JIMKI
JURNAL ILMIAH MAHASISWA
KEDOKTERAN INDONESIA

e-ISSN : 2721-1924 p-ISSN : 2302-6391

Penelitian Asli

Exploring The Role of Mitochondria in Alzheimer with Network Pharmacology: A Bioinformatics Analysis

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disease marked by the pathological accumulation of beta-amyloid peptide and hyperphosphorylated tau. Growing body of evidence indicates that mitochondrial dysfunction plays a pivotal role in AD pathogenesis, by inducing neurotoxicity through the formation of oxidative stress and reactive oxygen species (ROS). This study aims to investigate relevant mitochondrial proteins in Alzheimer by employing network pharmacology. Protein-coding genes associated with AD were identified from the GeneCards database, extracting only proteins scoring ≥1 in relevancy. Datasets associated with mitochondria were extracted from the STRING database. 632 overlapping proteins from both keywords were further enriched and topologically analyzed. This study employed enrichment analyses using ShinyGO to identify relevant biological, cellular, and molecular processes, in addition to disease pathways. Topology analyses were conducted through STRING and Cytoscape by implementing four different centrality parameters and clustering, the proteins were further curated to obtain pivotal proteins in AD and their dysregulation. Aligned with our enrichment analyses, the proteins topologically relevant were components of the mitochondrial oxidative phosphorylation (OXPHOS) pathway, crucial to the respiratory electron transport chain and ATP synthesis system. This study provides a foundation for the discovery of multi-target drugs in AD therapy.

Keywords: Alzheimer, bioinformatics, mitochondria, network analysis, protein-protein interaction.

1. INTRODUCTION

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease worldwide, characterized by progressive cognitive decline and distinct pathological hallmark.[1] Its prevalence rises steeply with age and the condition imposes a growing social and economic burden on millions of aging populations.[2] AD affect 1 out of 9

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elderly and is ultimately fatal, remaining as the seventh leading cause of global mortality.[3] AD neuropathology is classically defined by extracellular β-amyloid (Aβ) plaque and intracellular neurofibrillary tangles (NFTs) of aggregated hyperphosphorylated tau, directly correlating impaired protein homeostasis with synaptic loss and neuronal death.[4] However, AD phenotypes is further complexified by persistent oxidative stress, dysregulated cellular energy metabolism, and chronic neuroinflammation documented in affected brains.[5,6] These molecular processes synergistically heightens the susceptibility of neuronal damage in AD onset and progression.[7]

In recent years, mitochondria has been postulated as critical regulator implicated in various neurodegenerative diseases.[8] This dynamic organelle, tightly regulated by mitochondrial protein homeostasis, plays a pivotal role in cellular energy production, metabolic regulation, calcium homeostasis, apoptosis, and mitophagy.[9,10] Neurons are reliant on mitochondria function to meet high bioenergetic demands, sustain neurotransmission, and regulate long-range axonal transport.[11–13] Consequently, mitochondrial protein disturbances can directly precipitate synaptic failure and neuroinflammation leading to neuronal death.[14] Notably, mitochondrial damage has been revealed to precede Aβ accumulation and tau pathology, underscoring its critical role as the earliest detectable changes in AD pathogenesis.[15,16] The level of mitochondrial DNA (mtDNA), essential source of cellular proteins, is reduced and excessively fragmented in AD neurons. Similarly, the hyperactivation of dynamin-related protein 1 (Drp1) has been found to accelerate mtDNA fragmentation and alter mitochondria dynamics in axons, dendrites, and synapses of AD patients.[17] Thereby, investigating the underlying mechanism behind mitochondrial dysfunction is crucial to understand the sequential neuronal degeneration in AD.

AD is a multifactorial, polygenic disease that remains incompletely characterized despite substantial advances in pathological research.[18] Current management of AD largely remains symptomatic and no disease-modifying therapy have been conclusively established, underscoring the need for alternative therapeutic strategies addressing the heterogenous mechanism involved in AD.[19,20] Emerging evidence has elucidated mitochondrial dysfunction as a promising target in AD.[21] Protein-protein interaction

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(PPI) analysis provides a systematic approach to identify the common protein in AD and mitochondria biological pathway, therefore representing an actionable pharmacological target.[22] Such insights may guide the development AD therapy.

