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Transcriptional Profiling of Hippocampus Identifies Network Alterations in Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease characterized by rapid brain cell degeneration affecting different areas of the brain. Hippocampus is one of the earliest involved brain regions in the disease. Modern technologies based on high-throughput data have identified transcriptional profiling of several neurological diseases, including AD, for a better comprehension of genetic mechanisms of the disease. In this study, we investigated differentially expressed genes (DEGs) from six Gene Expression Omnibus (GEO) datasets of hippocampus of AD patients. The identified DEGs were submitted to Weighted correlation network analysis (WGCNA) and ClueGo to explore genes with a higher degree centrality and to comprehend their biological role. Subsequently, MCODE was used to identify subnetworks of interconnected DEGs. Our study found 40 down-regulated genes and 36 up-regulated genes as consensus DEGs. Analysis of the co-expression network revealed ACOT7, ATP8A2, CDC42, GAD1, GOT1, INA, NCALD, and WWTR1 to be genes with a higher degree centrality. ClueGO revealed the pathways that were mainly enriched, such as clathrin coat assembly, synaptic vesicle endocytosis, and DNA damage response signal transduction by p53 class mediator. In addition, we found a subnetwork of 12 interconnected genes (AMPH, CA10, CALY, NEFL, SNAP25, SNAP91, SNCB, STMN2, SV2B, SYN2, SYT1, and SYT13). Only CA10 and CALY are targets of known drugs while the others could be potential novel drug targets.

Keywords: Alzheimer's disease; bioinformatics; co-expression

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1. Introduction

First described by German neuropathologist Alois Alzheimer in 1906, Alzheimer's disease (AD) is an age-associated neurodegenerative disease characterized by progressive memory loss and deterioration of cognitive functions that ultimately leads to dementia [1,2].

Symptoms usually begin with progressive memory loss and then develop in other cognitive domains such as language, visuospatial skills, motor skills, and daily living activities [3]. Since AD usually has a silent onset, most patients with AD are already in an advanced stage by the time of diagnosis [4].

AD is a complex disease resulting from the interaction of genetic and environmental factors [5]. The primary pathogenesis of AD is known to be related to the accumulation of amyloid plaques and neurofibrillary tangles (NFTs), which are aggregates of β-amyloid

(Aβ) and hyperphosphorylated Tau protein [6]. Hence, the gradual intraneuronal accumulation of neurofibrillary tangles, the extracellular deposition of Aβ protein in the form of senile plaques, and massive neuronal death represent important neuropathological features of AD [6].

All these processes involve variations in the expression and regulation of numerous genes and genetic factors are estimated to attribute up to 79% of the risk of AD [7]. Mutations in genes that increase Aβ generation and aggregation, such as amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2), are considered to be the genetic causes of familial AD [8].

The identification of candidate genes could considerably increase our understanding of the biological mechanisms involved in the pathogenesis of AD and these could be used as diagnostic and predictive biomarkers.

AD affects various specific and vulnerable brain areas, and hippocampus is one of the first to be affected [9]. Hippocampus is a brain structure belonging to the limbic system embedded deep in the temporal lobe that plays a fundamental role in learning and memory processes. Some studies suggest that impaired neurogenesis in adult hippocampus could play a role in the onset of AD [9].

It has been shown that genes associated with neuronal loss, glial activation, and lipid metabolism increase with age [10]. However, in AD these genes are prematurely expressed along with genes related to protein folding and cell adhesion [10].

In recent years, bioinformatics analysis has been widely applied to molecular biology experiments revealing key pathways and pharmacological targets of complex diseases [11]. Therefore, the analysis of multiple high-throughput datasets of AD patients may be an optimal strategy [11–13].

A first model of AD progression was suggested by Blalock et al. [14] considering the biomarkers found in their study. Alterations in axons activate localized oligodendrocytes that secrete growth factor promoting adjacent neurons. In turn, these generate an up-regulation of tumor-suppressor-mediated protein aggregation and axon–myelin interactions along myelinated axons [14].

Previous studies evaluated several functional regions of human brains, such as entorhinal cortex, hippocampus, middle temporal gyrus, posterior cingulate cortex, superior frontal gyrus, and primary visual cortex. Each region is characterized by specific properties and gene expression changes [15,16].

Interspecies analyses to reveal alterations in AD brains were performed from human brain tissues and a transgenic mouse model. The study showed that genes implicated in insulin signaling and AD were altered in both tissues [17].

A similar study to investigate shared molecular mechanisms between AD and diabetes was conducted by Lee and Lee [18]. They found five common genes dysregulated in both diseases and disease-specific tissue in the brain and pancreas [18].

Our study, which is based on a computational approach, aims at the identification of novel AD biomarkers for diagnosis and treatment. We analyzed gene expression profiles of AD patients from several public datasets and obtained a consensus list of differentially expressed genes (DEGs) between AD and control samples. We investigated known drugs that interact with consensus DEGs using three different drug databases. Further, we investigated DEG interactions and their biological role using protein–protein interactions (PPIs), the co-expression network, and enrichment analysis.

2. Materials and Methods

2.1. Datasets

Six datasets (GSE1297, GSE5281, GSE36980, GSE29378, GSE48350, and GSE13214) were downloaded from the Gene Expression Omnibus (GEO), derived from the Affymetrix Human Genome U133A Array, the Affymetrix Human Genome U133 Plus 2.0 Array, the Affymetrix Human Gene 1.0 ST Array, the Illumina HumanHT-12 V3.0 expression beadchip, the Affymetrix Human Genome U133 Plus 2.0 Array, and Homo sapiens 4.8K 02-01

amplified cDNA, respectively. For all datasets, the gene expression levels of hippocampus of AD patients were analyzed as this is particularly vulnerable to damage in the early stages of AD [9].

