Drug Repurposing and Molecular Docking Analysis of Metformin Against Alzheimer's Disease Targets

Abstract

Alzheimer's disease is a progressive neurodegenerative disorder characterized by cognitive impairment, loss of motor function, widespread synaptic loss and neuronal damage and loss. Currently available treatments remain symptomatic and effective disease-modifying therapies remain limited.

Drug repurposing has emerged as a promising strategy for identifying new therapeutic applications of already existing drugs. Metformin, a widely prescribed antihyperglycemic agent, has demonstrated neuroprotective, anti-inflammatory, and antioxidant properties, which makes it a potential candidate for Alzheimer's treatment. The present study uses an integrated computational approach with network pharmacology and molecular docking to investigate the therapeutic potential of metformin in Alzheimer's disease treatment.

Potential metformin targets and Alzheimer's disease-associated genes were identified from publicly available databases, and overlapping targets were used to construct a protein-protein interaction network. Further, Gene Ontology (GO), and KEGG pathway enrichment analyses were carried out. Hub genes were identified based on network centrality and topology and selected for molecular docking studies to evaluate the binding affinity of metformin. Functional enrichment analysis indicated that the identified protein targets were mainly involved in inflammatory signalling, oxidative stress, apoptotic regulation, and metabolism, highlighting the multi-target function of metformin.

Docking analysis demonstrated favourable interactions between metformin and hub proteins, i.e. angiotensin-converting enzyme (ACE), moderate interactions were observed with MAPK1 and acetylcholinesterase (a primary target for current treatments). In contrast, SIRT1 exhibited comparatively weak binding, suggesting that its reported neuroprotective effects may be mediated through indirect signalling pathways rather than direct binding.

Overall, the findings suggest that metformin functions as a multi-target therapeutic agent capable of modulating several interconnected molecular targets and biological pathways relevant to Alzheimer's disease. Although further molecular dynamics simulations and experimental validation are required, this study provides computational evidence supporting the potential repurposing of metformin as a candidate therapeutic agent for Alzheimer's disease.

Keywords: Alzheimer's disease; metformin; drug repurposing; network pharmacology; molecular docking; protein-protein interaction; Gene Ontology; KEGG pathway analysis.

Introduction

Alzheimer's Disease is a progressive neurodegenerative disorder that results in the loss of memory, motor function, cognitive impairment and other basic functions required for day-to-day life. It is the most common type of dementia and is characterized by neuritic plaques and neurofibrillary tangles (NFTs) formed as a result of hyperphosphorylated tau proteins and accumulation of amyloid-beta (Aβ) peptides in the most affected areas of the brain– the medial temporal lobe and neocortical structures. This is linked to large-scale neuronal loss, synaptic dysfunction, and inflammation, which contribute to the progressive cognitive decline observed in affected individuals. Although the exact mechanisms of the disease are not yet completely understood, growing evidence suggests that a combination of genetic, environmental, and lifestyle factors contributes to its onset and progression. [1]

AD occurs in familial (~5% of total AD cases) or sporadic forms (>95% of cases). Familial AD is mainly characterized by autosomal dominant genetic mutations in APP, PSEN1, and PSEN2 genes, typically manifesting between 30 and 65 years and progressing rapidly. Sporadic AD, also known as late-onset AD, commonly manifests after the age of 65 and is influenced by genetics, lifestyle, and environmental factors, as well as various comorbidities.

As of 2023, an estimated 7.4% of adults aged 60 and older in India are living with dementia, accounting for approximately 8.8 million individuals, a number expected to rise from 5.3 million in 2020 to 11.4 million by 2050. As of 2026, around 55 million people globally are living with dementia, with Alzheimer's disease representing 60–70% of these cases (*WHO, 2023; Alzheimer's Association, 2026*). Prevalence increases sharply with age, affecting 5% of people aged 65–74, 13.1% aged 75–84, and 33.3% aged 85 or older. Women account for nearly two-thirds of cases, reflecting both longevity and higher susceptibility (*Alzheimer's Association, 2026*).

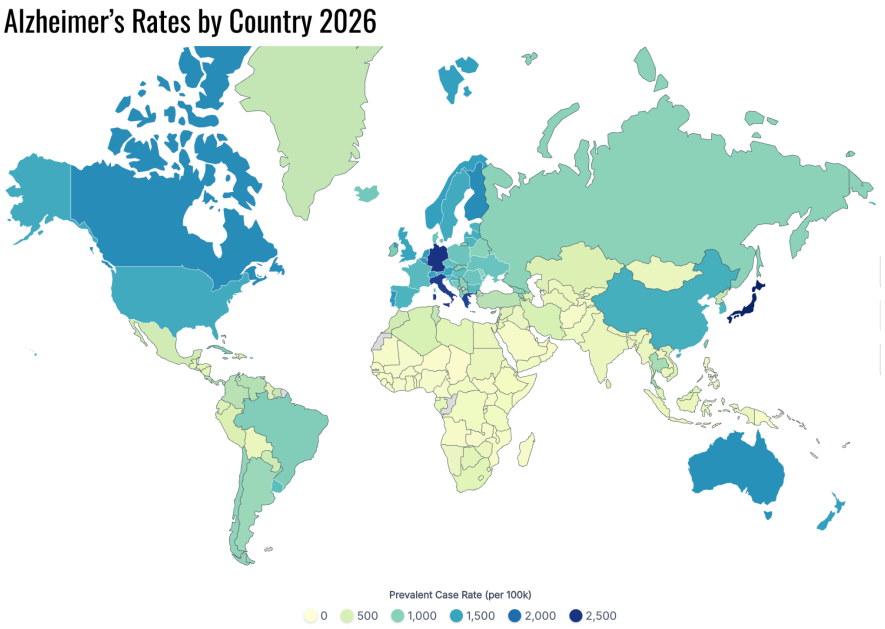

Fig 1: Alzheimer's Rates by Country 2026. World Population Review. Retrieved June 30, 2026, from https://worldpopulationreview.com/country-rankings/alzheimers-rates-by-country

Neuropathology of Alzheimer's Disease

The neuropathology of AD is characterized by two major hallmarks - amyloid plaques and neurofibrillary tangles - which are accompanied by widespread synaptic and neuronal loss, neuroinflammation, and oxidative stress, collectively driving progressive cognitive deterioration. [1][2]

Amyloid-β plaques arise from the sequential cleavage of amyloid precursor protein (APP) by beta and gamma secretases. Aβ peptides are soluble monomers that aggregate as amyloid fibrils, protofibrils, and oligomers during AD pathogenesis. They form large insoluble protease-resistant Aβ fibrils, which aggregate to form Aβ plaques. Additionally, Aβ peptides form diffusible Aβ oligomers (AβOs), which propagate throughout the brain. AβOs are prone to aggregation and propagation, and are considered the primary neurotoxic species of Aβ during AD development. Aβ peptides are predominantly released into the extracellular space and clump into Aβ fibrils or AβOs, giving rise to extraneuronal Aβ plaques. In the early stage of AD, the elevated levels of extraneuronal Aβ plaques, particularly near synapses, disrupt neuronal and synaptic communication and function. [21]

Microtubule-associated tau protein is majorly expressed in neurons and primarily localized within axons. Physiologically, tau plays a critical role in stabilizing the cytoskeleton by promoting the formation of microtubules from tubulin dimers, maintaining neuronal morphology, function, and axonal transport. NFTs are formed from hyperphosphorylated tau protein that, in some stages, can be twisted around each other to form paired helical filaments and accumulate in the cytoplasm, axons, and dendrites, causing a loss of cytoskeletal microtubules and tubulin-associated proteins, and disrupting neuronal cytoskeletal integrity. NFTs obstruct the axonal transportation of nutrients and chemicals, thereby impairing synaptic communications in the central nervous system. [1][21]

Chronic activation of microglia and astrocytes drives sustained neuroinflammation, releasing pro-inflammatory cytokines, reactive oxygen species, and complement factors that further worsen Aβ and tau pathology and accelerate synaptic and neuronal loss.

Major gene clusters involved in Alzheimer’s Disease development

In most cases, Alzheimer's does not have a single pathological or genetic cause. Instead, it is influenced by multiple genes in combination with lifestyle and environmental factors.

One well-known gene that influences Alzheimer's risk is the apolipoprotein E (APOE) gene, which is the strongest genetic risk factor for sporadic AD, and exists in three common allelic forms ε2, ε3, and ε4. The ε4 allele is associated with an elevated risk and earlier onset of the disease, whereas ε2 confers a protective effect. APOE ε4 disrupts the clearance of amyloid-beta (Aβ), promotes its accumulation, and enhances tau phosphorylation, contributing to neurofibrillary tangle formation, which ultimately disrupts protein homeostasis, impairs synaptic integrity and affects neuronal signaling. [22]

Three rare, highly penetrant mutations in gene variants are known to cause Early Onset Familial AD: Amyloid precursor protein (APP) on chromosome 21, Presenilin 1 (PSEN1) on chromosome 14, and Presenilin 2 (PSEN2) on chromosome 1. These mutations disrupt amyloid precursor protein processing, leading to the overproduction of amyloid-beta peptides and early neurodegenerative changes [22]. Mutations in PSEN1 cause the most severe forms of AD with complete penetrance, with disease onset occurring as early as 25 years of age. APP gene mutations cause an increase in the amyloid Aβ42/Aβ40 ratio, and levels of total and phosphorylated Tau protein. [23]

Additional risk-modifying genes include TREM2, SORL1, CLU, CR1, and PICALM, which influence amyloid processing, neuroinflammation, and synaptic function. These genes affect susceptibility but do not guarantee disease development, and their effects can vary across populations and ancestries.

The Current Treatment Landscape

Clinically approved (FDA approved) drugs used for AD can be broadly divided into the following classes:-

The *symptomatic treatment* of cognitive symptoms includes acetylcholinesterase inhibitors (AChEIs) and NMDA receptor antagonists (Memantine). **AChEIs** (Benzgalantamine, Donepezil, Galantamine, and Rivastigmine) boost postsynaptic stimulation by increasing both the level and duration of action of ACh by blocking esterase activity, thereby enhancing cognitive and behavioral functions in patients [24]. **Memantine** is used for the treatment of moderate to severe AD, modulating glutamate transmission and dopamine receptors to improve cognitive function, daily living abilities, and behavior. **Namzaric** is a fixed-dose combination of donepezil and memantine approved for moderate-to-severe AD. [24][27]

Management of non-cognitive symptoms including sleep disturbances, agitation, hallucinations, and delusions can be addressed by additional approved agents. **Suvorexant**, an orexin receptor antagonist, is prescribed for insomnia and has shown efficacy in people living with mild to moderate AD. **Brexpiprazole** is currently the only FDA-approved atypical antipsychotic indicated for the treatment of agitation associated with dementia due to AD. [27]

Disease-modifying anti-amyloid monoclonal antibodies targeting Aβare designed to slow cognitive and functional decline by removing beta-amyloid from the brain. Both **lecanemab** and **donanemab** are intravenous fusions approved for early AD, including mild cognitive impairment and mild dementia with confirmed elevated brain amyloid. [24][27]

Currently approved Alzheimer's drug treatments treat cognitive and behavioral symptoms but not neurodegeneration, especially in the later stages of AD. While memantine and cholinesterase inhibitors can help certain patients, and aducanumab and lecanemab reduce amyloid plaques, they have little effect on pathogenesis. Alzheimer's disease has a complex pathogenesis with amyloid and tau aggregation, neuroinflammation, vascular dysfunction, oxidative stress, and synaptic loss, and therefore, single target treatments are often ineffective. [25].

AChEIs and memantine remain symptomatic treatments that provide temporary cognitive benefit without modifying disease course, and are associated with adverse effects including gastrointestinal disturbances, bradycardia, and dizziness that limit tolerability in elderly patients [27]. The anti-amyloid monoclonal antibodies, though being the first approved disease-modifying therapies, are also associated with safety concerns such as amyloid-related imaging abnormalities, like cerebral edema and microhemorrhages, alongside prohibitive costs and limited accessibility in low and middle-income healthcare settings. [25][27]

Metformin

Metformin (*1,1-dimethylbiguanide hydrochloride*) is a biguanide antihyperglycemic agent and FDA-approved first-line pharmacotherapy used in the management of type II diabetes. Metformin and the related drug phenformin are derived from Galegine, a natural product from the plant *Galega officinalis*, often used in herbal medicine in medieval Europe [6]. The primary mechanism of action is inhibition of hepatic glucose production without simultaneous increase in plasma insulin concentrations. It reduces glucose absorption from the intestines, lowers liver glucose production, and improves insulin sensitivity.

