Blood-based molecular biomarker signatures in Alzheimer's disease: Insights from systems biomedicine analyses

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Abstract

Background: Alzheimer's disease (AD) is the progressive neurodegenerative disease characterized by dementia and cognitive dysfunction, and but no peripheral biomarkers available yet that can detect the AD. This study aimed to identify systems biomarkers signatures in the AD through integrative analyses. *Method:* We used two microarray datasets (GSE4226 and GSE4229, n=68) from blood of AD patients to identify differentially expressed genes (DEGs). Geneset and protein overrepresentation analysis, protein-protein interaction (PPI), DEGs-Transcription Factor interactions, DEGs-MicroRNAs interactions, protein-drug interactions, and protein subcellular localizations analyses were performed on common DEGs. *Result:* Total 25 DEGs were detected between the two datasets. Integration of genome scale transcriptome datasets with biomolecular networks revealed hub proteins (TUBB, ATF3, NOL6, UQCRC1, SND1, CASP2, BTF3, INPP5K, VCAM1, and CSTF1), TFs (FOXC1, ZNF3, GEMIN7, and SMG9), miRNAs (mir-20a-5p, mir-93-5p, mir-16-5p, let-7b-5p, mir-708-5p, mir-24-3p, mir-26b-5p, mir-17-5p, mir-4270, and mir-4441) as already known (BTF3, VCAM, FOXC1, mir-26b-5p, mir-20a-5p, mir-93-5p, let-7b-5p, mir-24-3p) and novel biomarkers candidates in AD. We evaluated the histone modifications of the identified biomolecules (hub genes and TFs). The hub genes and TFs revealed that they possess several histone modification sites associated with Alzheimer's disease. The protein-drug interactions revealed 10 candidate drugs consists of antineoplastic (Vinorelbine, Vincristine, Vinblastine, Epothilone D, Epothilone B, CYT997, and ZEN-012), dermatologicals (Podofilox), and immunosuppressive agents (Colchicine). The subcellular localization revealed that interactions of DEGs range from nucleus to plasma membrane through cytosol. *Conclusion:* This study presents blood based systems molecular biomarker signatures at RNA and protein levels which might be useful as peripheral biomarkers in the

AD. The candidate drugs, histone modification sites, and subcellular localizations will be useful in future drug design in the AD.

Keywords: Alzheimer's disease; blood biomarkers; systems biology; protein subcellular localizations, epigenetics.

1. Introduction

The Alzheimer's disease (AD) is the dynamic neurodegenerative disease with dementia and impairment of cognitive function in elderly people. Currently, 5.5 million peoples are afflicted with AD in USA (Association, 2017). The amyloid plaques deposition and neurofibrillary formation are hallmarks of the etiology of the AD [2]. The major fraction of AD patients diagnosed are late onset (over 65 years age) while only a small fraction (1-5%) are diagnosed early, but early diagnosis of AD is essential for management strategies of patients. Therefore, research directing towards exploring biomarkers may have impact on the AD diagnosis and treatment [3].

The neuroimaging techniques and cerebrospinal fluids biomarkers are used in everyday clinical practice to diagnose dementia [4–6]. These diagnostics costs money, low sensitivity and specificity making a challenge to implement in wider clinical setup. Thus, a simple blood test of the dementia patients may a valuable and implementable diagnostics assets for early diagnosis of the AD patients [7]. Considering the unavailability of the peripheral blood biomarkers [8–11]. Therefore, a blood based molecular biomarkers may be valuable in evaluating the diagnosis, prognosis, pathogenesis, and treatment of AD [12–16]

The epigenetic regulations in the pathogenesis of AD are manifested and accepted that it play role in the progression and development of the neurodegenerative disease. The epigenetic profiling (DNA methylation, non-coding RNAs, and histone modifications) is used to reveal the epigenetic regulation of gene expression in diseases [17]. Various external factors such as life style, age, environment, and disease state are contributor in epigenetic changes [17]. Recent studies explored the modifications of histones and methylation of the genes in AD [17], but the central mechanism of epigenetics in the AD is not clear.

The dysregulation of the miRNAs are implicated in AD [18]. Consequently, miRNAs are increasingly being studied in the exploration of biomarkers in the AD (Femminella et al., 2015; Shaik et al., 2018; Rahman et al., 2018). Therefore, systems biology analyses are used to elucidate the possible roles of biomolecules in different complex diseases (Ayyildiz and Arga, 2017; Calimlioglu et al., 2015; Guo et al., 2017; Islam et al., 2018; Karagoz et al., 2016; Kori and Arga, 2018; Rahman et al., 2018). Accordingly, to identify critical genes, we used two peripheral blood microarray gene expression datasets. Then, we performed functional annotation to get the important Gene Ontology and pathways enriched by the DEGs. Then, we integrated the DEGs with interactiona networks: (i) a PPI network of the proteins encoded by DEGs; (ii) DEG-TFs and DEGs-miRNAs interactions; (iii) protein-drug interaction networks to screen potential drugs; the binding affinity and mode of the drugs with target protein was evaluated by molecular docking simulations. Moreover, the subcellular localization prediction of the proteins encoded by the DEGs was performed intended to provide insights about the potential sites of drug targeting.

In present study, we used a systems biology pipeline to explore molecular biomarker signatures at RNA levels (mRNAs and miRNAs) and protein levels (hub proteins and TFs) to present valuable information in the mechanism of the peripheral blood molecular biomarkers of the AD that may provide much efficacious potential biomarkers for the early diagnosis and individualized prevention and therapy of the AD (Figure 1).

