Original Article

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Gene Ontology-based Functional Enrichment and PPI Network Mapping of APOE4-associated Genes in Alzheimer's Disease: A Method to Identify Potential Therapeutic Targets

Public Health Section

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ABSTRACT

Introduction: Apolipoprotein E (APOE) is a protein involved in cholesterol circulation and has been reported to play a role in neurodegenerative conditions such as Alzheimer's Disease (AD). Improper lipidation of APOE leads to aggregation of transmembrane amyloid-β (Aβ) proteins and hyperphosphorylation of tau proteins in AD. APOE4, a variant of the apolipoprotein E gene, affects lipid transport, amyloid-β clearance, and neuroinflammation, thereby increasing the risk of neurodegenerative diseases. It also exhibits distinct DNA methylation patterns and post-translational modifications that alter its stability and function compared to other isoforms.

Aim: To identify and evaluate APOE4-interacting proteins as potential therapeutic targets using functional enrichment tools.

Materials and Methods: This computational bioinformatics study was carried out using open-source, web-based databases between January and September 2024. APOE protein–protein interaction (PPI) networks were constructed using STRING database (v11.5), GeneMANIA (v3.6.0), and BioGRID (v4.4.212) with a confidence cut-off score of 0.5. Interacting proteins were ranked based on interaction scores, and the top 10 candidates were selected for further functional analysis. Results were summarised in tabular and graphical formats.

Results: A total of 32 genes were found to be associated with lipoprotein receptor activity, carbohydrate metabolism, cholesterol metabolism, and ribosome biogenesis, involving glucagon signalling and HIF-1 signalling pathways. Amyloid Precursor Protein (APP), Microtubule-Associated Protein Tau (MAPT), and Triggering Receptor Expressed on Myeloid cells 2 (TREM2) were repeatedly identified across multiple tools, with the highest confidence scores of 0.998, 0.997, and 0.995, respectively, in STRING database, and scores of 0.586 and 0.474 for APP and MAPT, in GeneMANIA. These three genes were present among other genes involved in apolipoprotein binding, with an enrichment score of 216.5 provided by the DAVID database.

Conclusion: APP and MAPT were identified as promising druggable targets, highlighting their importance in AD. In the present study, APOE4-interacting, drug-targetable, functionally enriched AD-related genes were shortlisted from hundreds of proteins reported across different Gene Ontology (GO) databases.

Keywords: Apolipoproteins, Biomarkers, Epigenesis, Neurodegeneration, Neuroinflammation, Polymorphism, Proteomics, Transcriptome

INTRODUCTION

The AD is a neurodegenerative disorder characterised by symptoms such as short-term memory loss, dementia, and difficulty with motor skills, often leading to severe cognitive decline [1,2]. The presence of extracellular Aβ protein aggregates and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein are hallmark pathological features of AD [3].

APOE is a glycoprotein consisting of 299 amino acid and is involved in various biological functions, including glucose metabolism, neuronal signalling pathways, and neuroinflammation [4]. It is primarily produced by astrocytes but can also be synthesised by microglial cells under stress conditions. APOE acts as a cofactor in enzyme activation and serves as a ligand for various receptors. It plays a crucial role in the synthesis, clearance, aggregation, and degradation of Aβ. Studies have shown that the association of APOE with Aβ is observed in cognitively healthy elderly individuals, while mild cognitive impairment exhibits the inevitable influence of APOE4 in AD [5].

The functions of APOE are not limited to amyloid-β regulation but also extend to lipid transport and redistribution between cells. It aids in neuronal repair by delivering lipids for membrane synthesis and is also involved in cytokine signalling. Additionally, APOE influences permeability and transport across the Blood-Brain Barrier (BBB).

The presence of one allele of APOE4 (E4/E3) increases the risk of AD by 2-3 fold, while APOE4 homozygous carriers exhibit a 9-15 fold increased risk. In contrast, a homozygous APOE2 genotype reduces the risk of AD by 66% compared to E2/E3, 87% compared to homozygous APOE3, and 99.6% compared to homozygous APOE4 alleles [6,7].

Lipidation of APOE is mediated by ATP-binding cassette transporter A1 (ABCA1) in astrocytes, neurons, and the choroid plexus [8]. Poor lipidation of APOE4 favours its binding to Aβ, leading to the formation of ApoE-Aβ heteromers [9]. The ApoE4-Aβ complex interacts with Very Low-Density Lipoprotein Receptors (VLDLR), resulting in internalisation with slower rates of endocytosis. Studies support the influence of APOE isoforms on increasing the rate of longitudinal Aβ accumulation in the order of E4 > E3 > E2, along with effects on lipid binding activity, tau hyperphosphorylation, and neuroinflammation [6].

The three APOE isoforms—APOE2, APOE3, and APOE4—differ by single amino acid substitutions at positions 112 and 158: APOE2 (Cys112, Cys158), APOE3 (Cys112, Arg158), and APOE4 (Arg112, Arg158), which significantly affect protein folding and function [7,8]. APOE binds as a ligand to the Low-Density Lipoprotein Receptor (LDLR) family, which includes VLDLR, LRP8, LRP4, LRP1, LRP1B, LRP2, and LR11/SorLA [6].To date, five drugs have been approved for the treatment of AD.

