Article

Blood-based molecular biomarker signatures in Alzheimer's disease: insights from systems biomedicine perspective

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Abstract: *Background and objectives*: Alzheimer's disease (AD) is the progressive neurodegenerative disease characterized by dementia, but no peripheral biomarkers available yet that can detect the AD. This study aimed to identify systems biomarker signatures in the AD through integrative analyses. *Materials and Methods:* We used two microarray transcriptomics datasets of blood from 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 done on common DEGs. *Results:* Total 25 DEGs were detected between the two datasets. Integration of DEGs 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). The analyses revealed candidate blood based biomarkers in the AD. We evaluated the histone modifications of the identified biomolecules. The hub genes and transcription factors (TFs) revealed that they possess several histone modification sites associated with Alzheimer's disease. The protein-drug interactions revealed 10 candidate drugs consisting of antineoplastic (Vinorelbine, Vincristine, Vinblastine, Epothilone D, Epothilone B, CYT997, and ZEN-012), dermatologicals (Podofilox), and immunosuppressive agents (Colchicine) that may target the candidate systems biomarkers. The subcellular localization analysis revealed the interactions of the DEGs range from nucleus to plasma membrane through cytosol. *Conclusions:* 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 [1]. 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 complex diseases including AD diagnosis and treatment [3-6].

The neuroimaging techniques and cerebrospinal fluids biomarkers are used in everyday clinical practice to diagnose dementia [7-9]. 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 [10]. Considering the unavailability of the peripheral blood biomarkers [11-14]. Therefore, a blood based molecular biomarkers may be valuable in evaluating the diagnosis, prognosis, pathogenesis, and treatment of AD [15–19].

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 [20]. Various external factors such as life style, age, environment, and disease state are contributor in epigenetic changes [20]. Recent studies explored the modifications of histones and methylation of the genes in AD [20], but the central mechanism of epigenetics in the AD is not clear.

The dysregulation of the miRNAs are implicated in AD [21-23]. Consequently, miRNAs are increasingly being studied in the exploration of biomarkers in the AD [4-5]. Therefore, systems biology analyses are used to elucidate the possible roles of biomolecules in different complex diseases [4-5]. 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).

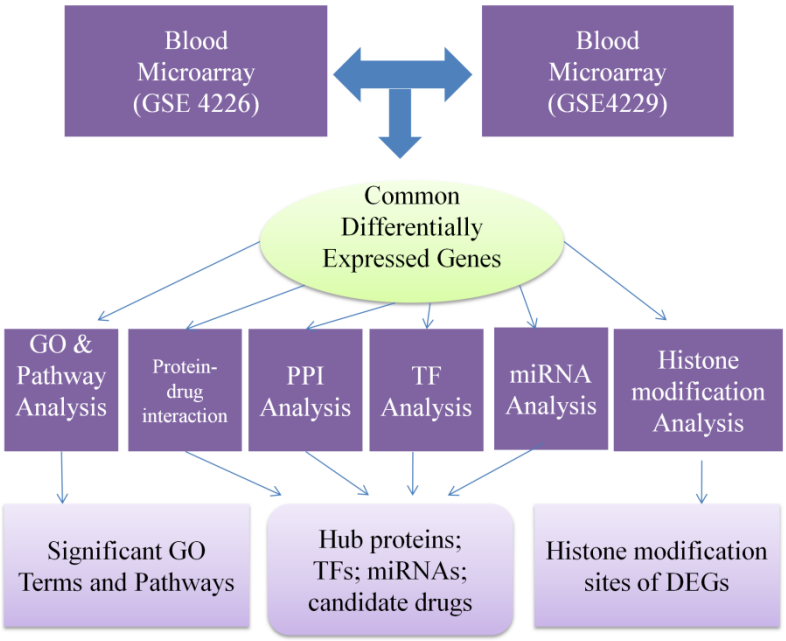

Figure 1: A multi-omics analysis approach used in this study. The blood based gene expression transcriptomics datasets of blood of Alzheimer's disease (AD) from NCBI-GEO database were analyzed to identify differentially expressed genes (DEGs). Significantly enriched pathways, Gene Ontology terms and disease overrepresentation were performed. To identify the hub proteins, regulatory biomolecules, protein-protein interaction networks, DEGs-TFS, DEGs-miRNAs were performed. The protein-drugs interaction analysis using DrugBank database and candidate small molecular drugs were identified. Candidate biomarkers at protein and microRNAs levels, drug target, candidate drugs, epigenetic regulatory patterns of the hub biomolecules, 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 datasets from peripheral blood mononuclear cells (GSE4226 and GSE4229) [24] in the AD compared to control was obtained from the NCBI-GEO database [25]. The datasets were analyzed in GEO2R of NCBI-GEO by log2 transformation and limma in hypothesis testing to identify DEGs in the AD compared to control. The Benjamini & Hochberg correction to control the false discovery rate, and p-value<0.01 was regarded as the cut-off criteria to identify DEGs with statistical significance. The Venn analysis was performed through the online tool—Jvenn [26] to identify common target DEGs from the two datasets.

2.2 Gene Ontology and Pathway Enrichment Analysis

The functional analysis of the DEGs was performed by gene over-representation analyses via Enrichr [27] to find out the Gene Ontology (GO) terminology and pathways. The protein class over representation of the DEGs was performed by PANTHER database [28] to identify the category of the proteins encoded by the DEGs. 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 the employed strategy linking the genotype and phenotype associations based disease overrepresentation analysis.

2.3 Protein-protein Interaction Analysis

We used the STRING protein interactome database to construct PPI networks of the proteins encoded by the DEGs [29]. We set medium confidence score (400) to construct the PPI since the number of DEGs were low. Network visualization and topological analyses were performed through NetworkAnalyst [30].

