

ORIGINAL RESEARCH ARTICLE

Key regulators of Alzheimer's disease:
Network biology and in silico analysis with
acetylcholinesterase and glutamate inhibitors

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Abstract

Alzheimer's disease (AD) is a complex, progressive neurodegenerative disorder driven by both genetic and environmental factors, with hallmark features including amyloid- β plaques and neurofibrillary tangles. Despite substantial research, the majority of currently licensed medications are still symptomatic, highlighting the need for multi-target and network-based treatment approaches. This study employed a systems biology approach to identify important regulatory proteins implicated in AD development and to assess their interaction patterns with approved glutamate and cholinesterase inhibitors. A protein-protein interaction network was developed using the 85 overlapping genes identified when AD-associated genes were selected from six significant biomedical databases. Seven major regulators were identified using centrality and hub analyses, including *APP*, *APOE*, *BDNF*, *VEGFA*, *PSEN1*, *CASP1*, and *NOTCH1*. Their participation in axon formation, signal transduction, neuroinflammatory processes, and neurodegenerative pathways was revealed by Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis. The binding affinities of donepezil, galantamine, rivastigmine, and memantine toward these targets were evaluated by molecular docking using AutoDock Vina. While *APP* demonstrated relatively weaker affinities, *BDNF* demonstrated the strongest overall binding interactions, especially with donepezil. Memantine showed significant binding to *PSEN1*, indicating that amyloidogenic processes may be indirectly modulated. These results demonstrate the usefulness of integrated computational techniques in identifying new therapeutic interaction networks and support a multi-target pharmacological paradigm. This study offers a systems-level basis for medication repurposing and precision intervention strategies in AD, although additional experimental validation is needed.

Keywords: Alzheimer's disease; Cholinesterase inhibitors; Glutamate inhibitors; Molecular docking; *In silico* studies; Network biology; Drug repurposing

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder known for damaging neurons in the brain and is characterized by memory loss, cognitive decline, and behavioral impairments.¹ Dementia, a more advanced manifestation of AD, currently affects over 55 million people worldwide, and this number is projected to triple by 2060.² The hallmark

pathological features of AD include the accumulation of amyloid- β (A β) plaques and neurofibrillary tangles formed by the tau protein, as well as the involvement of microglia and astrocytes.^{3,4} Owing to the multifactorial nature of the disease, early-stage (prodromal) diagnosis and treatment remain clinically challenging. Currently, there is no cure for AD; existing treatments only offer symptomatic relief.⁵ Hence, there is an urgent need for effective therapeutic agents capable of halting or reversing AD progression. Studies have shown that A β plaques and neurofibrillary tangles are present in approximately 85% of clinically diagnosed AD patients, suggesting that these features are central to disease pathology.⁶⁻⁸

Several genetic mutations are associated with AD pathogenesis⁹, including mutations in *APP*, *PSEN1/2*, *BACE1*, *MAPT*, and *APOE- ϵ 4*.¹⁰⁻¹² A β is a toxic peptide fragment of amyloid precursor protein (APP), produced via proteolytic cleavage by β - and γ -secretases^{13,14} and deposited in the brain as plaques.¹⁵ Mutations in the *PSEN1/2* genes (which encode γ -secretase) and the *BACE1* (which encodes β -secretase) lead to this abnormal cleavage process.^{10,16} Similarly, *MAPT*, which is associated with tau pathology, can give rise to paired helical filaments, neurofibrillary tangles, and hyperphosphorylated tau.¹⁷ Apolipoprotein E (ApoE), a cholesterol transporter, has

three isoforms— ϵ 2, ϵ 3, and ϵ 4, with ApoE- ϵ 4 being the most significant genetic risk factor for AD.¹⁸ Individuals carrying a single ϵ 4 allele (heterozygotes) have a 2–3-fold greater risk of developing AD, whereas those with two ϵ 4 alleles (homozygotes) have a 12–15-fold greater risk.¹⁹

In addition to United States Food and Drug Administration (FDA)-approved monoclonal antibodies²⁰, current treatments also include cholinesterase inhibitors (e.g., donepezil, rivastigmine, galantamine) and glutamate pathway modulators (e.g., memantine). Acetylcholine is a neurotransmitter essential for cognition, memory, synaptic transmission, neuromodulation, and the immune response.²¹ It is degraded by the enzyme acetylcholinesterase (AChE), and its reduction contributes to cognitive decline. On the other hand, glutamate, an excitatory neurotransmitter, chronically activates N-methyl-D-aspartate (NMDA) receptors, leading to Ca^{2+} influx upon removal of the Mg^{2+} block during depolarization.²² Excessive intracellular Ca^{2+} activates enzymes that produce reactive oxygen species, contributing to neurodegeneration. Cholinesterase and glutamate inhibitors function by binding to their respective enzymes or receptors to prevent these pathological processes, as illustrated in Figure 1.

Network biology is an interdisciplinary field that

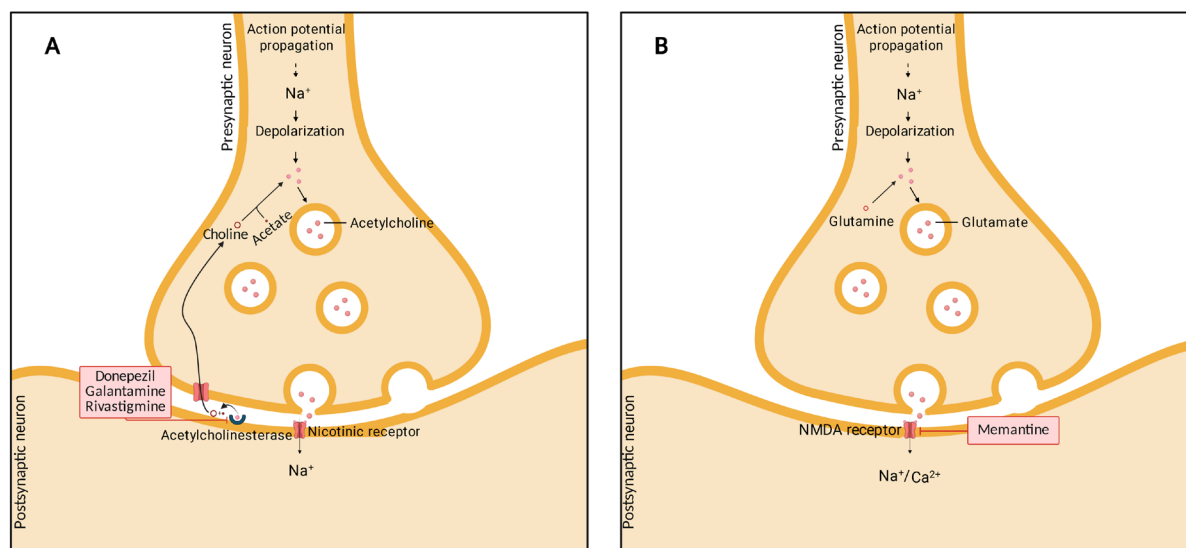


Figure 1. The synaptic mechanisms targeted by Food and Drug Administration-approved drugs for Alzheimer's disease. (A) Cholinergic synapse, where an action potential triggers acetylcholine release from the presynaptic neuron. Acetylcholine binds to nicotinic receptors on postsynaptic neurons, promoting signal transmission. Acetylcholinesterase rapidly breaks down acetylcholine into choline and acetate. Drugs (e.g., donepezil, galantamine, and rivastigmine) inhibit acetylcholinesterase, thereby increasing synaptic acetylcholine levels and improving cognitive function. (B) Glutamatergic synapse, where glutamate is released and binds to N-methyl-D-aspartate (NMDA) receptors, allowing Na^+ and Ca^{2+} influx. Excessive glutamate can cause excitotoxicity, contributing to neuronal damage. Memantine acts as an NMDA receptor antagonist, blocking abnormal Ca^{2+} influx while preserving normal neurotransmission. Together, these mechanisms represent key therapeutic strategies in the management of Alzheimer's disease through the modulation of cholinergic and glutamatergic pathways.

combines computational techniques with biological sciences. Modeling biological systems as interconnected networks rather than isolated components helps in deciphering complex biological functions across multiple levels (e.g., genes, proteins, cells, tissues, and organs). In such networks, nodes represent biomolecules (e.g., amino acid residues, proteins, or cells), and edges depict their physical, functional, or chemical interactions. Protein–protein interaction (PPI) data can be applied on a large scale to construct networks that reflect these associations.²³ For this purpose, Cytoscape serves as a comprehensive tool in network biology for the analysis and visualization of biomolecular interaction networks, integrating high-throughput expression data and other molecular states into a unified framework.²⁴

Despite extensive research, the molecular mechanisms underlying AD remain incompletely understood, particularly concerning key regulatory proteins and their potential therapeutic inhibitors. Although many drugs have been repurposed²⁵ and may act on multiple targets²⁶, AD remains incurable, largely because of its polygenic nature, and no molecule has yet been proven to comprehensively address this challenge. The intricacy of AD pathophysiology and the shortcomings of reductionist, single-target therapy approaches have been brought to light by subsequent clinical setbacks involving A β -targeting monoclonal antibodies and secretase inhibitors.

By utilizing established safety profiles and investigating novel mechanistic connections within disease-associated molecular networks, drug repurposing approaches, especially those utilizing FDA-approved agents, offer a translational advantage. This gap highlights the importance of system-level approaches to identify new intervention points and understand disease progression more comprehensively. This study aims to identify central protein regulators involved in the progression of AD by integrating PPI networks with computational analysis. The therapeutic potential of cholinesterase and glutamate inhibitors against these key targets was further evaluated via molecular docking and molecular dynamics simulation techniques.

2. Materials and methods

2.1. Identification of overlapping genes from various databases

In this study, several biological databases were utilized to identify the overlapping genes across all databases. Using “Alzheimer’s disease” as a search term, AD-related genes (gene symbols and descriptions) were mined from six existing databases: the DisGeNET database (<https://www.disgenet.org/>), the DrugBank database (<https://go.drugbank.com/>), the Ensembl database (<https://ensemblgenomes.org/>), the GeneCards database (<https://www.genecards.org/>), the OMIM database (<https://www.omim.org/>), and the TTD database (<https://db.idrblab.net/ttd/>). In the identification of overlapping genes, a traditional method, the “VLOOKUP” formula in Microsoft Excel (Version 2603, Microsoft, US) was used to identify the genes common to all the databases by comparing one database at a time with the remaining ones on the basis of gene symbols, and the process was repeated until a single consolidated sheet was obtained.