2. METHOD

2.1 Data Collection

A search on the GeneCards database was conducted using the keyword “Alzheimer” in the “Protein Coding”.[23] Proteins with confidence score ≥5 were extracted. Meanwhile, the STRING database was searched using the keyword “Mitochondria” and proteins forming high confidence interaction (≥0.700).[24] Venn diagram analysis was performed to obtain overlapping proteins between the two sets.

Protein Topology Analysis and Protein Target Centrality

Protein topology analysis was performed using the STRING database via the Cytoscape software using the confidence score cut-off of 0.700. The visualised protein-protein interaction network (PPIN) contains nodes (proteins) and edges (interaction between proteins). Proteins failing to form interactions or outliers were removed from the network.

Cluster analysis was performed with the CytoCluster plug-in.[25] Next, PPIN of the main network and clusters are analyzed with the “Analyze Network” tool and the CytoNCA plug-in to obtain four centrality parameters, namely betweenness centrality (BC), closeness centrality (CC), degree centrality (DC), and eigenvector centrality (EC).[26] Top 5% of the nodes based on four centrality parameters were identified as hub proteins. The top ten nodes within each cluster were also ranked based on four centrality parameters.

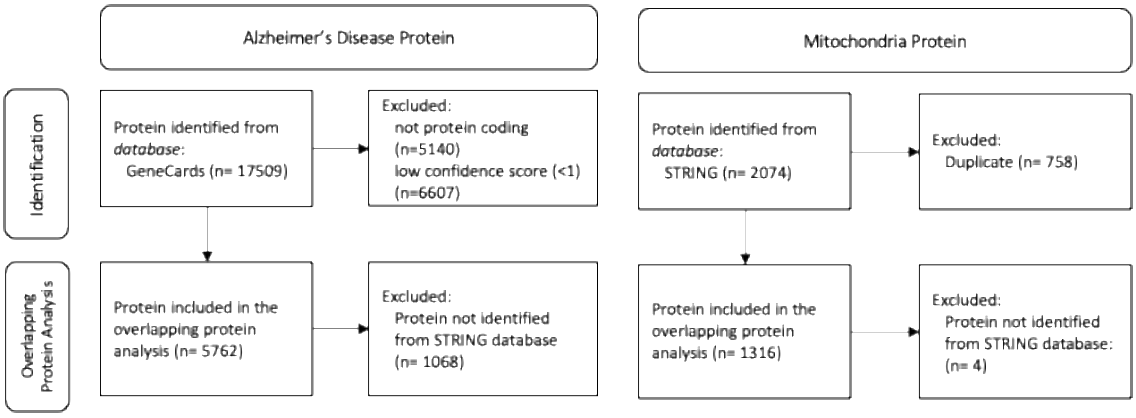

Figure 1. Flow diagram depicting the search result of alzheimer’s disease and mitochondria protein

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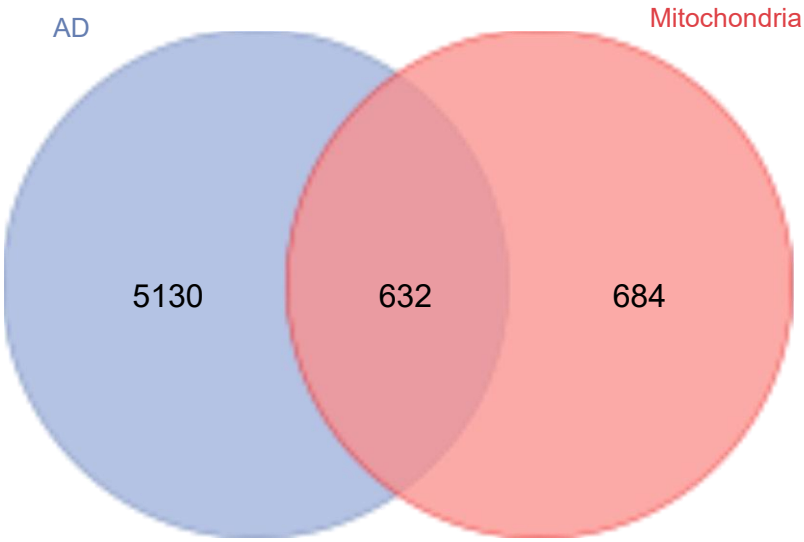

Figure 2. Venn diagram of protein related both to alzheimer's disease (AD) and mitochondria.

