RPL12;RPS5
	GO:0006614	SRP-dependent cotranslational protein targeting to membrane	0.014	RPS9;RPL12;RPS5
	GO:0000184	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	0.014	RPS9;RPL12;RPS5

Category	GO ID	Term	Adjusted P-value	Genes
	GO:0019083	viral transcription	0.014	RPS9;RPL12;RPS5
	GO:0019080	viral gene expression	0.014	RPS9;RPL12;RPS5
Cellular component	GO:0005840	ribosome	0.004	RPS9;RPL12;RPS5
	GO:0022626	cytosolic ribosome	0.01	RPS9;RPL12;RPS5
	GO:0044445	cytosolic part	0.014	RPS9;RPL12;RPS5
	GO:0022627	cytosolic small ribosomal subunit	0.015	RPS9;RPS5
	GO:0015935	small ribosomal subunit	0.015	RPS9;RPS5
Molecular function	GO:0019843	rRNA binding	0.0013	RPS9;RPL12;RPS5

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

Category	Pathways	Adj. P-value	Genes
KEGG	Ribosome	0.021	RPS9;RPL12;RPS5
BioCarta	Alternative Complement Pathway	0.02	C3
	Classical Complement Pathway	0.02	C3
	Lectin Induced Complement Pathway	0.02	C3
WikiPathways	Cytoplasmic Ribosomal Proteins	0.005	RPS9;RPL12;RPS5

3.2. 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, RPL12, RPS9, GNL2, PDE4D, and WDR1 were identified as the hub proteins. These are potential biomarkers and may lead to new AD therapeutic targets.