2.2. Network construction and analysis

A PPI network was constructed using the STRING database (version 12, <https://string-db.org/>), which displays multiple interactions between the selected genes. The search was restricted to the species “*Homo sapiens*” to construct the PPI network. The generated network was then visualized using Cytoscape (version 3.10.3, Institute for Systems Biology (ISB), US), an open-source software that provides visualization, analysis, modeling, and integration of interaction networks. The importance of each node was evaluated by calculating the mean of four centrality parameters and was assessed using CytoNCA in Cytoscape. These parameters included “Degree centrality (DC),” “Closeness centrality (CC),” “Betweenness centrality (BC),” and “EigenVector centrality (EvC).” DC represents the number of other nodes directly connected to a given node, and CC represents the shortest path between a node and other nodes. BC is calculated by considering several nodes and determining the number of shortest paths passing through the node of interest. EvC reflects the relative influence of a node within the network, on the basis that connections to high-scoring nodes contribute more to the score of a specific node than connections to low-scoring nodes. The top 10 genes with the highest mean values were selected from this process for further analysis. After centrality measurements, CytoHubba was applied to three parameters (degree, closeness, and betweenness) separately in Cytoscape, with the criterion of identifying the top 10 nodes.

2.3. Screening of target genes

To identify overlapping or target genes, genes that appeared in all four of the abovementioned datasets/processes were considered, and 10 genes were selected from each dataset. The overlapping genes were identified using VENNY version 2.1 (<https://bioinfo.gp.cnb.csic.es/tools/venny/>) and considered for further analysis. Moreover, a secondary PPI network was constructed with the identified target genes, followed by gene enrichment analysis and docking studies with AChE and glutamate inhibitors.

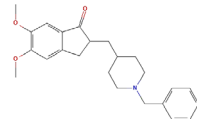
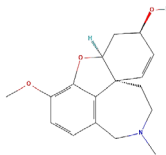
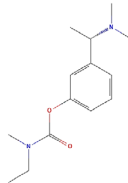
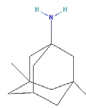
2.4. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses

The target genes were uploaded to the ShinyGO database (version 0.82, <http://bioinformatics.sdstate.edu/go/>), a web server used to generate enriched Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways on the basis of gene or protein lists. A threshold of false discovery rate < 0.05 and species restricted to "*Homo sapiens*" were applied, with the number of displayed pathways set to 20 and pathway sizes ranging from 2 to 2,000. Charts were generated on the same platform for data visualization and analysis.

2.5. Molecular docking and simulation

The target proteins were docked with four FDA-approved compounds, which include AChE inhibitors and glutamate inhibitors, and their information is provided in Table 1. Molecular docking was performed using AutoDock Vina (version 4.2.6, Scripps Research, US) to illustrate the binding activity and mechanism between the identified targets and the AChE and glutamate inhibitors. The three-dimensional structures of the ligand molecules were downloaded in SDF format from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>), and the SDF format was converted into PDBQT format using Discovery

Table 1. Information on four Food and Drug Administration-approved drug molecules collected from the PubChem database

Chemical ID	Molecule name	Molecular formula	Molecular weight	Structure
3152	Donepezil	$C_{24}H_{29}NO_3$	379.5 g/mol	
9651	Galantamine	$C_{17}H_{21}NO_3$	287.35 g/mol	
77991	Rivastigmine	$C_{14}H_{22}N_2O_2$	250.34 g/mol	
4054	Memantine	$C_{12}H_{21}N$	179.3 g/mol	

Studio Visualizer 2021 software (<https://discover.3ds.com/discovery-studio-visualizer-download>). The protein structures of the targets were downloaded from the RCSB database (<https://www.rcsb.org/>) in PDB format, and the docking sites were predicted using DeepSite (<https://www.playmolecule.com/deepsite/>) and are presented in Table 2. Discovery Studio Visualizer was used to remove water and ligand molecules, and AutoDock Tools were used to add hydrogen atoms and charges, while all other parameters were left at default settings. The modified protein structures were saved in PDBQT format. Finally, AutoDock Vina was used to perform the molecular docking analysis.

The binding affinities (kcal/mol) were predicted by docking scores, with the lowest score representing the strongest affinity. The docking results were visualized using the Discovery Studio Visualizer 2021 software to analyze the optimal binding pocket. Entropic contributions, solvent dynamics, and induced-fit conformational rearrangements may not be adequately captured by docking scores generated from empirical scoring functions, which are approximations of binding free energy.^{27,28} To prevent bias toward pre-defined pockets, grid box dimensions were chosen to cover the entire active or predicted interaction region. However, docking was carried out in blind mode to assess surface interaction tendencies of proteins lacking well-defined catalytic sites. This method does not replace dynamic simulations or experimental binding validation,

but it allows for comparative ranking of ligands.

3. Results

3.1. Construction of a common gene protein–protein interaction network

A total of 15,708 AD-related genes were extracted from the six databases, of which 85 were identified as overlapping genes using the “VLOOKUP” function in Microsoft Excel after removal of duplicate gene symbols. A PPI network was subsequently constructed to describe the interactions among the genes identified in the previous step. In this network, each protein is represented as a circular node, and the lines between two proteins represent edges. An increase in the number of edges indicates stronger interaction connectivity. A total of 85 nodes and 536 edges were obtained from the STRING database (Figure 2), which were further visualized and analyzed using Cytoscape.

3.2. Protein–protein interaction network analysis

From CytoNCA, the mean values of 85 AD-related genes based on the DC, CC, BC, and EvC parameters were obtained, and the top 10 nodes with the highest mean values were selected and ranked in descending order (Table 3). These parameters are crucial for identifying the relative importance of particular nodes in the network. Higher mean values indicate greater node importance. As a

Table 2. Protein characteristics and DeepSite binding site predictions

Protein	Protein Data Bank ID	Description	DeepSite binding site prediction			
			X	Y	Z	Score
APP	1IYT	Solution structure of the Alzheimer's disease amyloid β -peptide (1–42)	3.8	–0.0	–5.8	0.47
BDNF	1BND	Structure of the brain-derived neurotrophic factor/neurotrophin-3 heterodimer	20.4	–12.3	38.7	1.00
ApoE	2L7B	NMR structure of full length ApoE3	3.7	–10.6	4.7	0.99
VEGFA	1VPF	Structure of human vascular endothelial growth factor	35.0	10.0	12.4	0.94
NOTCH1	1YYH	Crystal structure of the human NOTCH1 ankyrin domain	27.6	33.7	–1.2	0.90
CASP1	1RWK	Crystal structure of human caspase-1 in complex with 3-(2-mercapto-acetyl-amino)-4-oxo-pentanoic acid	41.5	53.0	–1.3	0.97
PSEN1	2KR6	Solution structure of PSEN1 C-terminal fragment subunit	3.5	76.7	10.7	0.99

Abbreviations: ApoE: Apolipoprotein E; APP: Amyloid precursor protein; BDNF: Brain-derived neurotrophic factor; CASP1: Caspase-1; NMR: Nuclear magnetic resonance; NOTCH1: Neurogenic locus notch homolog protein 1; PSEN1: Presenilin 1; VEGFA: Vascular endothelial growth factor A.

result, *APP* had the highest mean centrality value, followed by *BDNF*, *APOE*, *VEGFA*, *CSTB*, *ADAM10*, *NOTCH1*, *CASP1*, *PSEN1*, and *ACE*.

Furthermore, 10 hub genes were identified using CytoHubba based on degree (Figure 3A), closeness (Figure 3B), and betweenness (Figure 3C) centrality measures and were arranged in a rank-wise manner in a clockwise direction. The rank was indicated by the size of the node, with larger nodes representing higher ranks. Notably, *APP*,

APOE, *BDNF*, and *VEGFA* consistently ranked highest across all three centrality measures.

3.3. Target gene screening and subnetwork formation

The results from the previous steps generated four different outputs, each containing 10 genes/nodes. These results were then compared using Venny version 2.1 to identify the genes with the greatest degree of overlap (Figure 4A). As a result, seven genes were identified, including *APOE*,

Table 3. Centrality measures of the top 10 genes

Gene	DC	CC	BC	EvC	Mean
<i>APP</i>	44	0.6614173	1075.3599	0.2749554	280.0740682
<i>BDNF</i>	38	0.6363636	1067.5322	0.23041376	276.5997443
<i>APOE</i>	41	0.6511628	769.1726	0.2734267	202.7742974
<i>VEGFA</i>	33	0.60431653	593.82007	0.19633302	156.9051799
<i>CSTB</i>	19	0.5283019	311.4442	0.12551706	82.77450474
<i>ADAM10</i>	19	0.5283019	278.20917	0.14023627	74.46942704
<i>NOTCH1</i>	25	0.5562914	263.93576	0.17393363	72.41649626
<i>CASP1</i>	24	0.5637584	255.20116	0.15443727	69.97983892
<i>PSEN1</i>	30	0.5915493	209.61992	0.22315016	60.10865487
<i>ACE</i>	21	0.5283019	187.96638	0.13246998	52.40678797

Abbreviations: BC: Betweenness centrality; CC: Closeness centrality; DC: Degree centrality; EvC: Eigenvector centrality.

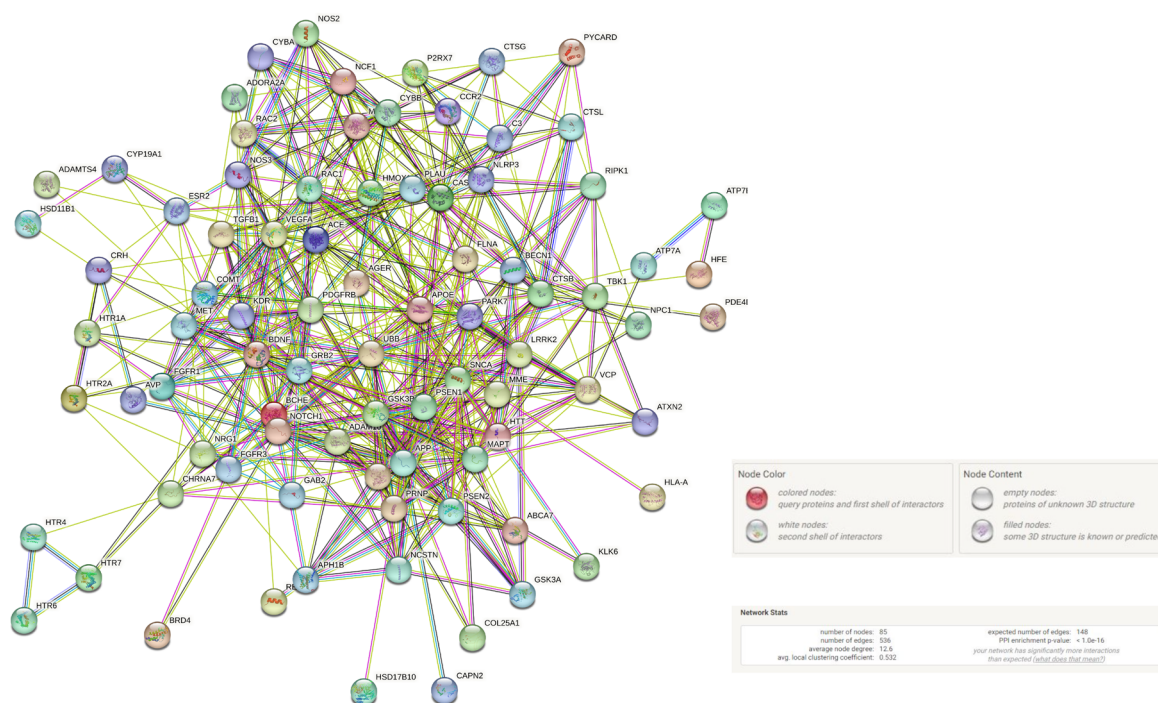


Figure 2. Protein-protein interaction network of 85 Alzheimer's disease-related genes