Metformin has an oral bioavailability of 50% to 60% and, following intestinal absorption, enters the portal vein and accumulates in the liver, which is a primary target. [5][6]

Metformin has been shown to act via several pathways – AMP-activated protein kinase (AMPK)-dependent and AMPK-independent mechanisms, inhibition of mitochondrial respiration, inhibition of mitochondrial glycerophosphate dehydrogenase, and also a mechanism involving the lysosome. At the molecular level, metformin inhibits the mitochondrial respiratory chain in the liver, leading to activation of AMPK, enhancing insulin sensitivity via effects on fat metabolism and lowering cAMP, thus reducing the expression of gluconeogenic enzymes. Metformin also has AMPK-independent effects on the liver that may include inhibition of fructose-1,6-bisphosphatase by AMP. [5][6]

Potential Therapeutic Effects of Metformin Against Alzheimer's Disease Targets

Metformin is being studied in central nervous system disorders due to its multiple effects, including activating the AMPK pathway, supporting neuroprotection and promoting re-myelination. [29]

Metformin is able to cross the blood-brain barrier, primarily through organic cation transporters such as OCT1. Research indicates that metformin is highly permeable and can enter the brain, potentially exerting effects on the central nervous system. Certain findings suggest that metformin can cross the BBB and have neuroprotective effects, neuronal regeneration and anti-inflammatory effects [20]. It has also shown anti-inflammatory effects through modified microglial activation and blocking NF-κB signalling, which is a major cause of neuroinflammation. [29]

Recent preclinical work has demonstrated that metformin treatment can reduce Aβ deposition, tau hyperphosphorylation, and tau pathology, and prevent neuronal cell death in the brain. It has also been shown to reduce learning and memory deficits in APP/PS1, SAMP8, and tau-seeded PS19 AD mouse models, as well as in streptozotocin-induced diabetic rodents exhibiting tau pathology and Aβ deposition. [30]

In addition, metformin treatment has demonstrated improved spatial memory in aged mice in an APOE genotype-dependent manner. In clinical studies, use of metformin in diabetic patients has been associated with slower cognitive decline and lower dementia risk, while in AD patients it has been shown to promote improved learning and memory. [30]

Network Pharmacology and Drug Repurposing

Network pharmacology is an advanced drug discovery approach that examines the interactions between drugs, targets and diseases within the context of biological networks. This approach assesses the influence of a one compound on multiple targets and the idea that several drugs can act on the same target within a biological system, unlike the conventional one drug-one target system. It incorporates various features from systems biology, bioinformatics, and computational network science to assess the cumulative effects of drugs, diseases, and therapeutic targets through combined analysis. [31]

Network pharmacology involves a series of steps including - *Data collection, Target Characterization, Network Construction, Network Analysis, and Drug repurposing.* [33]

Data collection involves proteomics, metabolomics, and genomics, and helps in collection of gene targets for selected disease, protein function, information about the drug itself and so on. Some of the major databases used for the same are Chembl, Drugbank, NCBI, canSAR, etc. Further, **target**

characterization is carried out to find out the major drug-target interactions. In this study, this is done using the SwissTargets and BindingDB databases. [33]

Characterization is followed by **network construction**, which is one of the most crucial steps of network pharmacology, and involves creating biological networks that represent the interactions between drugs, targets, and diseases. Network visualization and data integration platforms such as Cytoscape, STRING, and Pathway Commons facilitate the construction and analysis of protein-protein interaction networks, signaling cascades, and metabolic pathways. Artificial intelligence and machine learning methodologies are also increasingly being leveraged for tasks like drug-target interaction prediction, virtual screening, toxicity evaluation, and efficacy modeling. [32][33]

A network is a meticulously calculated computational model of many connected nodes and pathways. **Network analysis** usually consists of key attribute analysis, spatial analysis, network topology and robustness, energy balance analysis, and structural models. Network analysis helps to identify existing drugs whose target key nodes or pathways leading to potential new therapeutic effects. [33]

Drug Repurposing is the process of identifying new therapeutic uses of existing drugs that have been previously approved for a different target disease, and offers several advantages over traditional de novo drug discovery including reduced development time and cost, already established pharmacokinetics and toxicity profiles reducing the likelihood of failure in clinical trials, rapid response to medical needs and facilitates discovery of new mechanisms. [28]

In this study, this approach was applied to metformin, an FDA-approved antidiabetic agent with previously studied CNS penetrance and mechanistic overlap across multiple AD-associated pathways, to identify AD-associated protein targets. Molecular docking was then performed against selected hub targets to assess the binding affinity and interaction of metformin with the target, providing computational evidence for its multi-target therapeutic potential in AD.

Workflow & Methodology

Data Collection

A list of AD-associated genes was retrieved from the NCBI Gene and UniProt databases using "Alzheimer's disease" as the search query. The lists were combined and duplicates were removed, resulting in a list of a total of 808 genes with reported associations to AD [34]. Further, a targeted search of the UniProt Human Disease database using the same query returned 19 high-confidence core AD targets under the Uniprot DB Alzheimer's disease entry (KW-0026), which includes key proteins like APP, PSEN1/PSEN2, SORL1, MAPT, and GSK3A/B that are directly involved in amyloid processing, tau pathology, and AD pathogenesis. [35]

Gene Symbol	Gene Description	UniProt Protein Name	NCBI Gene ID	Aliases	Chromosome	Chromosomal Location	OMIM ID	UniProt Entry	Protein Length (aa)	UniProt AD Confirmed (KW-0026)
ABCA7	Phospholipid-transporting ATPase ABCA7	Phospholipid-transporting ATPase ABCA7						Q8IZY2	2146	Yes
ADAM10	Disintegrin and metalloproteinase domain-containing protein	Disintegrin and metalloproteinase domain-containing protein 10		KUZ | MADM				O14672	748	Yes
APOE	apolipoprotein E	Apolipoprotein E	348	AD2, APO-E, ApoE4, LDLCQ5, LPG	19	19q13.32	107741	P02649	317	Yes
APP	amyloid beta precursor protein	Amyloid-beta precursor protein	351	AAA, ABETA, ABPP, AD1I, CTFgamma, C	21	21q21.3	104760	P05067	770	Yes
CTSD	cathepsin D	Cathepsin D	1509	CLN10, CPSD, HEL-S-130P	11	11p15.5	116840	P07339	412	Yes
DBN1	Drebrin	Drebrin		D0S117E				Q16643	649	Yes
GSK3A	Glycogen synthase kinase-3 alpha	Glycogen synthase kinase-3 alpha						P49840	483	Yes
GSK3B	Glycogen synthase kinase-3 beta	Glycogen synthase kinase-3 beta						P49841	420	Yes
MAPT	Microtubule-associated protein tau	Microtubule-associated protein tau		MAPTL | MTBT1 | TAU				P10636	758	Yes
MT-ND1	NADH-ubiquinone oxidoreductase chain 1	NADH-ubiquinone oxidoreductase chain 1		MTND1 | NADH1 | ND1				P03886	318	Yes
MT-ND2	NADH-ubiquinone oxidoreductase chain 2	NADH-ubiquinone oxidoreductase chain 2		MTND2 | NADH2 | ND2				P03891	347	Yes
PPP5C	Serine/threonine-protein phosphatase 5	Serine/threonine-protein phosphatase 5		PPP5				P53041	499	Yes
PSEN1	Presenilin-1	Presenilin-1		AD3 | PS1 | PSNL1				P49768	467	Yes
PSEN2	Presenilin-2	Presenilin-2		AD4 | PS2 | PSNL2 | STM2				P49810	448	Yes
RALGPS2	Ras-specific guanine nucleotide-releasing factor RalGPS2	Ras-specific guanine nucleotide-releasing factor RalGPS2						Q86X27	583	Yes
SNCA	Alpha-synuclein	Alpha-synuclein		NACP | PARK1				P37840	140	Yes
SORL1	sortilin related receptor 1	Sortilin-related receptor	6653	C11orf32, LR11, LRP9, SORLA, SorLA-1,	11	11q24.1	602005	Q92673	2214	Yes
TREM2	triggering receptor expressed on myeloid cells 2	Triggering receptor expressed on myeloid cells 2	54209	AD17, PLOSL2, TREM-2, Trem2a, Trem2	6	6p21.1	605086	Q9NZC2	230	Yes
UNC5C	Netrin receptor UNC5C	Netrin receptor UNC5C		UNC5H3				O95185	931	Yes
AATF	apoptosis antagonizing transcription factor		26574	BFR2, CHE-1, CHE1, DED	17	17q12	608463			No
ABCA1	ATP binding cassette subfamily A member 1		19	ABC-1, ABC1, CERP, HDLCQTL13, HDL	9	9q31.1	600046			No
ABCB11	ATP binding cassette subfamily B member 11		8647	ABC16, BRIC2, BSEP, PFIC-2, PFIC2, PV	2	2q31.1	603201			No
ABCB4	ATP binding cassette subfamily B member 4		5244	ABC21, GBD1, ICP3, MDR2, MDR2/3, MI	7	7q21.12	171060			No
ABCC2	ATP binding cassette subfamily C member 2		1244	ABC30, CMOAT, DJS, MRP2, cMRP	10	10q24.2	601107			No
ABHD5	abhydrolase domain containing 5, lysophosphatidic acid acyltransferase		51099	CGI58, IECN2, NCIE2	3	3p21.33	604780			No
ACACA	acetyl-CoA carboxylase alpha		31	ACACD, ACACalpha, ACC, ACC1, ACCA	17	17q12	200350			No
ACE	angiotensin I converting enzyme		1636	ACE1, CD143, DCP, DCP1	17	17q23.3	106180			No
ACE2	angiotensin converting enzyme 2		59272	ACEH	X	Xp22.2	300335			No
ACKR1	atypical chemokine receptor 1 (Duffy blood group)		2532	CCBP1, CD234, DARC, DARC/ACKR1, D	1	1q23.2	613665			No
ACOX1	acyl-CoA oxidase 1		51	ACOX, AOX, MITCH, PALMCOX, SCOX	17	17q25.1	609751			No
ACP7	acid phosphatase 7, tartrate resistant (putative)		390928	PAPL, PAPL1	19	19q13.2	610490			No
ACSF3	acyl-CoA synthetase family member 3		197322		16	16q24.3	614245			No
ACVRL1	activin A receptor like type 1		94	ACVRLK1, ALK-1, ALK1, HHT, HHT2, OR	12	12q13.13	601284			No
ADA2	adenosine deaminase 2		51816	ADGF, CECR1, IDGFL, PAN, SNEDS, VA	22	22q11.1	607575			No
ADAMTS12	ADAM metallopeptidase with thrombospondin type 1 motif	12	81792	PRO4389	5	5p13.3-p13.2	606184			No
ADAMTS13	ADAM metallopeptidase with thrombospondin type 1 motif	13	11093	ADAM-TS13, ADAMTS-13, C9orf8, VWF(	9	9q34.2	604134			No
ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif	9	56999		3	3p14.1	605421			No
ADAR	adenosine deaminase RNA specific		103	ADAR1, AGS6, DRADA, DSH, DSRAD, D	1	1q21.3	146920			No
ADH1B	alcohol dehydrogenase 1B (class I), beta polypeptide		125	ADH2, HEL-S-117	4	4q23	103720			No
ADH1C	alcohol dehydrogenase 1C (class I), gamma polypeptide		126	ADH3	4	4q23	103730			No
ADIPOQ	adiponectin, C1Q and collagen domain containing		9370	ACDC, ACRP30, ADIPQTL1, ADPN, APM	3	3q27.3	605441			No
ADIPOR1	adiponectin receptor 1		51094	ACDCR1, CGI-45, CGI45, PAQR1, TESB	1	1q32.1	607945			No
ADIPOR2	adiponectin receptor 2		79602	ACDCR2, PAQR2	12	12p13.33	607946			No
ADRA2A	adrenoceptor alpha 2A		150	ADRA2, ADRA2R, ADRAR, ALPHA2AAR	10	10q25.2	104210			No
ADRB3	adrenoceptor beta 3		155	BETA3AR	8	8p11.23	109691			No
AFP	alpha fetoprotein		174	AFPD, FETA, HPAFP	4	4q13.3	104150			No
AGER	advanced glycosylation end-product specific receptor		177	RAGE, SCARJ1, sRAGE	6	6p21.32	600214			No
AGRP	agouti related neuropeptide		181	AGRT, ART, ASIP2	16	16q22.1	602311			No
AGT	angiotensinogen		183	ANHU, SERPINA8, hFLT1	1	1q42.2	106150			No
AGTR1	angiotensin II receptor type 1		185	AG2SB, AT1, AT1AR, AT1B, AT1R, AT1	3	3q24	106165			No
AGXT2	alanine--glyoxylate aminotransferase 2		64902	AGT2, BAIBA, DAIBAT	5	5p13.2	612471			No
AHNAK	AHNAK nucleoprotein		79026	AHNAK1RS, PM227, AHNAK	11	11q12.3	103390			No
AHR	aryl hydrocarbon receptor		196	FVH3, RP85, bHLHe76	7	7p21.1	600253			No
AHSG	alpha 2-HS glycoprotein		197	A2HS, AHS, APMR1, FETUA, HSGA	3	3q27.3	138680			No
AICDA	activation induced cytidine deaminase		57379	AID, ARP2, CDA2, HEL-S-284, HIGM2	12	12p13.31	605257			No
AKR1D1	aldo-keto reductase family 1 member D1		6718	3o5bred, CBAS2, SRD5B1	7	7q33	604741			No
AKT1	AKT serine/threonine kinase 1		207	AKT, PKB, PKB-ALPHA, PRKBA, RAC, R	14	14q32.33	164730			No
AKT2	AKT serine/threonine kinase 2		208	HIHGHH, PKBB, PKBBETA, PRKBB, RAC	19	19q13.2	164731			No
ALB	albumin		213	FDAHT, HSA, PRO0883, PRO0903, PRO	4	4q13.3	103600			No
ALDH2	aldehyde dehydrogenase 2 family member		217	ALDH-E2, ALDHI, ALDM	12	12q24.12	100650			No
ALG8	ALG8 alpha-1,3-glucosyltransferase		79053	CDG1H, PCLD3	11	11q14.1	608103			No
ALG9	ALG9 alpha-1,2-mannosyltransferase		79796	CDG1L, DIBD1, GIKANIS, LOH11CR1J	11	11q23.1	606941			No
ALOX12	arachidonate 12-lipoxygenase, 12S type		239	12-LOX, 12S-LOX, LOG12	17	17p13.1	152391			No
ALPL	alkaline phosphatase, biomineralization associated		249	AP-TNAP, APTNAP, HOPS, HPPA, HPPC	1	1p36.12	171760			No
AMACR	alpha-methylacyl-CoA racemase		23600	AMACRD, CBAS4, P504S, RACE, RM	5	5p13.2	604489			No
ANGPT1	angiopoietin 1		284	AGP1, AGPT, AGPT-1, ANG1, HAE5	8	8q23.1	601667			No
ANGPT2	angiopoietin 2		285	AGPT2, ANG2, LMPHM10	8	8p23.1	601922			No
ANGPTL2	angiopoietin like 2		23452	ARP2, HARP	9	9q33.3	605001			No
ANGPTL8	angiopoietin like 8		55908	C19orf80, PRO1185, PVPA599, RIFL, TD	19	19p13.2	616223			No
AOC3	amine oxidase copper containing 3		8639	HPAO, SSAO, VAP-1, VAP1	17	17q21.31	603735			No
APLN	apelin		8862	APEL, XNPEP2	X	Xq26.1	300297			No
APLNR	apelin receptor		187	AGTRL1, APJ, APJR, HG11	11	11q12.1	600052			No
APOA1	apolipoprotein A1		335	AMYLD3, HPALP2, apo(a)	11	11q23.3	107680			No
APOA5	apolipoprotein A5		116519	APOAV, RAP3	11	11q23.3	606368			No
APOB	apolipoprotein B		338	FCHL2, FLDB, LDLCQ4, apoB-100, apoB	2	2p24.1	107730			No
APOC3	apolipoprotein C3		345	APOCIII, Apo-C3, ApoC-3	11	11q23.3	107720			No
APOH	apolipoprotein H		350	B2G1, B2GP1, BG	17	17q24.2	138700			No
APPL1	adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1		26060	APPL, DIP13alpha, MODY14	3	3p14.3	604299			No
APPL2	adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 2		55198	DIP13B	12	12q23.3	606231			No
APTR	Alu-mediated CDKN1A/p21 transcriptional regulator		100505854	RSBN1L-AS1	7	7q11.23	616048			No
AQP1	aquaporin 1 (Colton blood group)		358	AQP-CHIP, CHIP28, CO	7	7p14.3	107776			No
AREG	amphiregulin		374	ARB, CRDGF, SDGF, AREG	4	4q13.3	104640			No
ARRB2	arrestin beta 2		409	ARB2, ARR2, BARR2	17	17p13.2	107941			No
ARRDC3	arrestin domain containing 3		57561	TLIMP	5	5q14.3	612464			No
ASL	argininosuccinate lyase		435	ASALD, ASL	7	7q11.21	608310			No
ATG7	autophagy related 7		10533	APG7-LIKE, APG7L, GSA7, SCAR31	3	3p25.3	608760			No
ATP6AP1	ATPase H+ transporting accessory protein 1		537	16A, ATP6IP1, ATP6S1, Ac45, CF2, VATF	X	Xq28	300197			No
ATP7B	ATPase copper transporting beta		540	PWD, WC1, WD, WND	13	13q14.3	606882			No
ATP8B1	ATPase phospholipid transporting 8B1		5205	ATPIC, BRIC, FIC1, ICP1, PFIC, PFIC1	18	18q21.31	602397			No