We generated for each GEO dataset a boxplot showing the distribution of genes for each sample after log transformation. Median-centered values were considered as indicators of normalized data. Thus, we verified that the GEO datasets had previously been normalized, as described in the works of the original datasets [12–17].

The computational approach is illustrated in Figure 1. A consensus list of DEGs among the 6 GEO datasets was investigated. Further, we performed a study to comprehend their biological role, including PPI, co-expression network, and Gene Ontology analyses.

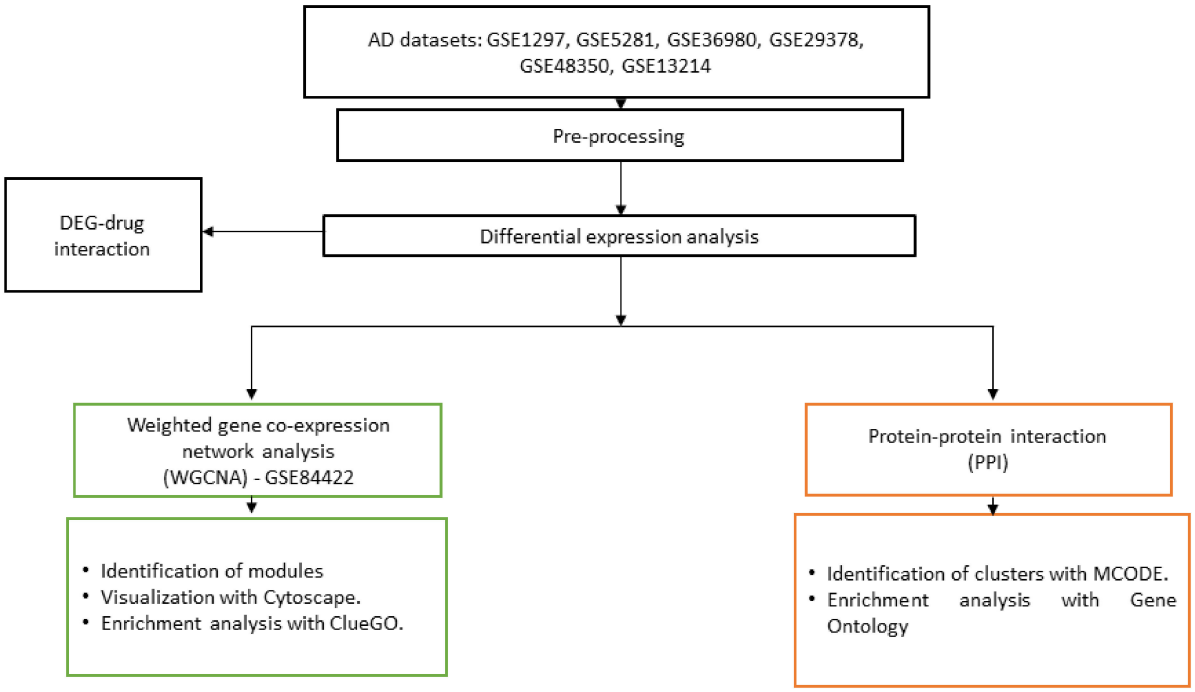

Figure 1. Computational workflow.

2.2. Differential Expression Analysis

The raw data of the GEO datasets were processed through GEO2R, which compares gene expression levels between AD and control subjects [19]. The differential expression analysis was performed considering GEO datasets separately with the limma package. We used as the cut-off a p-value < 0.05 and $|\log FC| > 1$ for GSE1297 and an adjusted p-value (false discovery rate) < 0.05 and $|\log FC| > 1$ for the other datasets. Probe ID mapping was performed with GEO2R.

We considered DEGs found in at least two datasets for further analyses.

2.3. Differentially Expressed Gene–Drug Interactions

We investigated whether DEGs are associated with known drugs. We used three drug databases: DGIdb [20], DrugCentral [21], and The Drug Repurposing Hub [22]. DEGs found in at least two GEO datasets were submitted to the drug databases. We selected drug–gene interactions that are present in at least two drug databases.

2.4. Co-Expression Network

Weighted correlation network analysis (WGCNA) [23] was used to create a network of co-expressed genes considering expression levels of DEGs in an independent dataset, GSE84422. We used GSE84422 as this dataset was not used in the previous analyses and contains gene expression levels of AD samples derived from hippocampus.

A correlation matrix was calculated by measuring Pearson's correlations among all DEG pairs. Furthermore, the genes were clustered into modules [23]. The modules are defined as groups of genes that are strongly interconnected with the genes of the same module and poorly connected with genes of other modules.

To visualize the modules obtained with WGCNA, we used Cytoscape [24]. In addition, for each gene in the network we calculated the degree centrality, defined as the number of edges that own a node, using the R-package igraph.

2.5. Protein–Protein Interaction

The study of interacting proteins can help us understand their biological role. In particular, for complex idiopathic diseases, such as AD, PPI networks are useful to comprehend the biological mechanism of the disease [25,26]. To evaluate the relationships among DEGs in AD, we built a PPI using STRING v11.0 with an average confidence interaction score of 0.4 to reduce the number of inconsistent PPIs [27]. The results obtained with STRING were submitted to Cytoscape using the MCODE plugin to reveal the interconnected regions (degree cut-off, 2; node score cut-off, 0.2; kappa score, 2; maximum depth, 100) [28].

2.6. Pathway and Gene Ontology Analysis

The genes associated with modules obtained with WGCNA were analyzed with ClueGO v2.5.8/CluePedia v1.5.8 [29]. ClueGO integrates Gene Ontology (GO) and pathway analysis to obtain a functional enrichment analysis. We set as the p-value $p \leq 0.01$. The genes identified by MCODE were analyzed with GO for biological processes.