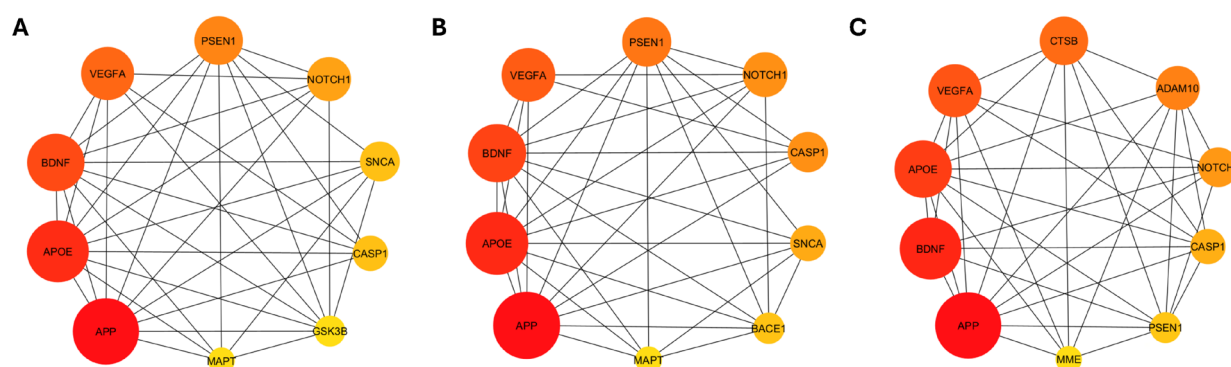


Figure 3. The top 10 nodes identified using Cytohubba, based on centrality measures: (A) degree, (B) closeness, and (C) betweenness

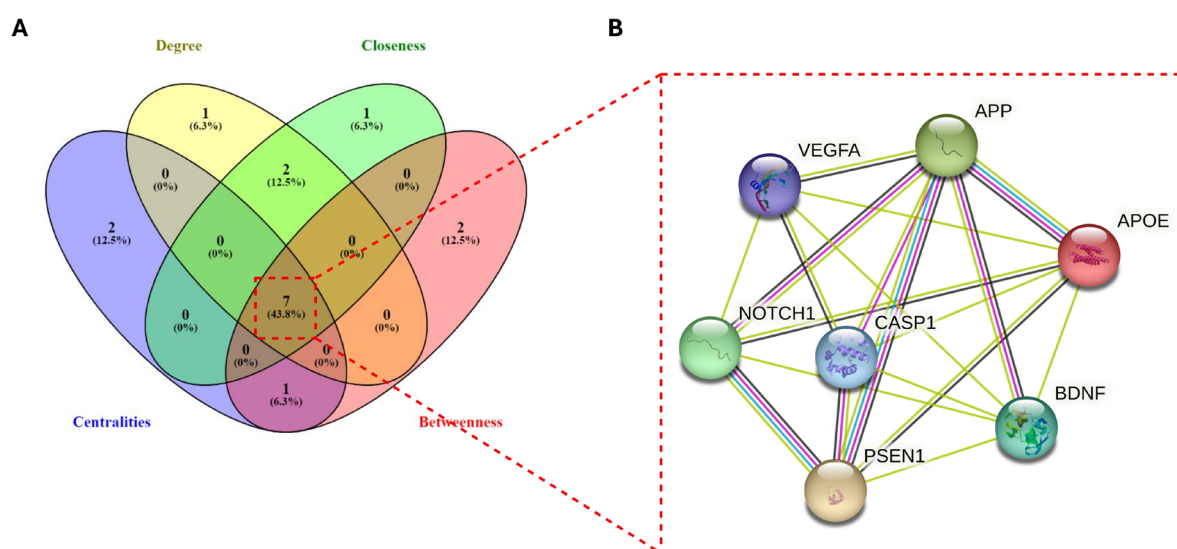


Figure 4. Target acquisition and sub-PPI network of the overlapping genes: (A) Venn diagram of the seven overlapping genes and (B) PPI network of the seven targets.

Abbreviation: PPI: Protein–protein interaction.

VEGFA, *NOTCH1*, *CASP1*, *PSEN1*, *APP*, and *BDNF*. Moreover, a secondary PPI network was generated using the identified target genes, as described previously (Figure 4B). The subnetwork comprised seven nodes with a total of 19 edges, where *APP*, *APOE*, *PSEN1*, *NOTCH1*, and *BDNF* are strongly interconnected. Overall, *APP* had the highest number of interactions, particularly with *APOE*, *PSEN1*, *NOTCH1*, and *BDNF*, followed by interactions involving *PSEN1*, *NOTCH1*, and *APOE*.

3.4. Gene enrichment analysis

To further elucidate the multiple mechanisms of the seven identified genes, GO enrichment and KEGG pathway analyses were conducted. The enriched GO categories included biological processes, cellular components, and molecular functions. The enriched biological processes

were primarily associated with axonogenesis, axon development, cell morphogenesis involved in neuron differentiation, neuron projection morphogenesis, plasma membrane-bound cell projection morphogenesis, cell projection morphogenesis, cell part morphogenesis, positive regulation of multicellular organismal processes, positive regulation of signal transduction, positive regulation of cell communication, and positive regulation of signaling, with all target genes contributing to these processes (Figure 5A).

The cellular component category was predominantly represented by secretory vesicles (Figure 5B), whereas the molecular function category was mainly associated with signaling receptor binding (Figure 5C). Secretory vesicles included five genes, while six genes were involved in signaling receptor binding. KEGG pathway analysis revealed associations of three genes with AD,

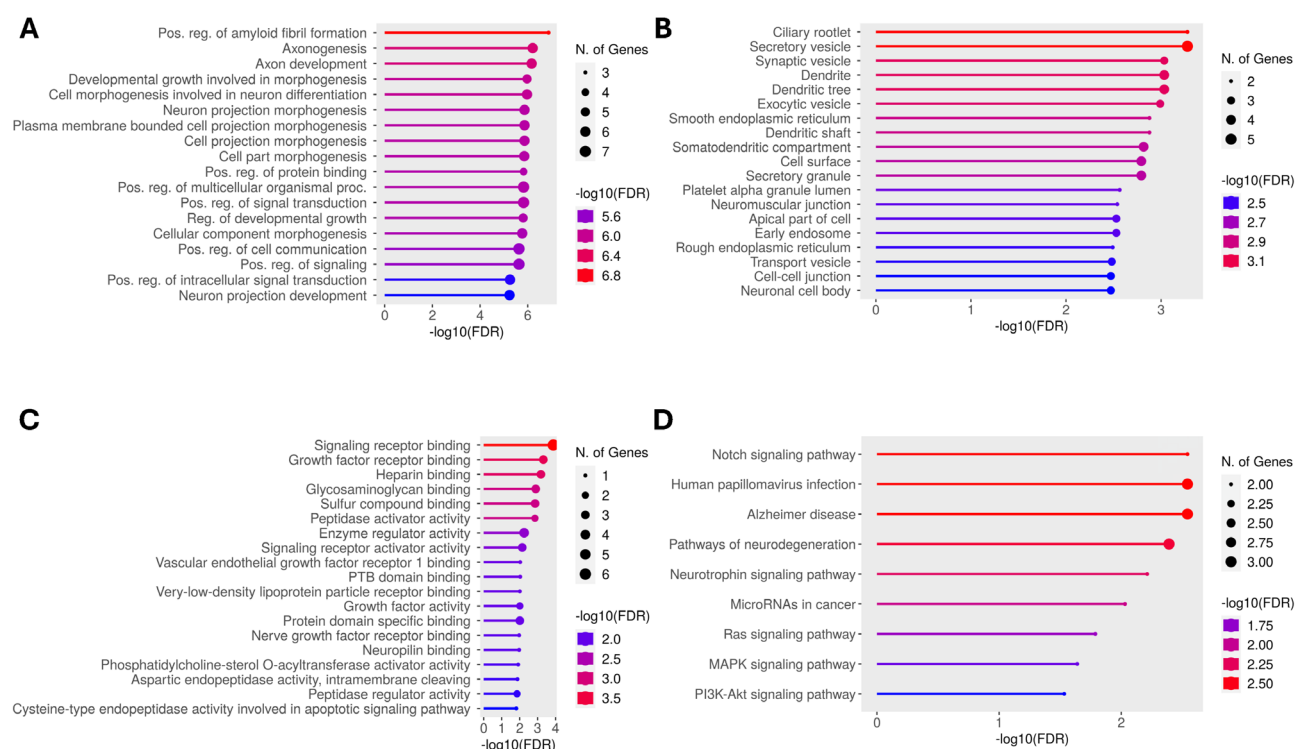


Figure 5. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses: (A) Biological processes, (B) cellular component, (C) molecular function, and (D) Kyoto Encyclopedia of Genes and Genomes pathways. Abbreviation: FDR: False discovery rate.

neurodegeneration-related pathways, and human papillomavirus infection pathways (Figure 5D).

3.5. Molecular docking analysis

The top seven target genes and FDA-approved drugs were subjected to molecular docking using AutoDock Vina version 4.2.6. The lowest binding energy indicates a strong binding affinity between the ligand and the receptor. In this study, the binding energies between all receptor–ligand pairs were negative, ranging from -3.72 kcal/mol to -10.16 kcal/mol (Figure 6). The findings suggest that the brain-derived neurotrophic factor (BDNF) protein exhibited the strongest overall binding affinity with donepezil, galantamine, rivastigmine, and memantine, whereas APP showed the weakest binding affinities among the targets. The binding energy of donepezil with BDNF was the strongest, followed by interactions with vascular endothelial growth factor A (VEGFA) > caspase-1 (CASP1) > presenilin 1 (PSEN1) > neurogenic locus notch homolog protein 1 (NOTCH1) > ApoE > APP (Figure 7).