2.4 DEG-TFs and DEGs-miRNAs Interaction Analysis

To identify regulatory TFs which regulate the DEGs at the transcriptional level, TF-target gene interactions were obtained from JASPAR database [31] to identify TFS based on topological parameters through NetworkAnalyst [30]. The regulatory miRNAs which regulate DEGs at the post-transcriptional level were identified from miRNAs-target gene interactions were obtained from TarBase [32] and miRTarBase [33] based on topological parameters.

2.5 Identitification of Histone Modification Sites

In order to identify histone modification patterns of the hub genes and TFs, we employed human histone modification database (HHMD)[34].

2.6 Cross Validation of the Candidate Biomarker Biomolecules

AlzGene database contains 618 genes published genetic association studies of AD [35] 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 [36].

2.7 Protein-drug Interactions Analysis

The protein-drug interaction was analyzed using DrugBank database (Version 5.0) [37] 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). The molecular docking analyses were performed using protein-small molecule docking server SwissDock [38]. The docking score and best-fit pose were selected for each ligand/drug.

2.8 Prediction for Protein Subcellular Localization

We used WoLF PSORT software [39] 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).

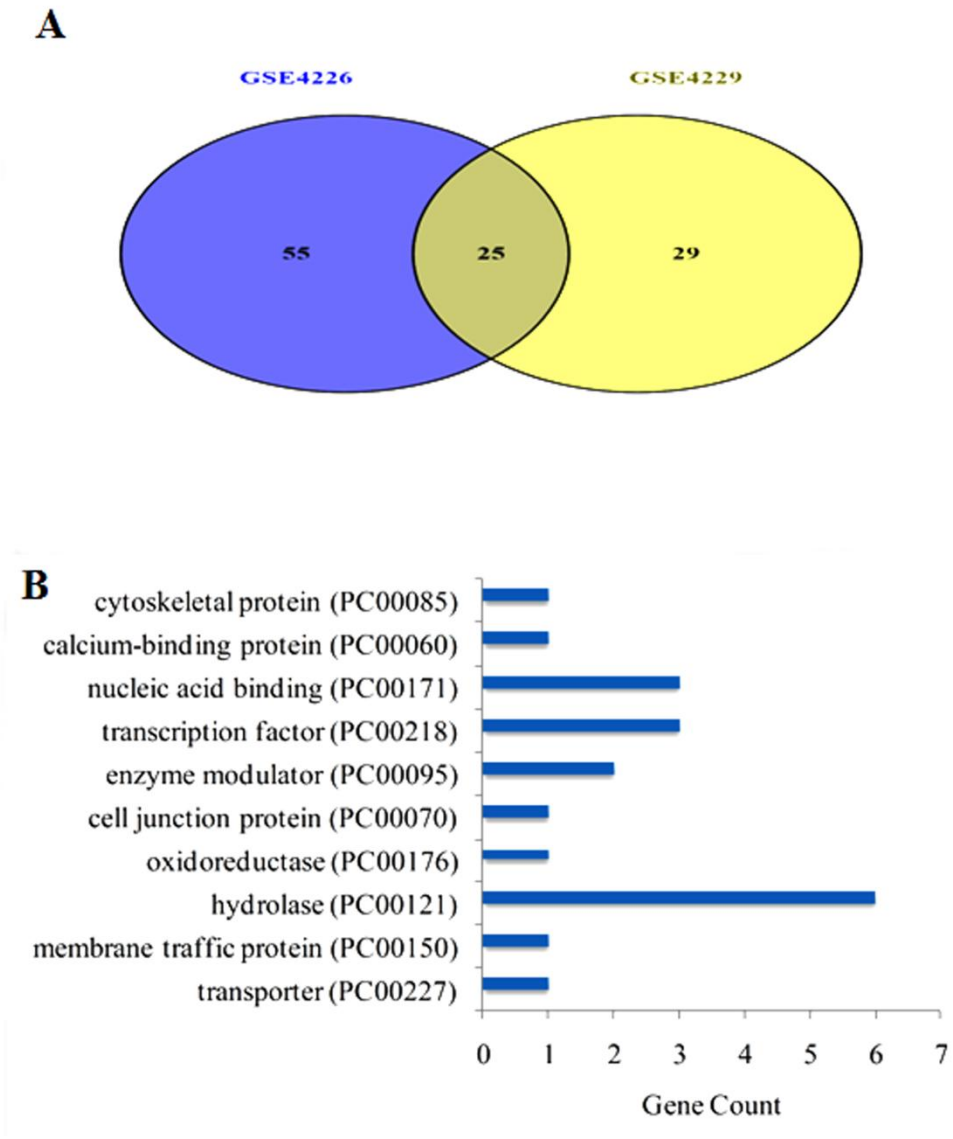

Figure 2. (A) Identification of DEGs in microarray datasets (GSE4226 and GSE4229). (A) The mutually expressed core DEGs identified between two datasets. (B) The protein class over representation by PANTHER protein class enriched by core DEGs.

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

Category	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**

Table 2. The molecular pathway enrichment in 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**

3.3. 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).