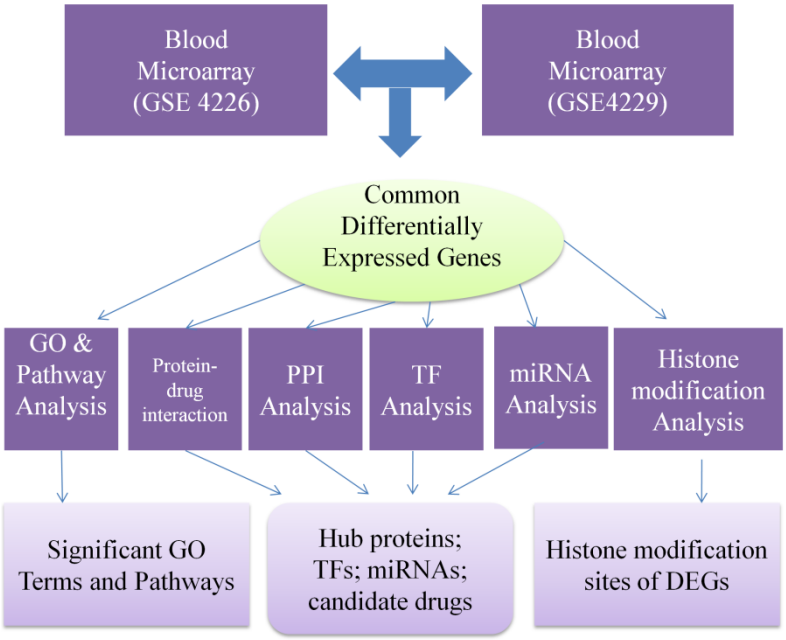

Figure 1: A multi-stage analysis methodology employed in this study. Gene expression transcriptomics datasets of blood of Alzheimer's disease (AD) were collected from the NCBI-GEO database. (B)The datasets were analyzed using GEO2R to identify differentially expressed genes (DEGs). Functional enrichment analyses of DEGs were performed to identify significantly enriched pathways, Gene Ontology terms, and disease overrepresentation terms. Protein-protein interaction networks were reconstructed around DEGs. TF-target gene interactions and miRNA-target gene and were downloaded from JASPAR and miRTarbase databases, respectively. The protein-drugs interaction analysis using DrugBank database and candidate small molecular drugs were identified. Significant pathways and GO terms, Candidate biomarkers at protein and TFs levels, drug target, candidate drugs, and drug targeting sites were identified.

2. Materials and Methods

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

The microarray gene expression high-throughput datasets (GSE4226 and GSE4229) in the AD was obtained from the NCBI-GEO database [27]. Peripheral blood mononuclear cells were collected from normal elderly control and AD subjects based on human MGC cDNA microarray was deposited in NCBI-GEO with the accession number GSE4226 and

GSE4229 [28]. The datasets were analyzed in NCBI-GEO2R to identify DEGs in the AD compared to control. Firstly, the gene expression dataset was normalized by log2 transformation. Then, we analyzed the datasets in NCBI's GEO2R tool using Limma in hypothesis testing and the Benjamini & Hochberg correction to control the false discovery rate. $P<0.01$ was regarded as the cut-off criteria to screen out significant DEGs. The Venn analysis was performed through the online tool—Jvenn [29] to identify common target DEGs from the two datasets.

2.2 Gene Ontology and Pathway Enrichment Analysis

Gene over-representation analyses were performed via bioinformatics resource Enrichr [30] to find out molecular function, biological process, and pathway annotations of the identified DEGs. In these analyses, the Gene Ontology (GO) terminology and KEGG pathway database were used as annotation sources. The protein class over representation was performed by PANTHER database [31]. The p-value<0.05 was considered significant for all the enrichment analyses. Furthermore, we also incorporated a gold bench mark verified dataset dbGaP (https://www.ncbi.nlm.nih.gov/gap) in our study for validating the proof of principle of our systems based approach linking the genotype and phenotype associations based disease overrepresentation analysis.

2.3 Protein-protein Interaction Analysis

We used the STRING protein interactome database [32] to construct a PPI networks of the proteins encoded by the DEGs. We set medium confidence score (400) to construct the PPI since the number of DEGs low. Network visualization and topological analyses were performed through NetworkAnalyst (Xia et al., 2015).

2.4 DEG-TFs Interaction Analysis

To identify regulatory TFs which regulate the DEGs at the transcriptional level, TF-target gene interactions were obtained from JASPAR database [34] to identify TFS based on topological parameters (i.e., degree and betweenness centrality) through NetworkAnalyst [33].

2.5 DEGs-miRNAs Interaction Analysis

The regulatory miRNAs which regulate DEGs at the post-transcriptional level, miRNAs-target gene interactions were obtained from TarBase [35] and miRTarBase [36] based on topological parameters (i.e., degree and betweenness centrality) analyzed in the NetworkAnalyst [33].

2.6 Identitification of Histone Modification Sites

We used human histone modification database (HHMD) to identify the histone modification sites of the hub genes and TFs [37].

2.7 Cross Validation of the Candidate Biomarker Biomolecules

AlzGene database contains 618 genes published genetic association studies of AD [38] was used to evaluate the interactions between AD-associated cellular alterations in the blood and the AD-associated alterations collected in AlzGene database. The miRNAs were also cross checked with a blood based miRNAs signatures which detected 12 diagnostic miRNAs of blood samples in AD (Leidinger et al., 2013).

2.8 Protein-drug Interactions Analysis

The protein-drug interaction was analyzed using DrugBank database (Version 5.0) [40] to identify potential drugs to be proposed in the AD. The three dimensional (3D) crystal structure of the target proteins TUBB (PDB ID:1TUB) was obtained from protein data bank

(PDB) [41]. The molecular docking analyses were performed using protein-small molecule docking server SwissDock [42]. The docking score and best-fit pose were selected for each ligand/drug.

2.9 Prediction for Protein Subcellular Localization

We used WoLF PSORT software [43] to predict the subcellular localizations of the proteins encoded by the DEGs. The WoLF PSORT software predicts subcellular localizations based on the amino acid sequence information.

3. Results

3.1 Transcriptomic Codes of Alzheimer's disease

The microarray datasets obtained from the blood tissues of AD were analyzed and 25 mutually expressed core DEGs were identified between the two datasets. This core 25 DEGs was considered as transcriptomic codes of AD (Figure 2A). The core DEGs of Alzheimer's disease were classified into diverse groups according to their functions and activities as transporter (4%), membrane traffic protein (4%), hydrolase (24%), oxidoreductase (4%), cell junction protein (4%), enzyme modulator (8%), transcription factor (12%), nucleic acid binding protein (12%), calcium-bidning protein (4%), and cytockletal protein (4%) (Figure 2B). The gene-set enrichment analysis showed that the DEGs were enriched in different biological process, molecular function and cellular component as summarized in the Table 1. The molecular pathway enrichment showed that Leukocyte transendothelial migration, Oxidative phosphorylation, Parkinson's disease, Cell adhesion molecules, Non-alcoholic fatty liver disease, Alzheimer's disease, and Huntington's disease pathways were altered in the AD (Table 2).