Table 1: List of 808 genes broadly associated with Alzheimer's Disease

Gene Symbol	Gene Description	UniProt Entry	Protein Length (aa)	NCBI Gene ID	OMIM ID	Chromosomal Locatior	Aliases
ABCA7	Phospholipid-transporting ATPase ABCA7	Q8IZY2	2146				
ADAM10	Disintegrin and metalloproteinase domain-containing protein 10	O14672	748				KUZ | MADM
APOE	apolipoprotein E	P02649	317	348	107741	19q13.32	AD2, APO-E, ApoE4, LDLCQ5, LPG
APP	amyloid beta precursor protein	P05067	770	351	104760	21q21.3	AAA, ABETA, ABPP, AD1I, CTFgamma,
CTSD	cathepsin D	P07339	412	1509	116840	11p15.5	CLN10, CPSD, HEL-S-130P
DBN1	Drebrin	Q16643	649				D0S117E
GSK3A	Glycogen synthase kinase-3 alpha	P49840	483				
GSK3B	Glycogen synthase kinase-3 beta	P49841	420				
MAPT	Microtubule-associated protein tau	P10636	758				MAPTL | MTBT1 | TAU
MT-ND1	NADH-ubiquinone oxidoreductase chain 1	P03886	318				MTND1 | NADH1 | ND1
MT-ND2	NADH-ubiquinone oxidoreductase chain 2	P03891	347				MTND2 | NADH2 | ND2
PPP5C	Serine/threonine-protein phosphatase 5	P53041	499				PPP5
PSEN1	Presenilin-1	P49768	467				AD3 | PS1 | PSNL1
PSEN2	Presenilin-2	P49810	448				AD4 | PS2 | PSNL2 | STM2
RALGPS2	Ras-specific guanine nucleotide-releasing factor RalGPS2	Q86X27	583				
SNCA	Alpha-synuclein	P37840	140				NACP | PARK1
SORL1	sortilin related receptor 1	Q92673	2214	6653	602005	11q24.1	C11orf32, LR11, LRP9, SORLA, SorLA-
TREM2	triggering receptor expressed on myeloid cells 2	Q9NZC2	230	54209	605086	6p21.1	AD17, PLOSL2, TREM-2, Trem2a, Trem
UNC5C	Netrin receptor UNC5C	O95185	931				UNC5H3

Table 2: List of 19 high-confidence core Alzheimer's Disease target proteins under the Uniprot DB Alzheimer's disease entry (KW-0026)

The molecular targets of metformin were collected by searching the SwissTargetPrediction, ChEMBL, and BindingDB databases. In ChEMBL, targets were retrieved using the search query "metformin" and manually filtered from the target activity summary to include only single proteins and protein complexes of *Homo sapiens*, returning 141 entries comprising proteins with documented interactions with metformin [36]. The SwissTargetPrediction database was searched by entering the canonical SMILES of metformin with *Homo sapiens* selected as the target organism; targets with probability scores above 0.05 were retained, with a lower threshold applied to account for the characteristically small molecular size of metformin, which typically yields reduced probability scores in structure-based prediction tools [37]. A parallel search on BindingDB using "metformin" as the query returned 12 significant targets [38]. The three target lists were subsequently combined and duplicate entries were removed.

SwissTargetPrediction (5)

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Nitric oxide synthase, inducible	NOS2	P35228	CHEMBL4481	Enzyme	0.08497809839310490	280 / 0
D(3) dopamine receptor	DRD3	P35462	CHEMBL234	Family A G protein-coupled receptor	0.07737228957533360	385 / 0
Nitric oxide synthase 1	NOS1	P29475	CHEMBL3568	Oxidoreductase	0.07737228957533360	528 / 0
Carbonic anhydrase 9	CA9	Q16790	CHEMBL3594	Lyase	0.07599613277156360	384 / 0
Carbonic anhydrase 2	CA2	P00918	CHEMBL205	Lyase	0.07599613277156360	959 / 0
Carbonic anhydrase 1	CA1	P00915	CHEMBL261	Lyase	0.07599613277156360	806 / 0
Carbonic anhydrase 12	CA12	O43570	CHEMBL3242	Lyase	0.07599613277156360	297 / 0
Dipeptidyl peptidase 2	DPP7	Q9UHL4	CHEMBL3976	Protease	0.0753165091413365	402 / 0
Dipeptidyl peptidase 8	DPP8	Q6V1X1	CHEMBL4657	Protease	0.0753165091413365	426 / 0
Nitric oxide synthase 3	NOS3	P29474	CHEMBL4803	Enzyme	0.07430753735288600	156 / 0
Diamine oxidase [copper-containing]	AOC1	P19801	CHEMBL2118	Oxidoreductase	0.07167744903596410	16 / 0
Dipeptidyl peptidase 4	DPP4	P27487	CHEMBL284	Protease	0.07135479276136810	2314 / 0
Lysyl oxidase homolog 2	LOXL2	Q9Y4K0	CHEMBL3714029	Oxidoreductase	0.07103347778724420	222 / 0
Histamine H3 receptor	HRH3	Q9Y5N1	CHEMBL264	Family A G protein-coupled receptor	0.06851069700970950	591 / 0
Histamine H4 receptor	HRH4	Q9H3N8	CHEMBL3759	Family A G protein-coupled receptor	0.06851069700970950	553 / 0
Histamine H1 receptor	HRH1	P35367	CHEMBL231	Family A G protein-coupled receptor	0.06851069700970950	110 / 0
Histamine H2 receptor	HRH2	P25021	CHEMBL1941	Family A G protein-coupled receptor	0.06851069700970950	62 / 0
CDGSH iron-sulfur domain-containing protein 1	CISD1	Q9NZ45	CHEMBL1795168	Unclassified protein	0.06820124859444620	7 / 0
Ribonucleoside-diphosphate reductase subunit M2	RRM2	P31350	CHEMBL1954	Oxidoreductase	0.06820124859444620	12 / 0
Ribonucleotide reductase	N/A	P23921&Q7LG56	CHEMBL3301398	Oxidoreductase	0.06820124859444620	11 / 0
Dipeptidyl peptidase 9	DPP9	Q86TI2	CHEMBL4793	Protease	0.06758623477299900	290 / 0
Tyrosine-protein kinase JAK3	JAK3	P52333	CHEMBL2148	Kinase	0.06284842707523530	1014 / 0
Non-receptor tyrosine-protein kinase TYK2	TYK2	P29597	CHEMBL3553	Kinase	0.06284842707523530	466 / 0
Tyrosine-protein kinase JAK1	JAK1	P23458	CHEMBL2835	Kinase	0.06284842707523530	1039 / 0
Tyrosine-protein kinase JAK2	JAK2	O60674	CHEMBL2971	Kinase	0.06284842707523530	965 / 0
Purine nucleoside phosphorylase	PNP	P00491	CHEMBL4338	Transferase	0.06256283619665020	117 / 0
Prothrombin	F2	P00734	CHEMBL204	Protease	0.06171331524367820	1212 / 0
Ribosomal protein S6 kinase beta-1	RPS6KB1	P23443	CHEMBL4501	Kinase	0.06143254432602630	404 / 0
Lysyl oxidase homolog 3	LOXL3	P58215	CHEMBL4105989	Oxidoreductase	0.05977284480483640	12 / 0
Protein-lysine 6-oxidase	LOX	P28300	CHEMBL2249	Oxidoreductase	0.05977284480483640	69 / 0
Gamma-aminobutyric acid receptor subunit rho-1	GABRR1	P24046	CHEMBL3561	Ligand-gated ion channel	0.05950034241174130	14 / 0
Carbonic anhydrase 5B, mitochondrial	CA5B	Q9Y2D0	CHEMBL3969	Lyase	0.05842193097815880	41 / 0
Carbonic anhydrase 14	CA14	Q9ULX7	CHEMBL3510	Lyase	0.05842193097815880	88 / 0
Carbonic anhydrase 7	CA7	P43166	CHEMBL2326	Lyase	0.05842193097815880	139 / 0
Carbonic anhydrase 5A, mitochondrial	CA5A	P35218	CHEMBL4789	Lyase	0.05842193097815880	49 / 0
Carbonic anhydrase 6	CA6	P23280	CHEMBL3025	Lyase	0.05842193097815880	35 / 0
Carbonic anhydrase 4	CA4	P22748	CHEMBL3729	Lyase	0.05842193097815880	163 / 0
Carbonic anhydrase 3	CA3	P07451	CHEMBL2885	Lyase	0.05842193097815880	19 / 0
Amine oxidase [flavin-containing] A	MAOA	P21397	CHEMBL1951	Oxidoreductase	0.05762518167380100	149 / 0
GABA-B receptor	N/A	Q9UBS5&O75899	CHEMBL2111463	Family C G protein-coupled receptor	0.05736187306277760	15 / 0
Indoleamine 2,3-dioxygenase 1	IDO1	P14902	CHEMBL4685	Oxidoreductase	0.05711610481182700	328 / 1
Sodium-dependent serotonin transporter	SLC6A4	P31645	CHEMBL228	Electrochemical transporter	0.05683864238785630	983 / 0
Serine/threonine-protein kinase pim-1	PIM1	P11309	CHEMBL2147	Kinase	0.05683864238785630	555 / 0

Table 3: List of metformin targets from SwissTargetsPrediction

BindingDB MonomerID	BindingDB Ligand Name	Target Name	Target Source Organism According to Curator or DataSource	Ki (nM)	IC50 (nM)	K
50229665	N,N-dimethylimidodicarbonimidic diamide::1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN	Dipeptidyl peptidase 4	Homo sapiens		29000	
50229665	N,N-dimethylimidodicarbonimidic diamide::1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN	Solute carrier family 22 member 1	Homo sapiens		1230000	
50229665	N,N-dimethylimidodicarbonimidic diamide::1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN	Bile salt export pump	Homo sapiens		>135000	
50229665	N,N-dimethylimidodicarbonimidic diamide::1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN	Bile salt export pump	Homo sapiens		>1000000	
50229665	N,N-dimethylimidodicarbonimidic diamide::1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN	ATP-binding cassette sub-family C member 4	Homo sapiens		>133000	
50229665	N,N-dimethylimidodicarbonimidic diamide::1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN	ATP-binding cassette sub-family C member 2	Homo sapiens		>133000	
50229665	N,N-dimethylimidodicarbonimidic diamide::1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN	ATP-binding cassette sub-family C member 3	Homo sapiens		>133000	
50229665	N,N-dimethylimidodicarbonimidic diamide::1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN	Bile salt export pump	Homo sapiens		>133000	
50229665	N,N-dimethylimidodicarbonimidic diamide::1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN	Solute carrier family 22 member 2	Homo sapiens		1700000	
50229665	N,N-dimethylimidodicarbonimidic diamide::1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN	Solute carrier family 22 member 1	Homo sapiens		2010000	
50229665	N,N-dimethylimidodicarbonimidic diamide::1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN	Multidrug and toxin extrusion protein 1	Homo sapiens		250000	
50229665	N,N-dimethylimidodicarbonimidic diamide::1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN	Dihydrofolate reductase	Homo sapiens	18000000		

Table 4: List of metformin targets from BindingDB

Cross-referencing metformin targets against genes associated with AD

To find AD-associated genes that can be targeted by metformin, we cross-reference the Metformin target list with the list of 808 broadly associated AD genes. The intersection of the returns a comprehensive list of 19 unique genes that are associated with Alzheimer's disease and can be targeted by metformin. Computational analysis and visualization for the same is carried out using numpy and matplotlib plots.