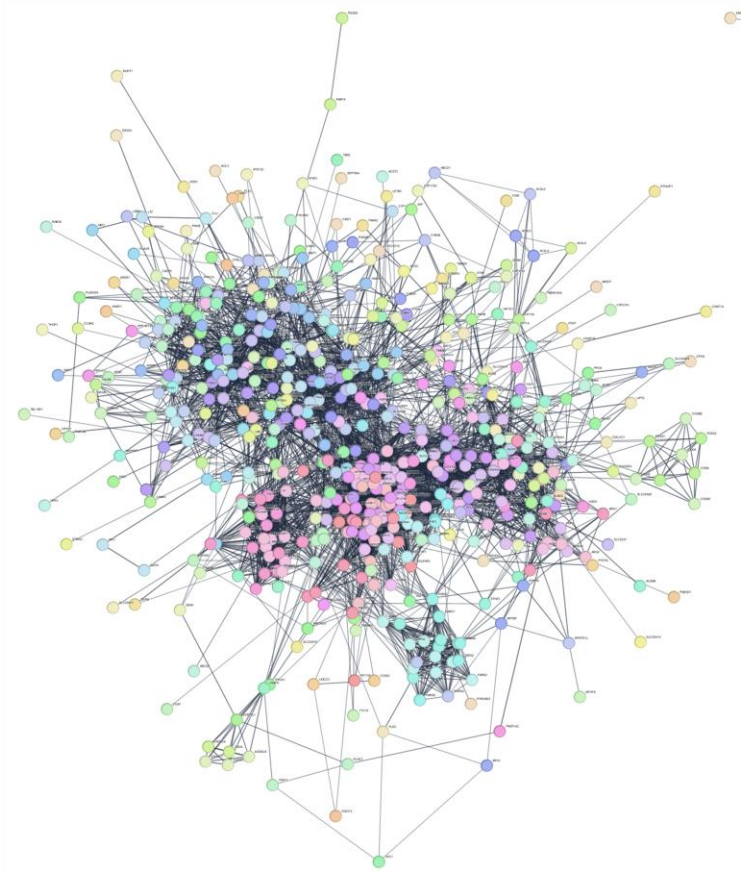

Figure 3. The PPIN of genes both related to Alzheimer's Disease and Mitochondria. Visualized through Cytoscape.