Similarly, galantamine showed strong binding with the BDNF protein, achieving a docking score of -7.63 kcal/mol, whereas the weakest binding was observed with APP (-4.52 kcal/mol), as presented in Figure 8. Based on the

binding energies for rivastigmine, the targets ranked (from strongest to weakest affinity) as follows: BDNF, PSEN1, APOE, VEGFA, CASP1, NOTCH1, and APP (Figure 9). Furthermore, the highest binding affinity of memantine was observed with PSEN1 (-7.85 kcal/mol), whereas the lowest affinity was observed with APP (-4.73 kcal/mol), as shown in Figure 10.

4. Discussion

Alzheimer's disease is increasingly understood not as a disease with a single cause, but as a complex network of interacting molecular failures spanning synaptic dysfunction²⁹, mitochondrial imbalance³⁰, neuroinflammation³¹, and vascular deterioration.³² The present study elucidates this intricate landscape with a systems biology approach, identifying seven critical regulators (APP, BDNF, APOE, VEGFA, PSEN1, NOTCH1, and CASP1) and exploring their molecular affinities with cholinergic and glutamatergic drugs via *in silico* docking. This dual-layered strategy highlights not only the molecular nodes central to the pathogenesis of AD but also the therapeutic versatility of established drugs such as donepezil, memantine, galantamine, and rivastigmine.

Instead of using a graphical Venn diagram approach,

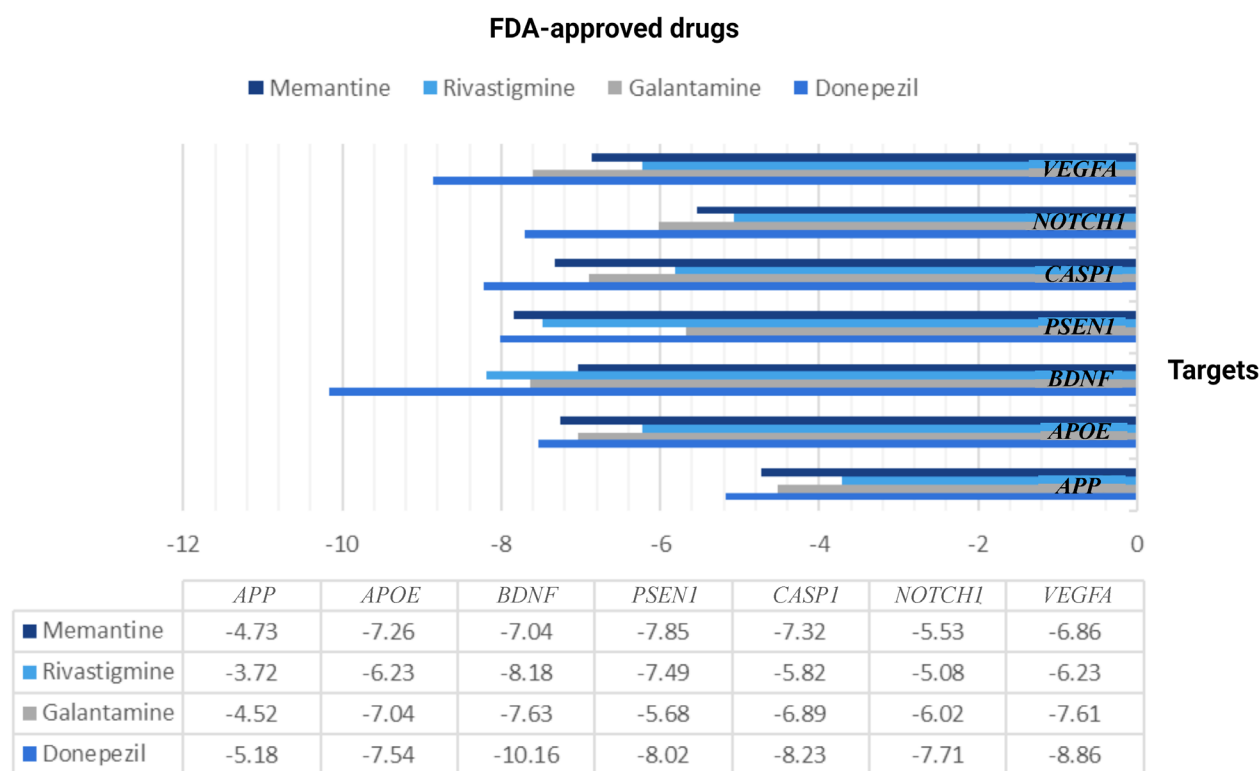


Figure 6. AutoDock results and the lowest binding energies (kcal/mol) between the targets and Food and Drug Administration (FDA)-approved drugs

a spreadsheet-based computational matching process was used to ensure rigorous identification of overlapping AD-associated genes across several biological sources. Although Venn diagrams are frequently used to visualize intersecting datasets, their scalability is intrinsically limited, and they are essentially descriptive rather than computational tools, especially when dealing with large gene lists.³³ Consequently, systematic pairwise comparisons of standardized gene symbols across datasets were carried out using the “VLOOKUP” function in Microsoft Excel. This method reduced uncertainty caused by synonyms or database-specific annotations by enabling accurate, symbol-based matching in compliance with accepted gene nomenclature standards.³⁴ Additionally, a transparent and repeatable framework for dataset integration is offered by spreadsheet-based lookup functions, which enable progressive validation and retention of annotation metadata for subsequent bioinformatic analysis.³⁵ This approach ensured computational accuracy, reduced manual selection bias, and made it easier to create a consolidated dataset suitable for further network and pathway analysis when compared to visualization-oriented overlap tools.

The strong binding affinity of donepezil for *BDNF*,

in particular, opens an intriguing therapeutic avenue. *BDNF*, a key modulator of synaptic plasticity and memory encoding, is consistently downregulated in the brains and cerebrospinal fluid of AD patients, contributing to synaptic failure and hippocampal atrophy.³⁶ A study confirmed that AChE inhibitors can modulate *BDNF* expression through NMDA receptor blockade, independent of their cholinesterase activity.³⁷ This suggests a hidden neurotrophic potential in drugs originally designed only to address acetylcholine depletion. Additionally, the interaction between *BDNF* and NMDA receptor signaling cascades creates a bridge between the cholinergic and glutamatergic systems.³⁸ By binding to *BDNF* and possibly stabilizing its function, donepezil may reinforce synaptic resilience beyond its canonical scope.

In contrast, *APP*, the canonical hallmark of AD, exhibited weak docking interactions, which aligns with the historical difficulty of pharmacologically modulating this protein. *APP* is primarily a transmembrane structural protein with minimal surface-accessible binding pockets, rendering it a poor direct drug target.³⁹ However, attention has shifted to its cleavage products, especially soluble A β oligomers (e.g., dimers, trimers, protofibrils), as neurotoxic agents, where A β 42 oligomer is more toxic than

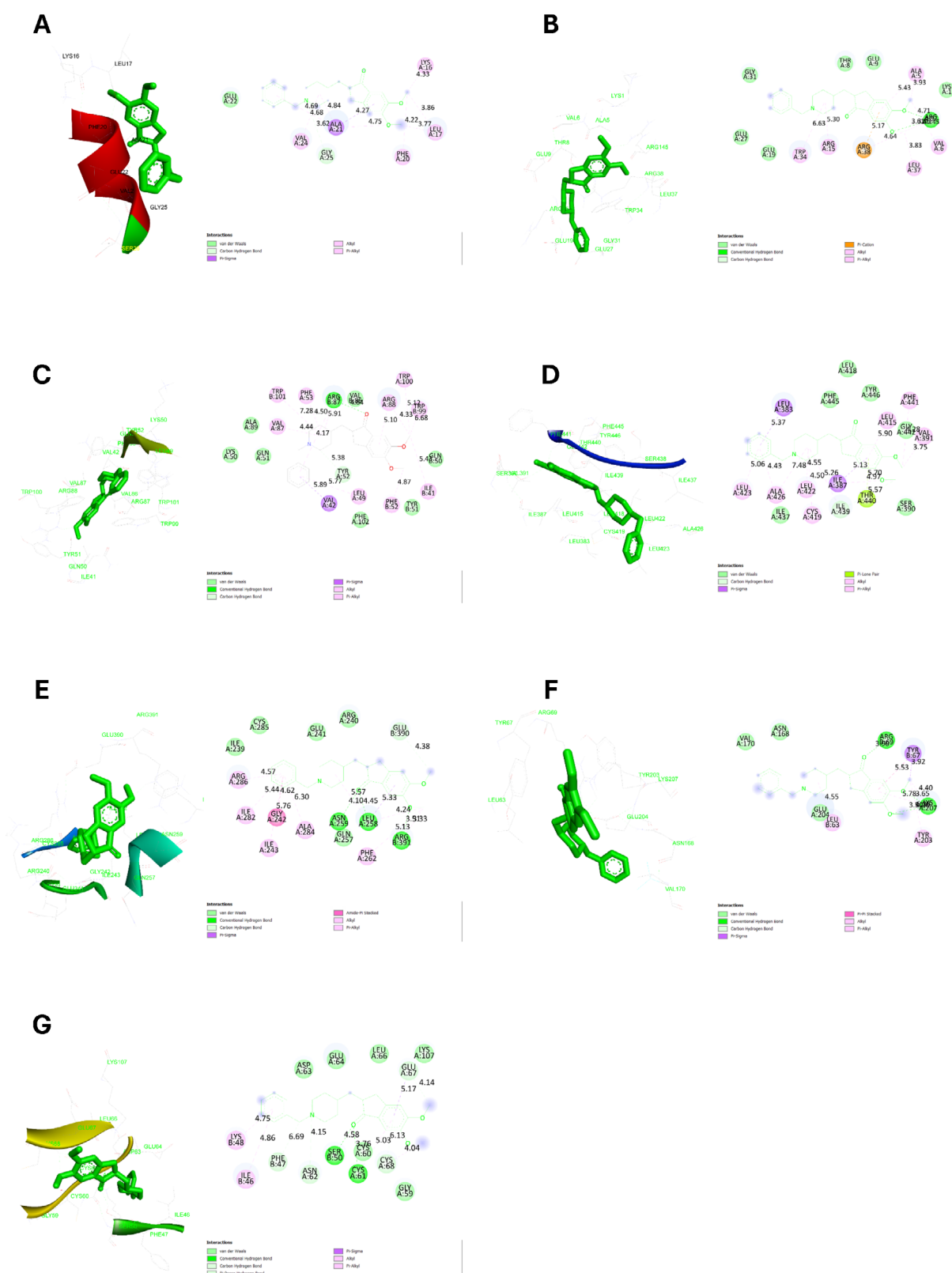


Figure 7. Two- and three-dimensional docking conformations of donepezil with target proteins encoded by (A) *APP*, (B) *APOE*, (C) *BDNF*, (D) *PSEN1*, (E) *CASP1*, (F) *NOTCH1*, and (G) *VEGFA*

A β 42 fibrils. These proteins are largely generated through PSEN1/2-mediated γ -secretase activity⁴⁰, placing PSEN1/2 at the therapeutic frontline.