Donepezil, galantamine, and rivastigmine are acetylcholinesterase inhibitors, whereas drugs such as memantine are NMDA receptor antagonists which is also used in combination with donepezil [10,11].

Limited information is available regarding APOE4 interactions with tau protein, and these interactions are often overlooked due to the strong presence of Aβ aggregates. However, earlier studies suggest that APOE4 binding to tau leads to phosphorylation, resulting in hyperphosphorylated tau proteins [3]. Overexpression of the C-terminal region of APOE results in various complications, including hyperphosphorylated tau and neuronal damage. The limited and sometimes contradictory studies on the relationship between APOE and Neurofibrillary Tangles (NFTs) caused by tau proteins have resulted in an incomplete understanding of this association. Previous studies suggest that APOE2 may contribute more significantly to tau pathology than APOE4; however, the role of APOE in tau-related pathologies remains unclear [12].

Given the versatile influence of APOE across multiple biological functions, the exact mechanism by which APOE affects tau pathology remains a subject of active investigation. Although studies have demonstrated an increased risk and faster disease progression of AD due to tau-mediated neurodegeneration independent of Aβ in mouse models, it remains undetermined whether these findings can be extrapolated to humans, and the underlying mechanisms are yet to be fully elucidated [13]. Additionally, studies reveal that selective removal of APOE4 from neurons significantly reduces tau-associated gliosis and neurodegeneration; however, it is unclear whether APOE4 influences tau pathology during the early or later stages of the disease [14].

In the present study, the model involves the selection of closely interacting APOE4 proteins through functional enrichment analysis and ranking of top-level functionally associated proteins using open-source, web-based Gene Ontology (GO) tools. For the selection of top-level APOE protein interactors through PPI analysis, the APOE gene name was used as a single-word search query across multiple GO databases, followed by additional databases for functional analysis to identify potential drug targets for AD.

MATERIALS AND METHODS

APOE PPI analysis: This in-silico bioinformatics-based study was conducted at our university between January and September 2024. This research did not involve human participants or animal subjects; therefore, ethical approval was not required. All methods utilised open-source, web-based GO databases. Stepwise selection of APOE protein interactors through PPI analysis and ranking based on interaction scores were performed as described in [Table/Fig-1]. The top-ranked interacting proteins from the top 10 strongly interacting protein lists were selected for functional enrichment analysis.

	Functional analysis	GO tools used	Results
Step 1	Selection of Top interactors of APOE protein	• string base • Genemania • BioGrid • ProteomeHD	[Table/Fig-2]
Step 2	Functional enrichment analysis and grouping	• David • ShinyGo • GoNet Dice	[Table/Fig-3-5]
Step 3	Ranking of the genes based on total number of molecular functions involved.		[Table/Fig-6]
Step 4	Functional enrichment analysis, gene clustering and APOE gene-drug interactions.		[Table/Fig-2-5,7,8]

[Table/Fig-1]: Step-by-step analysis of APOE PPI interactions in GO databases.

In STRING database, a confidence cut-off score of 0.50 was applied; GeneMANIA was used with its default settings (version 3.6.0); and BioGRID version 4.4.212 was employed. A confidence score cut-off of 0.5 indicates a 50% probability of false-positive interactions. While higher cut-off scores may increase precision, they may also exclude true interactions. Therefore, a 0.5 cut-off was considered optimal for this study.

PPI analysis using STRING database database: STRING database version 11.5 is an open-source online database (www.string-db.org) [15]. The data are represented as functional interaction scores derived from literature, curated databases, and experimental evidence. APOE was used as the keyword, and node-to-node interaction data were downloaded in tabular text format and saved as an Excel file. Top-level interacting proteins were selected based on the highest interaction scores [16,17].

PPI analysis using Genemania: GeneMANIA (version 3.6.0) is an open-source gene network search engine freely available at www.genemania.org [18]. It constructs gene interaction networks using functional interaction data obtained from multiple resources. APOE was used as the search keyword, and the resulting functional interaction data were downloaded in Excel format.

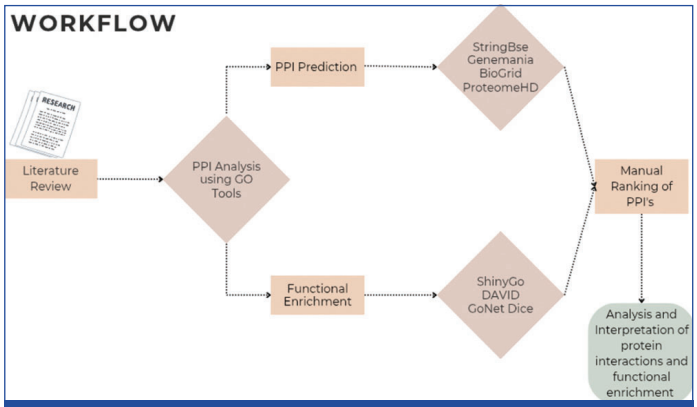
PPI analysis using BioGrid: BioGRID version 4.4.209 is a freely available open-source database that provides curated biological interaction data based on expert input and literature evidence [19]. Searching for a gene of interest yields information on protein interactions, chemical interactions, and network details. APOE was used as the search keyword, and the resulting data were downloaded in BioGRID format and converted into MS Excel for ranking strongly interacting APOE proteins.