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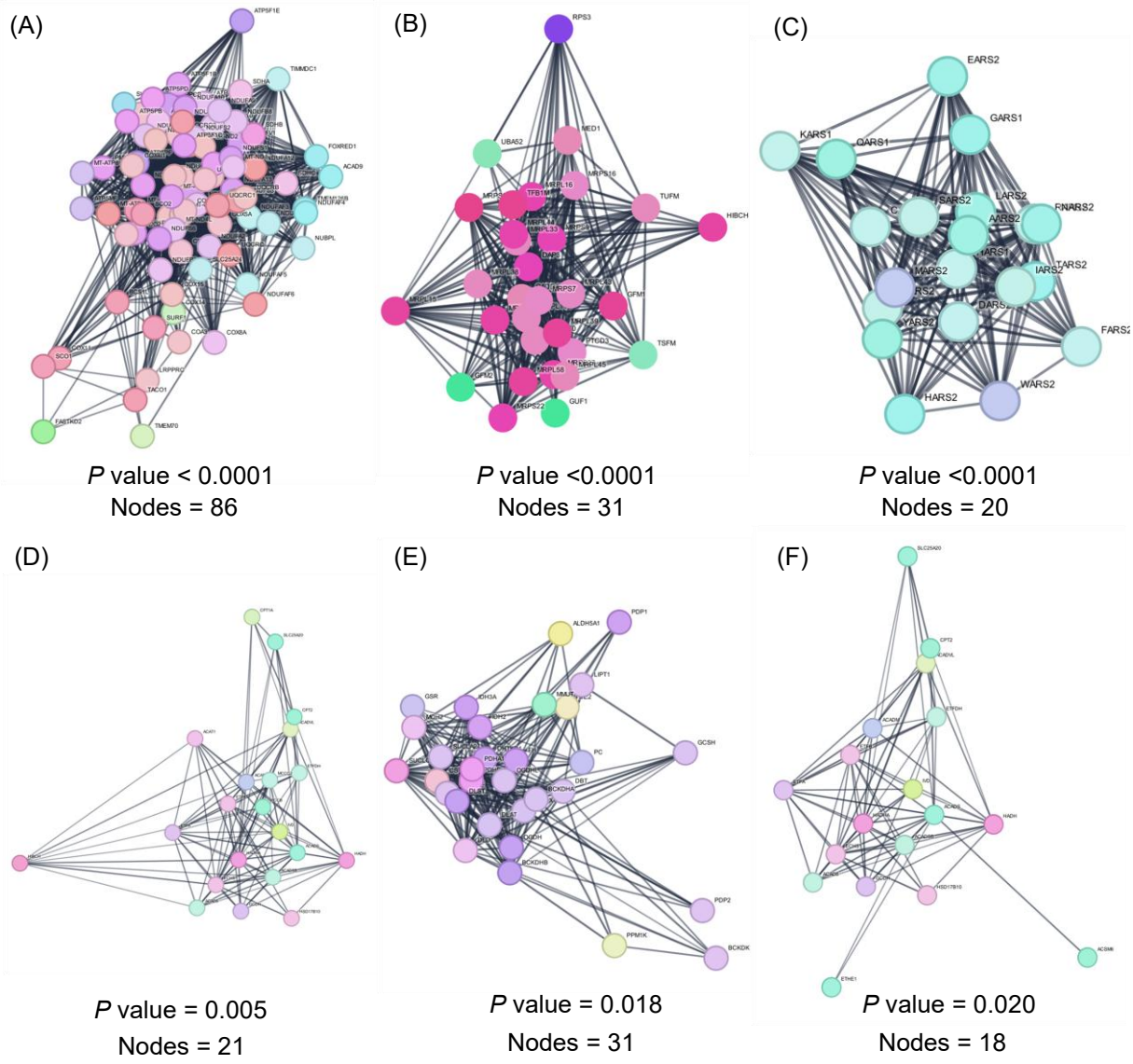

Figure 4. Prominent Clusters of the PPIN related to Alzheimer's Disease and Mitochondria. (A) Cluster 1, (B) Cluster 2, (C) Cluster 3, (D) Cluster 4, (E) Cluster 5, (F) Cluster 6. The *P* value less than 0.05 indicates significant results. Visualised through CytoCluster.

Protein Cluster Analysis

Six statistically significant ($p < 0.005$) clusters configured from the main network are defined by their primary biochemical role, then their components are further classified into specific functional categories. Protein classifications in this work were based on functional annotations from UniProtKB[27], Gene Ontology[28], KEGG Pathways[29], and the MitoCarta 3.0 mitochondrial proteome database[30], supported by established biochemical descriptions from Lehninger Principles of Biochemistry and standard reviews on oxidative phosphorylation, mitochondrial translation, aminoacyl-tRNA synthetases, fatty acid β-oxidation, and TCA cycle metabolism.[31]

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Enrichment Analysis

Hub proteins were enriched using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases on the ShinyGO 8.5 webserver.[32,33] GO annotation of biological processes, cellular components, and molecular functions, in addition to KEGG disease pathways were identified and visualised as bar plots.