3. Results

3.1. Differentially Expressed Genes

By performing a differential expression analysis, we obtained 67 down-regulated genes and 223 up-regulated genes in GSE1297. A total of 4002 DEGs (1450 down-regulated genes and 2552 up-regulated genes) were found in GSE5281. We found only 10 down-regulated genes in GSE36980 and 7 up-regulated genes in GSE29378. A total of 61 down-regulated genes and 13 up-regulated genes were found in GSE48350. No DEGs were obtained in GSE13214.

We found 40 down-regulated genes and 36 up-regulated genes as common DEGs in at least two GEO datasets. The Venn diagram in Figure 2 shows common DEGs among the GEO datasets.

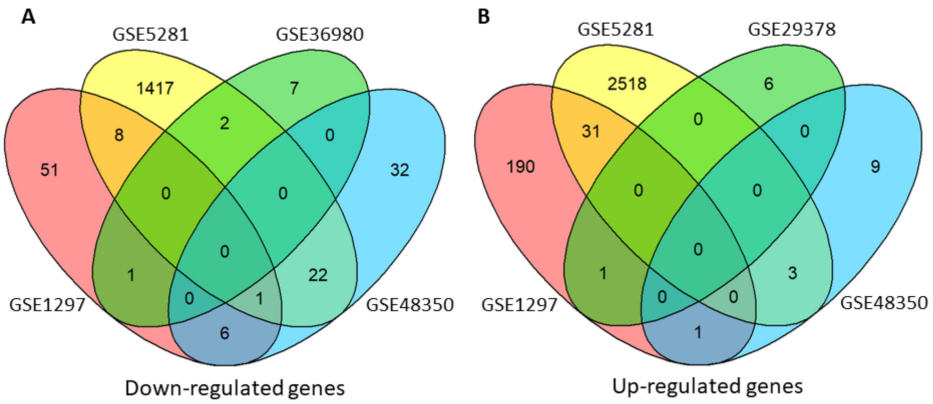

Figure 2. Venn diagram analysis of differentially expressed genes: (**A**) down-regulated genes; (**B**) up-regulated genes.

3.2. Drugs Interact with Differentially Expressed Genes in AD

We submitted the list of 76 DEGs to DGIdb, DrugCentral, and The Drug Repurposing Hub. We selected the drugs that interact with our DEGs in at least two of the drug databases.

DGIdb and DrugCentral revealed several drugs that have as targets two down-regulated genes: GOT1 and MET1. GOT1 is a target of aspartic acid and MET is a target of crizotinib, erlotinib, cabozantinib, gefitinib, pazopanib, and afatinib. In addition, the results from DGIdb and DrugCentral show that two up-regulated genes, COMT and PTAFR, act on common drugs in the two drug databases. Opicapone, tolcapone, and entacapone interact with COMT, and rupatadine interacts with PTAFR.

DGIdb and The Drug Repurposing Hub showed nine down-regulated genes (CA10, CACNG2, CALY, CKMT1A, GAD1, GLS2, GOT1, MAP4, and MET) interacting with known drugs. CA10 interacts with zonisamide, CACNG2 with gabapentin, CALY with apomorfina, clozapina, and trifluoperazine, CKMT1A with creatin, GAD1 and GLS2 with glutamic acid, GOT1 with aspartic acid, cysteine, and glutamic acid, and MAP4 with docetaxel and paclitaxel. MET is a target of alectinib, altiratinib, AMG-208, AMG-337, amuvatinib, BMS-777607, BMS-817378, cabozantinib, crizotinib, foretinib, golvatinib, JNJ-38877605, MGCD-265, MK-2461, MK-8033, PF-04217903, PHA-665752, savolitinib, and tivantinib. In addition, three up-regulated genes (COMT, PRKG1, and PTAFR) are targets of different drugs. COMT interacts with entacapone, nialamide, opicapone, tolcapone, and 2-metossiestradiolo, PRKG1 is a target of GSK-690693, and PTAFR is a target of apafant, foropafant, and rupatadina.

DrugCentral and The Drug Repurposing Hub revealed several drugs acting on two down-regulated genes (GOT1 and MET). GOT1 is a target of aspartic acid; MET is a target of cabozantinib, and crizotinib. In addition, we found two up-regulated genes: COMT interacts with entacapone, opicapone, and tolcapone; PTAFR interacts with rupatadine.

Tables 1 and 2 show the list of down-regulated and up-regulated genes that are targets of known drugs in at least two drug databases.

Overall, we obtained 34 drugs that interact with nine genes for down-regulated genes, and nine drugs that interact with three genes for up-regulated genes.

3.3. Network Construction and Module Detection

WGCNA was used to determine the expression levels of 76 differentially expressed genes in an independent dataset (GSE84422) containing 18 AD samples. A total of 5 out of 76 genes were not present in GSE84422. A total of 71 gene symbols were converted to probes obtaining 179 probes and used as an input for WGCNA.

WGCNA found a module of 131 probes related to 64 genes (Figure 3).

Then, we used Cytoscape to visualize the module obtained with WGCNA, obtaining a network of 64 genes and 1760 edges. ACOT7, ATP8A2, CDC42, GAD1, GOT1, INA, NCALD, and WWTR1 were the genes with the highest degree centrality. Figure 4 shows the co-expression network.

3.4. Enrichment Analysis

The 64 genes obtained with WGCNA were submitted to ClueGo for GO and pathway analysis. As shown in Figure 5A–D, the 64 genes are mainly enriched by the clathrin coat assembly, synaptic vesicle endocytosis, DNA damage response signal transduction by p53 class mediator, positive regulation of transforming growth factor beta receptor signaling pathway, glutamine family amino acid catabolic process, and neural nucleus development.