Interestingly, memantine, although primarily an NMDA receptor antagonist, demonstrated meaningful affinity for *PSEN1* in this study. This observation is supported by recent findings showing that NMDA modulation can indirectly alter γ -secretase activity and mitigate *PSEN1*-driven amyloidogenesis, likely via intracellular calcium regulation and synaptic stabilization.⁴¹ This indicates that over-activation of NMDA receptors leads to intracellular Ca²⁺ overload, which has been demonstrated to boost A β generation and γ -secretase activity, especially in models with mutations in *PSEN1*.^{42,43} Memantine limits amyloidogenic APP processing and indirectly modulates γ -secretase function by reducing calcium-dependent enzymatic dysregulation and pathological glutamatergic signaling.^{44,45} A molecular connection between NMDA modulation and decreased *PSEN1*-driven amyloidogenesis is also supported by the finding that stabilization of synaptic activity through NMDA receptor antagonism has been linked to a decrease in A β -induced excitotoxic feedback loops.⁴⁶ Furthermore, PSEN1/2 has also been implicated in mitochondrial fragmentation⁴⁷ and synaptic vesicle cycling⁴⁸, indicating that drugs that interact with these proteins could impact many neurodegenerative processes.

In support of the polypharmacological narrative, *VEGFA* has emerged as a strong interactor with multiple cholinergic drugs. *VEGFA* is best known for its role in vascular permeability and angiogenesis⁴⁹, but it also contributes to neuroprotection through enhanced cerebral blood flow⁵⁰, neurogenesis⁵¹, and antiapoptotic signaling⁵² in astrocytes and endothelial cells. The involvement of *VEGFA* in AD has been substantiated by evidence of its upregulation in early disease stages and depletion during later progression^{53,54}, indicating a biphasic compensatory role. Recent evidence suggests that AChE inhibitors may increase *VEGFA* expression and function by promoting nitric oxide production via endothelial nitric oxide synthase activation.^{55,56} The affinity of donepezil for *VEGFA* observed in docking studies could thus reflect not only symptomatic management but also vasoprotective effects that might alter disease progression.

APOE, which has long been established as the strongest genetic risk factor for late-onset AD through its ϵ 4 allele, presents a more nuanced pharmacological profile. While its docking affinity was moderate, its functional implications remain profound. ApoE modulates lipid metabolism, synaptic pruning, and amyloid clearance, and its isoforms directly affect blood–brain barrier integrity⁵⁷

and tau phosphorylation.⁵⁸ Although not traditionally viewed as druggable, allosteric modulators and gene editing approaches targeting *APOE* isoform balance are now under development.⁵⁹ Moreover, pharmacogenomic insights suggest that the *APOE* genotype may influence patient response to AChE inhibitors^{60–62}, underscoring the need for stratified treatment models.

Among the most compelling targets identified are CASP1, a proinflammatory protease that activates the NOD-, LRR- and pyrin domain-containing protein 3 inflammasome and drives pyroptotic cell death in neurons and glia.⁶³ The strong docking interactions of donepezil and rivastigmine with CASP1 suggest a possible anti-inflammatory role for these drugs, which is consistent with recent discoveries showing their capacity to inhibit inflammasome priming and cytokine release via α 7 nicotinic acetylcholine receptor signaling.^{64,65} CASP1 not only mediates interleukin 1 β maturation but also influences microglial polarization and neurovascular integrity⁶⁶, making it a multidimensional target in AD. Suppression of CASP1 activity could slow disease progression by dampening neuroinflammatory loops that accelerate tauopathy and synaptic degeneration.

The role of NOTCH1, a highly conserved signaling molecule involved in neurogenesis, stem cell maintenance, and angiogenesis⁶⁷, further enriches the therapeutic landscape. The observed affinity of memantine and galantamine for NOTCH1 supports recent models in which NMDA receptor modulation indirectly influences Notch signaling, affecting dendritic spine morphology and neural regeneration.^{68,69} Moreover, NOTCH1 cross-talks with *PSEN1* via the γ -secretase complex⁷⁰, suggesting that drugs affecting one may modulate the other, amplifying therapeutic effects through shared signaling nodes. Epigenetic regulation of NOTCH1 has also been implicated in tau pathology and neurogenic dysfunction, adding layers of potential intervention.⁷¹

The combined evidence of docking across multiple genes underscores a paradigm shift in AD pharmacology from single-target inhibition toward multi-target engagement. This is not merely a theoretical exercise; real-world drug discovery is now embracing hybrid molecules such as bis(7)-tacrine, combining AChE inhibition with NMDA antagonism⁷², and others that bridge neuroinflammation with neurotrophic support.⁷³ These drugs aim not only to slow symptoms but also to reshape the underlying molecular networks driving degeneration. This aligns with clinical findings that combination therapies outperform monotherapies in preserving cognition and delaying institutionalization in AD patients.^{74,75}

Importantly, APP, BDNF, ApoE, *VEGFA*, *PSEN1*,

NOTCH1, and CASP1 are not traditional small-molecule therapeutic targets. Enzymes or receptors with clearly defined catalytic or orthosteric binding pockets are the main targets of traditional molecular docking techniques.^{27,76} Static structural models may not adequately represent biologically relevant states because A β is a highly dynamic, aggregation-prone peptide with substantial conformational variability.⁷⁷ Similarly, rather than small-molecule binding, ApoE and secreted growth factors such as VEGFA and BDNF primarily affect biology through PPIs and receptor-mediated signaling.⁷⁸⁻⁸⁰ Shallow surface contact sites, significant conformational plasticity, and the reduced prediction reliability of scoring functions are some of the intrinsic limits of docking to soluble, non-enzymatic, or structurally flexible proteins.⁸¹ Therefore, rather than being conclusive proof of traditional orthosteric inhibition, binding energies derived from docking analyses against these targets should be interpreted cautiously as exploratory interaction estimates. These proteins were included in the current study to investigate, within a systems-level framework, possible surface interaction tendencies of approved AD treatments. However, any biologically significant connections indicated by docking scores would need to be further validated using molecular dynamics simulations and experimental binding assays.

Notably, drugs such as donepezil and galantamine may also regulate neurotransmitter levels through indirect mechanisms, such as the inhibition of glutamate-induced neurotoxicity⁸² and the enhancement of GABAergic tone.⁸³ These effects have been linked to the modulation of calcium channels and voltage-gated potassium channels, suggesting broader electrophysiological influences beyond the synapse.^{84,85} The convergence of cholinergic, glutamatergic, and neurotrophic signaling is therefore not incidental but mechanistically central to how these drugs provide cognitive and structural neuroprotection.

Taken together, the results of this study not only identify key AD regulators but also provide a credible framework for repurposing and improving existing therapeutics based on network biology principles. This study highlights how computational docking and centrality analysis can guide hypothesis-driven exploration of therapeutic targets. The finding that canonical drugs such as donepezil and memantine have high affinity for regulators such as BDNF, VEGFA, NOTCH1, and CASP1 suggests potentially untapped therapeutic value. These interactions, although *in silico*, align with *in vivo* observations reporting improved neurogenesis, vascular function, and reduced

inflammatory marker levels in treated patients. While experimental validation remains crucial, the concordance between molecular docking and emerging mechanistic insights underscores the power of integrated bioinformatics in advancing AD therapeutics.

Molecular docking is still a static approximation that does not fully account for protein flexibility, solvent dynamics, or entropic effects, but it offers useful initial insights into ligand-protein interactions and relative binding affinities. Since proteins are dynamic macromolecules by nature, rigid docking techniques are unable to fully capture the conformational rearrangements that ligand binding often induces. While post-docking free energy calculations, such as Molecular Mechanics-Poisson Boltzmann Surface Area or Molecular Mechanics-Generalized Born Surface Area, offer more accurate estimates of binding thermodynamics, molecular dynamics simulations enable the assessment of the temporal stability of protein-ligand complexes under near-physiological conditions. Therefore, it is difficult to definitively ascertain the long-term stability and physiological significance of the predicted complexes in the current investigation due to the lack of molecular dynamics simulations and binding free energy validation. However, docking remains a well-established first-line virtual screening method for early-stage drug development, providing rapid comparative binding assessments before more computationally demanding dynamic analyses. Future research using free energy estimates and molecular dynamics simulations would reinforce and corroborate the current findings.

5. Study limitations and future perspectives

Although the current computational framework offers useful initial insights, several limitations should be considered. First, the static crystalline structures used in docking simulations might not accurately reflect physiologically significant conformational ensembles. Second, the evaluation of the predicted conformational flexibility of complexes and temporal stability is constrained by the lack of molecular dynamics simulations. Third, thermodynamic validation was limited by the lack of binding free energy decomposition analyses (e.g., Molecular Mechanics-Poisson Boltzmann Surface Area and Molecular Mechanics-Generalized Born Surface Area). Future studies that incorporate improved sampling methods, long-timescale molecular dynamics, and *in vitro* binding experiments would enhance translational relevance and mechanistic interpretation. Network-level