3. RESULTS

Protein Identification

5.762 proteins related to AD and 1.316 mitochondrial proteins were identified **(Figure 1)**. A total of 632 proteins related to both AD and mitochondria were obtained from the Venn diagram analysis as shown on **Figure 2**. All overlapping proteins were successfully visualised with STRING database via Cytoscape (**Figure 3**). 52 outlier nodes were removed from the network.

Protein Topology and Centrality Analysis

The top 5% nodes of the overall PPIN and each statistically significant cluster was identified. The topology analysis of the six prominent clusters is illustrated in **Figure 4.** While the overall network centrality metrics is represented in **Table 1.** Notably, protein NDUFS1 and NDUFA9, representing the core components of mitochondrial oxidative phosphorylation (OXPHOS) machinery, are the central nodes with the highest value within the overall PPIN centrality analysis.

Within cluster 1, protein NDUFS1, NDUFS7, NDUFS3, and NDUFS8 consistently scored top across four centrality parameters, indicating their essential role in respiratory chain function. Meanwhile, protein MRPS7, MRPS9, MRPS16, MRPL33, MRPL11, MRPL16, and GFM1 are the top scoring nodes in cluster 2, comprising of ribosomal proteins and translation-associated factors. Protein QARS1, NARS2, IARS2, KARS1, AARS2, WARS2, EARS2, MARS2, and YARS2 characterized the centrality of cluster 3 as mitochondrial aminoacyl-tRNA synthetases. In cluster 4, protein with the highest centrality value included HADHA, ECHS1, ACADS, ACADM, HADH, ETFA, ETFB, ACADVL, ETFDH, and ACADSB, representing the fatty acid β-oxidation (FAO) enzymes and electron transfer flavoprotein system. Protein DLAT, DLD, PDHB, CS, DLST, PDHX, and SUCLG1 represents the nodes with consistently high centrality in cluster 5, consisting of pyruvate dehydrogenase, TCA cycle, α-ketoglutarate dehydrogenase family and related complexes in mitochondrial central carbon metabolism. Lastly, protein ACADS, HADHA, HADH, ETFB, ECHS1, ACADSB, ETFA, ETFDH, and ACADM are the highest nodes within the centrality analysis of cluster 6. This cluster mainly encompass acyl-

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Table 1. Centrality analysis of top 5% nodes within the PPIN based on four centrality parameters: degree centrality (DC), betweenness centrality (BC), closeness centrality (CC), and eigenvector centrality (EC).

Genes	Degree	Genes	Betweenness centrality	Genes	Closeness centrality	Genes
NDUFS3	87	TP53	28553.84	CYCS	0.455189	NDUFS3
TP53	84	CYCS	26509.52	VDAC1	0.444359	NDUFV1
NDUFS1	83	VDAC1	20161.75	TP53	0.440975	NDUFS2
NDUFV1	82	CS	18526.1	CS	0.439302	NDUFS1
CYCS	82	PPARGC1A	16380.36	SDHA	0.437311	NDUFS8
NDUFS2	81	HSPA9	12596.42	HSPA9	0.433059	NDUFB8
SDHA	80	PINK1	11724.08	ATP5F1A	0.431124	NDUFV2
NDUFS8	80	AKT1	11608.52	UQCRC2	0.430483	NDUFA9
UQCRC2	77	TUFM	11325.87	COX4I1	0.430163	UQCRC2
SDHB	76	BCL2	9758.649	ACO2	0.429844	NDUFS4
COX4I1	76	ACO2	9755.934	PPARGC1A	0.423555	NDUFA12
NDUFB8	75	MED1	9383.313	TFAM	0.422936	UQCRFS1
ATP5F1A	75	ATP5F1A	8755.368	SDHB	0.422628	CYC1
VDAC1	74	HSP90AA1	7674.66	SOD2	0.421704	NDUFS7
CYC1	74	MAPK8	7479.389	NDUFS3	0.419869	COX4I1
NDUFA9	73	SDHA	7430.074	TUFM	0.418958	COX5A
NDUFV2	73	PNPT1	6623.192	NDUFS1	0.417448	UQCRC1
UQCRFS1	73	SOD2	6491.087	ATP5F1C	0.416547	COX5B
CS	71	PRKN	6126.364	NDUFA9	0.415948	NDUFS6
NDUFS4	70	TFAM	5858.07	NDUFB8	0.415054	NDUFB9
NDUFS7	70	PPARA	5822.015	CYC1	0.412393	NDUFA13
COX5A	69	SRC	5781.65	PINK1	0.411807	UQCRB
UQCRC1	69	FXN	5636.263	NDUFS2	0.411222	NDUFA10
MT-CO1	68	IDH2	5414.828	UQCRFS1	0.409766	NDUFB10
COX5B	67	PLA2G6	5260.197	HSP90AA1	0.407746	NDUFA2
NDUFA12	67	KARS1	5097.068	NDUFS8	0.407746	UQCRQ
NDUFA13	66	TSFM	4915.185	HSPD1	0.407173	SDHB
ATP5F1C	66	NDUFS1	4898.115	NDUFV1	0.406316	MT-CO1
BCL2	65	GLS2	4856.356	MDH2	0.405746	MT-ND2
NDUFA10	63	DLD	4771.515	NDUFV2	0.405462	NDUFB3
NDUFS6	63	NDUFA9	4736.447	UQCRC1	0.405462	MT-ND1
MT-CO2	63	HSPA4	4697.66	MT-CO1	0.404895	COX6B1