3.5. Protein–Protein Interactions

We further explored the protein–protein interactions of 76 consensus DEGs using STRING and the network found was visualized with Cytoscape (Figure 6). We obtained a network of 39 nodes and 81 edges.

Table 1. Down-regulated genes and their interactions with known drugs in at least two of three drug databases (DGIdb, DrugCentral, and The Drug Repurposing Hub).

Down-Regulated Genes	Drugs
CA10	(1) ZONISAMIDE
CACNG2	(1) GABAPENTIN
CALY	(1) APOMORPHINE (2) CLOZAPINE (3) TRIFLUOPERAZINE
CKMT1A	(1) CREATINE
GAD1	(1) GLUTAMIC ACID
GLS2	(1) GLUTAMIC ACID
GOT1	(1) ASPARTIC ACID (2) CYSTEINE (3) GLUTAMIC ACID
MAP4	(1) DOCETAXEL (2) PACLITAXEL
MET	(1) CRIZOTINIB (2) ERLOTINIB (3) CABOZANTINIB (4) GEFITINIB (5) PAZOPANIB (6) AFATINIB (7) ALECTINIB (8) ALTIRATINIB (9) AMG-208 (10) AMG-337 (11) AMUVATINIB (12) BMS-777607 (13) BMS-817378 (14) FORETINIB (15) GOLVATINIB (16) JNJ-38877605 (17) MGCD-265 (18) MK-2461 (19) MK-8033 (20) PF-04217903 (21) PHA-665752 (22) SAVOLITINIB (23) TIVANTINIB

Table 2. Up-regulated genes and their interactions with known drugs in at least two of three drug databases (DGIdb, DrugCentral, and The Drug Repurposing Hub).

Up-Regulated Genes	Drugs
COMT	(1) OPICAPONE (2) TOLCAPONE (3) ENTACAPONE (4) NIALAMIDE (5) 2-METHOXYESTRADIOL
PRKG1	(1) GSK-690693
PTAFR	(1) RUPATADINE (2) APAFANT (3) FOROPAFANT

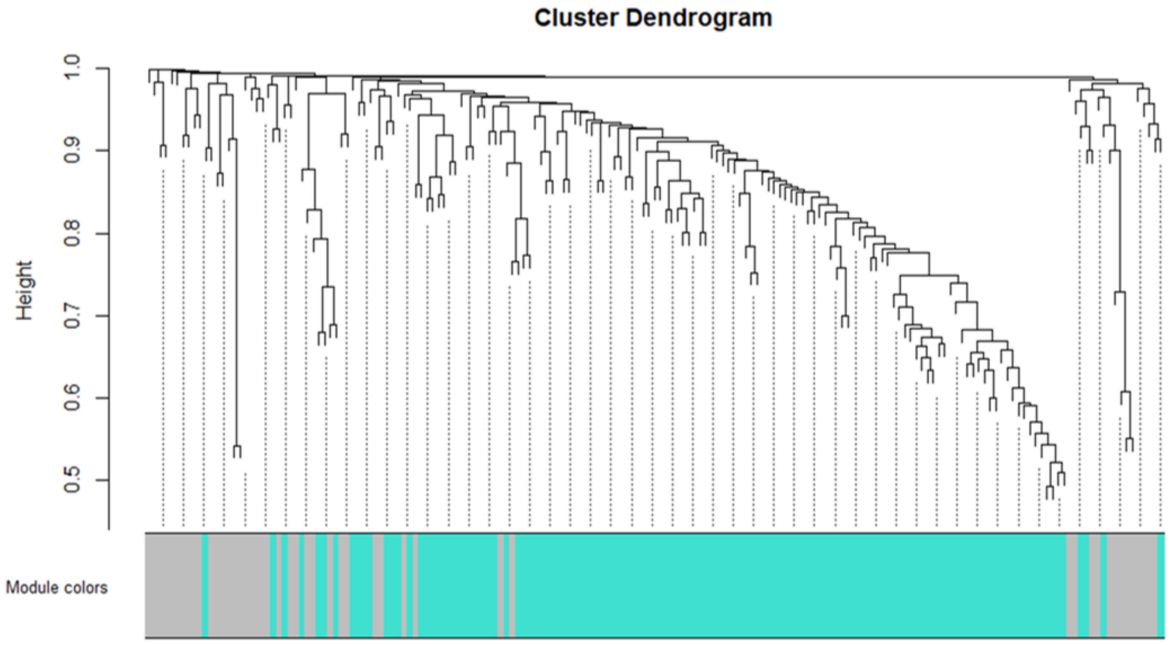

Figure 3. Hierarchical cluster dendrogram and gene module identified by weighted correlation network analysis (WGCNA). Gray genes are not included in any module.