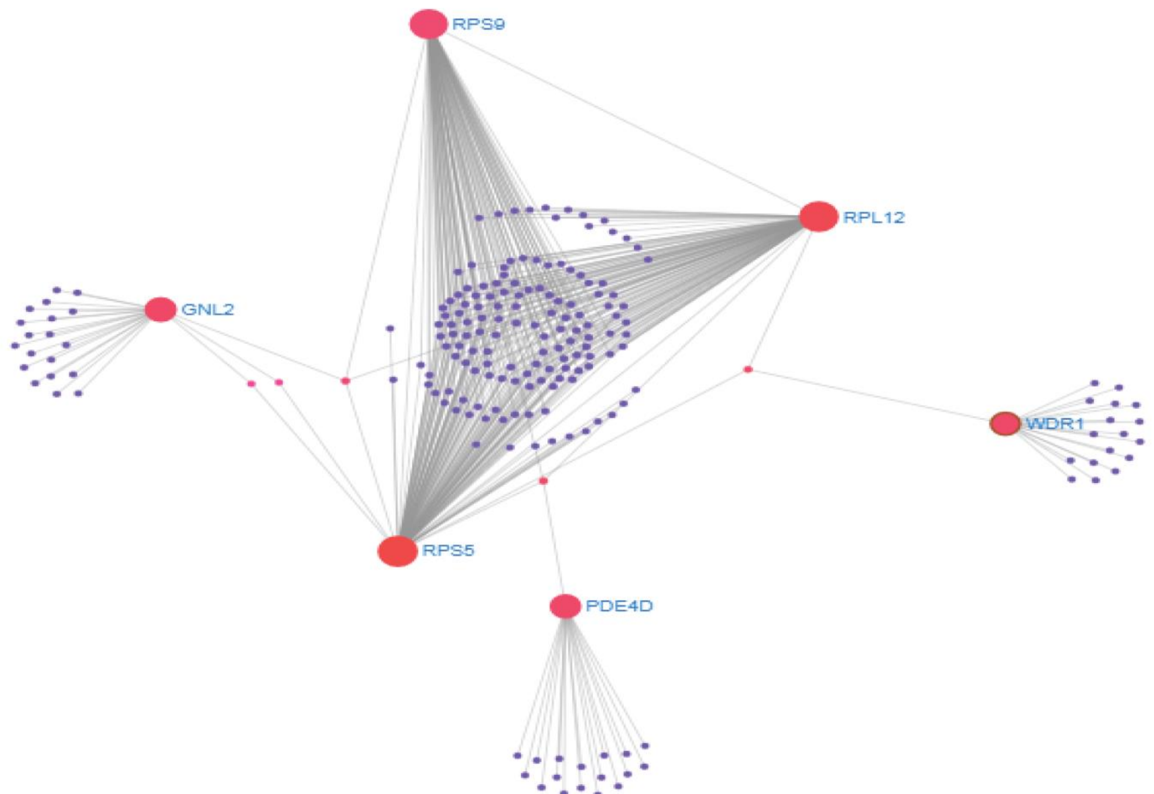

Figure 2. Protein-protein interaction network of the differentially expressed genes (DEGs) in Alzheimer's disease. The nodes indicate the DEGs and the edges indicate the interactions between two genes.

3.3. Epigenetic regulation of the differentially expressed genes.

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

Table 3. Histone modification of hub genes 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	✓	✓	✓	✓	✓
PDE4D	NM_001104631	✓	✓	✓	✓	✓
RPL12	NM_000976	✓	✓	✓	✓	✓
RPS9	NM_001013	✓	✓	✓	✓	✓
GNL2	NM_013285	✓	✓	✓	✓	✓
WDR1	NM_017491	✓	✓	✓	✓	✓

3.4. Identification of post-transcriptional regulator.

We identified TFs and miRNAs interacted with DEGs to reveal regulatory biomolecules that may regulate the expression of DEGs at transcriptional and post-transcriptional levels (Figure 3 and Table 4-5). The analysis revealed signi_cant TFs (SREBF2, NR1H2, NR1H3, 138 PRDM1, and XBP1) and miRNAs (miR-518e, miR-518a-3p, miR-518b, miR-518c, miR-139 518d-3p, and miR-518f) played signi_cant roles in the regulation of the DEGs identi_ed this study.