PPI analysis using ProteomeHD: ProteomeHD is an open web-based resource available at www.proteomeHD.net [20]. PPI data are curated from experimental proteomics datasets and cellular functional analyses. The co-regulation score threshold was set at 0.9, and interaction data were downloaded in MS Excel format to identify strongly interacting proteins based on score values.

Functional enrichment analysis using DAVID: The Database for Annotation, Visualisation and Integrated Discovery (DAVID) is an open-source functional annotation tool available at https://david.ncifcrf.gov/home.jsp [21]. The top-level APOE interactor gene list was converted using the DAVID Gene ID Conversion Tool, and the results were downloaded in MS Excel format for molecular functional analysis.

Functional enrichment analysis using ShinyGO: ShinyGO version 0.76 is an open-source functional enrichment analysis tool available at http://bioinformatics.sdstate.edu/go/ [22]. Groups of genes involved in high-level molecular functional enrichment were obtained and represented in tabular and PNG formats.

Functional enrichment analysis and grouping of functional gene partners using GoNET DICE: The Database of Immune Cell Expression (DICE) is a freely available web-based tool accessible at http://tools.dice-database.org/GOnet/ [23]. It performs GO term annotation and gene enrichment analyses using lists of human genes. The tool generates functional data along with graphical interactive network visualisations [Table/Fig-2].



[Table/Fig-2]: A flowchart representing the workflow and tools used for Protein Protein Interaction (PPI) and functional enrichment.

RESULTS

In the first step, APOE was used as a keyword in four GO web databases (STRING database, GeneMANIA, BioGRID, and ProteomeHD), and several hundred protein–protein interactions (PPIs) were identified. The PPI data were downloaded along with interaction scores and are presented in [Table/Fig-3]. The total number of PPIs identified in STRING database, GeneMANIA, BioGRID, and ProteomeHD were 75, 478, 107, and 1078, respectively. From each database, the top 10 strongly interacting proteins of APOE, with the highest PPI scores, were listed in descending order. This table summarises the total number of PPIs identified in STRING database, GeneMANIA, BioGRID, and ProteomeHD. These gene ontology tools do not provide direct confidence interval scores; instead, each uses its own algorithm to predict interaction scores or indirect confidence measures. STRING database employs confidence interval scores to indicate the likelihood of protein -protein interactions, whereas GeneMANIA provides a composite network score derived from multiple data sources. BioGRID incorporates confidence scores based on the quality and reliability of experimental evidence, and ProteomeHD similarly assigns interaction scores based on experimental proteomics data.

S. No.	STRING database		GeneMania		BioGrid		Promote HD	
	Gene	Score	Gene	Score	Gene	Score	Gene	Score
1	APOA1	0.997	CNTF	1	UQCRC2	1.813	WDR46	1.000
2	APOB	0.998	TMCC2	0.885	ATP5B	1.810	UTP18	0.048
3	APP	0.998	ZIC1	0.765	UQCRC1	1.785	UTP11	0.045
4	LDLR	0.998	LRP8	0.618	LRRC59	1.736	UTP3	0.037
5	LRP1	0.999	VLDLR	0.605	POR	1.707	NGDN	0.036
6	LRP8	0.999	APP	0.586	PDHA1	1.680	DNTTIP2	0.034
7	MAPT	0.997	APOA1	0.494	PHB2	1.677	DDX49	0.032
8	SORL1	0.995	LRP8	0.474	PDHB	1.675	NAT10	0.029
9	TREM2	0.995	MAPT	0.474	ACTB	1.675	NOP14	0.027
10	VLDLR	0.997	APOA5	0.333	CANX	1.668	RRP8	0.026

[Table/Fig-3]: Protein-Protein interactions of APOE.

A total of 32 APOE-interacting genes were identified. Among these, genes such as APOA1, APP, MAPT, VLDLR, and CNTF were found to be repeated twice, while LRP8 was repeated three times across different GO databases. These 32 genes (listed in [Table/Fig-4]) were selected for functional enrichment analysis using other GO databases.

The functional categorisation of gene clusters in various combinations and numbers, along with their respective GO terms and pathways, is represented in [Table/Fig-5-7]. The majority of gene clusters were associated with reelin receptor activity, lipoprotein receptor activity, carbohydrate metabolism, cholesterol metabolism, ribosome biogenesis, RNA binding, and enzyme binding. High-level functional enrichment was predominantly observed in reelin receptor activity, lipoprotein binding, and receptor activity.

Further, the top 32 APOE-interacting genes were ranked based on the number of times they were functionally involved, as reported by ShinyGO, DAVID, and GoNET databases. The ranked gene list, with the highest number of functional involvements, is shown in descending order in [Table/Fig-8].

PPI interaction analysis of the top-ranked 32 genes using STRING database resulted in a graphical network comprising two distinct gene clusters with varying interaction scores, yielding a total of 102 protein interactions. In ShinyGO, the functional network relationships of these 32 genes were described under 13 GO terms, involving various metabolic activities and signalling pathways [Table/Fig-9]. Different groups of 2-4 gene clusters exhibited up to 60-fold functional enrichment across multiple metabolic activities and signalling pathways [Table/Fig-10]. High-level functional enrichment (>40) was observed for vitamin digestion and absorption, cholesterol metabolism, and citric acid metabolism.