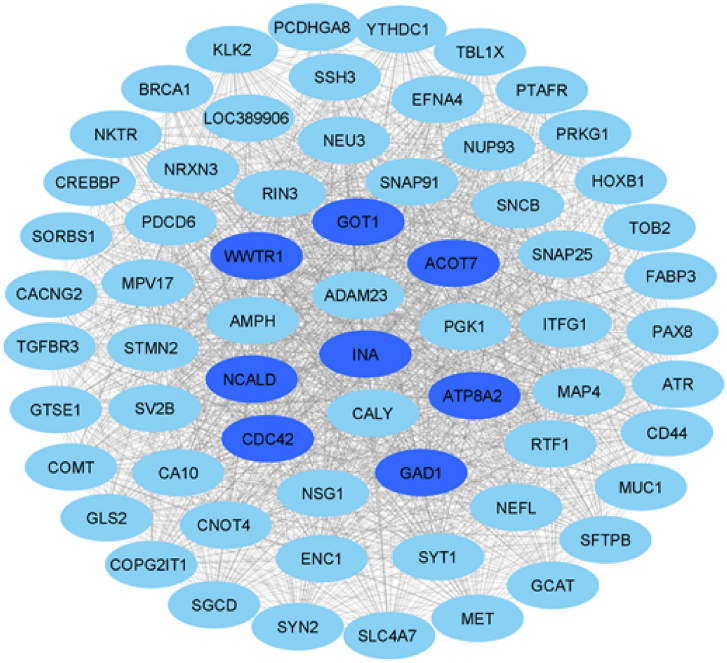

Figure 4. Co-expression network obtained with weighted correlation network analysis (WGCNA) and visualized with Cytoscape. Dark blue nodes correspond to the genes with a higher degree centrality. Grey colored edges indicate the gene interactions.

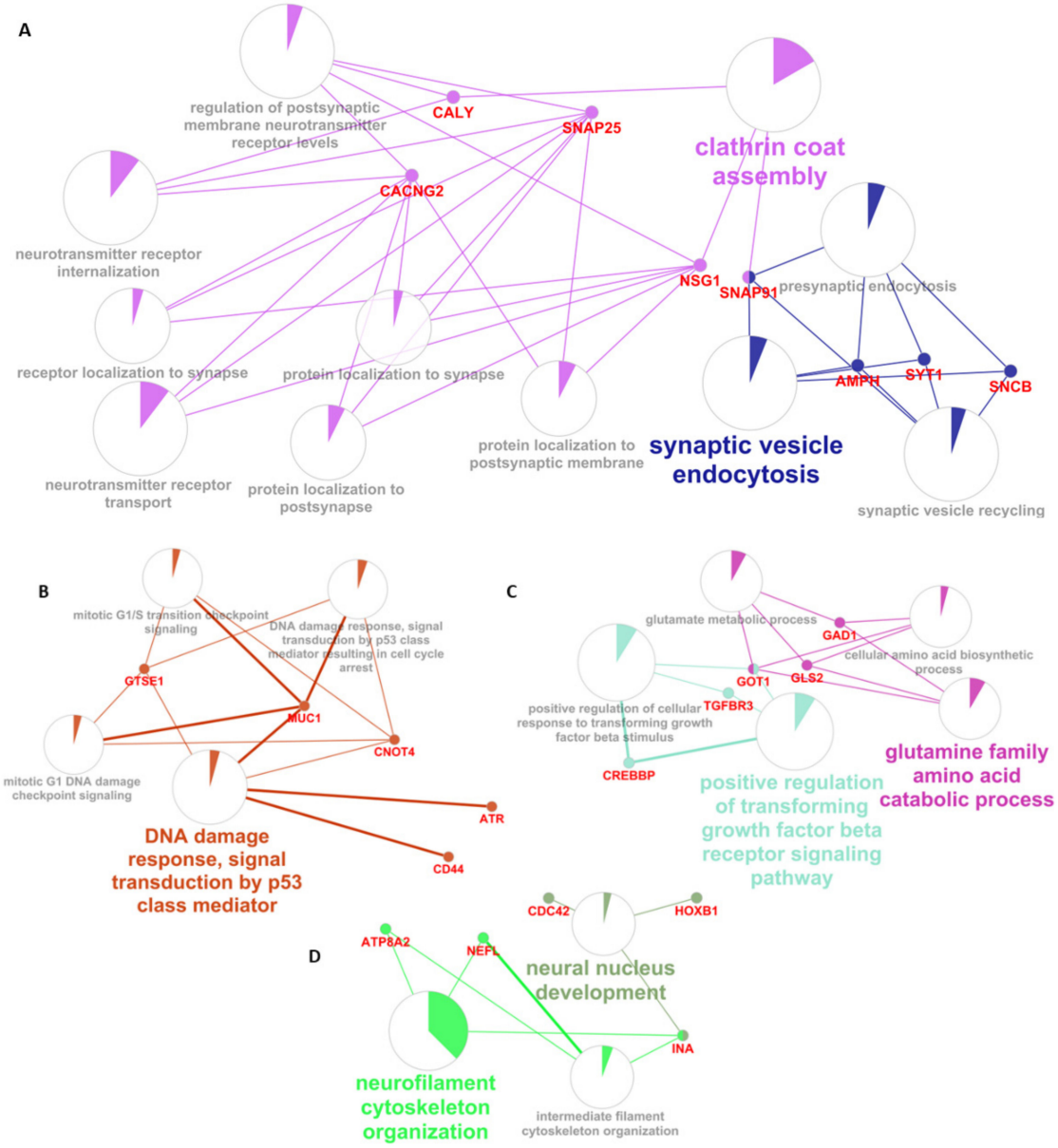

Figure 5. Pathway analysis using ClueGo from 64 genes obtained with WGCNA. Nodes represent GO terms and the node size represents the term's enrichment significance. (**A**) Clathrin coat assembly and synaptic vesicle endocytosis; (**B**) DNA damage response signal transduction by p53 class mediator; (**C**) positive regulation of transforming growth factor beta receptor signaling pathway and glutamine family amino acid catabolic process; (**D**) neurofilament cytoskeleton organization and neural nucleus development.

The MCODE plugin of Cytoscape was used to identify interconnected regions of the network identified with STRING and we obtained a cluster of 12 genes (AMPH, CA10, CALY, NEFL, SNAP25, SNAP91, SNCB, STMN2, SV2B, SYN2, SYT1, and SYT13) and 42 edges (Figure 7).