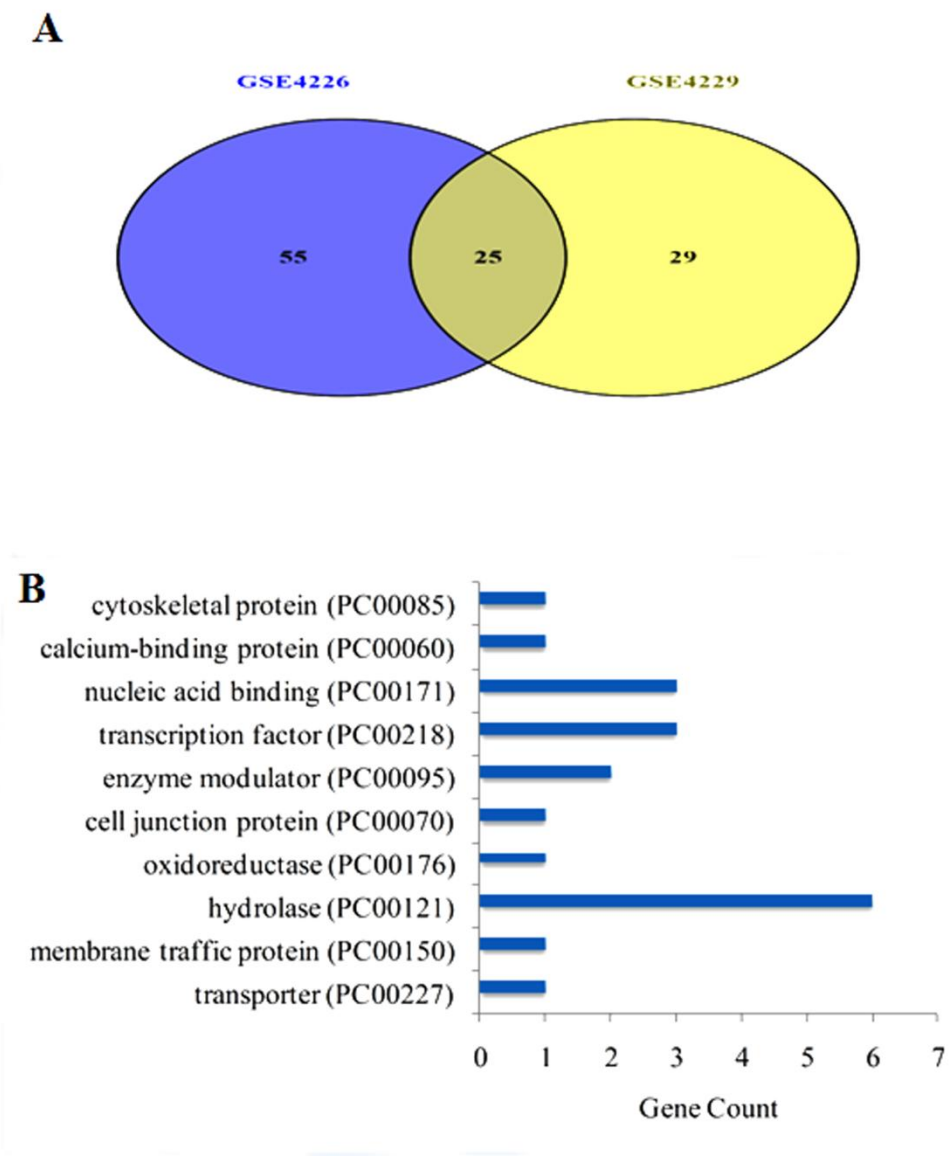

Figure 2: (A) Identification of DEGs in microarray datasets (GSE4226 and GSE4229). (A) The mutually expressed core DEGs identified between two datasets. (B) The protein class over representation by PANTHER protein class enriched by core DEGs.

Table 1: Gene set enrichment analysis of the differentially expressed genes identified from microarray data of blood of AD patients

	GO ID	Term	P-value	Gene
Biological Process	GO:1902043	positive regulation of extrinsic apoptotic signaling pathway via death domain receptors	0.0081	ATF3
	GO:0045869	negative regulation of single stranded viral RNA replication via double stranded DNA intermediate	0.0111	INPP5K
	GO:0006508	proteolysis	0.0004	CELA3A;CAPN6; UQCRC1;CASP2
	GO:0070973	protein localization to endoplasmic reticulum exit site	0.0087	BCAP29
	GO:0090315	negative regulation of protein targeting to membrane	0.0087	INPP5K
	GO:0046838	phosphorylated carbohydrate dephosphorylation	0.0099	INPP5K
	GO:0045719	negative regulation of glycogen biosynthetic process	0.0087	INPP5K
	GO:0035810	positive regulation of urine volume	0.0099	INPP5K
	GO:0046855	inositol phosphate dephosphorylation	0.0099	INPP5K
	GO:0009308	amine metabolic process	0.0087	VCAM1
Molecular Function	GO:0008131	primary amine oxidase activity	0.0087	VCAM1
	GO:0016681	oxidoreductase activity, acting on diphenols and related substances as donors, cytochrome as acceptor	0.0111	UQCRC1
	GO:0052866	phosphatidylinositol phosphate phosphatase activity	0.0087	INPP5K
	GO:0008121	ubiquinol-cytochrome-c reductase activity	0.0111	UQCRC1
	GO:0005000	vasopressin receptor activity	0.0087	INPP5K

	GO:0004439	phosphatidylinositol-4,5-bisphosphate 5-phosphatase activity	0.0111	INPP5K
	GO:0106019	phosphatidylinositol-4,5-bisphosphate phosphatase activity	0.0111	INPP5K
	GO:0046030	inositol trisphosphate phosphatase activity	0.0136	INPP5K
	GO:0042577	lipid phosphatase activity	0.0149	INPP5K
	GO:0016641	oxidoreductase activity, acting on the CH-NH2 group of donors, oxygen as acceptor	0.0198	VCAM1
Cellular Component	GO:0097504	Gemini of coiled bodies	0.0099	GEMIN7
	GO:0005750	mitochondrial respiratory chain complex III	0.0185	UQCRC1
	GO:0042599	lamellar body	0.0198	KLK5
	GO:0048471	perinuclear region of cytoplasm	0.0113	CAPN6;NOCT; INPP5K
	GO:0032040	small-subunit processome	0.0440	NOL6
	GO:0000794	condensed nuclear chromosome	0.0428	NOL6
	GO:0000793	condensed chromosome	0.0500	NOL6
	GO:0000228	nuclear chromosome	0.0642	NOL6
	GO:0032587	ruffle membrane	0.0653	INPP5K
	GO:0005876	spindle microtubule	0.0606	CAPN6

3.3. Proteomic Codes of Alzheimer's disease

To reveal central proteins, a protein-protein interaction of the DEGs was reconstructed around the DEGs (Figure 3). The topological analysis revealed 10 central hub proteins (TUBB, ATF3, NOL6, UQCRC1, SND1, CASP2, BTF3, INPP5K, VCAM1, and CSTF1) (Table 3).