S. No.	Description	Gene Ontology Term	Genes	Enrichment
1	Lipoprotein particle binding	GO:0030229~very-low-density lipoprotein particle receptor activity	LDLR, VLDLR, LRP8	440.8
		GO:0034189~very-low-density lipoprotein particle binding	VLDLR, TREM2	293.9
		GO:0071813~lipoprotein particle binding	LDLR, MAPT, TREM2	220.4
		GO:0034185~apolipoprotein binding	LRP1, CANX, MAPT, VLDLR, LRP8, TREM2, APP	216.5
		GO:0005041~low-density lipoprotein receptor activity	LDLR, LRP1, SORL1, VLDLR, LRP8	195.9
		GO:0008035~high-density lipoprotein particle binding	APOA1, LRP8, TREM2	135.6
		GO:0030169~low-density lipoprotein particle binding	LDLR, SORL1, TREM2	97.9
		GO:0050750~low-density lipoprotein particle receptor binding	APOB, APP	47
2	Pyruvate dehydrogenase activity	GO:0004739~pyruvate dehydrogenase (acetyl-transferring) activity	PDHB, PDHA1	391.8
		GO:0034604~pyruvate dehydrogenase (NAD+) activity	PDHB, PDHA1	195.9
3	Cargo receptor activity	GO:0038025~reelin receptor activity	VLDLR, LRP8	587.8
		GO:0038024~cargo receptor activity	LRP1, VLDLR, LRP8	88.1
4	Macromolecular complex binding	GO:0032050~clathrin heavy chain binding	LDLR, LRP1	130.6
		GO:0019894~kinesin binding	ACTB, LRP8	23.9
		GO:0019899~enzyme binding	APOA1, MAPT, POR, NOP14, APP	7.5
		GO:0044877~ macromolecular complex binding	UQCRC2, UQCRC1, LRP1, CNTF, TREM2	7
5	Specific Binding (Unique)	GO:0043395~heparan sulphate proteoglycan binding	LRP1, APP	61.8
		GO:0001540~beta-amyloid binding	LDLR, APOA1, LRP1, SORL1, LRP8, TREM2	41
		GO:0005543~phospholipid binding	APOA1, APOB, TREM2	14.6
6	Transmembrane	GO:0004888~ transmembrane signalling receptor activity	SORL1, LRP8, TREM2	9.3

[Table/Fig-4]: Functional Enrichment analysis of top ranked 32 APOE interacting gene clusters were grouped under 20 gene ontology terms in DAVID.

S. No.	Description	Pathways (click for details)	Fold Enrichment	Genes	Enrichment FDR
1	LDL Receptor-related	Low-density lipoprotein receptor repeats class B	438.7	5	0
		Low-density lipoprotein-receptor YWTD domain	361.3	5	0
		Mixed, incl. low-density lipoprotein receptor repeats class b and amnionless	263.2	3	0.00000094
		Mixed, incl. low-density lipoprotein-receptor ywtd domain and fasciclin	153.5	3	0.0000042
		Low-density lipoprotein receptor domain class A	139.6	5	0.0000000046
2	Amyloid/ Presenilin related	Mixed, incl. amyloid a4 and presenilin	223.3	2	0.0001
3	Apolipoprotein related	Mixed, incl. apolipoprotein a1/ a4/e domain and apolipoprotein c4	223.3	2	0.0001
		Mixed, incl. serum amyloid a proteins and apolipoprotein a1/a4/e domain	106.8	2	0.0004
		Mixed, incl. cholesterol metabolism and apolipoprotein I	94.5	3	0.000016
4	Coagulation related	Coagulation Factor Xa-inhibitory site	122.8	4	0.00000025
5	Ribosome biogenesis related	Ribosome biogenesis in eukaryotes and Sas10/Utp3/C1D family	149.8	5	0.0000000043
		Ribosome biogenesis in eukaryotes and soft-like domain	141.7	3	0.0000049
		Ribosome biogenesis in eukaryotes and Utp11 protein	140.4	4	0.00000016
		Mixed, incl. ribosome biogenesis in eukarvotes and sas10/uto3/c1d family	120.4	5	0.0000000067
		Dip2/Utp12 Family and ribosome biogenesis in eukaryotes	94.5	1	0.016
6	Cytokine related	Mixed, incl. lit/ osm family and interleukin 11	94.5	1	0.016
7	Complement/ EGF like	Complement Cir-like EGF-like	92.1	3	0.000016
8	Protease inhibitor-related	BPTI/Kunitz family of serine protease inhibitors	76.8	1	0.019
		Kunitz/Bovine pancreatic trypsin inhibitor domain	72.3	1	0.02
9	Immune receptor-related	SHP2-interacting transmembrane adaptor protein, SIT and Immunoglobulin V-set	61.4	1	0.023

[Table/Fig-5]: Functional enrichment analysis of top ranked 32 APOE interacting proteins in ShinyGO.