CoA dehydrogenases and auxiliary enzymes.

Protein Cluster Analysis

A functional categorization of mitochondrial proteins associated with AD was performed to delineate their biological roles within cellular metabolism **(Table 2).** Cluster 1, which is the largest cluster consisting of 86 nodes, includes the complete set of electron transport chain (ETC)

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Table 2. Functional categorization of mitochondrial proteins associated with AD

Classification	Functional Category	Proteins
	CLUSTER 1 (n = 86)	
Mitochondrial Oxidative Phosphorylation (OXPHOS) Machinery	ETC Complex I (NADH:Ubiquinone Oxidoreductase)	NDUFA1, NDUFA2, NDUFA4, NDUFA9, NDUFA10, NDUFA11, NDUFA12, NDUFA13; NDUFB3, NDUFB8, NDUFB9, NDUFB10, NDUFB11; NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8; NDUFV1, NDUFV2; MT-ND1, MT-ND2, MT-ND4, MT-ND4L, MT-ND5, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, NDUFAF6; TMEM126B, TIMMDC1, ACAD9, OXRED1, NUBPL
	ETC Complex II (Succinate Dehydrogenase)	SDHA, SDHB, SDHC
	ETC Complex III (Cytochrome bc1 Complex)	UQCRC1, UQCRC2, UQCRB, UQCRQ, UQCRFS1, CYC1, MT-CYB, BCS1L
	ETC Complex IV (Cytochrome c Oxidase)	COX4I1, COX5A, COX5B, COX6B1, COX7B, COX8A, COX10, COX11, COX14, COX15, SCO1, SCO2, COA3, SURF1, MT-CO1, MT-CO2, MT-CO3, TACO1
	ETC Complex V (ATP Synthase)	ATP5F1A, ATP5F1B, ATP5F1C, ATP5F1D, ATP5F1E, ATP5PF, ATP5PO, ATP5PB, ATP5PD, ATP5MF, TMEM70, MT-ATP6, MT-ATP8
	Mitochondrial Transport & Carrier Proteins	SLC25A3, SLC25A24
	Mitochondrial RNA Translation / mRNA Stability Factors	LRPPRC, FASTKD2
	Mitochondrial Protein Processing	PMPCA, PMPCB
	CLUSTER 2 (n = 31)	
Mitochondrial Translation System	Ribosomal Proteins MRPS	MRPS7, MRPS9, MRPS16, MRPS22, MRPS27, MRPS30, MRPS35
	Ribosomal Proteins MRPL	MRPL10, MRPL11, MRPL15, MRPL16, MRPL33, MRPL38, MRPL39, MRPL43, MRPL44, MRPL45, MRPL58
	Translation Factors	TUFM, TSFM, GFM1, GFM2, GUF1, PTCD3, DAP3
	RNA modifiers	TFB1M, GADD45GIP1, MED1
	Cytosolic Ribosomal or Ubiquitin Proteins	RPS3, UBA52
	Mitochondrial Metabolic Enzymes	HIBCH
	CLUSTER 3 (n = 20)	
Mitochondrial Aminoacyl-tRNA Synthetases	Mitochondrial Aminoacyl-tRNA Synthetases (mt-ARSs)	AARS2, CARS2, DARS2, EARS2, FARS2, HARS2, IARS2, LARS2, MARS2, NARS2, PARS2, RARS2, SARS2, TARS2, WARS2, YARS2
	Cytosolic Aminoacyl-tRNA Synthetases	GARS1, HARS1, QARS1, KARS1
	CLUSTER 4 (n = 21)	
Fatty Acid β-Oxidation System	FAO Enzymes	ACADS, ACADM, ACADVL, ACADSB, ACAD8, ECHS1, HADH, HADHA, ACAT1
	ETF/ETFDH Electron Transfer	ETFA, ETFB, ETFDH
	Carnitine Shuttle	CPT1A, CPT2, SLC25A20