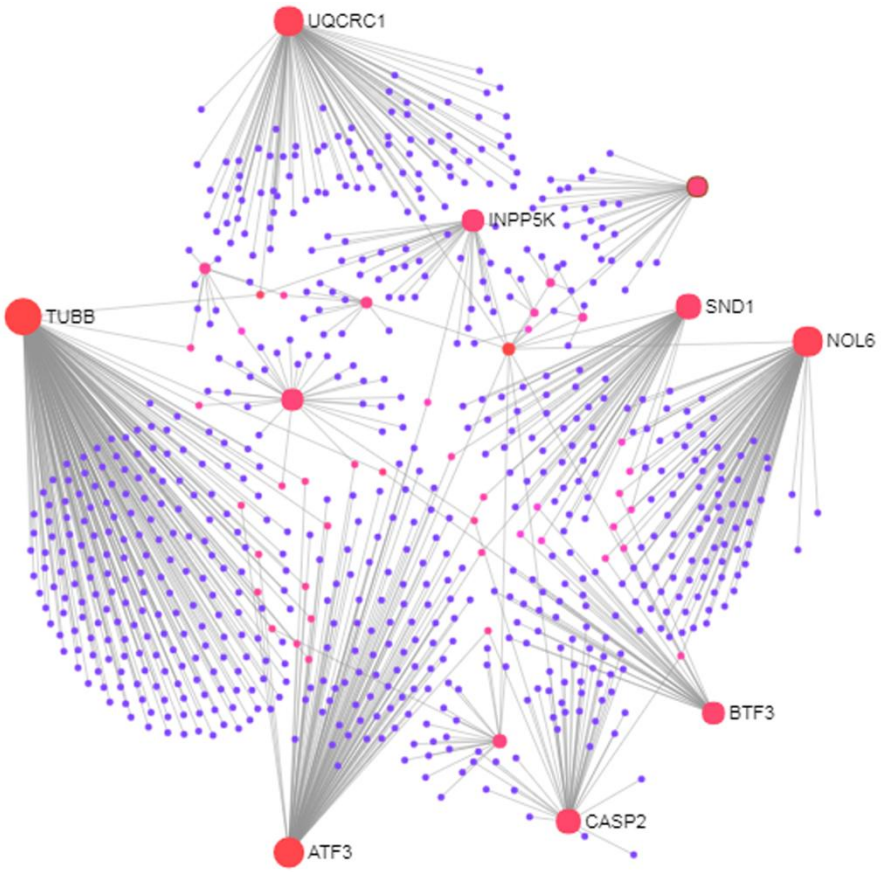

Figure 3: Protein-protein interaction network of the differentially expressed genes (DEGs) in Alzheimer's disease. The nodes indicate the DEGs and the edges indicate the interactions between two genes.

Table 3: Summary of hub proteins in Alzheimer's disease.

Symbol	**Description**	**Feature**
TUBB	Tubulin Beta Class	GTP binding *and* structural molecule activity
ATF3	Activating Transcription Factor 3	*DNA binding transcription factor activity* and *sequence-specific DNA binding*
NOL6	Nucleolar Protein 6	related to this gene include *RNA binding*
UQCRC1	Ubiquinol-Cytochrome C Reductase Core Protein 1	ubiquitin protein ligase binding *and* ubiquinol-cytochrome-c reductase activity
SND1	Staphylococcal Nuclease And Tudor Domain Containing 1	nucleic acid binding *and* transcription coregulator activity
CASP2	Caspase 2	enzyme binding *and* cysteine-type endopeptidase activity
BTF3	Basic Transcription Factor 3	this protein forms a stable complex with RNA polymerase IIB and is required for transcriptional initiation
INPP5K	Inositol Polyphosphate-5-Phosphatase K	vasopressin receptor activity *and* lipid phosphatase activity
VCAM1	Vascular Cell Adhesion Molecule 1	integrin binding *and* primary amine oxidase activity
CSTF1	Cleavage Stimulation Factor Subunit 1	RNA binding

3.4 The Regulatory Codes of Alzheimer's disease

We studied DEG-TFs interaction and DEGs-miRNAs interaction (Figure 4 and Figure 5) and detected central regulatory biomolecules (TFs and miRNAs) using topological parameters. 5 TFs (FOXC1, ZNF3, GEMIN7, SMG9, and BCAP29) and 10 miRNAs (mir-20a-5p, mir-93-5p, mir-16-5p, let-7b-5p, mir-708-5p, mir-24-3p, mir-26b-5p, mir-17-5p, mir-4270, and mir-4441) were detected from the DEGs-TFs (Figure 4) and DEGs-miRNAs interaction (Figure 5) networks respectively (Table 4). These biomolecules regulate genes at at transcriptional and post-transcriptional levels.