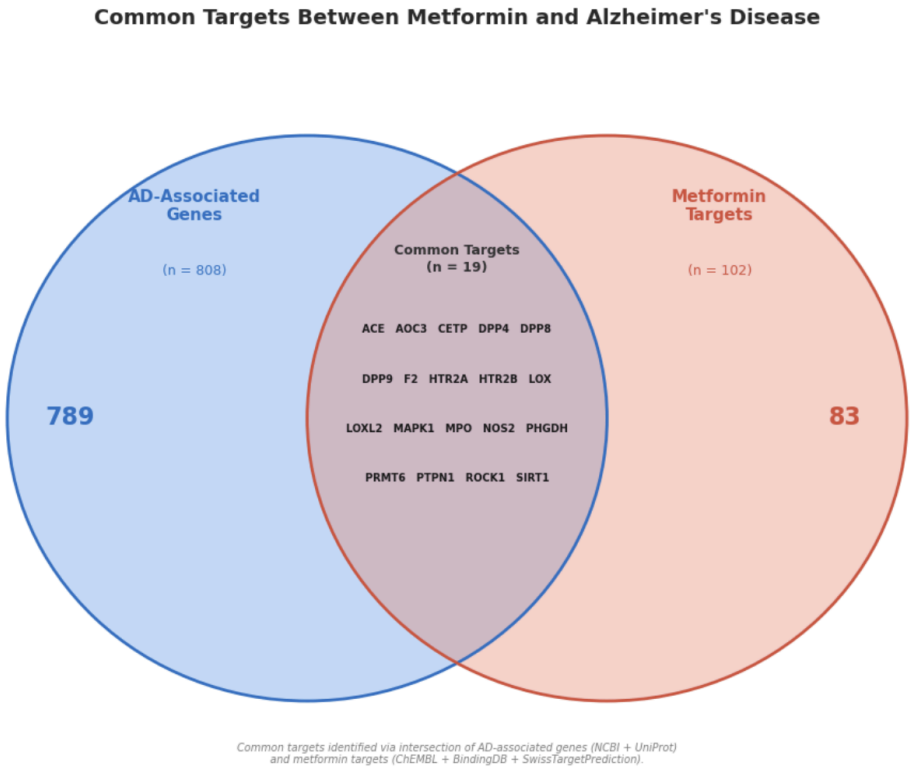

Figure 2: Venn diagram showing Alzheimer's disease-associated genes that can be targeted by metformin. (fig generated using matplotlib and numpy)

Identification of protein-protein interactions among selected genes using StringDB

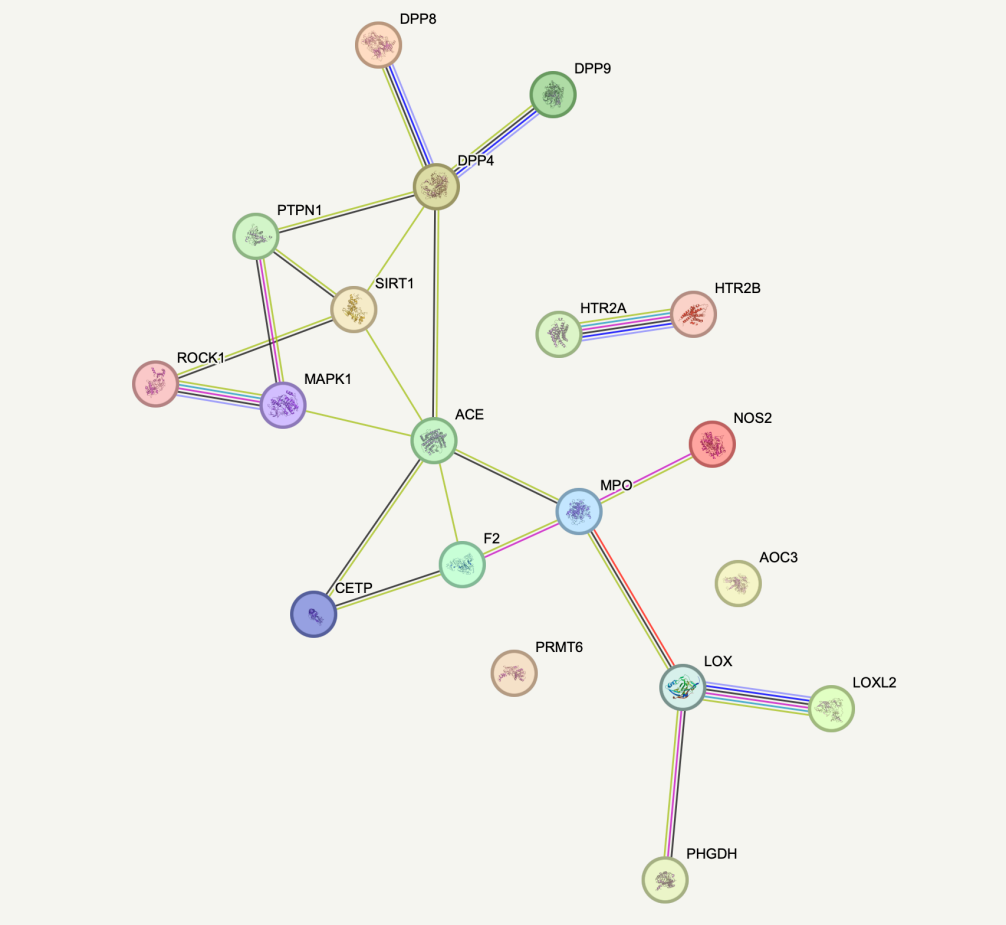

Fig 3(a): STRING Protein–Protein Interaction (PPI) Network (edges)

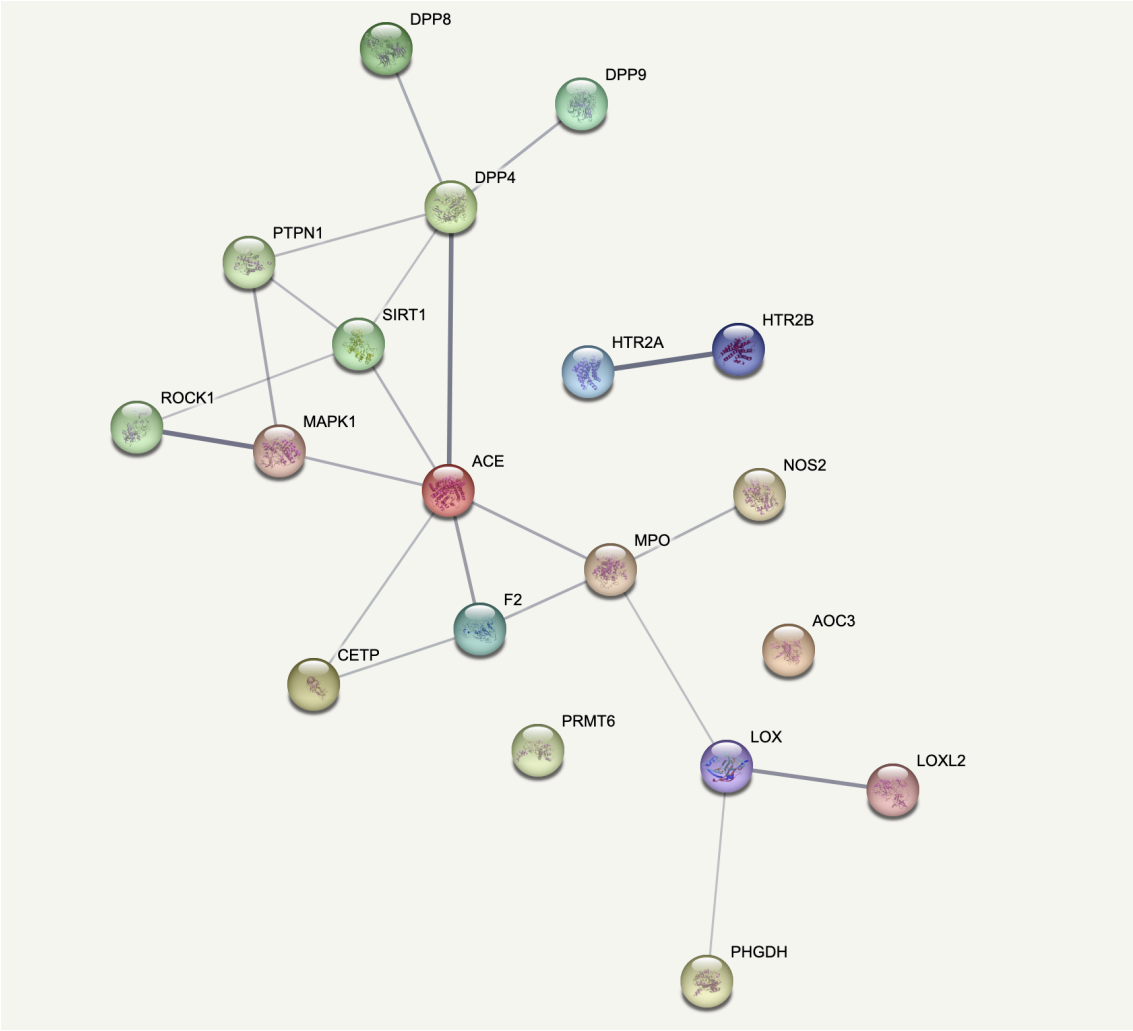

Fig 3(b): STRING Protein–Protein Interaction (PPI) Network (confidence)

Network Stats

number of nodes:	19	expected number of edges:	6
number of edges:	21	PPI enrichment p-value:	1.04e-06
average node degree:	2.21	*your network has significantly more interactions than expected (what does that mean?)*	
avg. local clustering coefficient:	0.521		

Fig 4: STRING Protein–Protein Interaction (PPI) Network statistics

A protein–protein interaction (PPI) network of the 19 common targets between metformin and Alzheimer's disease was constructed using the STRING database [7][37]. The network consists of 19 nodes representing the distinct proteins and multiple edges representing known or predicted functional interactions. The network has a density of 0.19, an average of 2.67 neighbors per node, a characteristic path length of 2.63, and a clustering coefficient of 0.193, indicating a sparse, moderately hub-centralized topology with network centralization = 0.275. This is analyzed using the cytoscape network analysis software [49].

Protein	Degree	Betweenness	Closeness	Hub_Score
ACE	6	0.444	0.51	100.0
MPO	4	0.358	0.438	88.235
DPP4	5	0.231	0.422	85.294
SIRT1	4	0.085	0.408	73.529
MAPK1	3	0.06	0.371	58.824
F2	3	0.021	0.395	58.824
LOX	3	0.208	0.331	58.824
PTPN1	3	0.015	0.322	44.118
CETP	2	0.0	0.34	32.353
ROCK1	2	0.003	0.299	26.471
NOS2	1	0.0	0.299	17.647
DPP8	1	0.0	0.292	11.765
DPP9	1	0.0	0.292	11.765
LOXL2	1	0.0	0.245	5.882
PHGDH	1	0.0	0.245	5.882
HTR2B	1	0.0	0.062	0.0
HTR2A	1	0.0	0.062	0.0

Table 5: PPI analysis - Degree, Betweenness, Closeness, and hub score

Further, the degree, closeness and betweenness measures are calculated for each node and a composite rank is deduced based on the average of the three measures. Here, ACE has the highest hub score (100.0), which reflects its position as the most highly connected and centrally located node in the network. MPO (88.2) and DPP4 (85.3) follow closely, and SIRT1 shows a score of 73.5. Together these 4 proteins form a central cluster, relative to the other proteins, whose scores are below 60.

Notably, HTR2A and HTR2B scored 0.0, reflecting their status as a topologically isolated pair disconnected from the main network. This is consistent with their separate functional association with serotonergic signaling rather than the core AD-metformin functionality as compared to ACE, MPO, DPP4, and SIRT1.

Several highly connected proteins are observed, indicating their potential in mediating the therapeutic effects of metformin against Alzheimer's disease. As observed earlier, ACE, DPP4, MPO, SIRT1, MAPK1 exhibit higher connectivity compared with other proteins, suggesting their central role in the network. Protein interactions form clusters based on functional association. The DPP4–DPP8–DPP9–PTPN1–SIRT1 cluster is associated with metabolic regulation and insulin signaling, while the MAPK1–ROCK1–SIRT1 cluster is involved in cellular signaling pathways linked to neuronal survival and neurodegeneration. Another cluster involving MPO, NOS2, and ACE is associated with neuroinflammation and oxidative stress, both of which are key pathological features of Alzheimer's disease.

KEGG Pathways					
pathway	description	count in network	strength	signal	false discovery rate
hsa04540	Gap junction	3 of 87	1.55	0.66	0.0284
hsa05142	Chagas disease	3 of 97	1.51	0.65	0.0284
hsa04726	Serotonergic synapse	3 of 108	1.46	0.64	0.0284
hsa04611	Platelet activation	3 of 122	1.41	0.64	0.0284
hsa00260	Glycine, serine and threonine metabolism	2 of 38	1.74	0.63	0.0376
hsa05206	MicroRNAs in cancer	3 of 159	1.29	0.59	0.0322
hsa05130	Pathogenic Escherichia coli infection	3 of 187	1.22	0.56	0.0376
hsa04020	Calcium signaling pathway	3 of 191	1.21	0.55	0.0376
hsa04810	Regulation of actin cytoskeleton	3 of 209	1.17	0.54	0.0390
hsa05200	Pathways in cancer	4 of 515	0.91	0.45	0.0451
					(less ...)

Table 6: KEGG pathway enrichment

KEGG pathway enrichment analysis is then performed via the STRINGDB functional enrichment tool, which identifies 10 significantly enriched pathways (FDR < 0.05). The most strongly enriched pathways include Glycine, Serine and Threonine metabolism (strength = 1.74, FDR = 0.038), Gap junction (strength = 1.55, FDR = 0.028), and Chagas disease (strength = 1.51, FDR = 0.028). Serotonergic Synapse enrichment (FDR = 0.028) corresponds to the HTR2A–HTR2B node pair, which formed a topologically isolated component distinct from the main hub-centered cluster.