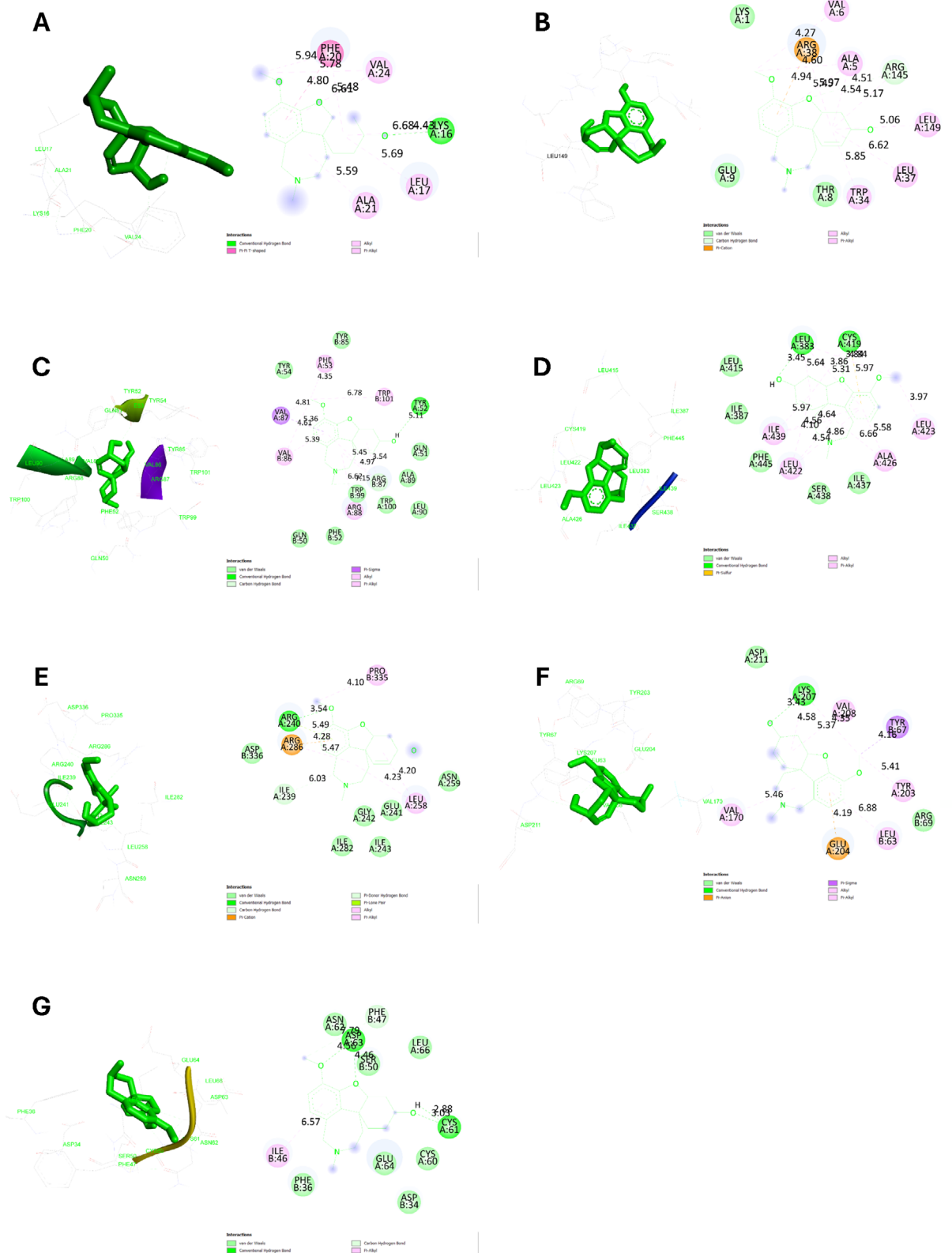


Figure 8. Two- and three-dimensional docking conformations of galantamine with target proteins encoded by (A) *APP*, (B) *APOE*, (C) *BDNF*, (D) *PSEN1*, (E) *CASP1*, (F) *NOTCH1*, and (G) *VEGFA*

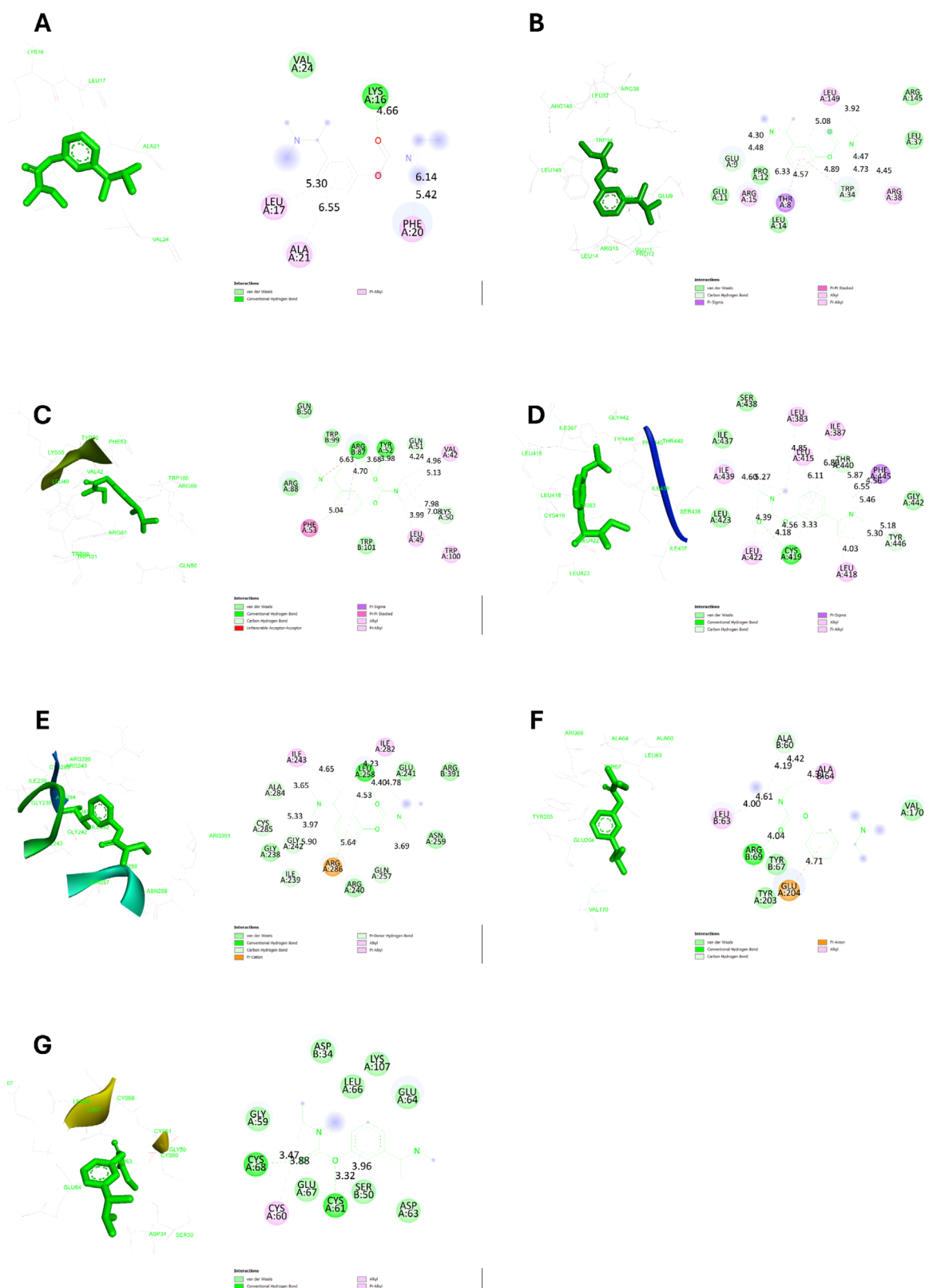


Figure 9. Two- and three-dimensional docking conformations of rivastigmine with target proteins encoded by (A) *APP*, (B) *APOE*, (C) *BDNF*, (D) *PSEN1*, (E) *CASP1*, (F) *NOTCH1*, and (G) *VEGFA*

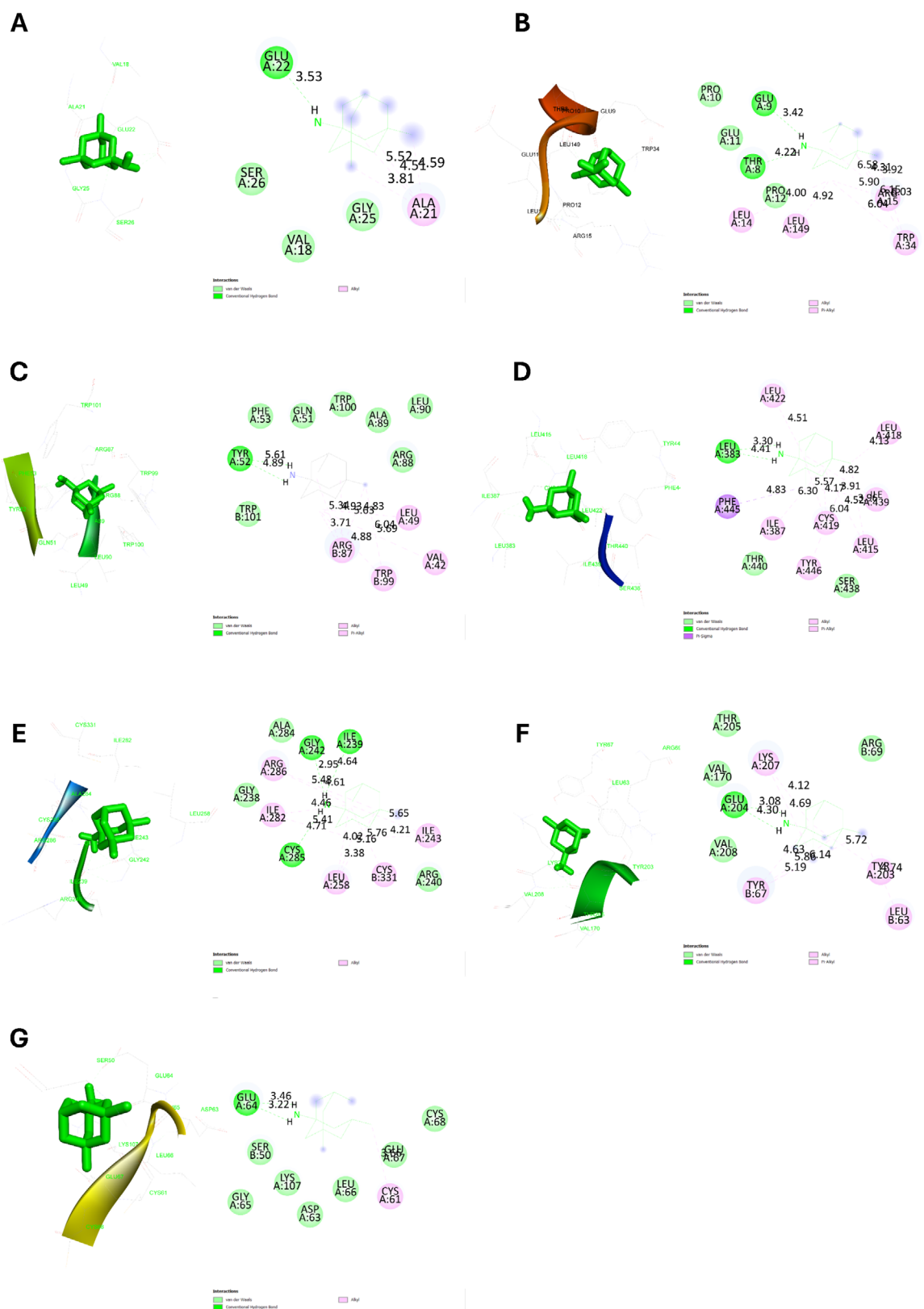


Figure 10. Two- and three-dimensional docking conformations of memantine with target proteins encoded by (A) *APP*, (B) *APOE*, (C) *BDNF*, (D) *PSEN1*, (E) *CASP1*, (F) *NOTCH1*, and (G) *VEGFA*

therapeutic target prioritization may also be improved by combining transcriptome and proteomic datasets.