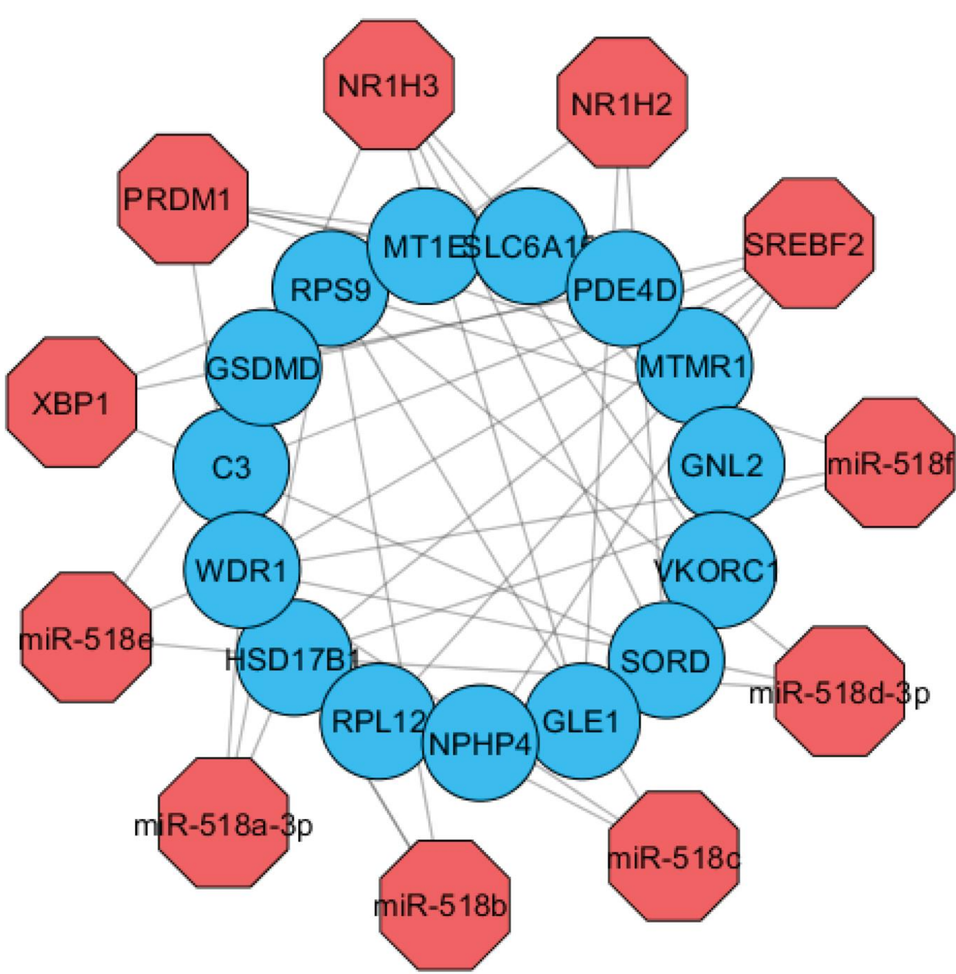

Figure 3. Interaction networks of gene with regulatory biomolecules (transcription factors and miRNAs).

Table 4. Biomolecules comprising transcription factors and MicroRNAs may regulate commonly dysregulated genes in Alzheimer's disease.

Biomolecules	Target Genes	P-value
Transcription Factor		
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
MicroRNA		
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

Table 5. A list of biomolecules proposed in the present study for Alzheimer's disease.

Biomarker Candidate	Name	Relevance with AD and neurodegenerative diseases	Novelty
Differentially Expressed Genes			
CNBD1	Cyclic Nucleotide Binding Domain Containing 1	Associated with alcoholism and Diabetes mellitus type 2	Novel
SUCLG2-AS1	SUCLG2 Antisense RNA 1	Associated with gastric cancer according	Novel
CCDC65	Coiled-Coil Domain Containing 65	Associated with ciliary dyskenesia	Novel
PDE4D	Phosphodiesterase 4D	PDE4D, which in preclinical research has been suggested to be of particular importance for cognition, in the hippocampus of a patient with AD	Known
MTMR1	Myotubularin Related Protein 1	Diseases associated with include Charcot-Marie-Tooth Disease	Novel
C3	Complement C3	Macular Degeneration, Hip, Cholesterol, Echocardiography	Novel
SLC6A15	Solute Carrier Family 6 Member 15	Diseases associated include Major Depressive Disorder	Novel
LINC01806	Long Intergenic Non-Protein Coding RNA 1806	a liated with the non-coding RNA class	Novel
FRG1JP	FSHD Region Gene 1 Family Member J, Pseudogene	Pseudogene	Novel
HSD17B1	Hydroxysteroid 17-Beta Dehydrogenase 1	Diseases associated with Acute T Cell Leukemia and Acute Closed-Angle Glaucoma	Novel
GAS5	Growth Arrest Speci c 5	Diseases associated with GAS5 include Autoimmune Disease and Malignant Pleural Mesothelioma	Novel
RPS5	Ribosomal Protein S5	Involved in retinitis pigmentosa	Novel
VKORC1	Vitamin K Epoxide Reductase Complex Subunit 1	Genetic polymorphism is associated with cardiovascular and neurodegenerative disease in AD	Novel
GLE1	GLE1, RNA Export Mediator	GLE1 mutations cause lethal congenital contracture syndrome, a severe autosomal recessive fetal motor neuron disease, and more recently, have been associated with amyotrophic lateral sclerosis.	Known
WDR1	WD Repeat Domain 1	associated with adaptive immunity highlighting its central role immunologic synapses	Novel
RPL12	Ribosomal Protein L12	Gene Ontology annotations related to gene RPS12 include structural constituent of ribosome	Novel
MORN1	MORN Repeat Containing 1	Diseases associated with MORN1 include Hemangioma of Lung	Novel
RAD52	RAD52 Homolog, DNA Repair Protein	high concentrations of amyloid-beta inhibit the expression and DNA damage response of RAD52	known
SDR39U1	Short Chain Dehydrogenase/Reductase Family 39U Member 1	Gene Ontology (GO) annotations related to this gene include oxidoreductase activity and coenzyme binding	Novel
NPHP4	Nephrocystin 4	Diseases associated with NPHP4 include Nephronophthisis 4	Novel
MT1E	Metallothionein 1E	related pathways are Metallothioneins bind metals and Metabolism	Novel