Several enriched pathways show direct mechanistic relevance to both AD pathology and metformin's pharmacological profile: *The Calcium Signaling pathway* (3 genes, FDR = 0.038) and *Regulation of Actin Cytoskeleton* (3 genes, FDR = 0.039) correspond to calcium dysregulation and cytoskeletal disruption in AD-associated neurodegeneration. Ca^{2+} signaling is involved in neurotransmitter release, synaptic plasticity, gene expression and other important neuronal functions. Increasing evidence suggests progressive Aβ accumulation causes the dysregulation of ionic homeostasis and influx of Ca^{2+} from the extracellular space, leading to Ca^{2+} dyshomeostasis. Intracellular Ca^{2+} levels can modulate APP processing, Aβ production as well as the formation of NFTs. Importantly, the genes associated with AD have also been found to modulate Ca^{2+} signaling. This can be corroborated by the Calcium hypothesis which shows the activation of the amyloidogenic pathway and its effect on neuronal Ca^{2+} signaling pathways responsible for cognition. According to this hypothesis, the depolarization of aged neurons causes the influx of Ca^{2+} from the extracellular space and excitotoxicity. Ca^{2+} dyshomeostasis has also been reported in both peripheral and central neurons during the aging process as well as in neurons of AD patients. [39] Further, recent studies indicate that the pathways regulating the stability and remodeling of the actin cytoskeleton are impaired in AD, disruption of which occurs at different levels of the signaling cascade, including direct regulators of actin and molecules upstream(GEFs and small GTPases). The actin cytoskeleton provides the structural scaffold for spines and synapses, and anchors neurotransmitter receptors at the synapse; degeneration of signaling that maintains spines leads to the eventual collapse of spines and loss of synapses. A stable synaptic cytoskeleton protects spiny synapses from elimination, and prevents the loss of their glutamate receptor content. [40]

The Platelet activation (FDR = 0.028) reflects the known vascular dysfunction linking AD and metabolism. Experimental studies have shown that activated platelets directly contribute to cerebral amyloid angiopathy by promoting the formation of Aβ aggregates which, in turn, activates platelets, creating a feed-forward loop. Excessive platelet activation has been observed to contribute to the pathogenesis of first or subsequent transient ischemic attack or stroke, perivascular or inflammatory

diseases and also neurodegenerative disorders. [41]

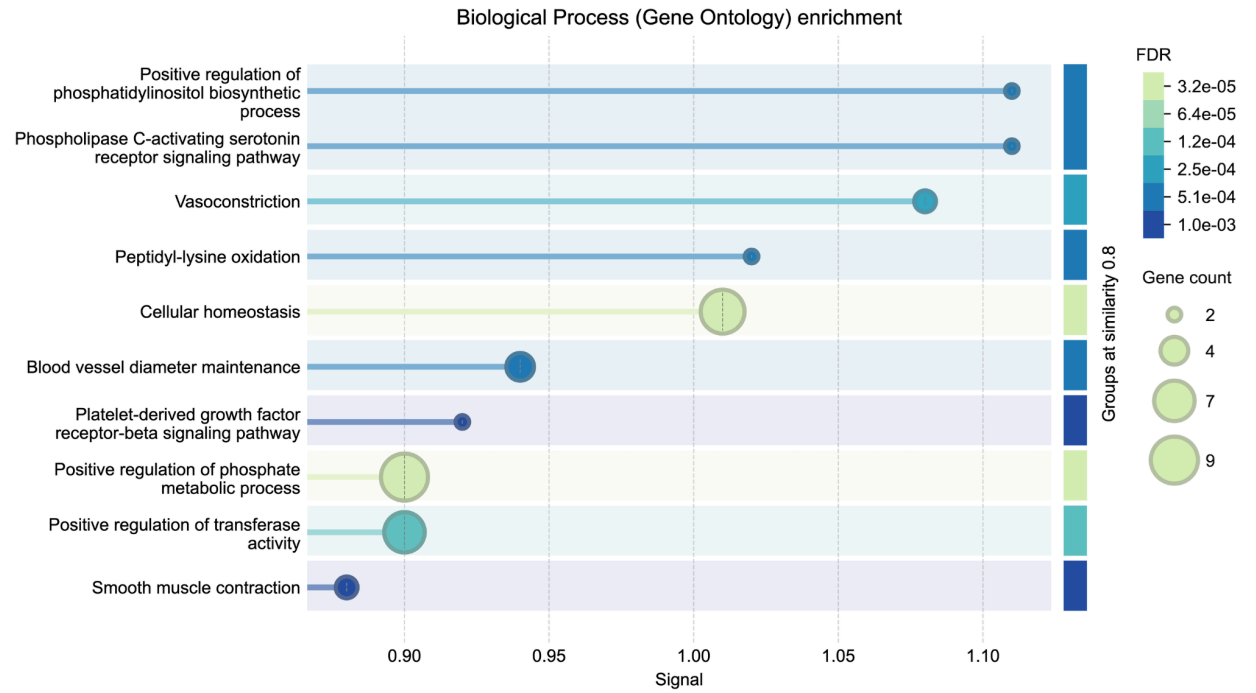

Fig 5: Gene Ontology Biological Process Enrichment graph

Gene Ontology Biological Process enrichment further supports the KEGG findings, with statistically significant terms including cellular homeostasis (largest gene overlap, 7–9 genes) and positive regulation of phosphate metabolic process. Several enriched terms also center around vascular function like vasoconstriction, blood vessel diameter maintenance, smooth muscle contraction, and PDGFR-beta signaling, which supports a shared vascular dysfunction axis linking AD cerebrovascular pathology with metformin's known vascular effects [29] . Serotonergic signaling was also enriched, corresponding to the HTR2A–HTR2B node pair identified in the network analysis. Peptidyl-lysine oxidation enrichment corresponded to the LOX/LOXL2 branch of the network, consistent with this pair forming a distinct functional module separate from the core ACE–SIRT1–DPP4–MPO hub cluster.

Based on the PPI network observed in StringDB, the following genes emerged as the central hubs

ACE (degree – 6)

Angiotensin-converting enzyme (ACE) converts angiotensin I to angiotensin II which acts as a vasoconstrictor and also plays a significant role in amyloid-beta metabolism. ACE is shown to degrade neurotoxic Aβ42 peptides. An increase in microglial ACE expression reduces amyloid plaque load, preserves synapses, and improves memory in AD mouse models. Dysregulation of the renin-angiotensin system (ACE-mediated signalling) promotes APP processing and tau pathology, making ACE significant in the vascular, neuroinflammatory, and amyloid-clearance pathways in AD. [8][9]

DPP4 (degree – 5)

Dipeptidyl peptidase 4 (DPP4) is primarily expressed by microglia in the hippocampus, where its activity contributes to neuroinflammatory dysregulation. DPP4 inhibition has demonstrated neuroprotective effects in AD models by reducing Aβ plaque deposition, decreasing tau hyperphosphorylation, and exerting anti-inflammatory and anti-oxidative effects on neuronal tissue. [10][11]

MPO (degree – 4)

Myeloperoxidase (MPO) is released by neutrophils and microglia and catalyzes the production of hypochlorous acid, a potent oxidant in neuroinflammation and oxidative stress. Aβ-driven disruption of the blood-brain barrier enables neutrophil infiltration and MPO release, which directly interact with amyloid plaques and increase neurotoxicity. Pathogenic variants in MPO have also been reported in familial Alzheimer disease, making it another central hub target in AD pharmacology. [12]

SIRT1 (degree – 4)

SIRT1 is an NAD^+-dependent deacetylase that promotes neuron survival, mitochondrial function, amyloid-beta clearance, and autophagy. Depletion of SIRT1 accelerates neurodegeneration, and its activation reduces Aβ toxicity and tau pathology in preclinical AD models. SIRT1 and AMPK (metformin's primary target) regulate each other reciprocally, positioning SIRT1 as a direct downstream effector of metformin's CNS mechanism of action. [14][15]

MAPK1 (degree – 3)

MAPK1 (ERK2) is a core component of the MAPK/ERK signalling cascade that contributes to tau hyperphosphorylation at specific microtubule-binding sites, impairing tau's ability to stabilize microtubules and promoting NFT formation. Its upstream activation by insulin resistance, oxidative stress, and neuroinflammation makes it a relevant target. [19]

Reference Based Docking Analysis

Molecular docking analyses the conformation and orientation of molecules into the binding site of a macromolecular target [47]. To assess the direct binding potential of metformin against the identified target proteins, reference-based molecular docking was performed. For every target, a high-resolution crystal structure co-complexed with a known and validated small-molecule activator/inhibitor was retrieved from the RCSB Protein Data Bank. The co-crystallized reference ligand was removed from each structure, along with solvent molecules, while catalytically essential cofactors and metal ions (e.g., zinc, heme) were retained to preserve the native active-site conformation. Polar hydrogens and appropriate atomic charges were added using meeko, and protein and ligand structures were converted to the PDBQT format required for docking, assisted by meeko and openbabel libraries [43d][43e].

Molecular docking was performed using AutoDock Vina [43a], which employs a gradient-based conformational search algorithm coupled with an empirical scoring function to estimate binding affinity. For each target, the grid box was centered on the coordinates of the original co-crystallized inhibitor, limiting metformin to be evaluated within the established relevant binding sites. This approach allows the predicted binding pose and affinity of metformin to be directly compared with the binding of a validated inhibitor at the same site.

Docking scores are reported in kcal/mol, with more negative values indicating more favorable predicted binding affinity, where values more negative than approximately −6 to −7 kcal/mol are generally considered indicative of favorable binding. Accounting for metformin's small size and high polarity is

also necessary, which tend to yield less negative scores than larger, more hydrophobic drug-like ligands regardless of true binding potential. It should be noted that docking scores reflect predicted binding affinity and pose compatibility only, and do not confirm functional enzymatic activation or inhibition. For functional validation, further clinical study is required.

Docking Analysis Of Metformin On A Major Alzheimer's Disease Target - Acetylcholine Esterase Enzyme

Acetylcholinesterase (AChE) is an enzyme responsible for breaking down acetylcholine (ACh), a neurotransmitter crucial for memory and cognitive functions. In AD, there is a notable decline in cholinergic neurons leading to reduced ACh levels, which is closely associated with cognitive decline and memory loss. AChE is commonly found in association with β-amyloid plaques and neurofibrillary tangles, which are hallmark features of Alzheimer's pathology. The presence of AChE around these plaques suggests a potential role in the disease's progression. [18]

The cholinergic hypothesis suggests that the cognitive deficits observed in Alzheimer's disease are attributed to a loss of cholinergic function, caused by severe damage to cholinergic neurons leading to significant loss of choline acetyltransferase (ChAT) activity within the cerebral cortex and hippocampus. The decline in ChAT activity, combined with the detrimental effects of Aβ on ACh synthesis, release, and degradation, leads to a reduction in ACh levels. This decrease impairs its physiological functions in learning, memory, motor regulation, and sleep cycle regulation, and has led to the development of AChE inhibitors such as donepezil as a therapeutic strategy to improve cholinergic transmission and reduce cognitive decline. [18][24]

For this study, reference-based docking was employed, wherein a protein structure co-crystallized with a bound ligand of known function is used to identify the active site for docking. The crystal structure of AChE in complex with donepezil (an FDA-approved AChE inhibitor used in AD therapy) was selected for this purpose (PDB ID: 4EY7, resolution: 2.35 Å, *Homo sapiens*) [42.a]. Metformin was docked into the active site defined by the donepezil binding pose using AutoDock Vina, and its binding interactions were compared against the reference ligand. Protein preparation and visualization were performed using UCSF ChimeraX [43.c]. Molecular docking was carried out using AutoDock Vina 1.2.0 [43.a], and docking poses were analyzed using PyMOL [43.b].

A docking score of -5.546 is observed, showing that metformin exhibited moderate binding affinity toward acetylcholinesterase (AChE). The docking grid was centered on the binding pocket generally occupied by the donepezil inhibitor. Metformin thus exhibits a moderate interaction with the active site of AChE. Multiple docking poses showed similar binding energies ranging from −5.3 to −4.5 kcal/mol, indicating the stable placement of the ligand within the binding pocket. For process validation, donepezil was also re-docked onto the A chain of ACHE, which showed high binding affinity and docking score of -12.21 kcal/mol, consistent with previous results of the approved drug molecule.

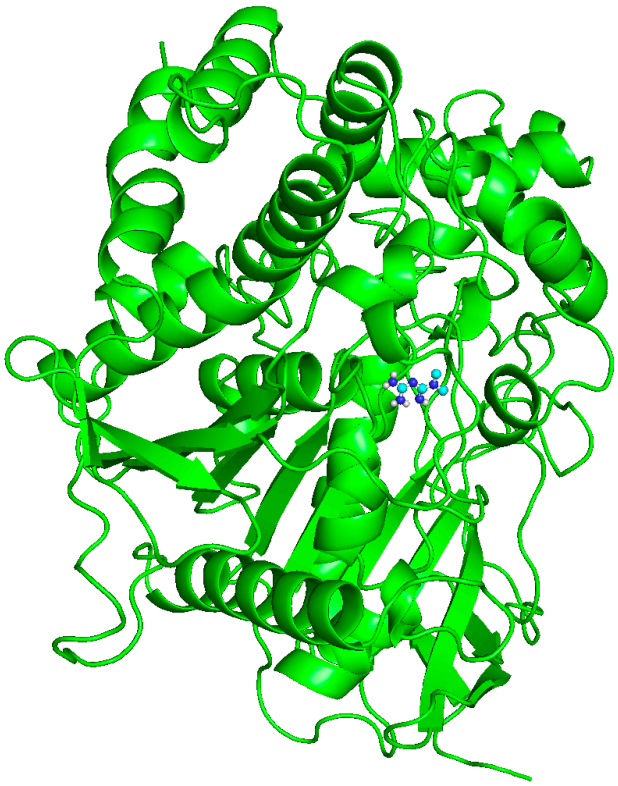

Fig 6: Metformin docked at the AChE active site (blue). Visualization using PyMOL (Open-Source).