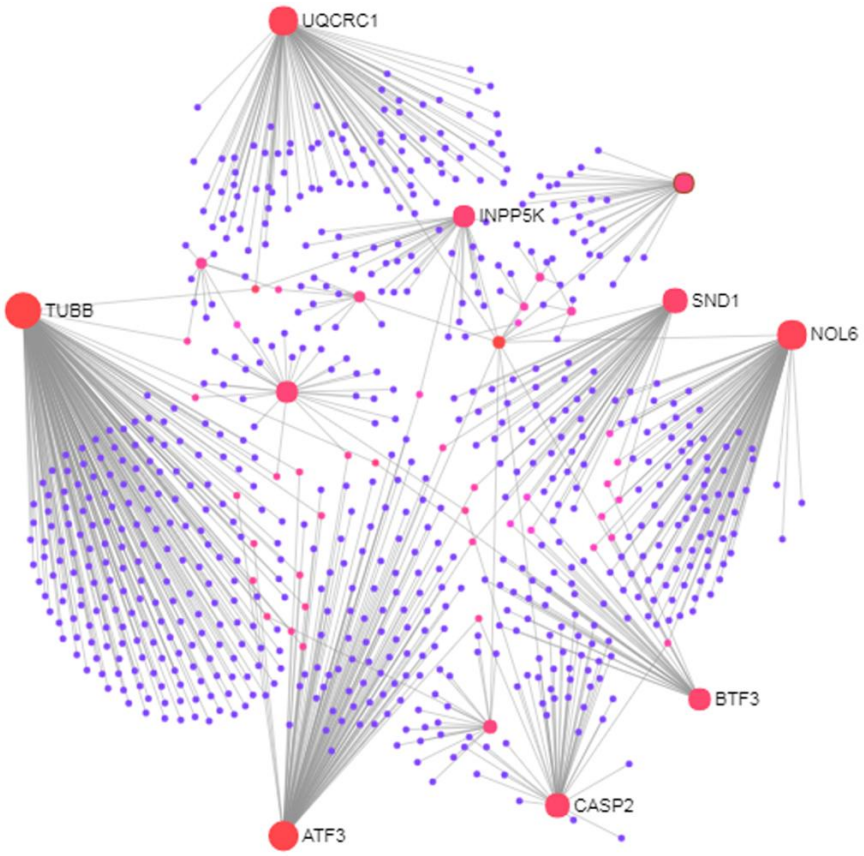

Figure 3. Protein-protein interaction network of the differentially expressed genes (DEGs) in Alzheimer's disease. The nodes indicate the DEGs and the edges indicate the interactions between two genes.

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.

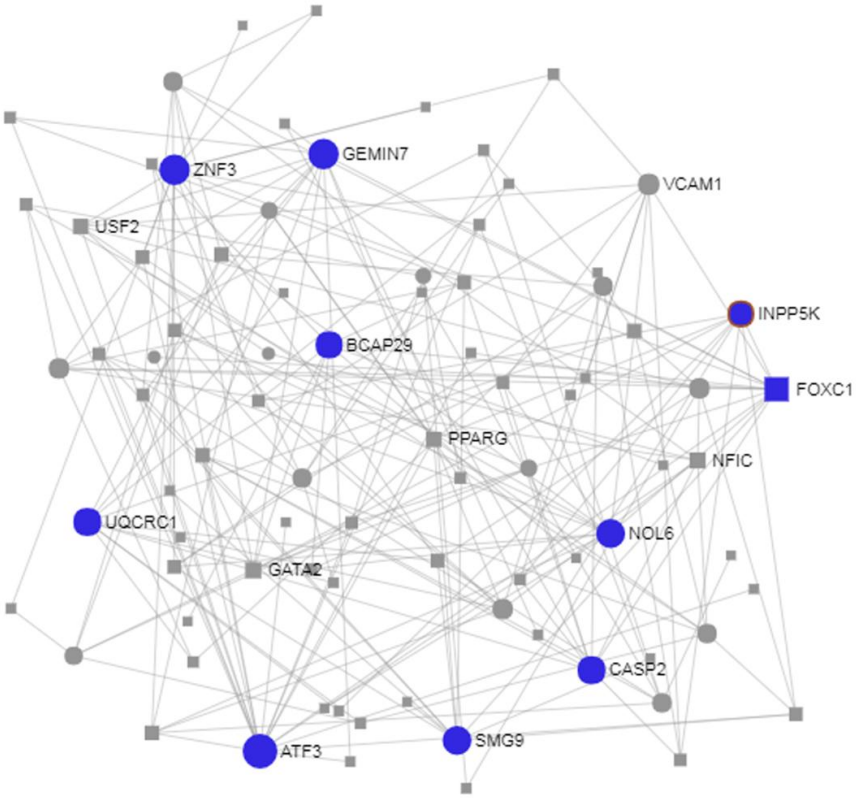

Figure 4: The differentially expressed genes and transcription factor interactions was analyzed to identify the transcription factors that regulate the differentially expressed genes in Alzheimer's disease.

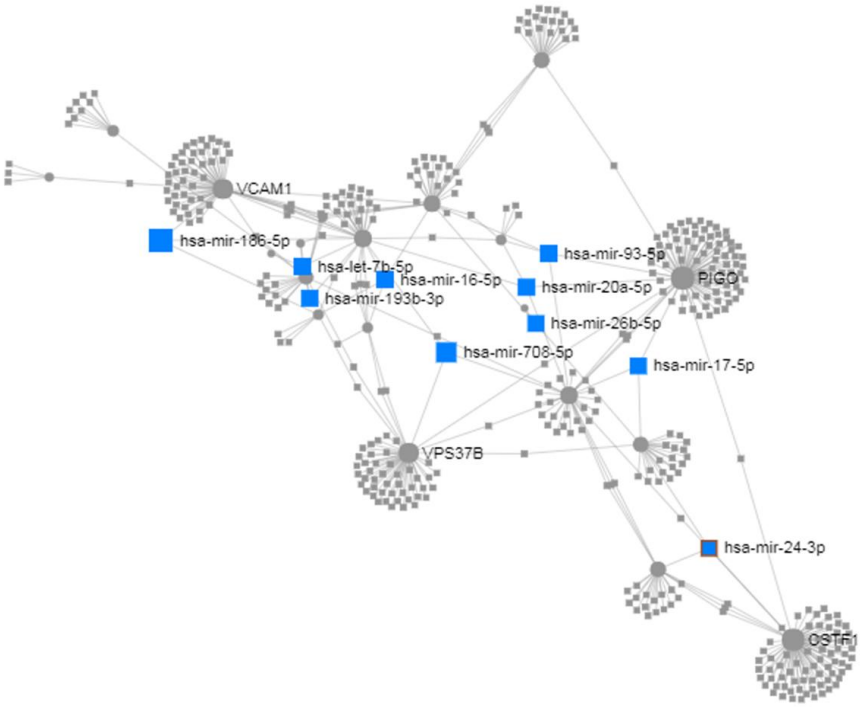

Figure 5: The differentially expressed genes and microRNAs interactions was analyzed to identify the microRNAs that regulate the differentially expressed genes in Alzheimer's disease.