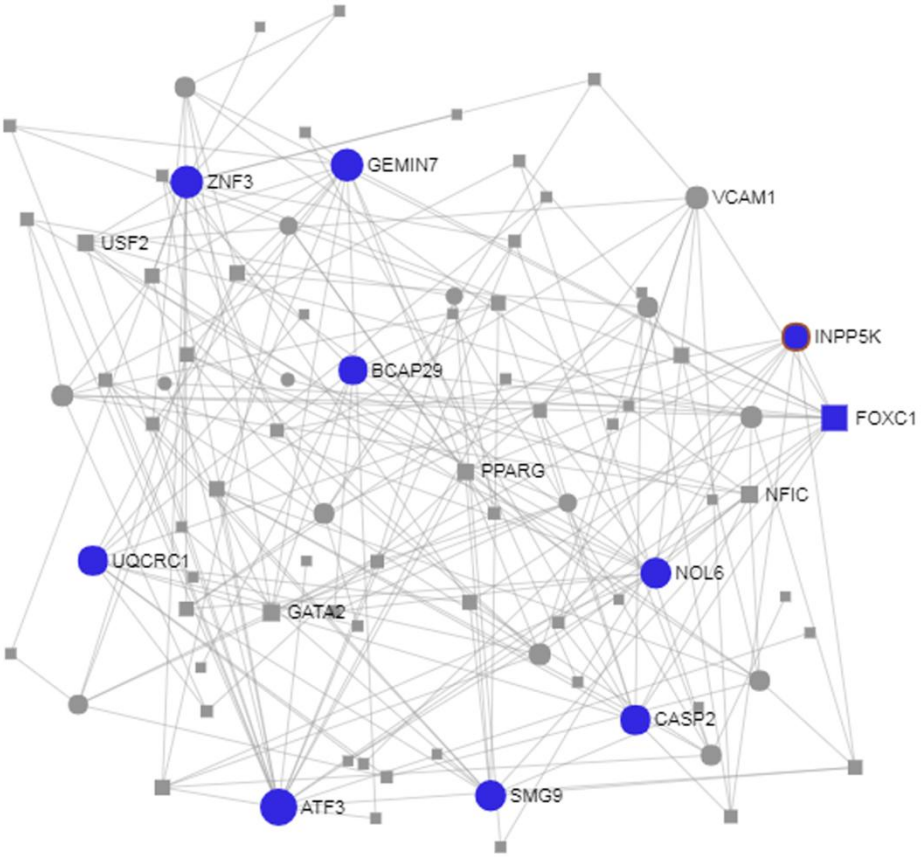

Figure 4: Construction of regulatory networks of DEGs-TFs interaction.

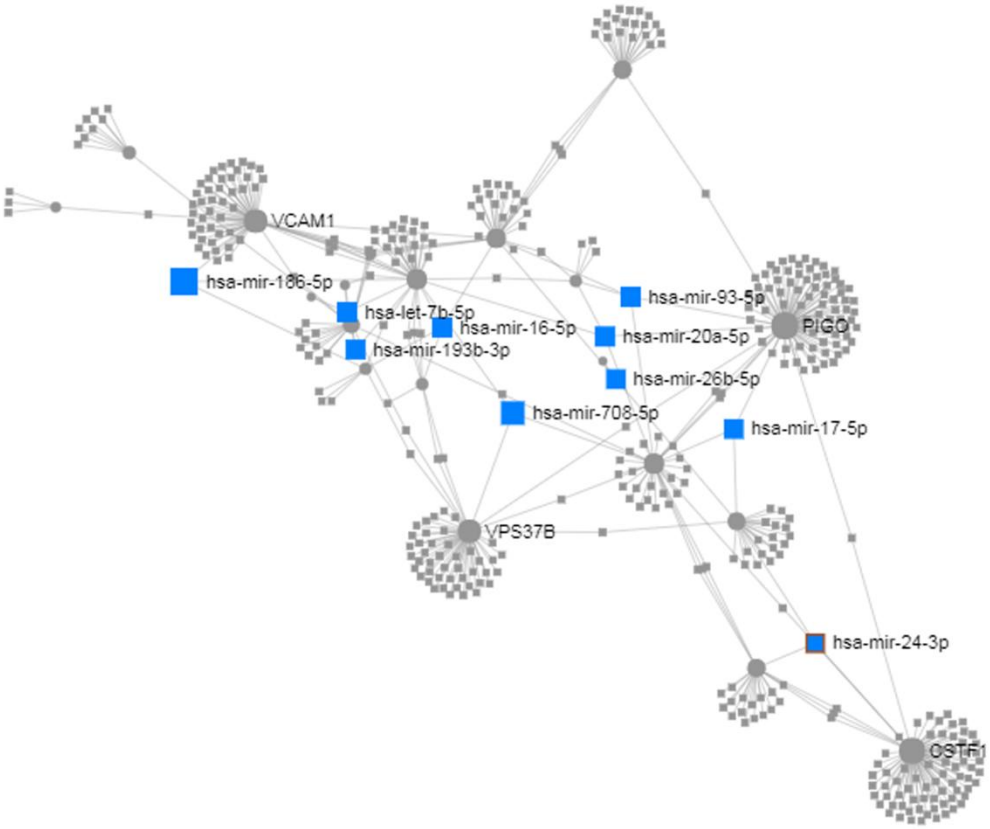

Figure 5: Construction of regulatory networks of DEGs-miRNAs interaction.

Table 4: Summary of regulatory biomolecules (TFs, miRNAs) in Alzheimer's disease

Symbol	Description	Feature
FOXC1	Forkhead Box C1	DNA binding transcription factor activity and transcription factor binding
ZNF3	Zinc Finger Protein 3	nucleic acid binding and identical protein binding
GEMIN7	Gem Nuclear Organelle Associated Protein 7	Transport of the SLBP independent Mature mRNA and RNA transport.
SMG9	SMG9, Nonsense Mediated MRNA Decay Factor	Viral mRNA Translation and Gene Expression.
BCAP29	B Cell Receptor Associated Protein 29	B Cell Receptor Signaling Pathway (sino) and AKT Signaling Pathway
	miRNAs	
mir-20a-5p	MicroRNA 20	Parkinsons Disease Pathway and cancer.
mir-93-5p	MicroRNA 93	miRNAs involved in DNA damage response.
mir-16-5p	MicroRNA 16	sudden infant death syndrome susceptibility pathways and in cancer
let-7b-5p	MicroRNA let-7b	MicroRNAs in cancer and metastatic brain tumor
mir-708-5p	MicroRNA 708	respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins.
mir-24-3p	MicroRNA 24	Peptide hormone metabolism and hepatitis C and hepatocellular carcinoma.
mir-26b-5p	MicroRNA 26	Parkinsons Disease Pathway and cancer.
mir-17-5p	MicroRNA 17	Parkinsons Disease Pathway and cancer.
mir-4270	MicroRNA 4270	affiliated with the miRNA class
mir-4441	MicroRNA 4441	affiliated with the miRNA class.