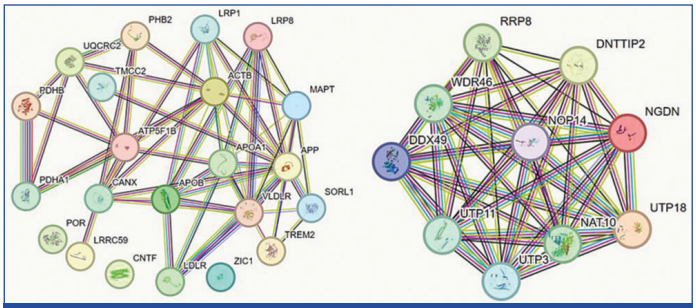
S. No.	Description	GO_Term_det	Genes	P_FDR_adj
1	Lipoprotein related	Low-density lipoprotein particle receptor activity	LDLR, LRP1, LRP8, SORL1, VLDLR	0.0000000001
		Lipoprotein particle receptor activity	LDLR, LRP1, LRP8, SORL1, VLDLR	0.0000000004
		Apolipoprotein binding	CANX, LRP1, LRP8, MAPT, TREM2, VLDLR	0
		Protein-lipid complex binding	APOA1, LDLR, LRP8, MAPT, SORL1, TREM2, VLDLR	0
		Lipoprotein particle binding	APOA1, LDLR, LRP8, MAPT, SORU, TREM2, VLDLR	0
		Very-low-density lipoprotein particle receptor activity	LOLR, LRP8, VLOLR	0.000000909
		High-density lipoprotein particle binding	APOA1, LRP8, TREM2	0.0000181
		Low-density lipoprotein particle binding	LOLR, SORU, TREM2	0.0000689
		Lipoprotein particle receptor binding	APOA1, APOB, LRP1	0.000292
		Very-low-density lipoprotein particle binding	TREM2, VLOLR	0.00041
2	Cargo receptor	Cargo receptor activity	LOLR, LRP1, LRP8, SORU, VLOLR	0.00000162
3	Special binding (Unique)	Amyloid-beta binding	APOA1, LDLR, LRP1, SORU, TREM2	0.00000196
4	Macromolecuar complex	Clathrin heavy chain binding	LOLR, LRP1	0.00295
		Enzyme binding	APOA1, APOB, APP, LDLR, LRP1, MAPT, NAT10, NOP14, SORL1, TREM2	0.00051
		Protein-containing complex binding	APOA1, CNTF, LOLR, LRP1, LRP8, MAPT, SORL1, TREM2, VLOLR	0.0000247
5	RNA related	RNA binding	CANX, LRP1, MAPT, NAT10, NGDN, NOP14, UTP3, WDR46	0.0144
6	Amide/Peptide binding	Amide binding	APOA1, LDLR, LRP1, SORU, TREM2	0.00174
		Peptide binding	APOA1, LDLR, LRP1, SORU, TREM2	0.000749
7	Lipid/Sterol transfer	Cholesterol transfer activity	APOA, APOB	0.0144
		Sterol transfer activity	APOA, APOB	0.0152

[Table/Fig-6]: Functional enrichment analysis of top ranked 32 APOE interacting proteins in GoNET –DICE.

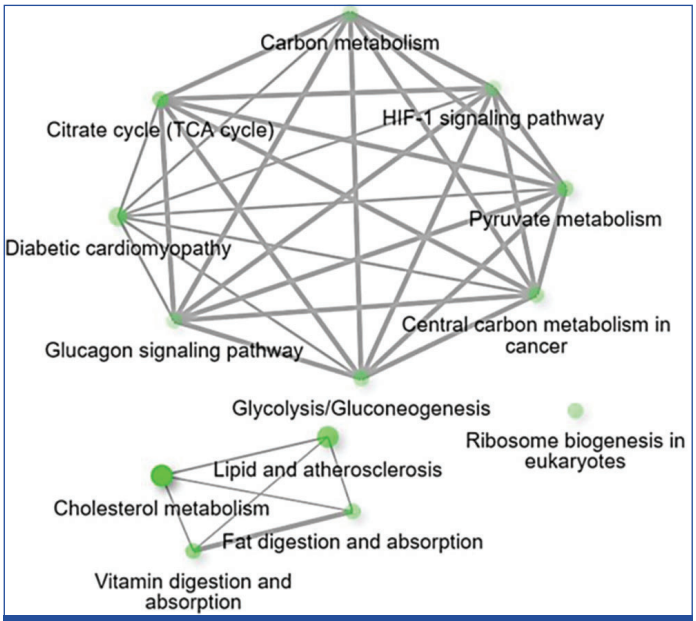
S. No.	Gene	Shiny GO Rank	Gene	David Rank	Gene	Go Net Rank
1	LDLR	12	LDLR	12	LRP1	126
2	APP	9	LRP8	11	LDLR	118
3	CNTF	9	LRP1	10	APP	106
4	UTP3	9	TREM2	10	APOA1	98
5	LRP1	8	BLDLR	8	MAPT	91
6	NAT10	8	APOAI	5	TREM2	89
7	NGDN	8	APP	5	VLDLR	74
8	NOP14	8	MAPT	5	APOB	72