Table 3. Summary of regulatory biomolecules (TFs, miRNAs) in Alzheimer's disease.

Symbol	Description	Feature
TFs		
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 4. 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**	✓	✓	✓	✓	✓
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.

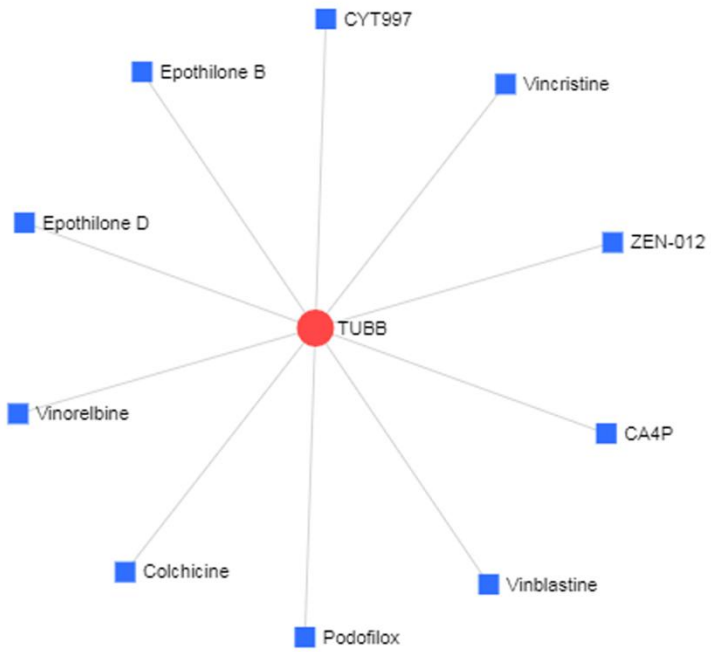

Figure 6: Protein-drug interactions network. The interactions between drugs and hub node (TUBB) were represented. The area of the node represents the degree of interaction in the network.

Table 5: 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**	**-**	**-**

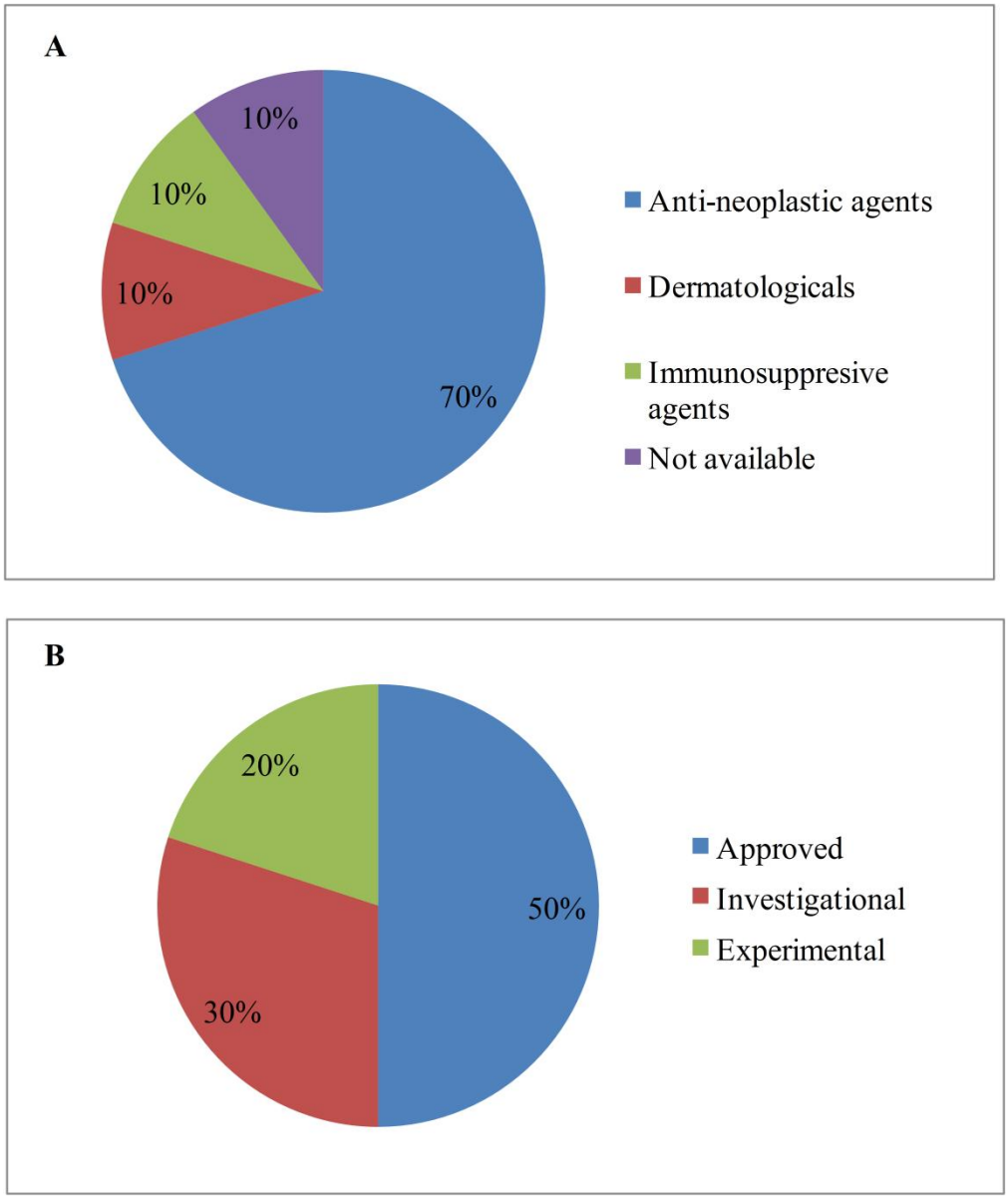

Figure 7: (A) Distribution of drugs into anatomical therapeutic chemical classes. (B) Classification of repositioned drugs according to developmental stages.