3.5 Epigenomics Codes of Alzheimer's disease: Histone Modification Sites of Biomolecules

To provide insights about the epigenetic regulations of the biomolecules, we studied the histone modifications patterns. We observed that eight hub genes and TFs related with several histone modification places (Table 5).

Table 5: Histone modification patterns (obtained from HHMD) of novel hub genes and TFs with respect to the already known histone modification sites in neurodegenerative diseases.

Official symbol of Biomeolecules	**RefSeq ID**	**Histone modification sites already known in neurodegenerative diseases**				
		H3K27	**H3K4**	**H3K9**	**H3K9/H4K20**	**H4R3**
Hub protein						
TUBB	NM_178014	✓	✓	✓	✓	✓
ATF3	NM_001030287	✓	✓	✓	✓	✓
NOL6	NM_139235	✓	✓	✓	✓	✓
UQCRC1	NM_003365	✓	✓	✓	✓	✓
SND1	NM_014390	✓	✓	✓	✓	✓
CASP2	NM_032983	✓	✓	✓	✓	✓
BTF3	NM_001037637	✓	✓	✓	✓	✓
INPP5K	NM_016532	✓	✓	✓	✓	✓
VCAM1	NM_080682	✓	✓	✓	✓	✓
CSTF1	NM_001324	✓	✓	✓	✓	✓
TFs						
FOXC1	NM_001453	✓	✓	✓	✓	✓
ZNF3	NM_017715	✓	✓	✓	✓	✓
GEMIN7	NM_001007269	✓	✓	✓	✓	✓
SMG9					✓	✓
BCAP29	NM_001008407	✓	✓	✓	✓	✓

3.6 Cross-validation of the Identified Potential Biomarkers

The cross checking of the biomolecules with AlzGene database revealed no overlapped of the 25 DEGs with the AD-related biomarkers in AlzGene database. Moreover, The present study identified miR-26a-5p which was consistent with the previous study of 12 blood based miRNAs signatures of AD.

3.7 Protein-drug Interactions

In the subnetwork, TUBB connected to the drugs Vinorelbine, Vincristine, Vinblastine, Podofilox, Colchicine, Epothilone D, Epothilone B, Cyt997, Ca4p, and Zen-012 (Figure 6). The identified drugs were categorized into different classes based on anatomical sites (Figure 7A) and further classified according to developmental stages (Figure 7B). Considering the statistical significance of the drug-protein interactions and the possible role of the targeted protein in Alzheimer's disease pathogenesis, a set protein-drug interactions selected, and a series of molecular docking simulations were performed to analyze the binding affinities of identified drugs with their target protein (Table 6). The resultant energetic state and docking scores indicated the presence of thermodynamically feasible confirmations for all these interactions.

Figure 6: Protein-drug interactions network. The interactions between drugs and hub node (TUBB) were represented. The area of the node represents the degree of interaction in the network.

Table 6: Protein-drug interactions and their binding affinity by molecular docking statistics.

Target protein	**Drug**	**Description**	**FullFitness (kcal/mol)**	**Estimated ΔG (kcal/mol)**
TUBB	Vinorelbine	Approved/Antineoplastic Agents	-4874.85	-8.43
	Vincristine	Approved/Antineoplastic Agents	-4848.09	-8.39
	Vinblastine	Approved/Antineoplastic Agents	-4757.89	-8.41
	Podofilox	Approved/Dermatologicals	-4947.47	-7.90
	Colchicine	Approved/Immunosuppressive Agents	-4906.52	-7.13
	Epothilone D	Experimental/Antineoplastic Agents	-5007.01	-7.29
	Epothilone B	Experimental/Antineoplastic Agents	-4782.27	-8.32
	CYT997	Investigational/Antineoplastic Agents	-5048.60	-7.14
	CA4P	Investigational/No available	-4951.83	-8.19
	ZEN-012	Investigational/Antineoplastic Agents	-	-