9	WDR46	8	SORL1	5	LRP8	67
10	APOA1	7	CANX	4	CNTF	65
11	MAPT	7	APOB	3	SORL1	62
12	TREM2	7	PDHA1	3	POR	52
13	APOB	6	PDHB	3	ACTB	41
14	DDX49	6	ACTB	2	NAT10	34
15	RRP8	6	CNTF	2	PHB2	34
16	UTP11	6	DDX49	2	UTP3	29
17	UTP18	6	DNTT1P2	2	NGDN	28
18	POR	6	LRRC59	2	NOP14	26
19	ACTB	3	NAT10	2	UTP11	26
20	CANX	2	NGDN	2	WDR46	26
21	LRP8	2	NOP14	2	RRP8	24
22	SORL1	2	POR	2	DDX49	23
23	ZIC1	2	RRP8	2	UTP18	23
24	ATP5B	0	UQCRC2	2	ATP5B	22
25	DNTTIP2	0	UTP11	2	PDHA1	18
26	LRRC59	0	UTP3	2	PDHB	18
27	PDHA1	0	PHB2	1	UQCRC2	14
28	PDHB	0	TMCC2	1	UQCRC1	9
29	PHB2	0	UTP18	1	CANX	8
30	TMCC2	0	WDR46	1	ZIC1	8
31	UQCRC2	0	ZIC1	1	TMCC2	5
32	VLDLR	0	ATP5B	0	DNTTIP2	0

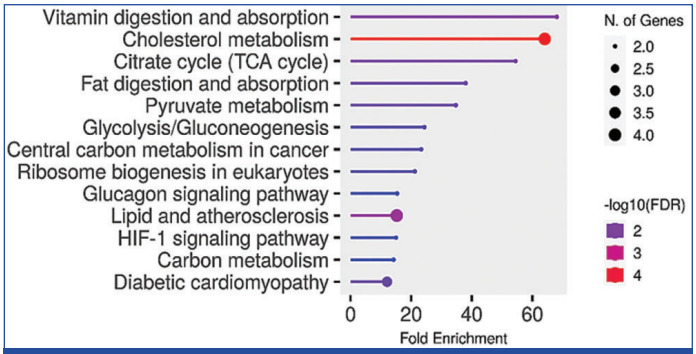
[Table/Fig-7]: Ranking of top ranked APOE genes based on their involvements in total number of functions.



[Table/Fig-8]: (Source: STRING database [15]) PPI interactions of top ranked 32 APOE interacting proteins in STRING database. A total of 102 proteins were found interacting in two different clusters with enrichment p-value of <1.0e-16.



[Table/Fig-9]: The above image obtained from ShinyGO site showcases the signalling pathways and their network of functional relationship of top ranked 32 genes in ShinyGO [22].



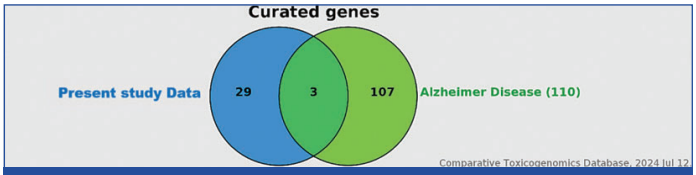
[Table/Fig-10]: Bar chart of ShinyGO, showing functional enhancement of 32 genes in a group of 2 to 4 genes involved in various disease related metabolic functions.

In STRING database, clusters comprising 2-5 genes were associated with specific human diseases, as shown in [Table/Fig-11]. Almost all gene clusters were related to brain or neurological disorders, including AD.

S. No.	Term description	Matching proteins in network
1	Alzheimer's Disease (AD)	APP, MAPT, TREM2
2	Familial hypercholesterolemia	APOB, LDLR
3	Lewy body dementia	APP, MAPT
4	Lipid metabolism disorder	APOB, APOA1, LDLR
5	Amyloidosis	APOA1, APP, ACTB
6	Hypolipoproteinemia	APOB, APOA1
7	Frontotemporal dementia	APP, MAPT
8	Familial visceral amyloidosis	APOA1, ACTB
9	Atherosclerosis	APOB, APOA1
10	Brain disease	APP, MAPT, TREM2, VLDLR, ACTB

[Table/Fig-11]: Different clusters of 32 genes associated with various diseases as reported in STRING database.

Disease-gene interaction analysis of the 32 genes was performed using www.ctdbase.org [24], which identified three genes—APP, MAPT, and TREM2—associated with 110 reported AD-related genes [Table/Fig-12]. In www.dgidb.org [25], the APP gene was reported to have 65 drug interactions across various human disorders, including 14 approved and 51 unapproved drugs. Of these, 14 drugs were considered for AD treatment, with Aducanumab and Inositol being approved [Table/Fig-13].



[Table/Fig-12]: Out of 32 top ranked genes, 3 curated genes namely APP, MAPT and TREM2 were reportedly associated with AD (110 genes) in MyGene Venn analysis. (courtesy: www.ctdbase.org).

The MAPT protein was associated with 65 approved and 376 unapproved drugs for human disorders; however, only Phenserine, an unapproved drug, was reported to be considered for AD treatment. No drug interactions were identified for the TREM2 protein. Through PPI analysis and cross-verification of top-ranked genes using a well-established drug–disease database, APP and MAPT emerged with high functional rankings, suggesting their potential as promising drug targets in AD.