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.

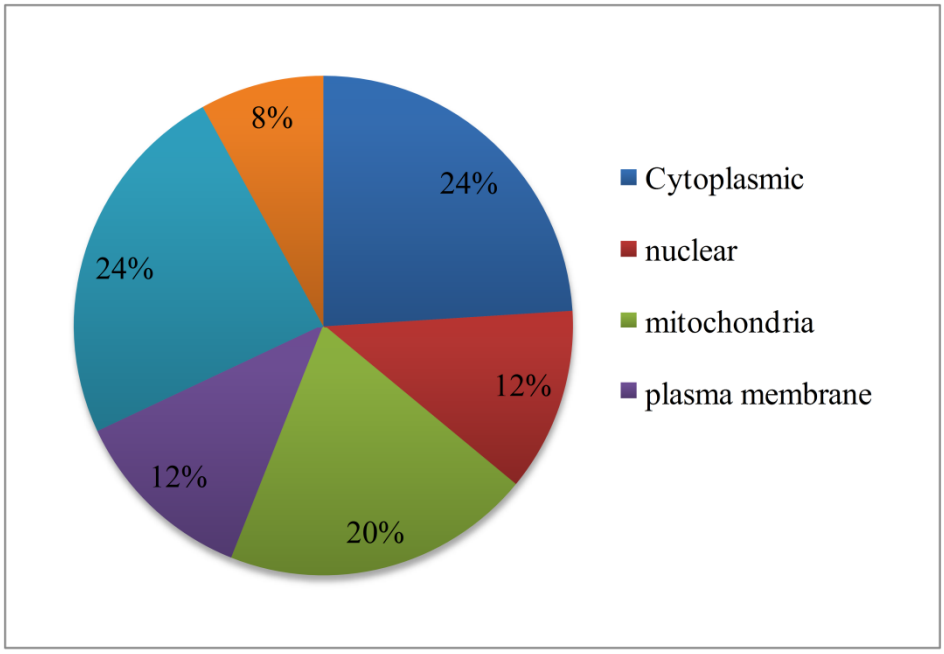

Figure 8: The distribution and percentages of the DEGs is shown to be the protein subcellular localization.

4. Discussion

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 [14]. 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 and RNA-seq are extensively used in biomedical research as the main resources of biomarker candidates form complex diseases [4-6,40]. Microarray gene expression profiling is widely used to identify DEGs in various diseases including AD [4-6,40]. 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. The over-representation analyses revealed AD and neurodegeneration associated molecular pathways (Oxidative phosphorylation and Alzheimer's disease's disease pathway) [41]. 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 [42,43]. Plasma lipoproteins play role in modulating the integrity of vascular systems in brain and neuroinflammation; consequently lipoproteins may modulate the pathogenesis of the AD [44,45]. The neoplasms were found significant, but inverse relations suggested between cancer and Alzheimer's disease by some researchers [46-48]. 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 [49]. 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 [50]. 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. The diseases associated with TUBB include cortical dysplasia with other brain malformations (https://www.genecards.org/). The ATF3 pathway is implicated in brain vascular damage [51]; it may be associated with amyloid deposition in AD [52]. 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 [53]; dysregulation of CASP2 may be implicated in AD. BTF3 is a blood biomarkers of AD [54]. 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 [55]. 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 [56,57]. Our previous study also revealed FOXC1 as regulatory TF in AD [4]. ZNF is implicated critically in the pathogenesis of neuronal diseases [58], but its role in AD is not clear yet. GEMN6 was reported to be associated with AD [59], but its role in AD is not reported yet. The role of SMG9 is not reported in AD yet [60]. BCAP29 was suggested as diagnostic biomarkers in peripheral blood gene expression analysis [61].

The microRNA (miRNAs) is single stranded non-coding RNAs and regulate genes [22]. In recent years, characterization of such regulatory miRNAs is considered crucial in the context of molecular biology [62]. The dysregulated miRNAs have been reported in the pathobiology of the AD [22]. Since miRNAs may serve as biomarkers for diagnosis and therapeutic target for breakthrough treatment strategies in the AD [23]; therefore, we identified the miRNAs as regulatory component of the DEGs. The identified mir-20a-5p is associated with aging [63] and proposed as peripheral blood biomarker in AD [13]. The upregulation of mir-93-5p was reported as biomarkers in serum of AD patients and qRT validation was also performed [13]. The mir-16-5p and mir-708-5p was identified as differentially expressed in CSF in AD [64,65]. The let-7b-5p was down-regulated in AD as a member of 12 blood based miRNA biomarker signature [65]. The dysregulation of mir-24-3p was also reported in lung cancer [13]. Decreased expression of mir-24-3p was identified as candidate that can discriminate AD from control CSF [66]. The mir-26b-5p was down-regulated in AD as a member of 12 blood based miRNA biomarker signature [54]. Elevated levels of miR-26-b may thus contribute to the AD neuronal pathology [67]. The mir-17-5p was found to be deregulated in circulatory biofluids in lung cancer [13] and was dysregulated in the AD [68]. 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 (Table 4) [69].

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 [70]. 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. Conclusions

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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References

[1] Association A. 2017 Alzheimer's disease facts and figures. Alzheimer's Dement 2017;13:325–73. doi:10.1016/j.jalz.2017.02.001.

[2] Dunckley T, Beach TG, Ramsey KE, Grover A, Mastroeni D, Walker DG, et al. Gene expression correlates of neurofibrillary tangles in Alzheimer's disease. Neurobiol Aging 2006;27:1359–71. doi:10.1016/j.neurobiolaging.2005.08.013.