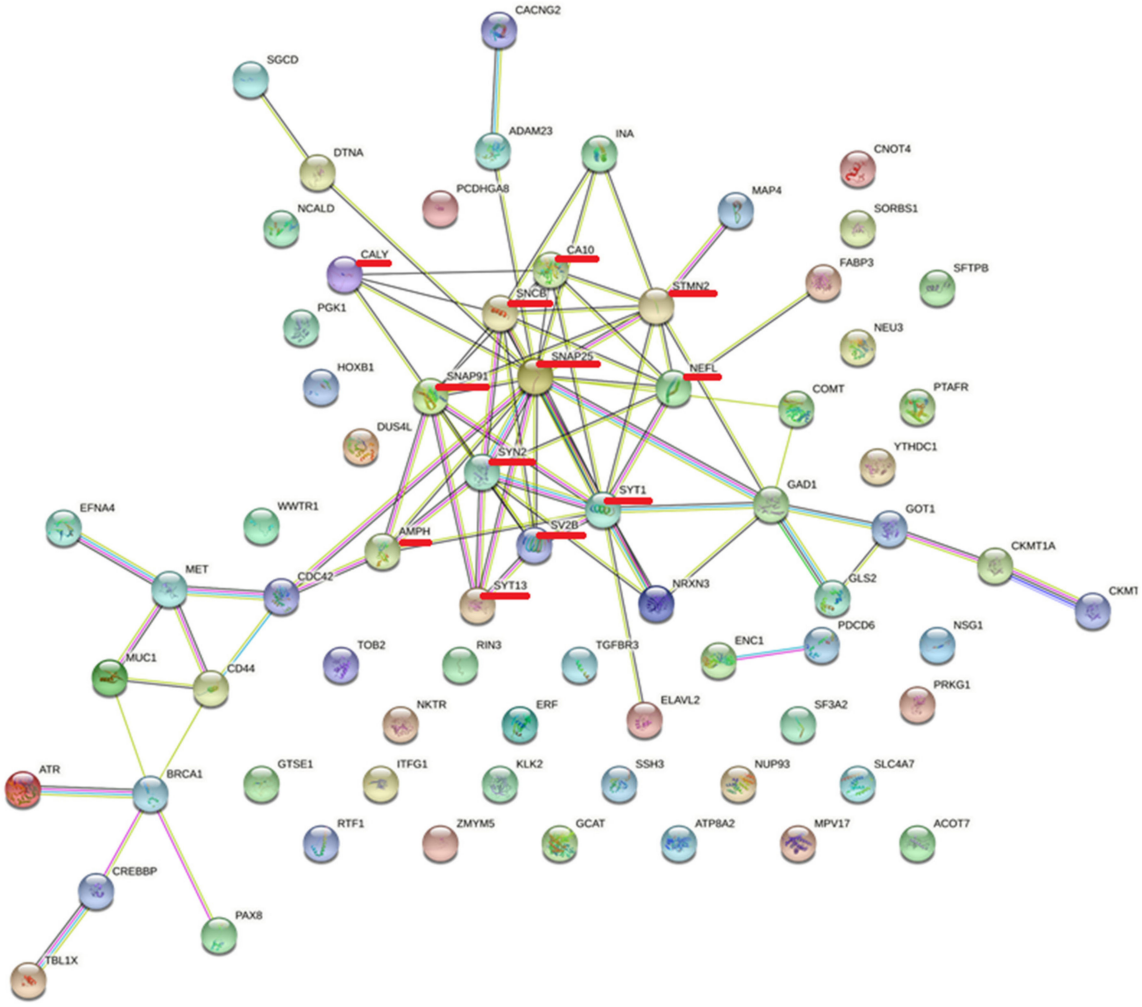

Figure 6. Protein–protein interactions of 76 consensus differentially expressed genes in Alzheimer's disease. Twelve genes identified with MCODE are highlighted.

The 64 genes of the module obtained with WCGNA and the 12 genes present in the cluster found with MCODE have in common 11 genes (AMPH, CA10, CALY, NEFL, SNAP25, SNAP91, SNCB, STMN2, SV2B, SYN2, and SYT1). We also verified whether the 64 genes present in the module obtained with WGCNA are also targets of known drugs and found that the AD down-regulated genes CA10, CACNG2, CALY, GAD1, GLS2, GOT1, MAP4, and MET interact with known drugs. GOT1 and GAD1 are also genes with a higher degree centrality. We also found that 2 out of 12 genes present in the MCODE cluster are also targets of known drugs (CA10 and CALY).

All three upregulated genes (COMT, PRKG1, and PTAFR) that are targets of known drugs are present in the WGCNA module. No up-regulated gene present in the MCODE cluster is a target of known drugs.