DISCUSSION

Recently, web-based GO databases have expanded significantly, accumulating extensive disease gene data and functional interaction relationships. Much of this information is available as open-source data, providing promising approaches for identifying novel drug targets and disease-specific genes. It can be inferred that certain

S. No.	Drug	Stage	Regulatory approval	Interaction score
1	Aducanumab	Approved (FDA 2021, limited use)	Approved	3.23
2	Gantenerumab	Clinical Trial (Phase III, discontinued 2022)	Not Approved	2.42
3	Bapineuzumab	Clinical Trial (Phase III, failed)	Not Approved	2.42
4	Ponezumab	Clinical Trial (Phase II)	Not Approved	2.42
5	Solanezumab	Clinical Trial (Phase III, discontinued)	Not Approved	2.42
6	Tramiprosate	Clinical Trial (Phase III, discontinued)	Not Approved	2.42
7	Valiltramiprosate	Clinical Trial (Phase III – ongoing in some countries)	Not Approved	1.62
8	Lecanemab	Approved (FDA 2023)	Approved	1.62
9	Crenezumab	Clinical Trial (Phase III, negative results)	Not Approved	0.81
10	Caprospinol	Preclinical	Not Approved	0.81
11	Vanutide cridificar	Clinical Trial (Phase II)	Not Approved	0.81
12	Methylinositol	Preclinical	Not Approved	0.27
13	Posiphen	Clinical Trial (Phase II)	Not Approved	0.27
14	Inositol	Approved (for other indications, not AD)	Approved	0.11

[Table/Fig-13]: List of APP-interacting drugs considered in the treatment of Alzheimer's Disease (AD) [25].

proteins consistently appear with high interaction scores across multiple GO databases. Furthermore, the functional relevance of top-interacting proteins can be assessed and grouped based on their degree of involvement in various biological processes.

In the present study, the approach involved grouping APOE-interacting proteins through functional network analysis and assigning rankings based on the frequency of their involvement in different functional roles across multiple GO databases. Identifying suitable therapeutic targets remains challenging due to the complexity of target properties. Although several drugs for AD are currently in various phases of clinical trials, existing treatments primarily delay disease progression rather than provide a cure. Recently, multiple anti-APOE drugs have entered development, considering the crucial intrinsic roles of APOE and its isoforms in AD pathology. The rapid growth in AD research suggests that effective treatment may require combination therapies targeting multiple pathways.

In the present PPI analysis, hundreds of APOE-interacting proteins were identified across updated GO databases, making the task of narrowing down top-ranked, druggable functional genes particularly challenging. Initially, 32 APOE-interacting proteins were shortlisted, and their functional roles were further explored using ShinyGO, DAVID, and GoNET. These tools provided insights into gene clusters, molecular pathways, functional networks, enrichment scores, and their potential significance in lipoprotein and carbohydrate metabolism, particularly in brain and neurological disorders, including AD.

The endocytosis of APOE particles is primarily mediated by LDLR and LRP1, which ranked highly in the present study analysis. Both proteins contribute to Aβ clearance and have been previously implicated in its transport across the BBB [26,27]. In addition to LDLR, which ranked highest in ShinyGO and DAVID analyses, other key genes such as APP and TREM2 were also identified as important contributors to AD pathology. Although the precise mechanisms underlying APOE interactions with these proteins remain unresolved, the ranking data provide valuable support for prioritising key proteins involved in AD disease mechanisms.

Previous findings suggest that overexpression of LDLR protects against tau protein–mediated pathology in AD by suppressing microglial activation and neurodegeneration [26,27], thereby contributing to the preservation of myelin integrity. Both LRP1 and LDLR play pivotal roles in regulating APOE-Aβ complexes across the BBB, while LDLR controls APOE metabolism and cholesterol transport in neurons and astrocytes. Studies using LDLR-deficient experimental mouse models have monitored APOE levels and demonstrated that functional LDLR activity reduces APOE levels. Similarly, studies involving LDLR-overexpressing mouse models of tau pathology have shown a 90% reduction in APOE levels, reinforcing the crucial role of LDLR in AD [26,27].

In tau-related pathology of AD, APOE induces microglial activation, leading to neurodegeneration. LDLR overexpression results in a reduced subset of activated microglial cells, including Disease-Associated Microglial (DAM) genes such as APOE and TREM2 [26,27]. LRP8, also known as APOER2, is an APOE receptor with high affinity for APOE-rich β-VLDL and comparatively lower affinity for LDL and VLDL. Binding of APOE to LRP8 increases Aβ production and APP endocytosis in an isoform-dependent manner [26,27]. The presence of the APOE4 allele disrupts the recycling of APOE receptors such as APOER2 (LRP8), leading to increased Aβ production. LRP8, along with VLDLR, has previously been reported as a core component of the Reelin signalling pathway [26,27].

APOE has also been shown to enhance β-secretase activity, which is crucial for the amyloidogenic cleavage of APP and can influence Aβ production through its association with cell-surface receptors of the LDL receptor (LRP) family [26,27]. APP processing occurs via two distinct pathways: amyloidogenic and non amyloidogenic [28,29].

In the non amyloidogenic pathway, α-secretase cleaves APP to generate sAPPα and α-CTF. γ-Secretase subsequently cleaves α-CTF to produce P3 peptides and the APP intracellular domain (AICD). Because γ-secretase cleaves within the Aβ region, no Aβ monomers are formed in this pathway. In the amyloidogenic pathway, sequential cleavage of APP by β-secretase and γ-secretase results in the formation of Aβ. β-site APP-cleaving enzyme 1 (BACE1) facilitates β-cleavage of APP at the N-terminus, generating CTF99, which is further cleaved by γ-secretase to release Aβ peptides and AICD [28,29]. The resulting Aβ peptide is typically 40 amino acids long and is generally considered less toxic; however, under certain conditions, cleavage results in longer Aβ species that are more aggregation-prone [28,29]. Due to their hydrophobic nature, Aβ peptides have a high tendency to aggregate. Accumulation of Aβ monomers leads to the formation of oligomers, which subsequently aggregate into amyloid plaques [28,29].