[3] Li X, Jiang W, Zhang Y, Meng F, Lian B, Chen X, et al. Identification of active transcription factor and miRNA regulatory pathways in Alzheimer's disease. Bioinformatics 2013;29:2596–602. doi:10.1093/bioinformatics/btt423.

[4] Rahman, MR; Islam, T; Turanli, B; Zaman, T; Faruquee, HM; Rahman, MM; Mollah, MNH; Nanda, RK; Arga, KY; Gov, Esra et al. Network-Based Approach to Identify Molecular Signatures and Therapeutic Agents in Alzheimer's Disease. Comput Biol Chem., 2018; In press, DOI: https://doi.org/10.1016/j.compbiolchem.2018.12.011.

[5] Rahman, M.R., Islam, T., Shahjaman, M., Quinn, J.M.W., Holsinger, R.M.D., Moni, M.A. Common molecular biomarker signatures in blood and brain of Alzheimer ' s disease. BioRxiv482828 [Preprint] 1–19, 2018. doi:http://dx.doi.org/10.1101/482828.

[6] Rahman, M.R., Islam, T., Gov, E., Turanli, B., Shahjaman, M., Banu, N.A., Mollah, M.N.H., Arga, K.Y., Moni, M.A. Identification of Prognostic Biomarker Signatures and Candidate Drugs in Colorectal Cancer: Insights from Systems Biology Analysis. *Preprints* 2018; 2018120018, doi:http:// 10.20944/preprints201812.0018.v1

[7] McKhann G, Knopman DS, Chertkow H, Hymann B, Jack CR, Kawas C, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging- Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263–9. doi:10.1016/j.jalz.2011.03.005.

[8] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on. Alzheimer's Dement 2011;7:270–9. doi:10.1016/j.jalz.2011.03.008.The.

[9] Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging- Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's Dement 2011;7:280–92. doi:10.1016/j.jalz.2011.03.003.Toward.

[10] Lista S, Faltraco F, Prvulovic D, Hampel H. Blood and plasma-based proteomic biomarker research in Alzheimer's disease. Prog Neurobiol 2013;101–102:1–17. doi:10.1016/j.pneurobio.2012.06.007.

[11] Schneider P, Hampel H, Buerger K. Biological Marker Candidates of Alzheimer's Disease in Blood, Plasma, and Serum. CNS Neurosci Ther 2009;15:358–74. doi:10.1111/j.1755-5949.2009.00104.x.

[12] Shi L, Baird AL, Westwood S, Hye A, Dobson R, Thambisetty M, et al. A Decade of Blood Biomarkers for Alzheimer's Disease Research: An Evolving Field, Improving Study Designs, and the Challenge of Replication. J Alzheimer's Dis 2018;62:1181–98. doi:10.3233/JAD-170531.

[13] Kumar S, Reddy PH. Are circulating microRNAs peripheral biomarkers for Alzheimer's disease? Biochim Biophys Acta - Mol Basis Dis 2016;1862:1617–27. doi:10.1016/j.bbadis.2016.06.001.

[14] Sharma N, Singh AN. Exploring biomarkers for Alzheimer's disease. J Clin Diagnostic Res 2016;10:KE01-KE06. doi:10.7860/JCDR/2016/18828.8166.

[15] Fehlbaum-Beurdeley P, Jarrige-Le Prado AC, Pallares D, Carriere J, Guihal C, Soucaille C, et al. Toward an Alzheimer's disease diagnosis via high-resolution blood gene expression. Alzheimers Dement 2010;6:25–38. doi:10.1016/j.jalz.2009.07.001.

[16] Rye PD, Booij BB, Grave G, Lindahl T, Kristiansen L, Andersen H-M, et al. A novel blood test for the early detection of Alzheimer's disease. J Alzheimers Dis 2011;23:121–9. doi:10.3233/JAD-2010-101521.

[17] Maes OC, Schipper HM, Chertkow HM, Wang E. Methodology for discovery of Alzheimer's disease blood-based biomarkers. J Gerontol A Biol Sci Med Sci 2009;64:636–45. doi:10.1093/gerona/glp045.

[18] Han G, Wang J, Zeng F, Feng X, Yu J, Cao H-Y, et al. Characteristic transformation of blood transcriptome in Alzheimer's disease. J Alzheimers Dis 2013;35:373–86. doi:10.3233/JAD-121963.

[19] Bai Z, Stamova B, Xu H, Ander BP, Wang J, Jickling GC, et al. Distinctive RNA expression profiles in blood associated with Alzheimer disease after accounting for white matter hyperintensities. Alzheimer Dis Assoc Disord 2014;28:226–33. doi:10.1097/WAD.0000000000000022.

[20] Wen KX, Milic J, El-Khodor B, Dhana K, Nano J, Pulido T, et al. The role of DNA methylation and histone modifications in neurodegenerative diseases: A systematic review. PLoS One 2016;11:1–31. doi:10.1371/journal.pone.0167201.

[21] Maciotta S, Meregalli M, Torrente Y. The involvement of microRNAs in neurodegenerative diseases. Front Cell Neurosci 2013;7:1–17. doi:10.3389/fncel.2013.00265.

[22] Femminella GD, Ferrara N, Rengo G. The emerging role of microRNAs in Alzheimer's disease. Front Physiol 2015;6:1–5. doi:10.3389/fphys.2015.00040.

[23] Shaik MM, Tamargo IA, Abubakar MB, Kamal MA, Greig NH, Gan SH. The role of microRNAs in Alzheimer's disease and their therapeutic potentials. Genes (Basel) 2018;9. doi:10.3390/genes9040174.

[24] Maes OC, Xu S, Yu B, Chertkow HM, Wang E, Schipper HM. Transcriptional profiling of Alzheimer blood mononuclear cells by microarray. Neurobiol Aging 2007;28:1795–809. doi:10.1016/j.neurobiolaging.2006.08.004.