Biomarker Candidate	Name	Relevance with AD and neurodegenerative diseases	Novelty
		Differentially Expressed Genes	
SORD	Sorbitol Dehydrogenase	Diseases associated include Cataract and Microvascular Complications of Diabetes	Novel
LINC00638	Long Intergenic Non-Protein Coding RNA 638	the non-coding RNA class	Novel
MCM3AP-AS1	MCM3AP Antisense RNA 1	Diseases associated with MCM3AP-AS1 include Glioblastoma	Novel
GSDMD	Gasdermin D	related pathways are Apoptosis and Autophagy and Innate Immune System	Novel
RPS9	Ribosomal Protein S9	Gene Ontology annotations related to gene RPS9 include structural constituent of ribosome	Novel
GNL2	G Protein Nucleolar 2	GNL2 plays a role in the neurogenesis of retina	Novel
		Transcription Factors	
SREBF2	Sterol Regulatory Element Binding Transcription Factor 2	Increased expression at mRNA levels in AD	Known
NR1H3	Nuclear Receptor Subfamily 1 Group H Member 3	The genetic variant was studied to determine the e ects of rs7120118 variation in the NR1H3 gene on the progression of AD	Known
NR1H2	Nuclear Receptor Subfamily 1 Group H Member 2	The genetic polymorphism in NR1H2 may contribute to the pathogenesis of AD	Novel
PRDM1	PR/SET Domain 1	The exome sequencing and functional studies revealed the genetic variants of PRDM1 in Crohn's. associated with systemic lupus erythematosus	Novel
WDR1	WD Repeat Domain 1	WDR1 is associated with adaptive immunity highlighting its central role immunologic synapses	Novel
GNl2	G Protein Nucleolar 2	GNL2 plays a role in the neurogenesis of retina in Zebra sh	Novel
XBP1	X-Box Binding Protein 1	The role of XBP1 in neurodegeneration remains controversial and appears to be disease-specific	Novel
		MicroRNAs	
miR-518e	MicroRNA 518	roles have been suggested for miR-518e and miR-518a-3p in AD	Known
miR-518a-3p	MicroRNA 518a	roles have been suggested for miR-518e and miR-518a-3p in AD	Known
miR-518b	MicroRNA 518b	dysregulated in esophageal carcinoma	Novel
miR-518c	MicroRNA 518c	biomarker for Parkinson's disease	Known
miR-518d-3p	MicroRNA 518d	Predicted as a therapeutic target in Huntington's disease	Known
miR-518f	MicroRNA518f	RNA gene an icted with RNA class	Novel

3.5. Cross-validation of the differentially expressed genes in independent blood gene expression data.

The differential expression of identified genes that show common deregulation in blood and brain gene expression dataset GSE97760, we performed cross-validation with an independent gene expression data of advanced AD patients compared to a matched control. 13 DEGs were consistently identi_ed in this independent advanced stage AD dataset overlapped with our results (Figure 4 and Table 6).

3.6. Discussion.

The lack of peripheral blood biomarkers for AD has led to a race to identify much-needed evidence for the early diagnosis of this debilitating disease. The identification of peripheral biomarkers may also shed light on molecular mechanisms of AD and enable the monitoring of treatment. Advances in biomedical technology have spurred discoveries in numerous research areas. Microarray analysis is widely used in biomedical research and is considered the main resource for candidate biomarkers. Microarray databases contain a wealth of untapped genomic information. We analyzed two gene expression datasets from peripheral

blood and brain of the AD patients in an attempt to identify potential biomarker candidates. Our analysis revealed 27 DEGs common dysregulated to the blood and brain of AD patients. We cross-compared the differential expression of these identified DEGs in available blood gene expression from advanced AD cases compared to controls. The comparison showed 13 DEGs were consistently deregulated in the cross-validation dataset suggesting the reliability of the identified candidate biomarkers and corroborates the employed approach.