AChE (4EY7) – Metformin & Donepezil (positive control):
Docking Parameters — AChE (4EY7): Grid center: [−14.02, −43.067, 26.783] | Box size: 18 × 16 × 18 Å | Exhaustiveness: 16 | Poses: 10

Molecular interaction analysis shows 3 hydrogen bonds between metformin and key AChE active site residues - GLU 202 (×2, distances: 2.913Å and 3.142Å) and TYR 133 (3.173Å) - alongside hydrophobic and van der Waals contacts with TRP 86, GLY 120, GLY 121, and SER 125. Metformin binds at the peripheral region of the active site gorge, engaging TRP 86 and GLU 202, whereas donepezil binds deeper within the gorge through extensive aromatic interactions with TRP 286, TYR 337, PHE 338, and TYR 341. This difference in binding mode suggests metformin may interact with the peripheral anionic site of AChE unlike donepezil which binds deep in the catalytic gorge, which may account for the difference in binding affinity relative to donepezil.

Reference Based Docking Analysis of Metformin on Secondary Alzheimer's Disease Targets as Identified by Network Pharmacology

Angiotensin Converting Enzyme (ACE)

Angiotensin converting enzyme is a dipeptidyl carboxypeptidase that is best known for its role in blood pressure regulation, where it cleaves angiotensin I into angiotensin II which is a potent vasoconstrictor. Increasing evidence shows ACE exerting significant effects in the central nervous system, beyond its

classical homeostatic function. ACE is widely expressed in multiple brain regions implicated in memory and learning, including the hippocampus, cortex, and basal ganglia. It is a key modulator of cognitive processes through enzymatic activity on a diverse set of neuroactive substrates and influence on oxidative balance, inflammatory signaling, and synaptic function. [8][9]

ACE has been implicated in AD pathogenesis through the brain renin-angiotensin system (RAS). Angiotensin II signaling also contributes to cerebrovascular dysfunction, oxidative stress, and neuroinflammation, all of which accelerate amyloid-β accumulation and tau pathology. ACE itself has also been reported to directly participate in Aβ degradation, positioning it at the intersection of vascular and amyloid-clearance pathways relevant to AD. [9]

Central-acting ACE inhibitors have been reported to prevent cognitive impairment and decrease β-amyloid-dependent neurodegeneration in animal models of Alzheimer's disease. Clinical observations have also suggested that ACE inhibitors reduce the rate of functional decline in patients with Alzheimer's disease and dementia. However, the role of ACE in AD remains uncertain as ACE was also found to have hydrolytic activity capable of degrading β-amyloid. Overexpression of catalytically active ACE has been shown to reduce the burden of neurotoxic β-amyloid and preserve cognitive function in mice. It was also suggested that ACE may be protective since elevated ACE levels reduce the risk of global brain atrophy and Alzheimer's disease. [45]

Here, we test metformin's binding ability as a potential inhibitor by reference based docking analysis. The structure PDB 1O86 is selected for the same, which represents the crystal structure of human angiotensin converting enzyme in complex with lisinopril, which is a known ACE inhibitor. The grid box is constructed based on the binding pocket of lisinopril and metformin is docked onto the same pocket to assess its binding affinity at the inhibitor site. Metformin exhibited a binding affinity of −7.446 kcal/mol toward ACE, comparable to that of the reference inhibitor lisinopril (−7.420 kcal/mol) docked under identical conditions as a positive control. The similar binding affinities suggest that metformin occupies the ACE active site with similar energetic favorability to a clinically validated ACE inhibitor. Molecular interaction analysis revealed 6 hydrogen bonds between metformin and key ACE active site residues, including HIS 387 (×2), TYR 523 (bidirectional), GLU 384, and ALA 354 residues which constitute the zinc-binding catalytic region and substrate binding pocket of ACE. This suggests metformin may interfere with ACE's catalytic activity and act as a potent inhibitor. Further functional and in vitro studies are required to validate the inhibitory potential of metformin on ACE activity.

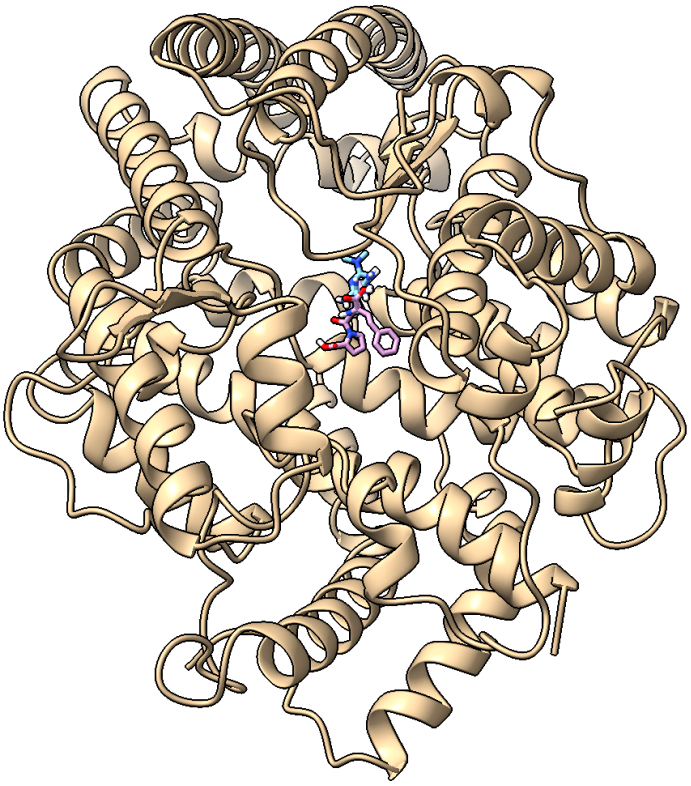

Fig 7: Metformin docked, observed in blue, with reference to lisinopril (pink) onto ACE.

ACE (1O86) – Metformin & Lisinopril (positive control):
Docking Parameters — ACE (1O86): Grid center: [40.60, 37.20, 43.40] | Box size: 20 × 20 × 20 Å | Exhaustiveness: 20 | Poses: 10

Mitogen Activated Protein Kinases (MAPK1/ERK2)

Mitogen-activated protein kinases (MAPKs), which are serine/threonine protein kinases, play major roles in cell signal transduction. In mammals, MAPKs are comprised of extracellular signal-regulated kinase 1/2 (ERK), P38 kinases (P38), and c-Jun N-terminal kinases. [44]

Many studies have illustrated that aberrant activation of the MAPK pathway is involved in the pathogenesis of AD via induction of neurotoxicity, synaptic dysfunction, autophagy dysfunction, and apoptosis. [19]

Irregular MAPK1/ERK2 activity is associated with increased Aβ-induced neuronal apoptosis. ERK1/2 negatively regulates β-secretase expression, which promotes the production of Aβ. It can mediate downstream oxidative stress responses to Aβ accumulation, exacerbating synaptic dysfunction and neuronal death. MAPK1 also interacts with kinases and phosphatases that regulate Tau protein

phosphorylation. Hyperactivation of the ERK/MAPK1 pathway contributes to NFT formation by facilitating abnormal Tau phosphorylation, leading to microtubule destabilization and cell loss. [19][44]

MAPK1 is activated by reactive oxygen species and inflammatory cytokines. Aberrant MAPK1 activity amplifies oxidative damage and enhances proinflammatory signaling, accelerating neurodegeneration. Overactivated ERK is associated with NFT formation and early AD-related protein deposition, leading to hippocampal function impairment cognitive deficits, including memory loss observed in early and progressive stages of AD in both patients and mouse models. Prevention of MAPK overactivation can reduce Aβ deposition, tau hyperphosphorylation, neuronal apoptosis, and memory impairment. [44]

For MAPK1, the crystal structure of ERK2 in complex with the small-molecule inhibitor FR180204 (PDB ID: 1TVO) was used [42.b]. Metformin was docked at the FR180204-occupied ATP-binding pocket, yielding a docking score of −5.313 kcal/mol, indicating moderate predicted binding affinity at this site.

Molecular interaction analysis of the best docking pose shows a close polar contact between metformin and ASP 167 (2.893Å) suggests a near-hydrogen bond interaction at the DFG motif of the MAPK1 kinase domain. Van der Waals and hydrophobic contacts were observed with TYR 36, ARG 67, LYS 54, GLU 71, THR 68, and TYR 64 residues lining the ATP-binding pocket.

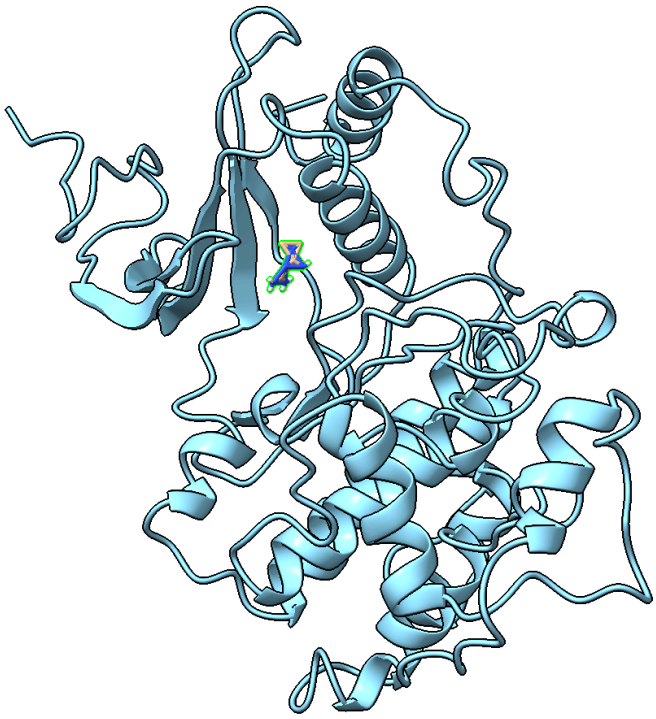

Fig 8: Metformin docked onto MAPK1/ERK2.

MAPK1/ERK2 (1TVO) – Metformin:
Docking Parameters — MAPK1/ERK2 (1TVO): Grid center: [6.429, −4.372, 16.444] | Box size: 22 × 22 × 22 Å | Exhaustiveness: 10 | Poses: 10

The binding affinity of metformin at the MAPK1 active site is lower than scores typically reported for established kinase inhibitors. This may be attributed to metformin's small molecular size and highly hydrophilic nature, which limit its ability to form extensive hydrophobic and van der Waals interactions within the ATP-binding cleft. This is consistent with metformin's effects on MAPK1 signalling that arise through indirect pathway modulation via AMPK crosstalk rather than direct competitive inhibition of the kinase active site. [6][29][30]

Sirutin 1

Sirtuin-1 is an NAD^+-dependent deacetylase that acts as a regulator of cellular stress responses and metabolic homeostasis. In the brain, SIRT1 activity promotes neuroprotective effects like enhanced synaptic plasticity, mitochondrial function, antioxidant defences and reduced inflammation. As SIRT1 activity reduces with age, its overexpression has been linked to reduction in AD symptoms, making it a potential target in AD treatment. Small-molecule SIRT1 activators like resveratrol have shown positive outcomes in preclinical models. [15]

SIRT1 is known to mitigate the amyloid pathway by upregulating the α-secretase that precludes Aβ formation and downregulating β-secretase. In AD mouse models, treatment with SIRT1 activators results in lower Aβ production and plaque burden, as well as enhanced clearance of Aβ peptides. It also helps control tau pathology by directly deacetylating tau protein, promoting tau degradation via the proteasome, and indirectly reducing abnormal tau phosphorylation. Active SIRT1 can deacetylate the NF-κB transcription factor and suppress pro-inflammatory cytokine production, activate the transcriptional coactivator PGC-1α to bolster mitochondrial biogenesis and antioxidant gene expression. [15][46]

To test metformin's binding ability at the activator site, it was docked onto SIRT1 (PDB ID: 4ZZH) in place of previously bound activator 4TO [42.c]. Molecular docking of metformin with the SIRT1 activator-binding site yielded a relatively weak binding affinity of −3.1 kcal/mol, suggesting limited direct interaction at this site. This indicates that metformin is unlikely to be a direct activator of SIRT1 at this site. Redocking of the activator 4TO at the activator binding site shows a binding affinity of approximately -6.5 kcal/mol, which validates the docking protocol.