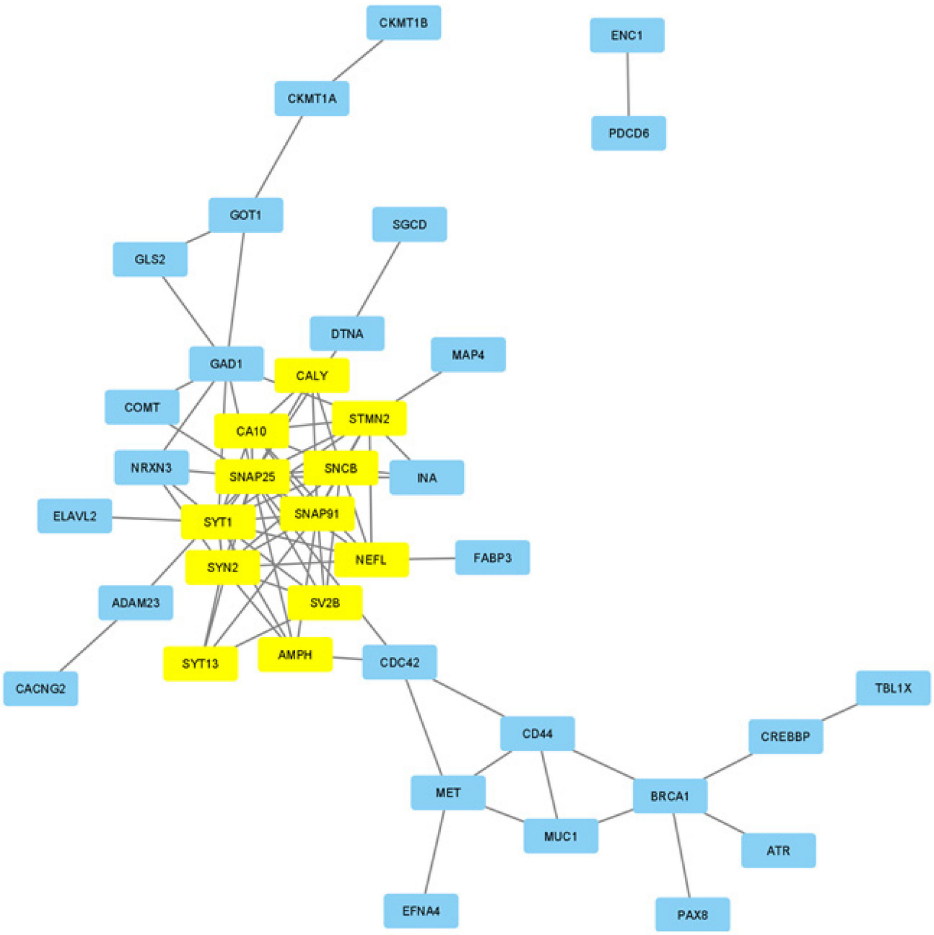

Figure 7. Interconnected regions using MCODE from the network obtained with STRING.

The 12 genes obtained with MCODE were analyzed with GO to study their biological processes (Table 3). Biological processes found include neurotransmitter internalization and secretion, synapse processes, and calcium-ion-regulated exocytosis. The genes mainly involved in these processes are SNAP25, SNAP 91, SYN2, and SYT1.

Table 3. Gene Ontology analysis of the 12 genes obtained with MCODE.

GO: Biological Processes	# Tot Genes	Genes Obtained with MCODE	FDR
neurotransmitter receptor internalization	9	CALY SNAP25	0.0111
clathrin coat assembly	18	CALY SNAP91	0.0329
synaptic vesicle endocytosis	52	SNCB AMPH SYT1 SNAP91	0.0000933
calcium-ion-regulated exocytosis	43	SYT1 SYT13 SYN2	0.00221
neurotransmitter secretion	81	SYT1 SYN2 SNAP25	0.00997
organelle localization	475	NEFL SYT1 SYN2 SNAP25 SNAP91	0.00405

#: number.

4. Discussion

Computational analyses based on high-throughput technologies such as microarray and Next-Generation Sequencing (NGS) have grown rapidly in recent years. Nevertheless, few analyses have been performed on AD, and those often involved a single dataset and only a few samples [30].

Our study used six open public GEO datasets containing gene expression levels of AD samples from hippocampus and normal samples and a hypothesis-based research design. We performed a differential expression analysis for each dataset comparing gene expression data on AD vs. healthy samples. Then, we generated a list of consensus DEGs found in at least two GEO datasets, obtaining 76 DEGs (40 down-regulated genes and 36 up-regulated genes). We verified whether the 76 DEGs are targets of known drugs in at least two of three drug databases (DGIdb, DrugCentral, and The Drug Repurposing Hub). Overall, we found that 9 out of the 40 down-regulated genes interact with 34 known drugs and 3 out of the 36 up-regulated genes interact with 9 drugs.

In particular, we found that the down-regulated gene MET is the gene with the highest number of interacting drugs. Among the drugs, we found Crizotinib, Gefitinib, and Pazopanib.

Crizotinib, an anticancer drug inhibitor of the receptor tyrosine kinase, acts on the down-regulated MET gene. It has been observed to be a potent inhibitor of SHIP2 [31]. Inosito 5′-phosphatase 2 containing the SH2 domain of SHIP2 is a lipid phosphatase that produces fosfatidilinositolo-3,4-bisfosfato from fosfatidilinositolo-3,4,5-trifosfato and is involved in many neurodegenerative diseases [31]. A recent study showed that SHIP2 inhibition decreased β-amyloid-induced tau hyperphosphorylation and reduced memory impairment in a transgenic mouse model of AD [32]. This indicates that SHIP2 may be a promising target for AD.

Gefitinib, an epidermal growth factor receptor (EGFR) inhibitor approved for the treatment of cancer, also acts on the MET gene. It was recently identified as a potential lead compound for β-secretasi (BACE-1), a potential target for the treatment of AD [33].

Another drug that acts on the MET gene is Pazopanib, whose role has been examined in mouse models that express mutant human tau P301L (TauP301L) or the precursor protein of triple mutant amyloid (3x-AβPP) [34]. Pazopanib can cross the blood–brain barrier without detectable peripheral side effects and reduces the expression of p-tau in TauP301L mice [26]. Pazopanib achieves a brain concentration sufficient for the inhibition of various tyrosine kinases, including vascular endothelial growth factor receptors (VEGFRs) [34]. Furthermore, pazopanib does not alter β-amyloid or astrocyte levels in 3x-AβPP mice, but modulates some inflammatory markers (IP-10, MIP-1α, MIP1β, and RANTES) [34]. All this suggests that Pazopanib may be involved in the clearance of p-tau and in the modulation of astrocytic activity in taupathies.