Gene set enrichment analyses also revealed AD-associated molecular signaling pathways that included the ribosome and complement systems. Employing protein-protein interaction networks, we also identified dysregulated central hub proteins that control many cellular processes. These hub proteins are considered key drivers in the mechanisms underlying the disease [22]. Therefore, we reconstructed the protein interaction network focusing on the DEGs in an attempt to identify related hub proteins. Such proteins have the potential to contribute to the formation and progression of AD. Of the DEGs we identified, mRNA levels of RPS5, a ribosomal protein, has been shown to be increased in the frontal cortex of AD subjects and AD transgenic mice [23].

Epigenetic alterations are present in different tissues during aging, as well as in neurodegenerative disorders such as AD. AD-related genes exhibit epigenetic changes, indicating that epigenetics might contribute to pathogenic changes observed in dementia. Epigenetic modifications are reversible and may potentially be targeted by pharmacological intervention [24]. We have identified epigenetic changes in hub genes (Table 3) and have investigated histone modification patterns of DEGs. Histone modifications are posttranslational modifications of the amino-terminal tails of histone proteins that affect nucleosome structures and gene accessibility to TFs. Histone modification thus affects downstream molecular interactions, thereby affecting patterns of gene expression. We report several histone modification sites present within the hub genes, many of which are already known to be associated with several neurodegenerative diseases [25]. The identification of these known modifications in genes further validates the discovery of the novel DEGs and hub genes that we have identified in this investigation. Our analysis also revealed that differentially expressed DEGs, regulatory TFs, and miRNAs that strongly influence gene expression at the transcriptional and post-transcriptional levels (Table 4-5).

The SREBF2 is a cholesterol regulating genes and significantly increased mRNA levels expression were observed in the late-onset AD in the brain and blood microarray observations suggesting SREBF2 as biomarkers of AD at pathological and gene expression levels [26]. In another study evaluated the SREBF2 mRNA level expression in neurodegenerative prion disease. Significantly increased expression of SREBF2 was in prion-infected neuron cells suggesting cholesterogenic upregulation as a neuronal response to prion infection, emphasizing cholesterol biosynthesis critical pathways in prion disease [27]. The genetic variant was studied to determine the effects of rs7120118 variation in the NR1H3 gene on the progression of AD. A significant increase in the mRNA levels of NRIH3 among the AD patients was found by qPCR analysis. Overall, these data suggest that the CT genotype of rs7120118 associated with increased mRNA levels of NR1H3, but the disease severity does not affect NR1H3 expression [28]. Additionally, association analysis of common variants in NR1H3 identified rs2279238 conferring a 1.35-fold increased risk of developing progressive MS. Protein expression analysis revealed that mutant NR1H3 alters gene expression profiles, suggesting a disruption in transcriptional regulation as one of the mechanisms underlying MS pathogenesis. Novel medications based on NR1H3 models are expected to provide symptomatic relief and halt