[25] Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: Archive for functional genomics data sets - Update. Nucleic Acids Res 2013;41:991–5. doi:10.1093/nar/gks1193.

[26] Bardou P, Mariette J, Escudie F, Djemiel C, Klopp C. jvenn: an interactive Venn diagram viewer. BMC Bioinformatics 2014;15:293. doi:10.1186/1471-2105-15-293.

[27] Kuleshov M V., Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res 2016;44:W90–7. doi:10.1093/nar/gkw377.

[28] Mi H, Muruganujan A, Thomas PD. PANTHER in 2013: Modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. Nucleic Acids Res 2013;41:377–86. doi:10.1093/nar/gks1118.

[29] Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, et al. The STRING database in 2017: Quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2017;45:D362–8. doi:10.1093/nar/gkw937.

[30] Xia J, Gill EE, Hancock REW. NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data. Nat Protoc 2015;10:823–44. doi:10.1038/nprot.2015.052.

[31] Khan A, Fornes O, Stigliani A, Gheorghe M, Castro-Mondragon JA, Van Der Lee R, et al. JASPAR 2018: Update of the open-access database of transcription factor binding profiles and its web framework. Nucleic Acids Res 2018;46:D260–6. doi:10.1093/nar/gkx1126.

[32] Sethupathy P, Corda B, Hatzigeorgiou AG. TarBase : A comprehensive database of experimentally supported animal microRNA targets TarBase : A comprehensive database of experimentally supported animal microRNA targets. Rna 2006;12:192–7. doi:10.1261/rna.2239606.scale.

[33] Hsu S Da, Lin FM, Wu WY, Liang C, Huang WC, Chan WL, et al. MiRTarBase: A database curates experimentally validated microRNA-target interactions. Nucleic Acids Res 2011;39:163–9. doi:10.1093/nar/gkq1107.

[34] Zhang Y, Lv J, Liu H, Zhu J, Su J, Wu Q, et al. HHMD: The human histone modification database. Nucleic Acids

Res 2009;38:149–54. doi:10.1093/nar/gkp968.

[35] Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: The AlzGene database. Nat Genet 2007;39:17–23. doi:10.1038/ng1934.

[36] Leidinger P, Backes C, Deutscher S, Schmitt K, Mueller SC, Frese K, et al. A blood based 12-miRNA signature of Alzheimer disease patients. Genome Biol 2013;14:R78. doi:10.1186/gb-2013-14-7-r78.

[37] Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res 2018;46:D1074–82. doi:10.1093/nar/gkx1037.

[38] Grosdidier A, Zoete V, Michielin O. SwissDock, a protein-small molecule docking web service based on EADock DSS. Nucleic Acids Res 2011;39:270–7. doi:10.1093/nar/gkr366.

[39] Horton P, Park K-J, Obayashi T, Fujita N, Harada H, Adams-Collier CJ, et al. WoLF PSORT: protein localization predictor. Nucleic Acids Res 2007;35:W585-7. doi:10.1093/nar/gkm259.

[40] Islam T, Rahman MR, Gov E, Turanli B, Gulfidan G, Haque MA, et al. Drug Targeting and Biomarkers in Head and Neck Cancers : OMICS 2018;22:422–36. doi:10.1089/omi.2018.0048.

[41] Li H, Hong G, Lin M, Shi Y, Wang L, Jiang F, et al. Identification of molecular alterations in leukocytes from gene expression profiles of peripheral whole blood of Alzheimer's disease. Sci Rep 2017;7:1–10. doi:10.1038/s41598-017-13700-w.

[42] Paul J, Strickland S, Melchor JP. Fibrin deposition accelerates neurovascular damage and neuroinflammation in mouse models of Alzheimer's disease. J Exp Med 2007;204:1999–2008. doi:10.1084/jem.20070304.

[43] Ahn HJ, Zamolodchikov D, Cortes-Canteli M, Norris EH, Glickman JF, Strickland S. Alzheimer's disease peptide-amyloid interacts with fibrinogen and induces its oligomerization. Proc Natl Acad Sci 2010;107:21812–7. doi:10.1073/pnas.1010373107.

[44] Chen X. Role of LDL Cholesterol and Endolysosomes in Amyloidogenesis and Alzheimer's Disease. J Neurol Neurophysiol 2014;5:1–15. doi:10.4172/2155-9562.1000236.

[45] Hone E, Pedrini S, Gupta VB, Russell A, Laws SM, Villemagne VLL, et al. the Influence of Low-Density Lipoproteins on Neocortical Amyloid Load in the Australian Imaging Biomarker and Lifestyle Study. Alzheimer's Dement 2017;13:P1023–4. doi:10.1016/j.jalz.2017.06.1437.

[46] Roe CM, Behrens MI, Xiong C, Miller JP, Morris JC. Alzheimer disease and cancer. Neurology 2005;64:895–8. doi:10.1212/01.WNL.0000152889.94785.51.

[47] Roe CM, Fitzpatrick AL, Xiong C, Sieh W, Kuller L, Miller JP, et al. Cancer linked to Alzheimer disease but not vascular dementia. Neurology 2010;74:106–12. doi:10.1212/WNL.0b013e3181c91873.

[48] Ou S-M, Lee Y-J, Hu Y-W, Liu C-J, Chen T-J, Fuh J-L, et al. Does Alzheimer's disease protect against cancers? A nationwide population-based study. Neuroepidemiology 2013;40:42–9. doi:10.1159/000341411.

[49] Freedman DM, Wu J, Chen H, Kuncl RW, Enewold LR, Engels EA, et al. Associations between cancer and Alzheimer's disease in a U.S. Medicare population. Cancer Med 2016;5:2965–76. doi:10.1002/cam4.850.

[50] Sevimoglu T, Arga KY. The role of protein interaction networks in systems biomedicine. Comput Struct Biotechnol J 2014;11:22–7. doi:10.1016/j.csbj.2014.08.008.