SIRT1 (4ZZH) — Metformin & 4TO (reference activator):

Docking Parameters — SIRT1 (4ZZH): Grid center: [25.637, −53.996, 2.231] | Box size: 16 × 16 × 16 Å | Exhaustiveness: 20 | Poses: 10

Results Table 1: Comparative Binding Affinity Summary

Target Protein	Metformin Binding Affinity (kcal/mol)	Reference Ligand Dock - Binding affinity (kcal/mol)	Biological Relevance in AD	Interpretation
AChE	−5.546	−12.21 (donepezil)	Promotes acetylcholine hydrolysis and may accelerate Aβ aggregation. Inhibition improves cognitive symptoms in AD.	Moderate binding at the catalytic gorge of approved Donepezil inhibitor.
ACE	−7.446	−7.420 (lisinopril)	Contributes to Aβ metabolism, neuroinflammation and cerebrovascular dysfunction. ACE inhibition has been associated with neuroprotective effects in AD.	Strongest result with respect to metformin docking and comparable to validated ACE inhibitor; Strong predicted binding - metformin may directly interact with the ACE active site.
MAPK1	−5.313	—	Regulates neuronal survival, tau phosphorylation and inflammatory signalling. Dysregulation contributes to AD progression.	Consistent with indirect AMPK-mediated modulation; Moderate favourable binding - potential direct target.
SIRT1	−3.1	−6.5 (4TO)	Regulates neuroinflammation, mitochondrial function, autophagy and neuronal survival. Activation is considered neuroprotective in AD.	Excluded; Weak binding at the allosteric activator site - direct activation by metformin unlikely at this site

Results Table 2: Summary of Molecular Docking Results for Metformin Against AD-Associated Target Proteins

Target Protein	PDB ID	Reference Ligand	Reference Ligand Score (kcal/mol)	Metformin Score (kcal/mol)	Pose Range (kcal/mol)	No. of H-bonds	Key Interacting Residues	Interaction Type
AChE (Acetyl Choline Esterase Enzyme)	4EY7	Donepezil (inhibitor)	-12.21	-5.546	-5.3 to -4.5	3	GLU202 (×2), TYR133	H-bonds; hydrophobic contacts with TRP86, GLY120, GLY121, SER125
ACE (Angiotensin converting enzyme)	1O86	Lisinopril (inhibitor)	-7.420	-7.446	-6.4 to -6.0	6	HIS387 (×2), TYR523 (×2), GLU384, ALA 354	H-bonds at zinc-binding catalytic region and substrate binding pocket
MAPK1/ ERK2 (Mitogen-activated protein kinases)	1TVO	FR180204(inhibitor)	—	-5.313	-5.3 to -4.5	0	ASP167, TYR36, ARG67, LYS54, GLU71, THR68, TYR64	Near H-bond at DFG motif; van der Waals and hydrophobic contacts at ATP-binding pocket
SIRT1 (Sirutin 1)	4ZZH	4TO (STAC activator)	-6.5	-3.1	-3.1 to -2.8	—	—	Weak binding affinity; no further analysis

Results & Discussion

This study employs an integrated network pharmacology approach to investigate the potential therapeutic effects of metformin as a drug against Alzheimer's Disease. Network pharmacology assisted drug discovery allows for evaluation of complex interactions between drugs, genes and biological pathways, making it particularly suitable for multifactorial disorders such as Alzheimer's disease. By evaluating the major genes and pathways involved in AD development and drug targets of metformin, the study identified several common proteins that may mediate the neuroprotective effects of metformin.

AD-associated genes that can be targeted by metformin were identified by cross-referencing the Metformin target list with the list of 808 broadly associated AD genes, which returned a comprehensive list of 19 unique genes. The identified targets were predominantly associated with inflammatory signalling, oxidative stress, apoptosis, response to metabolic stress, and protein phosphorylation, which are some of the key pathways implicated in AD pathophysiology and contributing to amyloid-β accumulation and tau hyperphosphorylation and finally neuronal degeneration.

PPI network analysis further revealed ACE, DPP4, MPO, MAPK1 and SIRT1 as the most highly connected hub proteins among the previously identified genes, whose activity may affect several connected biological pathways. KEGG pathway enrichment analysis showed their involvement in major mechanistic pathways including calcium signalling, actin cytoskeleton regulation, and platelet activation, which are commonly associated with AD progression. This was further supported by Gene Ontology Biological Process enrichment. These findings support the hypothesis that metformin may exert therapeutic effects against AD through coordinated regulation of several disease-associated pathways rather than on a single molecular target.

Proteins occupying central positions within interaction networks may contribute to drug activity through either direct or indirect regulatory mechanisms to exert activator or inhibitor functionality. But, PPI network centrality by itself does not necessarily guarantee protein interaction with selected therapeutic compounds, and thus, molecular docking was performed to evaluate whether metformin could bind these proteins. Molecular docking analysis was done for a primary AD target – Acetyl Choline Esterase enzyme, and for 3 secondary targets – ACE, MAPK1, SIRT1 – which were previously identified as major hub genes via network pharmacology. Docking was performed using the reference based docking methodology, using the docking parameters of validated ligands, to assess the binding of metformin relative to the validated activator or inhibitor complexes for each of the proteins.

Acetylcholinesterase (AChE) is the primary target of FDA-approved AD treatments, including donepezil, which acts as a competitive inhibitor at the enzyme's active site gorge. Reference-based docking of metformin was performed using donepezil as the reference ligand, with the docking grid centered on the donepezil binding pocket. Metformin exhibited a docking score of -5.546 kcal/mol, showing moderate predicted binding affinity at the AChE active site. Further molecular interaction analysis revealed 3 hydrogen bonds with interacting active site residues – GLU 202 (×2, 2.913Å and 3.142Å) and TYR 133 (3.173Å) – and hydrophobic and van der Waals contacts with TRP 86, GLY 120, GLY 121, and SER 125. Metformin binds at the peripheral region of the active site gorge unlike the deep catalytic gorge engaged by donepezil, suggesting a distinct but potentially complementary binding mode, providing a computational idea for a potential inhibitory interaction of metformin at the AChE active site.

Among the secondary AD targets identified from PPI network analysis, molecular docking was performed for three proteins with established mechanistic relevance in AD pathophysiology: Angiotensin-Converting Enzyme (ACE), Mitogen-Activated Protein Kinase 1 (MAPK1), and Sirtuin-1 (SIRT1).

ACE showed the strongest docking result, with metformin exhibiting comparable binding affinity to the validated ACE inhibitor lisinopril at the same inhibitor binding site, with the docking grid centred on the lisinopril binding pocket (PDB: 1O86). Both lisinopril and metformin yielded binding affinities of approximately −7.4 kcal/mol, suggesting direct inhibitory potential of metformin at a target increasingly recognised for its role in Aβ degradation and neuroinflammatory regulation. Molecular interaction analysis revealed 6 significant hydrogen bonds at the zinc-binding catalytic region and substrate binding pocket, alongside hydrophobic and van der Waals interactions.

MAPK1 docking with metformin, performed with respect to the validated inhibitor FR180204 at the FR180204 binding site (PDB: 1TVO), showed moderate favourable binding with a best docking score of −5.313 kcal/mol, consistent with indirect AMPK-mediated modulation rather than direct competitive inhibition. Molecular interaction analysis revealed van der Waals and hydrophobic contacts with residues lining the ATP-binding pocket, with no classical hydrogen bonds detected, though a near-contact with ASP 167 (2.893Å) at the DFG motif was observed.

The consistently moderate scores, in the range of -5 kcal/mol, can be attributed to metformin's characteristically small molecular size and high polarity, which limit extensive hydrophobic interactions within active sites which is a known limitation of structure-based docking for small hydrophilic compounds.

In contrast, docking against the SIRT1 activator-binding site (PDB: 4ZZH) yielded a comparatively weak binding affinity of −3.1 kcal/mol, relative to the −6.5 kcal/mol binding affinity of the validated activator 4TO. Although SIRT1 was identified as one of the highest-degree hub proteins in the PPI network, the docking results suggest that metformin is unlikely to directly occupy the same allosteric activator pocket represented by the selected crystal structure. This indicates that the potential activation of SIRT1 by metformin observed in experimental studies may occur indirectly through upstream signalling mechanisms such as the AMPK–SIRT1 axis, rather than through direct ligand binding at this site. Alternatively, direct activation could occur through binding at a distinct site on the protein. Further computational and experimental studies are required to validate the role of metformin in SIRT1 activation in the context of Alzheimer's disease therapeutics.

Overall, the findings support the hypothesis that metformin functions as a multi-target therapeutic agent capable of modulating several interconnected biological pathways relevant to Alzheimer's disease. Metformin appears to influence an extensive network of signalling pathways associated with neuroinflammation, metabolic homeostasis, oxidative stress and neuronal survival. Such a mechanism is particularly helpful for complex neurodegenerative disorders wherein multiple pathological pathways contribute simultaneously to disease progression.

Computational analysis including network pharmacology, molecular docking and interaction analysis, though promising, is not complete evidence of therapeutic validation of a drug molecule. Several limitations of the same must be acknowledged. Network pharmacology relies on publicly available databases, whose completeness and accuracy depend on current experimental evidence and periodic updates. Consequently, potentially relevant drug-target interactions or disease-associated genes may not be captured, leading to incomplete interaction networks. Discrepancies in curation standards and update frequencies across databases also lead to inconsistent network construction and conflicting interpretations [32]. Further, Topology-based methods tend to favor highly interconnected hub proteins, often identifying generic signaling nodes rather than disease-specific ones, for instance, the MAPK family, which is significant in several pathways [32].

Molecular docking predicts the most favourable binding poses based on static three-dimensional protein structures and scoring functions, not necessarily accounting for protein flexibility, solvent effects,

conformational dynamics, or the complex physiological environment. Scores are often undermined by oversimplified scoring functions and should thus be interpreted as computational predictions rather than definitive measures of biological activity. [32][47] In addition, this study does not include molecular dynamics simulations or binding free-energy calculations, which could provide further insights into the stability and persistence of the predicted protein-ligand interactions.

Despite these limitations, present literature supports growing evidence of metformin's potential neuroprotective effects through multiple AD relevant mechanisms. Beyond its well-established peripheral metabolic actions, it is capable of crossing the blood-brain barrier to directly influence brain function. It has been reported to promote neuroprotection, neural regeneration, angiogenesis, and anti-inflammatory responses through modulation of multiple signalling pathways, including AMPK signalling, PI3K–protein kinase B (Akt)–mTOR signalling, lipid metabolism, inflammatory signalling, and mitochondrial-related pathways [30]. These diverse mechanisms further support the potential of metformin as a candidate for drug repurposing in Alzheimer's disease.

Further studies are required to validate the computational findings of the present study. Molecular dynamics simulations are needed to assess the structural stability of the docked protein-ligand complexes over time. Subsequent estimation of binding free energies using approaches such as MM/PBSA or MM/GBSA could further improve confidence in the predicted interactions [48]. Experimental validation through *in vitro* enzyme inhibition assays, neuronal cell culture models, and *in vivo* Alzheimer's disease models will be essential to confirm the biological relevance of the identified targets. In addition, transcriptomic and proteomic analyses may provide further insight into the downstream molecular mechanisms by which metformin modulates Alzheimer's disease-associated signalling pathways. Such integrated computational and experimental approaches will be critical for evaluating the therapeutic potential of metformin as a repurposed treatment for Alzheimer's disease. [28][30][32]

Conclusion

This study employed an integrated network pharmacology and molecular docking approach to assess the therapeutic potential of metformin in Alzheimer's disease. The findings identified several significant overlapping targets and key signalling pathways associated with neuroinflammation, oxidative stress, apoptosis, and metabolic regulation, highlighting the multi-target mechanism of metformin. Molecular docking further supported favourable interactions with AChE and selected hub proteins, particularly ACE and MAPK1. Computational evidence supporting the potential of metformin as a candidate for drug repurposing in Alzheimer's disease is provided and further establishment of a foundation for future molecular dynamics simulations and experimental validation to confirm the therapeutic efficacy.

References:-

1) Breijyeh, Z., & Karaman, R. (2020). Comprehensive review on Alzheimer's disease: Causes and treatment. Molecules, 25(24), 5789. https://doi.org/10.3390/molecules25245789

2) Prabhu, Anirudh. (2023). Alzheimer's Disease: A Comprehensive Review of its Causes, Diagnosis, and Treatment. Journal of Student Research. 12. 10.47611/jsrhs.v12i4.5770.

3) National Institute on Aging. (2026, January 28). Alzheimer's Disease Genetics Fact Sheet. https://www.nia.nih.gov/health/alzheimers-causes-and-risk-factors/alzheimers-disease-genetics-fact-sheet

4) Chandana Yesudas, Neethu P, Illakkiam Devaraj, Genetic insights and molecular pathways in Alzheimer's disease: Unveiling the complexity of neurodegeneration, Brain Disorders, Volume 17, 2025, 100178, ISSN 2666-4593, https://doi.org/10.1016/j.dscb.2024.100178.

5) Traci E LaMoia, Gerald I Shulman, Cellular and Molecular Mechanisms of Metformin Action, Endocrine Reviews, Volume 42, Issue 1, February 2021, Pages 77–96, https://doi.org/10.1210/endrev/bnaa023

6) Rena, G., Hardie, D. G., & Pearson, E. R. (2017). The mechanisms of action of metformin. Diabetologia, 60(9), 1577–1585. https://doi.org/10.1007/s00125-017-4342-z

7) Genes in PPI network

    a) https://www.genecards.org/

    b) https://string-db.org/cgi/network?taskId=bRgj72gsumZs&sessionId=b6yDH8MigGXy

8) Gomez, A. R., Byun, H. R., Wu, S., Muhammad, A. K. M. G., Ikbariyeh, J., Chen, J., Muro, A., Li, L., Bernstein, K. E., Ainsworth, R., & Tourtellotte, W. G. (2025). Boosting angiotensin-converting enzyme (ACE) in microglia protects against Alzheimer's disease in 5xFAD mice. Nature Aging, 5(7), 1280–1294. https://doi.org/10.1038/s43587-025-00879-1

9) Futran-Sheinberg, R., Salvo, M., & Quitterer, U. (2026). Role of angiotensin-converting enzyme in cognitive aging: Evidence from preclinical and clinical studies. Frontiers in Drug Discovery, 6, 1855021. https://doi.org/10.3389/fddsv.2026.1855021

10) Jiang, X., Li, J., Yao, X., Ding, H., Gu, A., & Zhou, Z. (2024). Neuroprotective effects of dipeptidyl peptidase 4 inhibitor on Alzheimer's disease: A narrative review. Frontiers in Pharmacology, 15, 1361651. https://doi.org/10.3389/fphar.2024.1361651

11) Zhuge, F., Zheng, L., Pan, Y., Ni, L., Fu, Z., Shi, J., & Ni, Y. (2024). DPP-4 inhibition by linagliptin ameliorates age-related mild cognitive impairment by regulating microglia polarization in mice. Experimental Neurology, 373, 114689. https://doi.org/10.1016/j.expneurol.2024.114689