6. Conclusion

To assess the interaction landscape of well-established cholinergic and glutamatergic treatments and to clarify the molecular architecture underlying AD, this study offers a comprehensive systems biology approach. Seven major regulators—*APP*, *APOE*, *BDNF*, *VEGFA*, *PSEN1*, *CASPI*, and *NOTCH1*—were identified as key nodes in the AD interactome through multi-database gene mining and PPI network analysis. Their involvement in axonogenesis, synaptic signaling, neuroinflammation, and neurovascular control was validated by functional enrichment analysis, highlighting the complex nature of AD pathology. FDA-approved medications and these targets exhibited variable binding affinities according to molecular docking analyses. BDNF consistently showed strong interactions, especially with donepezil, while APP showed relatively lower binding affinity. The strong affinity of memantine for PSEN1 adds to the growing body of research that connects amyloidogenic regulation and NMDA receptor modulation. Crucially, these results support the idea that network-level modulation rather than single-target suppression may provide a therapeutic advantage in AD. While docking offers useful initial insights, confirming physiological relevance will require further integration of molecular dynamics simulations and experimental validation. Overall, this research demonstrates the translational potential of network-guided drug repurposing approaches in developing AD precision therapies.

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Conflict of interest

The author declares no competing interests.

Author contributions

This is a single-authored paper.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

No data was generated or used in the research described in this article.

Further disclosure

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References

1. Scheltens P, De Strooper B, Kivipelto M, *et al.* Alzheimer's disease. *Lancet*. 2021;397(10284):1577-1590.
doi: 10.1016/S0140-6736(20)32205-4
2. 2024 Alzheimer's disease facts and figures. *Alzheimers Dement*. 2024;20(5):3708-3821.
doi: 10.1002/alz.13809
3. Montine TJ, Monsell SE, Beach TG, *et al.* Multisite assessment of NIA-AA guidelines for the neuropathologic evaluation of Alzheimer's disease. *Alzheimers Dement*. 2016;12(2):164-169.
doi: 10.1016/j.jalz.2015.07.492
4. Di Benedetto G, Burgaletto C, Bellanca CM, Munafò A, Bernardini R, Cantarella G. Role of Microglia and Astrocytes in Alzheimer's Disease: From Neuroinflammation to Ca²⁺ Homeostasis Dysregulation. *Cells*. 2022;11(17):2728.
doi: 10.3390/cells11172728
5. Varadharajan A, Davis AD, Ghosh A, *et al.* Guidelines for pharmacotherapy in Alzheimer's disease - A primer on FDA-approved drugs. *J Neurosci Rural Pract*. 2023;14(4):566-573.
doi: 10.25259/JNRP_356_2023
6. Beach TG, Monsell SE, Phillips LE, Kukull W. Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005-2010. *J Neuropathol Exp Neurol*. 2012;71(4):266-273.
doi: 10.1097/NEN.0b013e31824b211b
7. Schneider JA, Arvanitakis Z, Leurgans SE, Bennett DA. The neuropathology of probable Alzheimer disease and mild cognitive impairment. *Ann Neurol*. 2009;66(2):200-208.
doi: 10.1002/ana.21706
8. Serrano-Pozo A, Qian J, Monsell SE, *et al.* Mild to moderate Alzheimer dementia with insufficient neuropathological changes. *Ann Neurol*. 2014;75(4):597-601.
doi: 10.1002/ana.24125

9. Andrade-Guerrero J, Santiago-Balmaseda A, Jeronimo-Aguilar P, *et al.* Alzheimer's Disease: An Updated Overview of Its Genetics. *Int J Mol Sci.* 2023;24(4):3754.
doi: 10.3390/ijms24043754
10. Bekris LM, Yu CE, Bird TD, Tsuang DW. Genetics of Alzheimer disease. *J Geriatr Psychiatry Neurol.* 2010;23(4):213-227.
doi: 10.1177/0891988710383571
11. Strang KH, Golde TE, Giasson BI. MAPT mutations, tauopathy, and mechanisms of neurodegeneration. *Lab Invest.* 2019;99(7):912-928.
doi: 10.1038/s41374-019-0197-x
12. Hampel H, Vassar R, De Strooper B, *et al.* The β -Secretase BACE1 in Alzheimer's Disease. *Biol Psychiatry.* 2021;89(8):745-756.
doi: 10.1016/j.biopsych.2020.02.001
13. Selkoe DJ. Biochemistry and molecular biology of amyloid beta-protein and the mechanism of Alzheimer's disease. *Handb Clin Neurol.* 2008;89:245-260.
doi: 10.1016/S0072-9752(07)01223-7
14. Hardy J. Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci.* 1997;20(4):154-159.
doi: 10.1016/s0166-2236(96)01030-2
15. Surguchov A, Emamzadeh FN, Titova M, Surguchev AA. Controversial Properties of Amyloidogenic Proteins and Peptides: New Data in the COVID Era. *Biomedicines.* 2023;11(4):1215.
doi: 10.3390/biomedicines11041215
16. Cole SL, Vassar R. The role of amyloid precursor protein processing by BACE1, the beta-secretase, in Alzheimer disease pathophysiology. *J Biol Chem.* 2008;283(44):29621-29625.
doi: 10.1074/jbc.R800015200
17. Wang Y, Mandelkow E. Tau in physiology and pathology. *Nat Rev Neurosci.* 2016;17(1):5-21.
doi: 10.1038/nrn.2015.1
18. Husain MA, Laurent B, Plourde M. APOE and Alzheimer's Disease: From Lipid Transport to Physiopathology and Therapeutics. *Front Neurosci.* 2021;15:630502.
doi: 10.3389/fnins.2021.630502
19. Farrer LA, Cupples LA, Haines JL, *et al.* Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA.* 1997;278(16):1349-1356.
doi: 10.1001/jama.278.16.1349
20. Kim BH, Kim S, Nam Y, Park YH, Shin SM, Moon M. Second-generation anti-amyloid monoclonal antibodies for Alzheimer's disease: current landscape and future perspectives. *Transl Neurodegener.* 2025;14(1):6.
doi: 10.1186/s40035-025-00465-w
21. Sam C, Bordoni B. Physiology, Acetylcholine. In: *StatPearls.* Treasure Island (FL): StatPearls Publishing. 2023. Available from <https://www.ncbi.nlm.nih.gov/books/NBK557825/> [Last accessed on].
22. Puranik N, Song M. Glutamate: Molecular Mechanisms and Signaling Pathway in Alzheimer's Disease, a Potential Therapeutic Target. *Molecules.* 2024;29(23):5744.
doi: 10.3390/molecules29235744
23. Barabási AL, Oltvai ZN. Network biology: understanding the cell's functional organization. *Nat Rev Genet.* 2004;5(2):101-113.
doi: 10.1038/nrg1272
24. Shannon P, Markiel A, Ozier O, *et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 2003;13(11):2498-2504.
doi: 10.1101/gr.1239303
25. Kulkarni VS, Alagarsamy V, Solomon VR, Jose PA, Murugesan S. Drug Repurposing: An Effective Tool in Modern Drug Discovery. *Russ J Bioorg Chem.* 2023;49(2):157-166.
doi: 10.1134/S1068162023020139
26. Talevi A. Multi-target pharmacology: possibilities and limitations of the "skeleton key approach" from a medicinal chemist perspective. *Front Pharmacol.* 2015;6:205.
doi: 10.3389/fphar.2015.00205
27. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. *Nat Rev Drug Discov.* 2004;3(11):935-949.
doi: 10.1038/nrd1549
28. Genheden S, Ryde U. The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. *Expert Opin Drug Discov.* 2015;10(5):449-461.
doi: 10.1517/17460441.2015.1032936
29. De Strooper B, Karran E. The Cellular Phase of Alzheimer's Disease. *Cell.* 2016;164(4):603-615.
doi: 10.1016/j.cell.2015.12.056
30. Swerdlow RH. Mitochondria and Mitochondrial Cascades in Alzheimer's Disease. *J Alzheimers Dis.* 2018;62(3):1403-1416.
doi: 10.3233/JAD-170585
31. Heneka MT, Carson MJ, El Khoury J, *et al.* Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* 2015;14(4):388-405.
doi: 10.1016/S1474-4422(15)70016-5
32. Xu LZ, Li FY, Xu J, Li BQ, Li Y, Jia JP. Vascular endothelial

- growth factor is an effective biomarker for vascular dementia, not for Alzheimer's disease: A meta-analysis. *Alzheimers Dement.* 2024;16(2):e12612.
doi: 10.1002/dad2.12612
33. Heberle H, Meirelles GV, da Silva FR, Telles GP, Minghim R. InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinform.* 2015;16(1):169.
doi: 10.1186/s12859-015-0611-3
 34. Braschi B, Denny P, Gray K, *et al.* Genenames.org: the HGNC and VGNC resources in 2019. *Nucleic Acids Res.* 2019;47(D1):D786-D792.
doi: 10.1093/nar/gky930
 35. Barrett T, Wilhite SE, Ledoux P, *et al.* NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Res.* 2013;41(Database issue):D991-D995.
doi: 10.1093/nar/gks1193
 36. Budni J, Bellettini-Santos T, Mina F, Garcez ML, Zugno AI. The involvement of BDNF, NGF and GDNF in aging and Alzheimer's disease. *Aging Dis.* 2015;6(5):331-341.
doi: 10.14336/AD.2015.0825
 37. Bendix I, Serdar M, Herz J, *et al.* Inhibition of acetylcholinesterase modulates NMDA receptor antagonist mediated alterations in the developing brain. *Int J Mol Sci.* 2014;15(3):3784-3798.
doi: 10.3390/ijms15033784
 38. Mishchenko TA, Mitroshina EV, Usenko AV, *et al.* Features of Neural Network Formation and Their Functions in Primary Hippocampal Cultures in the Context of Chronic TrkB Receptor System Influence. *Front Physiol.* 2019;9:1925.
doi: 10.3389/fphys.2018.01925
 39. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol.* 2007;8(2):101-112.
doi: 10.1038/nrm2101
 40. Xiao X, Liu H, Liu X, Zhang W, Zhang S, Jiao B. APP, PSEN1, and PSEN2 Variants in Alzheimer's Disease: Systematic Re-evaluation According to ACMG Guidelines. *Front Aging Neurosci.* 2021;13:695808.
doi: 10.3389/fnagi.2021.695808
 41. Targa Dias Anastacio H, Matosin N, Ooi L. Familial Alzheimer's Disease Neurons Bearing Mutations in PSEN1 Display Increased Calcium Responses to AMPA as an Early Calcium Dysregulation Phenotype. *Life.* 2024;14(5):625.
doi: 10.3390/life14050625
 42. Cheung KH, Shineman D, Müller M, *et al.* Mechanism of Ca²⁺ disruption in Alzheimer's disease by presenilin regulation of InsP3 receptor channel gating. *Neuron.* 2008;58(6):871-883.
doi: 10.1016/j.neuron.2008.04.015
 43. Bezprozvanny I, Mattson MP. Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. *Trends Neurosci.* 2008;31(9):454-463.
doi: 10.1016/j.tins.2008.06.005
 44. Tang BC, Wang YT, Ren J. Basic information about memantine and its treatment of Alzheimer's disease and other clinical applications. *Ibrain.* 2023;9(3):340-348.
doi: 10.1002/ibra.12098
 45. Parsons CG, Stöffler A, Danysz W. Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system--too little activation is bad, too much is even worse. *Neuropharmacology.* 2007;53(6):699-723.
doi: 10.1016/j.neuropharm.2007.07.013
 46. Shankar GM, Li S, Mehta TH, *et al.* Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med.* 2008;14(8):837-842.
doi: 10.1038/nm1782
 47. Sarasija S, Norman KR. A γ -Secretase Independent Role for Presenilin in Calcium Homeostasis Impacts Mitochondrial Function and Morphology in *Caenorhabditis elegans*. *Genetics.* 2015;201(4):1453-1466.
doi: 10.1534/genetics.115.182808
 48. Zhang C, Wu B, Beglopoulos V, *et al.* Presenilins are essential for regulating neurotransmitter release. *Nature.* 2009;460(7255):632-636.
doi: 10.1038/nature08177
 49. Venkatraman L, Claesson-Welsh L. The role of VEGF in controlling vascular permeability. In: *Tumor Angiogenesis: A Key Target for Cancer Therapy*. Cham, Switzerland: Springer; 2019; 33-50.
doi: 10.1007/978-3-319-31215-6_37-1
 50. Quaggin SE. A half-century of VEGFA: from theory to practice. *J Clin Invest.* 2024;134(15):e184205.
doi: 10.1172/JCI184205
 51. Lee C, Kim MJ, Kumar A, Lee HW, Yang Y, Kim Y. Vascular endothelial growth factor signaling in health and disease: from molecular mechanisms to therapeutic perspectives. *Signal Transduct Target Ther.* 2025;10(1):170.
doi: 10.1038/s41392-025-02249-0
 52. Shibuya M. Vascular Endothelial Growth Factor (VEGF) and Its Receptor (VEGFR) Signaling in Angiogenesis: A Crucial Target for Anti- and Pro-Angiogenic Therapies. *Genes Cancer.* 2011;2(12):1097-1105.
doi: 10.1177/1947601911423031
 53. Yang HS, Yau WW, Carlyle BC, *et al.* Plasma VEGFA and