disease progression by reducing the inflammatory response and promoting remyelination [29]. The genetic polymorphism in NR1H2 may contribute to the pathogenesis of AD [30]. The exome sequencing and functional studies revealed the genetic variants of PRDM1 in Crohn's disease [31]. PRDM1 was associated with systemic lupus erythematosus (SLE) [32]. There is a link between cerebral inflammation and degeneration in SLE [33], but inverse relations suggested for SLE and Parkinson's disease patients since SLE had a decreased risk of subsequent Parkinson's disease [34]. However, the study indicates that the risk of dementia may be elevated in individuals with SLE, an autoimmune disease affecting a range of systems including the peripheral and central nervous system concluding SLE is significantly associated with dementia [35]. WDR1 is associated with adaptive immunity highlighting its central role in immunologic synapses [36] and cardiovascular diseases [36, 37]. GNL2 plays a role in the neurogenesis of retina in Zebrafish [38]. Gene Ontology (GO) annotations related to gene RPS9 and RPS12 include structural constituent of ribosome according to genecards database, but the role of these ribosomal proteins in the neurodegenerative disease is obscure. The role of XBP1 in neurodegeneration remains controversial and appears to be disease-specific. XBP1 occupancy was observed on the promoters of genes linked to neurodegenerative pathologies, including AD [39], although the relevance of these events remains speculative. Indeed, XBP1 activates a plethora of target genes involved in a variety of physiological functions, including neuronal plasticity [39, 40, 41], suggesting an important role during the branching and maturation of developing neurons. Accumulation of unfolded or misfolded proteins in the ER leads to an ER stress response, which is characteristic of cells with a high level of secretory activity and is implicated in a variety of disease conditions such as AD [42]. Hub protein PDE4D was particularly noteworthy since recent studies have suggested that phosphodiesterases are promising therapeutic drug targets in AD [43].

miRNAs play important roles in gene regulation, and there is emerging evidence demonstrating their potential for use as biomarkers for AD and other diseases; it is likely, therefore, that miRNAs play significant roles in the pathogenic process underlying AD [44, 45]. Indeed, such roles have been suggested for miR-518e and miR-518a-3p in AD [45,46]. Similarly, miR-518c may also be a useful biomarker for Parkinson's disease [46], while miR-518b is dysregulated in esophageal carcinoma [47].

3.6.1. Limitation of the study.

Due to various bioinformatics analyses in the data provided in this report, consideration should be given in the interpretation of these findings. Probably, in future clinical trials with samples from patients with AD, the findings achieved are therefore, will be validated. Such specimens are sadly not available at present. Furthermore, considering the nature of this study relies on information gathered at different sites, site-to-site disparities are likely to affect the results like differences in microarray platforms, blood gathering, and RNA extraction techniques. Because the population is different in both datasets, they may influence the results. Although none of the studies documented mixed pathologies, comorbidities diseases and the use of drugs may also have affected the findings.