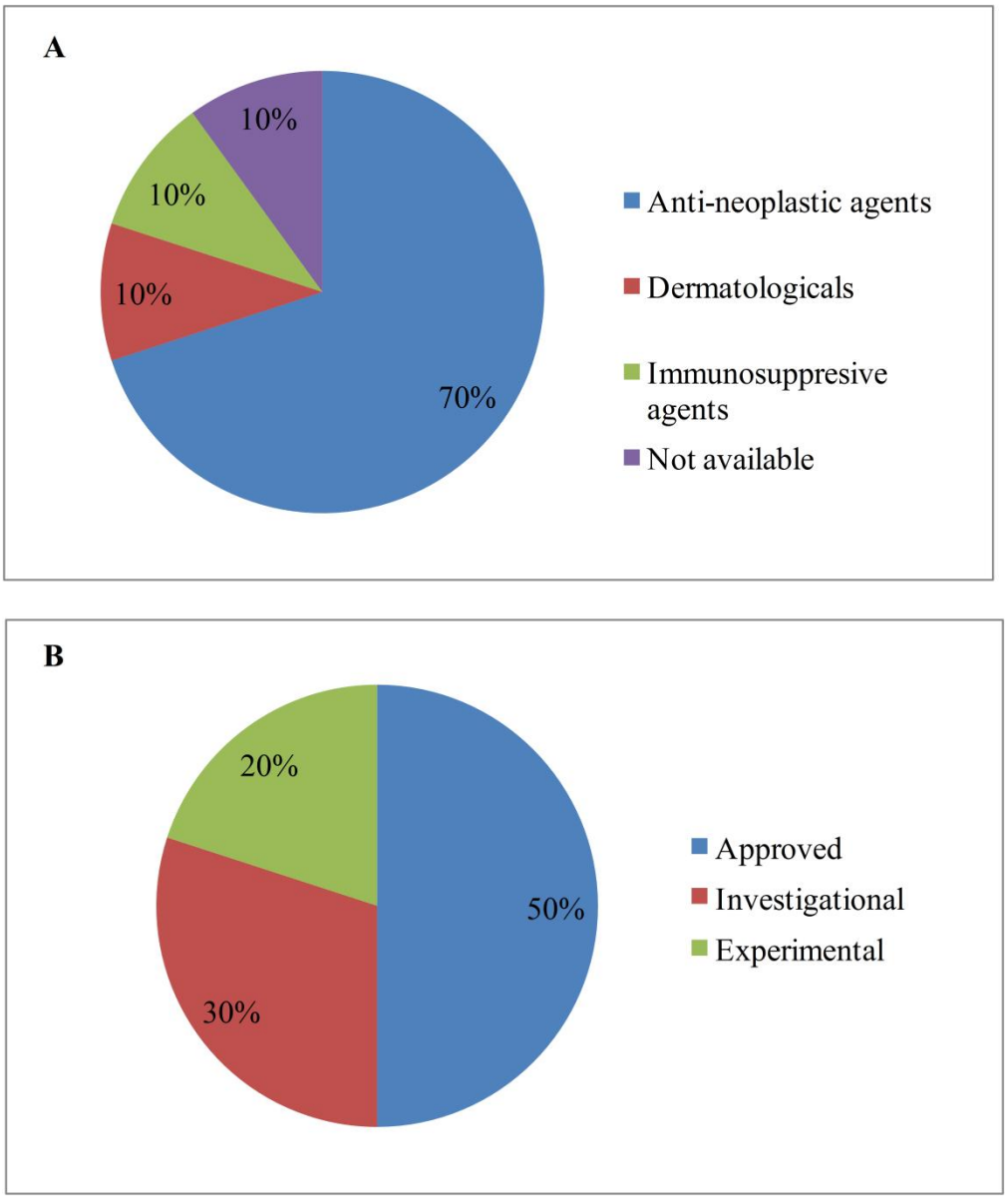

Figure 7: (A) Distribution of drugs into anatomical therapeutic chemical classes. (B) Classification of repositioned drugs according to developmental stages.

3.8 Protein Subcellular Localization

We studied the subcellular localization of proteins encoded by the DEGs in the AD was predicted. The subcellular levels of proteins were displayed in Figure 8. Particularly, nodes of TUBB, SND1 CASP2 were localized into cytoplasm; ATF3, BTF3 were localized into nucleus; NOL6, UQCRC1, INPP5K were localized into mitochondria; VCAM1 was localized into plasma membrane; and CSTF1 was localized into cytoplasmic and nucleus.

These analyses suggested these DEGs may be genes may be associated with the regulation of AD in a stepwise manner from nucleus to extracellular position via cytosol.

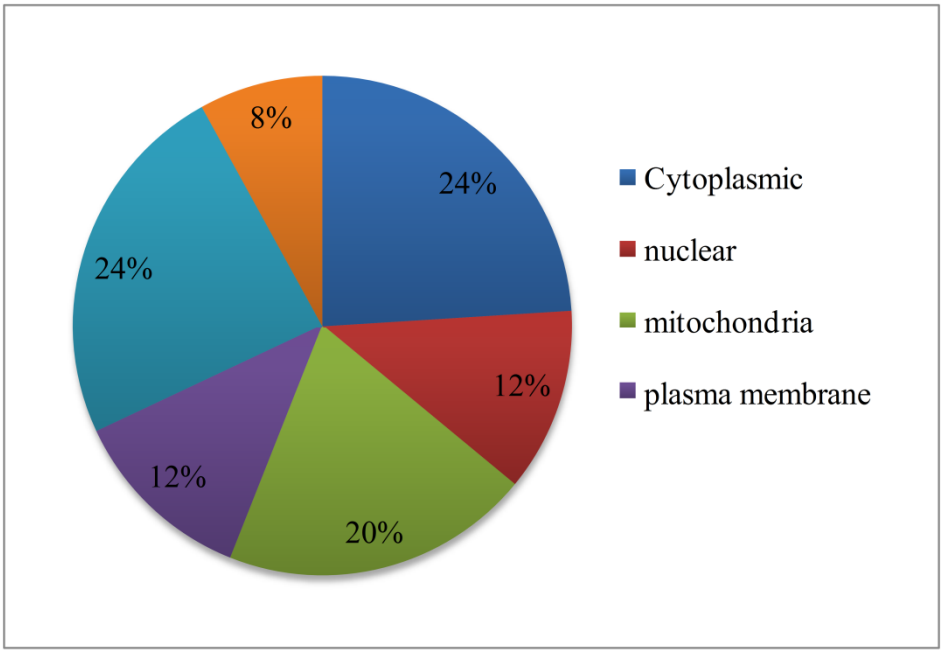

Figure 8: The distribution and percentages of the DEGs is shown to be the protein subcellular localization.

4. Discussion

Much research is directed towards exploration of the biomarkers to elucidate the molecular mechanisms in the AD. The diagnosis of the AD is currently performed based on neuropsychological evaluation and neuroimaging, but the robust and specific biomarkers for diagnosis and prognosis of AD is an unmet challenge [11]. In this study, we comprehensively studied gene expressions obtained from peripheral blood of the AD patients aimed to identify robust candidate biomarkers that may serve as potential therapeutic targets or biomarkers of the AD.

Microarrays are extensively used in biomedical research and also the main resources of biomarker candidates [44]. Microarray gene expression profiling is widely used to identify

DEGs in various diseases (Gov et al., 2017; Guo et al., 2017; Islam et al., 2018; Rahman et al., 2018). Analyzing the gene expression patterns in the blood of the AD patients revealed significant alterations in the expression profiles of 25 common DEGs in two transcriptomics datasets (GSE4226 and GSE4229). The over-representation analyses revealed AD and neurodegeneration associated molecular pathways (Oxidative phosphorylation and Alzheimer's disease's disease pathway) [46]. The disease over representation analysis by dbGaP resulted with statistically significant fibrin fragment D, Lipoproteins LDL, and Neoplasms pathways. The fibrin deposition enhance the neurovascular damage and neuroinflammation in the Alzheimers disease [47,48]. Plasma lipoproteins play role in modulating the integrity of vascular systems in brain and neuroinflammation; consequently lipoproteins may modulate the pathogenesis of the AD [49,50]. The neoplasms were found significant, but inverse relations suggested between cancer and Alzheimer's disease by some researchers [51–53]. The under diagnosis of the AD may be the cause of the lower AD risk in cancer survivors. Moreover, a relationship between cancer and AD was not supported in few studies [54]. We think aging is related both to increase risk of AD and cancer.