Interestingly, for up-regulated genes, we found COMT as a gene with more interacting drugs. Among the drugs, we found tolcapone and entacapone. Tolcapone and entacapone act on the up-regulated gene COMT. They are used with levodopa for the symptomatic treatment of Parkinson's disease. In a previous study, tolcapone and entacapone were studied for their potential ability to inhibit hexapeptide derived from tau [35]. It was found that tolcapone and entacapone show antiplatelet properties against β-amyloid and α-synuclein. Tolcapone showed a better inhibition of aggregation of the tau-derived hexapeptide and could be used for the treatment of AD due to its greater penetration into the central nervous system compared with entacapone [35].

Then, we built a network of co-expressed genes using WGCNA on an independent GEO dataset. The network was constructed considering gene expression levels of 76 DEGs. We obtained a module of 64 genes with ACOT7, ATP8A2, CDC42, GAD1, GOT1, INA, NCALD, and WWTR1 as the genes with a higher degree centrality. A previous study identified CDC42 as one of the genes associated with AD that, along with other genes, is able to predict AD and cardiovascular disease in blood samples [26].

The 64 genes are mainly enriched in the clathrin coat assembly, synaptic vesicle endocytosis, and DNA damage response signal transduction by p53 class mediator pathways.

There is a correlation between clathrin coat assembly and Aβ protein. The extracellular deposition of Aβ protein is one of the most important neuropathological features of AD. APP is the gene that produces Aβ after it is internalized by clathrin-mediated endocytosis. Thus, an increase in extracellular amyloid deposition reflects an increase in the production of Aβ [36]. In addition, alterations in endocytosis can lead to the over-production of Aβ protein [37].

We further investigated the protein functional network that involves the 76 consensus DEGs. We found a network of 39 interacting proteins consisting of 81 interactions. Among them, MCODE identified AMPH, CA10, CALY, NEFL, SNAP25, SNAP91, SNCB, STMN2, SV2B, SYN2, SYT1, and SYT13 as a cluster of interconnected genes. AMPH, CA10, CALY, NEFL, SNAP25, SNAP91, SNCB, STMN2, SV2B, SYN2, and SYT1 are common genes between the WGCNA module and the 12 genes found with MCODE.

AMPH encodes a protein modulator of endocytosis in synapses [38]. AMPH knockout mice showed a reduced turnover of synaptic vesicle and cognitive deficits [39]. In addition, a reduced expression of AMPH was reported in AD brain regions [40].

The protein encoded by CA10 is involved in brain development and the central nervous system [41]. Moreover, a bioinformatics study revealed 15 genes, including CA10, to be hub genes and able to predict the occurrence of AD [42].

CALY regulates different aspects of the endosomal and synaptic processes. It is needed for endocytosis, degradation, and synaptic plasticity. CALY could be an interesting drug target for neurological diseases such as AD and schizophrenia [43].

Previous studies revealed that elevated levels of NEEFL are a known indicator of early neuronal injury and axonal damage in preclinical AD [44].

SNAP25 is a well-established biomarker of functional synapses as it is a component of the SNARE complex that regulates synaptic communication via synaptic vesicles [45]. In a previous study, elevated levels of SNAP25 were present in cerebrospinal fluid (CSF) of AD patients, suggesting it as a biomarker for early diagnosis [46].

We also found another synaptosome-associated protein, SNAP91. SNAP91 regulates synaptic vesicle transport, and its dysregulation could damage synaptic transmission [47]. SNAP91 is involved in several neurodegenerative diseases, such Parkinson's disease and AD, where low levels of SNAP91 have been found [48,49].

SNCB encodes a protein involved in neuronal plasticity. High levels of SNCB were observed in AD in CSF and plasma [50].

STMN2 belongs to the stathmin family and plays a role in microtubule dynamics. Microtubules are involved in axonal signaling transduction and neuronal growth [51]. However, there is not a clear association between STMN2 and AD.

Previous studies found a decrease in SV2B and SYN2 in AD patients [52,53].

SYT1 and SYT13 are components of a synaptotagmin family with a role in neurotransmitter release. High levels of SYT1 were found in CSF of AD patients.

The down-regulated genes CA10 and CALY are targeted by known drugs. CA10 interacts with zonisamide, and CALY with apomorfina, clozapina, and trifluoperazine. The up-regulated genes COMT, PRKG1, and PTAFR present in the WGCNA module are targets of known drugs. No known drug acts on genes present in the MCODE module.

Several works suggested that the primary pathological hallmarks of AD are Aβ plaques and NFTs. Additional changes could occur in the brain of AD patients as secondary responses. However, we could not determine whether the alterations found in our study are directly linked to the primary or secondary AD changes. Further analyses considering the clinical relevance of each AD sample should be performed to well characterize our findings [54,55].

The gene alterations found in our hypothesis-based research study are limited by the small number of samples utilized in our approach. Although our results are mostly

consistent with the literature, future studies should test our gene network to demonstrate its efficacy.

5. Conclusions

In conclusion, our study identified 76 DEGs in AD that regulate several de-regulated pathways. We identified several genes with a higher degree centrality (ACOT7, ATP8A2, CDC42, GAD1, GOT1, INA, NCALD, and WWTR1). In addition, MCODE revealed 12 interconnected genes (AMPH, CA10, CALY, NEFL, SNAP25, SNAP91, SNCB, STMN2, SV2B, SYN2, SYT1, and SYT13) that could play a crucial role in AD. Further studies should investigate how the alteration of these genes may be linked to the evolution of AD.

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