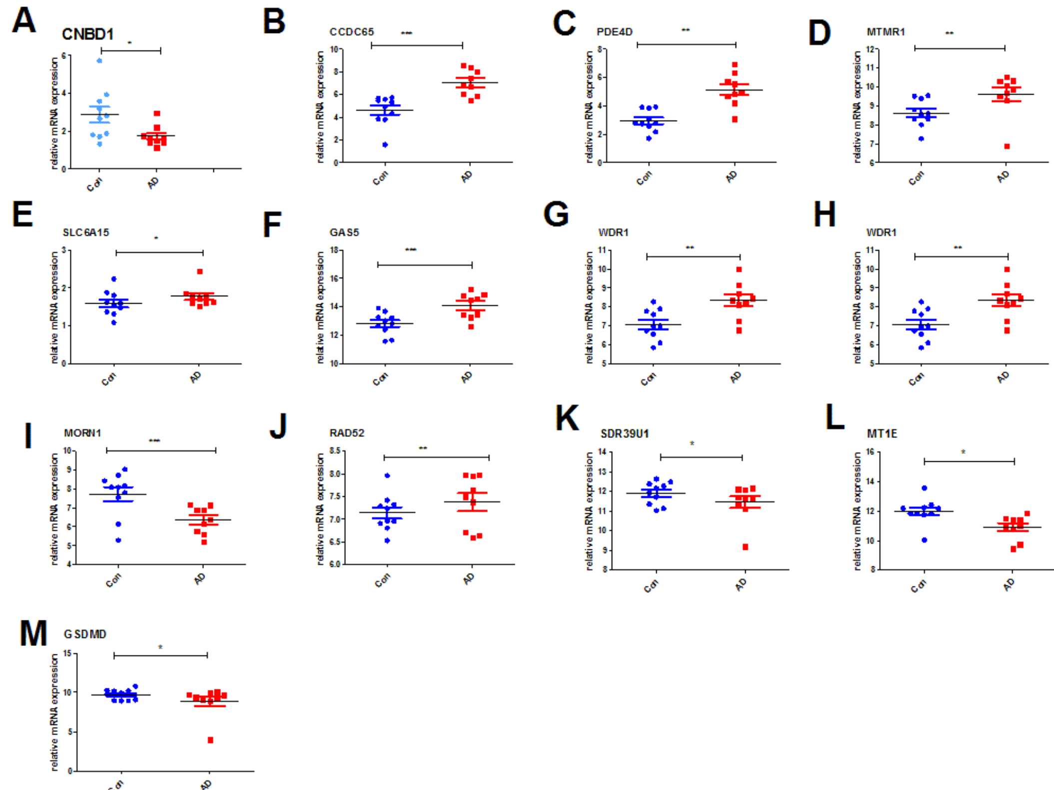

Figure 4. Fig shows significant differential expression of proposed candidate blood and brain genes in an independent blood gene expression data (GSE97760) of advanced Alzheimer's disease (p-value < 0:05). Note: Con=control, AD=Alzheimer's disease, A=CNBD1, B=CCDC65, C=PDE4D, D=MTMR1, E=SLC6A15, F=GAS5, G=WDR1, H=MORN1, I=RAD52, J=SDR39U1, K=NPHP4, L=MT1E, M=GSDMD.

Table 6. Cross-validation of proposed candidate blood and brain genes in an independent blood gene expression data (GSE97760) of advanced Alzheimer's disease.

Gene Symbol	P-value in GSE97760 (blood AD data)	Log2 Fold Change
CNBD1	0.0202	-1.004
SUCLG2-AS1	-	-
CCDC65	0.0002	2.454
PDE4D	0.0043	0.992
MTMR1	0.0027	0.789
C3	-	-
SLC6A15	0.0441	0.290
LINC01806	-	-
FRG1JP	-	-
HSD17B1	0.0577	-0.438
GAS5	0.0003	1.424
RPS5	0.3599	0.157
VKORC1	0.4937	0.230
GLE1	0.7641	-0.046
WDR1	0.0118	1.229
RPL12	0.6454	0.054
MORN1	0.0009	-1.487
RAD52	0.0088	1.661
SDR39U1	0.0484	-0.371
NPHP4	0.0012	-1.518
MT1E	0.0138	-1.0481
SORD	0.7501	0.102
LINC00638	-	-
MCM3AP-AS1	0.0703	0.505
GSDMD	0.0456	-1.0147
RPS9	0.2854	-0.320
GNL2	0.1440	0.381

4. Conclusions

In the present study, we analyzed blood and brain transcriptomic and eQTL data to identify common DEGs between these two tissues in Alzheimer's disease. We integrated these common DEGs into pathway analysis for protein-protein interactions, TFs, and miR-NAs. Nine common DEGs were identified from microarray data of blood and brain. We also identified 18 eQTL genes common to blood cells and brain cells. Neurodegeneration associated molecular signaling pathways and several miRNAs were identified as putative transcriptional and post-transcriptional regulators of the DEGs we identified. In addition, several histone modification sites of hub proteins were also identified. Thus, we have identified potential biomarker transcripts that are commonly dysregulated in both blood cells and brain tissues. We propose that these biomarkers may enable the rapid and cost-effective assessment of blood sample analysis for the diagnosis of AD. This novel approach to identify markers can be employed in easily accessible tissue (blood) to assess its expression in an inaccessible tissue (brain) and is one that could be applied to other related clinical problems. We now propose a more detailed validation of this approach and of the putative biomarker transcripts we have identified with clinical-based investigations.

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Conflicts of Interest

The authors declare no conflict of interest.

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