- PGF impact longitudinal tau and cognition in preclinical Alzheimer's disease. *Brain*. 2024;147(6):2158-2168.
doi: 10.1093/brain/awae034
54. Zhang M, Zhang Z, Li H, *et al.* Blockage of VEGF function by bevacizumab alleviates early-stage cerebrovascular dysfunction and improves cognitive function in a mouse model of Alzheimer's disease. *Transl Neurodegener*. 2024;13(1):1.
doi: 10.1186/s40035-023-00388-4
 55. Fukumura D, Gohongi T, Kadambi A, *et al.* Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. *Proc Natl Acad Sci USA*. 2001;98(5):2604-2609.
doi: 10.1073/pnas.041359198
 56. Figueroa XF, González DR, Martínez AD, Durán WN, Boric MP. ACh-induced endothelial NO synthase translocation, NO release and vasodilatation in the hamster microcirculation in vivo. *J Physiol*. 2002;544(3):883-896.
doi: 10.1113/jphysiol.2002.021972
 57. Jackson RJ, Meltzer JC, Nguyen H, *et al.* APOE4 derived from astrocytes leads to blood-brain barrier impairment. *Brain*. 2022;145(10):3582-3593.
doi: 10.1093/brain/awab478
 58. Wang C, Xiong M, Gratuze M, *et al.* Selective removal of astrocytic APOE4 strongly protects against tau-mediated neurodegeneration and decreases synaptic phagocytosis by microglia. *Neuron*. 2021;109(10):1657-1674.e7.
doi: 10.1016/j.neuron.2021.03.024
 59. Williams T, Borchelt DR, Chakrabarty P. Therapeutic approaches targeting Apolipoprotein E function in Alzheimer's disease. *Mol Neurodegener*. 2020;15(1):8.
doi: 10.1186/s13024-020-0358-9
 60. Waring JF, Tang Q, Robieson WZ, *et al.* APOE-ε4 Carrier Status and Donepezil Response in Patients with Alzheimer's Disease. *J Alzheimers Dis*. 2015;47(1):137-148.
doi: 10.3233/JAD-142589
 61. De Beaumont L, Pelleieux S, Lamarre-Thérout L, Dea D, Poirier J. Butyrylcholinesterase K and Apolipoprotein E-ε4 Reduce the Age of Onset of Alzheimer's Disease, Accelerate Cognitive Decline, and Modulate Donepezil Response in Mild Cognitively Impaired Subjects. *J Alzheimers Dis*. 2016;54(3):913-922.
doi: 10.3233/JAD-160373
 62. Cheng YC, Huang YC, Liu HC. Effect of Apolipoprotein E ε4 Carrier Status on Cognitive Response to Acetylcholinesterase Inhibitors in Patients with Alzheimer's Disease: A Systematic Review and Meta-Analysis. *Dement Geriatr Cogn Disord*. 2018;45(5-6):335-352.
doi: 10.1159/000490175
 63. Han YH, Liu XD, Jin MH, Sun HN, Kwon T. Role of NLRP3 inflammasome-mediated neuronal pyroptosis and neuroinflammation in neurodegenerative diseases. *Inflamm Res*. 2023;72(9):1839-1859.
doi: 10.1007/s00011-023-01790-4
 64. Lu B, Kwan K, Levine YA, *et al.* α7 nicotinic acetylcholine receptor signaling inhibits inflammasome activation by preventing mitochondrial DNA release. *Mol Med*. 2014;20(1):350-358.
doi: 10.2119/molmed.2013.00117
 65. Ke P, Shao BZ, Xu ZQ, Chen XW, Wei W, Liu C. Activating α7 nicotinic acetylcholine receptor inhibits NLRP3 inflammasome through regulation of β-arrestin-1. *CNS Neurosci Ther*. 2017;23(11):875-884.
doi: 10.1111/cns.12758
 66. Ye X, Song G, Huang S, *et al.* Caspase-1: A Promising Target for Preserving Blood-Brain Barrier Integrity in Acute Stroke. *Front Mol Neurosci*. 2022;15:856372.
doi: 10.3389/fnmol.2022.856372
 67. Sachan N, Sharma V, Mutsuddi M, Mukherjee A. Notch signalling: multifaceted role in development and disease. *FEBS J*. 2024;291(14):3030-3059.
doi: 10.1111/febs.16815
 68. Brai E, Marathe S, Astori S, *et al.* Notch1 Regulates Hippocampal Plasticity Through Interaction with the Reelin Pathway, Glutamatergic Transmission and CREB Signaling. *Front Cell Neurosci*. 2015;9:447.
doi: 10.3389/fncel.2015.00447
 69. Giniger E. Notch signaling and neural connectivity. *Curr Opin Genet Dev*. 2012;22(4):339-346.
doi: 10.1016/j.gde.2012.04.003
 70. Lleó A, Berezovska O, Ramdya P, *et al.* Notch1 competes with the amyloid precursor protein for gamma-secretase and down-regulates presenilin-1 gene expression. *J Biol Chem*. 2003;278(48):47370-47375.
doi: 10.1074/jbc.M308480200
 71. Kim WY, Snider WD. Functions of GSK-3 Signaling in Development of the Nervous System. *Front Mol Neurosci*. 2011;4:44.
doi: 10.3389/fnmol.2011.00044
 72. Liu YW, Luo JL, Ren H, *et al.* Inhibition of NMDA-gated ion channels by bis(7)-tacrine: whole-cell and single-channel studies. *Neuropharmacology*. 2008;54(7):1086-1094.
doi: 10.1016/j.neuropharm.2008.02.015
 73. Bajda, M., Guzior, N., Ignasik, M., & Malawska, B. Multi-target-directed ligands in Alzheimer's disease treatment. *Curr Med Chem*. 2011; 18(32), 4949–4975.

- doi: 10.2174/092986711797535245
74. Kabir MT, Uddin MS, Mamun AA, *et al.* Combination Drug Therapy for the Management of Alzheimer's Disease. *Int J Mol Sci.* 2020;21(9):3272.
doi: 10.3390/ijms21093272
 75. Cummings JL, Osse AML, Kinney JW, Cammann D, Chen J. Alzheimer's Disease: Combination Therapies and Clinical Trials for Combination Therapy Development. *CNS Drugs.* 2024;38(8):613-624.
doi: 10.1007/s40263-024-01103-1
 76. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. *Molecules.* 2015;20(7):13384-13421.
doi: 10.3390/molecules200713384
 77. Tycko R. Amyloid polymorphism: structural basis and neurobiological relevance. *Neuron.* 2015;86(3):632-645.
doi: 10.1016/j.neuron.2015.03.017
 78. Mahley RW. Apolipoprotein E: from cardiovascular disease to neurodegenerative disorders. *J Mol Med.* 2016;94(7):739-746.
doi: 10.1007/s00109-016-1427-y
 79. Ferrara N, Kerbel RS. Angiogenesis as a therapeutic target. *Nature.* 2005;438(7070):967-974.
doi: 10.1038/nature04483
 80. Chao MV. Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat Rev Neurosci.* 2003;4(4):299-309.
doi: 10.1038/nrn1078
 81. Pagadala NS, Syed K, Tuszynski J. Software for molecular docking: a review. *Biophys Rev.* 2017;9(2):91-102.
doi: 10.1007/s12551-016-0247-1
 82. Kim SH, Kandiah N, Hsu JL, Suthisisang C, Udommongkol C, Dash A. Beyond symptomatic effects: potential of donepezil as a neuroprotective agent and disease modifier in Alzheimer's disease. *Br J Pharmacol.* 2017;174(23):4224-4232.
doi: 10.1111/bph.14030
 83. Jelinek M, Jurajda M, Duris K. Oxidative Stress in the Brain: Basic Concepts and Treatment Strategies in Stroke. *Antioxidants.* 2021;10(12):1886.
doi: 10.3390/antiox10121886
 84. Urrutia J, Arrizabalaga-Iriondo A, Sanchez-Del-Rey A, *et al.* Therapeutic role of voltage-gated potassium channels in age-related neurodegenerative diseases. *Front Cell Neurosci.* 2024;18:1406709.
doi: 10.3389/fncel.2024.1406709
 85. Baumgartner TJ, Haghighijoo Z, Goode NA, Dvorak NM, Arman P, Laezza F. Voltage-Gated Na⁺ Channels in Alzheimer's Disease: Physiological Roles and Therapeutic Potential. *Life.* 2023;13(8):1655.
doi: 10.3390/life13081655