[51] Aung HH, Altman R, Nyunt T, Kim J, Nuthikattu S, Budamagunta M, et al. Lipotoxic brain microvascular injury is mediated by activating transcription factor 3-dependent inflammatory and oxidative stress pathways. J Lipid Res 2016;57:955–68. doi:10.1194/jlr.M061853.

[52] Zhu M, Allard JS, Zhang Y, Perez E, Spangler EL, Becker KG, et al. Age-Related Brain Expression and Regulation of the Chemokine CCL4 / MIP-1 A in APP / PS1 Double-Transgenic Mice. J Neuropathol Exp Neurol 2014;73:362–74.

[53] Jariwal N, Rajasekaran D, Srivastava J, Gredler R, Akiel MA, Robertson CL, et al. Role of the staphylococcal nuclease and tudor domain containing 1 in oncogenesis (Review). Int J Oncol 2015;46:465–73.

doi:10.3892/ijo.2014.2766.

[54] Lunnon K, Sattlecker M, Furney SJ, Coppola G, Simmons A, Proitsi P, et al. A blood gene expression marker of early Alzheimer's disease. J Alzheimer's Dis 2013;33:737–53. doi:10.3233/JAD-2012-121363.

[55] Huang C-W, Tsai M-H, Chen N-C, Chen W-H, Lu Y-T, Lui C-C, et al. Clinical significance of circulating vascular cell adhesion molecule-1 to white matter disintegrity in Alzheimer's dementia. Thromb Haemost 2015;114:1230–40. doi:10.1160/TH14-11-0938.

[56] French CR, Waskiewicz AJ, Ordan J, French CR, Seshadri S, Destefano AL, et al. Mutation of FOXC1 and PITX2 induces cerebral small-vessel disease Find the latest version : Mutation of FOXC1 and PITX2 induces cerebral small-vessel disease 2014;124:4877–81. doi:10.1172/JCI75109.same.

[57] Haffner C, Malik R, Dichgans M. Genetic factors in cerebral small vessel disease and their impact on stroke and dementia. J Cereb Blood Flow Metab 2016;36:158–71. doi:10.1038/jcbfm.2015.71.

[58] Cassandri M, Smirnov A, Novelli F, Pitolli C, Agostini M, Malewicz M, et al. Zinc-finger proteins in health and disease. Cell Death Discov 2017;3:17071. doi:10.1038/cddiscovery.2017.71.

[59] Wong J. Altered Expression of RNA Splicing Proteins in Alzheimer's Disease Patients: Evidence from Two Microarray Studies. Dement Geriatr Cogn Dis Extra 2013;3:74–85. doi:10.1159/000348406.

[60] da Costa PJ, Menezes J, Romão L. The role of alternative splicing coupled to nonsense-mediated mRNA decay in human disease. Int J Biochem Cell Biol 2017;91:168–75. doi:10.1016/j.biocel.2017.07.013.

[61] Hsu WC, Denq C, Chen SS. A diagnostic methodology for Alzheimer's disease. J Clin Bioinforma 2013;3:1–13. doi:10.1186/2043-9113-3-9.

[62] Goodall EF, Heath PR, Bandmann O, Kirby J, Shaw PJ. Neuronal dark matter: the emerging role of microRNAs in neurodegeneration. Front Cell Neurosci 2013;7:1–16. doi:10.3389/fncel.2013.00178.

[63] Hackl M, Brunner S, Fortschegger K, Schreiner C, Micutkova L, Mück C, et al. miR-17, miR-19b, miR-20a, and miR-106a are down-regulated in human aging. Aging Cell 2010;9:291–6. doi:10.1111/j.1474-9726.2010.00549.x.

[64] Burgos K, Malenica I, Metpally R, Courtright A, Rakela B, Beach T, et al. Profiles of extracellular miRNA in cerebrospinal fluid and serum from patients with Alzheimer's and Parkinson's diseases correlate with disease status and features of pathology. PLoS One 2014;9:e94839. doi:10.1371/journal.pone.0094839.

[65] McKeever PM, Schneider R, Taghdiri F, Weichert A, Multani N, Brown RA, et al. MicroRNA Expression Levels Are Altered in the Cerebrospinal Fluid of Patients with Young-Onset Alzheimer's Disease. Mol Neurobiol 2018:10–2. doi:10.1007/s12035-018-1032-x.

[65] Leidinger P, Backes C, Deutscher S, Schmitt K, Mueller SC, Frese K, et al. A blood based 12-miRNA signature of Alzheimer disease patients. Genome Biol 2013;14:R78. doi:10.1186/gb-2013-14-7-r78.

[66] Lusardi TA, Phillips JI, Wiedrick JT, Harrington CA, Lind B, Lapidus JA, et al. MicroRNAs in Human Cerebrospinal Fluid as Biomarkers for Alzheimer's Disease. J Alzheimers Dis 2017;55:1223–33. doi:10.3233/JAD-160835.

[67] Absalon S, Kochanek DM, Raghavan V, Krichevsky AM. MiR-26b, upregulated in Alzheimer's disease, activates cell cycle entry, tau-phosphorylation, and apoptosis in postmitotic neurons. J Neurosci 2013;33:14645–59. doi:10.1523/JNEUROSCI.1327-13.2013.

[68] Hu Y-B, Li C-B, Song N, Zou Y, Chen S-D, Ren R-J, et al. Diagnostic Value of microRNA for Alzheimer's Disease: A Systematic Review and Meta-Analysis. Front Aging Neurosci 2016;8:1–9. doi:10.3389/fnagi.2016.00013.

[69] Pattaroni C, Jacob C. Histone methylation in the nervous system: functions and dysfunctions. Mol Neurobiol 2013;47:740–56. doi:10.1007/s12035-012-8376-4.

[70] Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. Molecules 2015;20:13384–421. doi:10.3390/molecules200713384.