TREM2, ranked eleventh in ShinyGO, eighth in DAVID, and fifth in GoNET, plays an essential role in both amyloid- and tau-related pathologies in AD. Its functions include microglial sensing, regulation of phagocytosis, and modulation of inflammatory responses in microglia [28,29].

Apart from APOE interactions with Aβ, other significant interactions also contribute to AD progression. TREM2, a major genetic risk factor in AD, is an innate immunomodulatory receptor expressed on the microglial cell membrane and is one of the top interactors of APOE. The pathway involving APOE and TREM2 is a key regulator of microglial functions across several neurodegenerative diseases [30]. The hinge region of the APOE protein and the CDR1 and CDR2 regions of TREM2 have previously been identified as being involved in molecular recognition, with interaction strength varying depending on the APOE isoform. APOE4 exhibits greater binding affinity and stability with TREM2 than the E3 and E2 variants [30,31], which is reflected in the strong interaction scores observed between APOE and TREM2.

Neurons acquire cholesterol primarily through endocytosis of HDL-like APOE-containing lipoproteins. This process is mediated by multiple lipid-binding receptors, including LDLR, VLDLR, LRP8,

LRP4, LRP1, LRP1B, LRP2, and LR11/SorLA, which facilitate the internalisation of APOE–lipid complexes [32,33]. However, the precise mechanism of Aβ clearance mediated by APOE remains incompletely understood. Some studies suggest that APOE4 mediates Aβ clearance across BBB at a slower rate via LDL receptors. APOE interacts with specific LDL receptor–related proteins (LRPs) expressed on neuronal and glial cells, thereby facilitating the transport of extracellular lipoproteins between cells. Regulation of cholesterol homeostasis in both AD and cardiovascular diseases has been shown to be influenced by the structural organisation of APOE within HDL particles [30,31]. Additionally, APOE interacts with receptors expressed on myeloid cells, such as TREM2, to regulate Aβ plaque dynamics, while microglial TREM2-mediated barriers protect neurons from Aβ-induced damage by modulating inflammatory responses [34].

Although TREM2 emerged as a top interactor in the present study functional network analysis, very few drug candidates are currently available that specifically target this receptor. This limitation likely reflects existing knowledge gaps and mechanistic challenges rather than a lack of biological relevance. TREM2 functions as an innate immune receptor in microglia, and its signalling is complex, context-dependent, and strongly influenced by APOE isoforms [35]. Structural variability in APOE–TREM2 interactions, combined with limited understanding of downstream signalling pathways, has constrained drug discovery efforts to date. Future studies focusing on ligand-binding domains, signalling modulation, and the development of selective agonists or antibodies may help overcome these challenges [36].

Another important consideration is the potential curation bias in publicly available gene ontology and PPI databases. Genes such as APP and MAPT have been extensively studied, which may inflate their interaction scores compared with less-characterised but biologically relevant candidates. While this overrepresentation does not undermine their established roles in AD, it underscores the importance of validating highly ranked proteins using complementary datasets and experimental approaches to avoid overlooking less-explored yet promising therapeutic targets [37].

APP, whose cleavage leads to amyloid plaque formation through the action of β- and γ-secretases, has long been considered a potential therapeutic target. Numerous attempts have been made to target γ-secretase in order to inhibit the amyloidogenic pathway [38-40]. However, these interventions have not achieved the expected therapeutic efficacy, partly due to late-stage intervention when extensive amyloid aggregation has already occurred. Additionally, amyloid plaque formation may represent an early pathogenic event in AD, whereas MAPT (tau protein) pathology becomes dominant during later stages and drives disease progression. Importantly, APOE4 exhibits strong interactions with both major pathological targets of AD—APP and MAPT. Targeting APOE4 may therefore provide a strategy to modulate both amyloid and tau pathways; however, whether APOE4 regulation can simultaneously downregulate both remains to be determined.

Limitation(s)

This study involved ranking potential therapeutic targets based on the frequency of repeated protein interactions across multiple gene ontology tools. The confidence scores provided by each database are calculated using different algorithms. While this approach enables prioritisation of top interactors and potential therapeutic targets, additional analyses—such as protein docking, in-vitro functional assays, and in-vivo validation studies—are required to assess their true biological and therapeutic relevance.

CONCLUSION(S)

APP, MAPT, and TREM2 were identified as the top-ranked functionally enriched genes associated with AD. With the exception of TREM2, both APP and MAPT are established druggable targets in multiple human disorders. Present study findings highlight the potential of APP and MAPT as complementary targets to existing anti-Aβ and anti-tau therapies, suggesting that modulation of these proteins may enhance therapeutic efficacy or help overcome resistance in AD treatment. This study provides a prioritised list of functionally enriched, potentially drug-targetable AD-related proteins. Future investigations should include in-vitro validation of key PPIs, target-binding assays, and pathway modulation studies to bridge the gap between in-silico predictions and experimental drug discovery.

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