12) Rivera Antonio, A. M., Padilla Martínez, I. I., Torres-Ramos, M. A., & Rosales-Hernández, M. C. (2025). Myeloperoxidase as a therapeutic target for oxidative damage in Alzheimer's disease. Journal of Enzyme Inhibition and Medicinal Chemistry, 40(1), 2456282. https://doi.org/10.1080/14756366.2025.2456282

13) Cao, Y., Tang, K., Ma, P., Zhang, R., Yang, Y., Li, T., Zhang, Y., & Peng, X. (2025). The role of peripheral innate immune cells in Alzheimer's disease progression. Frontiers in Immunology, 16, 1616939. https://doi.org/10.3389/fimmu.2025.1616939

14) Agarwal, P., & Mishra, A. K. (2024). The role of sirtuin 1 in ageing and neurodegenerative disease: A molecular perspective. Ageing Research Reviews, 101, 102464. https://doi.org/10.1016/j.arr.2024.102464

15) Mubarak, M., Khan, A., & Bashir, S. (2025). Dual GSK3β/SIRT1 modulators for Alzheimer's: Mechanisms, drug discovery and future perspectives. Frontiers in Pharmacology, 16, 1662241. https://doi.org/10.3389/fphar.2025.1662241

16) Zhang, Z., Liu, X., Zhang, S., Song, Z., Lu, K., & Yang, W. (2024). A review and analysis of key biomarkers in Alzheimer's disease. Frontiers in Neuroscience, 18, 1358998. https://doi.org/10.3389/fnins.2024.1358998

17) Singh, R., Jain, S., Paliwal, V., Verma, K., Paliwal, S., & Sharma, S. (2024). Does metabolic manager show encouraging outcomes in Alzheimer's? Challenges and opportunity for protein tyrosine phosphatase 1B inhibitors. Drug Development Research, 85, e70026. https://doi.org/10.1002/ddr.70026

18) García-Ayllón M-S, Small DH, Avila J and Sáez-Valero J (2011) Revisiting the Role of Acetylcholinesterase in Alzheimer's Disease: Cross-Talk with P-tau and β-Amyloid. Front. Mol. Neurosci. 4:22. doi: 10.3389/fnmol.2011.00022

19) Suad Hamdan Almasoudi, Hayder M. Al-kuraishy, Ali I. Al-Gareeb, Duaa Eliwa, Athanasios Alexiou, Marios Papadakis, Gaber El-Saber Batiha, Role of mitogen-activated protein kinase inhibitors in Alzheimer's disease: Rouge of brain kinases, Brain Research Bulletin, Volume 224, 2025, 111296, ISSN 0361-9230, https://doi.org/10.1016/j.brainresbull.2025.111296.

20) Sharma, S., Zhang, Y., Akter, K. A., Nozohouri, S., Archie, S. R., Patel, D., Villalba, H., & Abbruscato, T. (2023). Permeability of Metformin across an In Vitro Blood–Brain Barrier Model during Normoxia and Oxygen-Glucose Deprivation Conditions: Role of Organic Cation Transporters (Octs). Pharmaceutics, 15(5), 1357. https://doi.org/10.3390/pharmaceutics15051357

21) Amyloid-β and Tau in Alzheimer's disease: pathogenesis, mechanisms, and interplay. (2025). In Cell Death and Disease (Vol. 17, p. 21). https://doi.org/10.1038/s41419-025-08186-8

22) Tiwari, P., Dwivedi, R., Kaushik, M. et al. Genetics and Epigenetics of Alzheimer's Disease: Understanding Pathogenesis and Exploring Therapeutic Potential. J Mol Neurosci 75, 72 (2025). https://doi.org/10.1007/s12031-025-02363-2

23) Andrade-Guerrero, J., Santiago-Balmaseda, A., Jeronimo-Aguilar, P., Vargas-Rodríguez, I., Cadena-Suárez, A. R., Sánchez-Garibay, C., Pozo-Molina, G., Méndez-Catalá, C. F., Cardenas-Aguayo, M.-d.-C., Diaz-Cintra, S., Pacheco-Herrero, M., Luna-Muñoz, J., & Soto-Rojas, L. O. (2023). Alzheimer's Disease: An Updated Overview of Its Genetics. International Journal of Molecular Sciences, 24(4), 3754. https://doi.org/10.3390/ijms24043754

24) Zhang, J., Zhang, Y., Wang, J. et al. Recent advances in Alzheimer's disease: mechanisms, clinical trials and new drug development strategies. Sig Transduct Target Ther 9, 211 (2024). https://doi.org/10.1038/s41392-024-01911-3

25) Mostafa, R. E., & Asaad, G. F. (2026). Bridging the gaps in Alzheimer's disease: a comprehensive review of current and emerging therapies. Inflammopharmacology, 34(4), 2219–2241. https://doi.org/10.1007/s10787-026-02151-3

26) Engelhardt, E., Resende, E. P. F., & Gomes, K. B. (2024). Physiopathological mechanisms underlying Alzheimer's disease: a narrative review. Dementia & neuropsychologia, 18, e2024VR01. https://doi.org/10.1590/1980-5764-DN-2024-VR01

27) Alzheimer's Association. (updated April 2025). FDA-Approved treatments for Alzheimer's. https://www.alz.org/getmedia/4f4ca289-a2c6-4df9-8cdf-390365bd477e/alzheimers-dementia-fda-approved-treatments-for-alzheimers-ts.pdf

28) Pinzi L, Bisi N and Rastelli G (2024) How drug repurposing can advance drug discovery: challenges and opportunities. Front. Drug Discov. 4:1460100. doi: 10.3389/fddsv.2024.1460100

29) R, Y., Sharma, S., C, M., Rangari, G. M., Misra, A. K., Rao, K. S., Nayak, B. S., Vennela, J., & Sandhyarani, S. (2026). Metformin Repurposing in Neurological Disorders: A Clinical Trial Landscape. Annals of neurosciences, 09727531261421807. Advance online publication. https://doi.org/10.1177/09727531261421807

30) Loan, Allison1,2,#; Syal, Charvi1,#; Lui, Margarita1; He, Ling3,*; Wang, Jing1,4,5,*. Promising use of metformin in treating neurological disorders: biomarker-guided therapies. Neural Regeneration Research 19(5):p 1045-1055, May 2024. | DOI: 10.4103/1673-5374.385286

31) Sneha Saha1, Anuranjita Kundu*, Network Pharmacology in Drug Discovery: Concepts, Methodology, Applications and Future Perspectives, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 6, 7044-7057. https://doi.org/ 10.5281/zenodo.20963918

32) Thombre, K.R., Gabhane, V., Gupta, K.R. et al. Network pharmacology in the multi-omics era: uncovering novel therapeutic strategies. Discov. Chem. 3, 111 (2026). https://doi.org/10.1007/s44371-026-00567-y

33) Berad, P., Vikas Dhole, Burli, S., & Kumbhar, P. (2025). Network Pharmacology in Drug Repurposing and Reprofiling: Unraveling the Interplay between Drugs and Targets. International Journal of Pharmaceutical Sciences, 3(3), 677–688. https://doi.org/10.5281/zenodo.14996147

34) (Alzheimer's Disease) AND "Homo sapiens"[porgn:__txid9606] - Gene - NCBI. (n.d.). https://www.ncbi.nlm.nih.gov/gene

35) UniProt. (n.d.). UniProt. https://www.uniprot.org/uniprotkb?query=(keyword:KW-0026)

36) ChEMBL.(n.d.). https://www.ebi.ac.uk/chembl/explore/targets/STATE_ID:TBdd_-fiaFoM0Jf2KCdkEA%3D%3D

37) Molecular Modelling Group of the Swiss Institute of Bioinformatics. (n.d.). SwissTargetPrediction. https://swisstargetprediction.ch/

38) Liu, T. (n.d.). BindingDB BDBM50229665 1,1-Dimethylbiguanide::CHEMBL1431::METFORMIN::N,N-dimethylimidodicarbonimidic diamide.

https://www.bindingdb.org/rwd/bind/chemsearch/marvin/MolStructure.jsp?monomerid=50229665#

39) Cascella, R., & Cecchi, C. (2021). Calcium Dyshomeostasis in Alzheimer's Disease Pathogenesis. International Journal of Molecular Sciences, 22(9), 4914. https://doi.org/10.3390/ijms22094914

40) Penzes, P., & Vanleeuwen, J. E. (2011). Impaired regulation of synaptic actin cytoskeleton in Alzheimer's disease. Brain research reviews, 67(1-2), 184–192. https://doi.org/10.1016/j.brainresrev.2011.01.003

41) Carbone, M. G., Pomara, N., Callegari, C., Marazziti, D., & Imbimbo, B. P. (2022). Type 2 Diabetes Mellitus, Platelet Activation and Alzheimer's Disease: A Possible Connection. Clinical neuropsychiatry, 19(6), 370–378. https://doi.org/10.36131/cnfioritieditore20220604

42) RCSB PDB crystal structures used

a) Cheung, J., Rudolph, M., Burshteyn, F., Cassidy, M., Gary, E., Love, J., Height, J., & Franklin, M. (2012). Crystal structure of recombinant human acetylcholinesterase in complex with donepezil (PDB ID: 4EY7). RCSB Protein Data Bank. https://doi.org/10.2210/pdb4EY7/pdb

b) Ohori, M., Kinoshita, T., Okubo, M., Sato, K., Yamazaki, A., Arakawa, H., Nishimura, S., Inamura, N., Nakajima, H., Neya, M., Miyake, H., & Fujii, T. (2005). Identification of a selective ERK inhibitor and structural determination of the inhibitor–ERK2 complex. Biochemical and Biophysical Research Communications, 336(1), 357–363. https://doi.org/10.1016/j.bbrc.2005.08.082

   i) Kinoshita, T. (2005). The structure of ERK2 in complex with a small molecule inhibitor (PDB ID: 1TVO). RCSB Protein Data Bank. https://doi.org/10.2210/pdb1TVO/pdb

c) Dai, H., Case, A. W., Riera, T. V., Considine, T., Lee, J. E., Hamuro, Y., Zhao, H., Jiang, Y., Sweitzer, S. M., Pietrak, B., Schwartz, B., Blum, C. A., Disch, J. S., Caldwell, R., Szczepankiewicz, B., Oalmann, C., Ng, P. Y., White, B. H., Casaubon, R., … Ellis, J. L. (2015). Crystallographic structure of a small molecule SIRT1 activator–enzyme complex. Nature Communications, 6, 7645. https://doi.org/10.1038/ncomms8645

   i) Dai, H. (2015). SIRT1/Activator complex (PDB ID: 4ZZH). RCSB Protein Data Bank. https://doi.org/10.2210/pdb4ZZH/pdb

43) Softwares in Docking

a) AutoDock Vina 1.2.0

   i) Eberhardt, J., Santos-Martins, D., Tillack, A. F., & Forli, S. (2021). AutoDock Vina 1.2.0: New docking methods, expanded force field, and Python bindings. Journal of Chemical Information and Modeling, 61(8), 3891–3898. https://doi.org/10.1021/acs.jcim.1c00203

b) Open-Source PyMOL build

   i) Schrödinger, LLC. (2025). Open-Source PyMOL (Version 3.2). https://github.com/schrodinger/pymol-open-source

c) UCSF ChimeraX

   i) Pettersen, E. F., Goddard, T. D., Huang, C. C., Meng, E. C., Couch, G. S., Croll, T. I., Morris, J. H., & Ferrin, T. E. (2021). UCSF ChimeraX: Structure visualization for researchers, educators, and developers. Protein Science, 30(1), 70–82. https://doi.org/10.1002/pro.3943

d) Meeko

i) Forli, S., & the AutoDock development team. (2024). Meeko (Version 0.6.1) [Computer software]. GitHub. https://github.com/forlilab/Meeko

e) OpenBabel

i) O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. Journal of Cheminformatics, 3, 33. https://doi.org/10.1186/1758-2946-3-33

44) Du, Y., Du, Y., Zhang, Y. et al. MKP-1 reduces Aβ generation and alleviates cognitive impairments in Alzheimer's disease models. Sig Transduct Target Ther 4, 58 (2019). https://doi.org/10.1038/s41392-019-0091-4

45) Yang, M.-H., Ho, T.-C., Chang, C.-C., Su, Y.-S., Yuan, C.-H., Chuang, K.-P., & Tyan, Y.-C. (2023). Utilizing Proteomic Approaches to Uncover the Neuroprotective Effects of ACE Inhibitors: Implications for Alzheimer's Disease Treatment. Molecules, 28(16), 5938. https://doi.org/10.3390/molecules28165938

46) Mehramiz, M., Porter, T., O'Brien, E. K., Rainey-Smith, S. R., & Laws, S. M. (2023). A potential role for Sirtuin-1 in Alzheimer's disease: Reviewing the biological and environmental evidence. Journal of Alzheimer S Disease Reports, 7(1), 823–843. https://doi.org/10.3233/adr-220088

47) Torres, P. H. M., Sodero, A. C. R., Jofily, P., & Silva-Jr, F. P. (2019). Key Topics in Molecular Docking for Drug Design. International Journal of Molecular Sciences, 20(18), 4574. https://doi.org/10.3390/ijms20184574

48) Genheden, S., & Ryde, U. (2015). The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. Expert Opinion on Drug Discovery, 10(5), 449–461. https://doi.org/10.1517/17460441.2015.1032936

49) Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Research, 13(11), 2498–2504. https://doi.org/10.1101/gr.1239303

Supplementary Materials:-

1) Supplementary Table S1: Full list of 808 AD-associated genes
2) Supplementary Table S2: Metformin targets combined - ChEMBL, SwissTargetPrediction, BindingDB
3) Supplementary Table S3: 19 common targets
4) Supplementary Table S4: Full PPI network topology metrics
5) Supplementary Table S5: Full GO & KEGG enrichment results
6) Supplementary Table S6: All docking poses for AChE, ACE, MAPK1, SIRT1
7) Supplementary Code: Full docking notebook (metformin_dock-3.ipynb)