The integrative analysis of protein-protein interaction network is widely used to identify the central proteins which are considered as the key players in mechanisms behind the disease [55]. Thus, studied PPI network around DEGs identified key hub proteins (Table 2). These identified hub proteins have the potential to contribute to the formation and progression of neurodegenerative diseases. The identified hub proteins are associated with different biological phenomena. According to GeneCards database (https://www.genecards.org/), diseases associated with TUBB include cortical dysplasia with other brain malformations. The ATF3 pathway is implicated in brain vascular damage [56]; it may be associated with amyloid deposition in AD [57]. The NOL6 is associated with ribosome biogenesis according to GeneCards database. Among the related pathways of

UQCRC1 are metabolism and respiratory electron transport, but its association is not reported in the AD yet. The multifunctional protein SND1 is deregulated in various cancers [58]; dysregulation of CASP2 may be implicated in AD. BTF3 is a blood biomarkers of AD [59]. INPP5K is associated with the disease muscular dystrophy, and intellectual disability, but no relation with the AD is reported yet. VCAM1 expression was found to be associated significantly with AD dementia [60]. The relation of hub protein CSTF1 with the AD is not clear yet.

We identified biomolecules (TFs and miRNAs) since alteration in these molecules may provide key information on dysregulation of gene expression in the AD. One study evaluated the hypothesis that FOXC1 contributes to cerebral vascular disease in the AD [61,62]. Our previous study also revealed FOXC1 as regulatory TF in AD (Rahman et al., 2018). ZNF is implicated critically in the pathogenesis of neuronal diseases [63], but its role in AD is not clear yet. GEMN6 was reported to be associated with AD [64], but its role in AD is not reported yet. The role of SMG9 is not reported in AD yet [65]. BCAP29 was suggested as diagnostic biomarkers in peripheral blood gene expression analysis [66].

The microRNA (miRNAs) is single stranded non-coding RNAs and regulate genes [19]. In recent years, characterization of such regulatory miRNAs is considered crucial in the context of molecular biology [67]. The dysregulated miRNAs have been reported in the pathobiology of the AD [19]. Since miRNAs may serve as biomarkers for diagnosis and therapeutic target for breakthrough treatment strategies in the AD [20]; therefore, we identified the miRNAs as regulatory component of the DEGs. The identified mir-20a-5p is associated with aging [68] and proposed as peripheral blood biomarker in AD [10]. The upregulation of mir-93-5p was reported as biomarkers in serum of AD patients and qRT validation was also performed [10]. The mir-16-5p and mir-708-5p was identified as differentially expressed in CSF in AD [69,70]. The let-7b-5p was down-regulated in AD as a

member of 12 blood based miRNA biomarker signature [71]. The dysregulation of mir-24-3p was also reported in lung cancer [10]. Decreased expression of mir-24-3p was identified as candidate that can discriminate AD from control CSF [72]. The mir-26b-5p was down-regulated in AD as a member of 12 blood based miRNA biomarker signature [59]. Elevated levels of miR-26-b may thus contribute to the AD neuronal pathology [73]. The mir-17-5p was found to be deregulated in circulatory biofluids in lung cancer [10] and was dysregulated in the AD [74]. The role of mir-4270 and mir-4441 in the AD is not reported yet.

To decipher the epigenetic regulations, we analyzed and detected several histone modification sites of biomolecules implicated with neurodegenerative disease [75] (Table 4).

We studied the protein-drug interactions to identify candidate drugs in the AD. Total 10 drugs were identified from the interaction network. Various molecular methods are used in pharmaceutical research to investigate complex biological systems; molecular docking broadly used in drug design to elucidate the ligand conformation within the binding site of the target protein. The molecular docking methods estimate free energy by evaluating critical phenomena involved in the intermolecular recognition process in ligand-receptor binding [76]. Consequently, in the present study, we evaluated the binding mode of the ligands/drugs with the target protein TUBB and energetically stable conformations were obtained. Therefore, discovering associations between the identified drugs and AD are required for further investigations.

We studied the interactions of the DEGs by protein subcellular prediction of the DEGs to provide insights about the potential sites of the protein localizations. The interactions of DEGs broaden from nucleus to extracellular position via cytosol. The screening of drugs to relieve AD, the protein subcellular localization potentially renders targeting sites for particular drugs. For example, the identified drugs target TUBB may be

required to enter into cytosol. Thus, the present study provides insights about biomolecules and candidate drugs which have high potential in peripheral blood biomarkers in the AD.

5. Conclusion

In the present study, we have analyzed the blood-based transcriptomics profiles using integrative multi-omics analyses to reveal systems level biomarkers at protein (hub proteins, TFs) and RNA level (mRNAs, miRNAs). A number of key hub genes significantly enriched in leukocyte transendothelial migration, oxidative phosphorylation, Parkinson's disease, cell adhesion molecules, non-alcoholic fatty liver disease, Alzheimer's disease, and Huntington's disease were identified. We also identified significant hub proteins (TUBB, ATF3, NOL6, UQCRC1, SND1, CASP2, BTF3, INPP5K, VCAM1, and CSTF1), TFs (FOXC1, ZNF3, GEMIN7, SMG9, and BCAP29), and miRNAs (mir-20a-5p, mir-93-5p, mir-16-5p, let-7b-5p, mir-708-5p, mir-24-3p, mir-26b-5p, mir-17-5p, mir-4270, mir-4441) that regulate the function of the DEGs in the AD. These biomolecules may be considered systems biomarkers at protein level and RNA levels. The hub genes possess several histone modification sites. The protein subcelleular localizations prediction of the DEGs provides cellular sites to screen drugs to relieve AD. The interactions of DEGs extend from nucleus to plasma membrane via cytosol. A number of drugs have been identified from protein-drug interaction networks that might be considered for the treatment of the AD. The interaction between drugs and target protein was studied by molecular docking simulation to estimate the ligand-receptor binding free energy. In this study, we have identified potential peripheral biomarkers for the AD which can be detected as transcripts in blood cells. The candidate biomarkers identified for the AD using blood cell transcripts warrant clinical investigations in AD patients to evaluate their utility. The nature of these biomarkers and the pathways they participate in may reveal new aspects of AD development and progression since these biomarkers are evident in cells outside the central nervous system.

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Conflict of interest

The authors declare no conflict of interest.