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CLUSTER 5 (n = 31)		
Mitochondrial Central Carbon Metabolism	Pyruvate Dehydrogenase Complex	PDHA1, PDHB, DLAT, DLD, PDHX, PDP1, PDP2, BCKDK
	TCA Cycle	CS, MDH2, FH, IDH2, IDH3A, SUCLG1, SUCLG2, SUCLA2, OGDH, DLST, ME2, DHTKD1
	Anaplerotic/Linked Pathways	PC, ALDH5A1, GCSH, LIPT1
	BCAA Catabolism	BCKDHA, BCKDHB, BCKDK, PPM1K, DBT, DLD
	α-Ketoglutarate Dehydrogenase Family & Related Complexes	OGDH, DLST, DLD, DHTKD1, OGDHL
	Organic Acid Metabolism	MMUT
	GSH/GSSG recycling	GSR
CLUSTER 6 (n = 18)		
Mitochondrial Fatty Acid Oxidation and Electron Transfer Pathway Proteins	Acyl-CoA Dehydrogenases	ACADS, ACADM, ACADVL, ACADSB, ACAD8
	Auxiliary Enzymes	ECHS1, HADH, HADHA
	Fatty Acid Transport and Carnitine-Dependent Entry	CPT2, SLC25A20
	Electron Transfer Flavoprotein System	ETFA, ETFB, ETFDH
	Amino Acid Catabolism	IVD, GCDH, ETHE1, ACSM6, HSD17B10

protein subunits and auxiliary assembly factors required for oxidative phosphorylation (OXPHOS). Subsequent clusters align with mitochondrial protein production (Clusters 2–3), and energy substrate degradation pathways (Clusters 4–6).

Enrichment Analysis

Enrichment analysis of 59 hub proteins in AD related to mitochondria revealed several biological roles. KEGG pathway enrichment analysis identified 100 significantly associated pathways (FDR <0.05), including oxidative phosphorylation, citrate cycle, and chemical carcinogenesis-reactive oxygen species (**Figure 4A**). Pathways associated with metabolic conditions and mitochondrial dysfunction (non-alcoholic fatty liver disease and diabetic cardiomyopathy), along with pathways associated with neurodegeneration (Parkinson disease, prion disease, and Huntington disease) also emerge as significantly enriched.[34,35]

The GO analysis revealed 803 significantly enriched GO terms (FDR <0.05), classified into biological processes (638), cellular components (41), and molecular functions (124). Key biological processes are related to the electron transport chain (ETC) and energy metabolism, such as mitochondrial electron transport from ubiquinol to cytochrome C and aerobic ETC (**Figure 4B**). Similarly, enriched cellular components are primarily associated with the ETC, including complex I, III, and IV of the ETC (**Figure 4C**). Lastly, top-ranked molecular functions are in the oxidative phosphorylation, ROS detox, and stress-apoptosis signalling.

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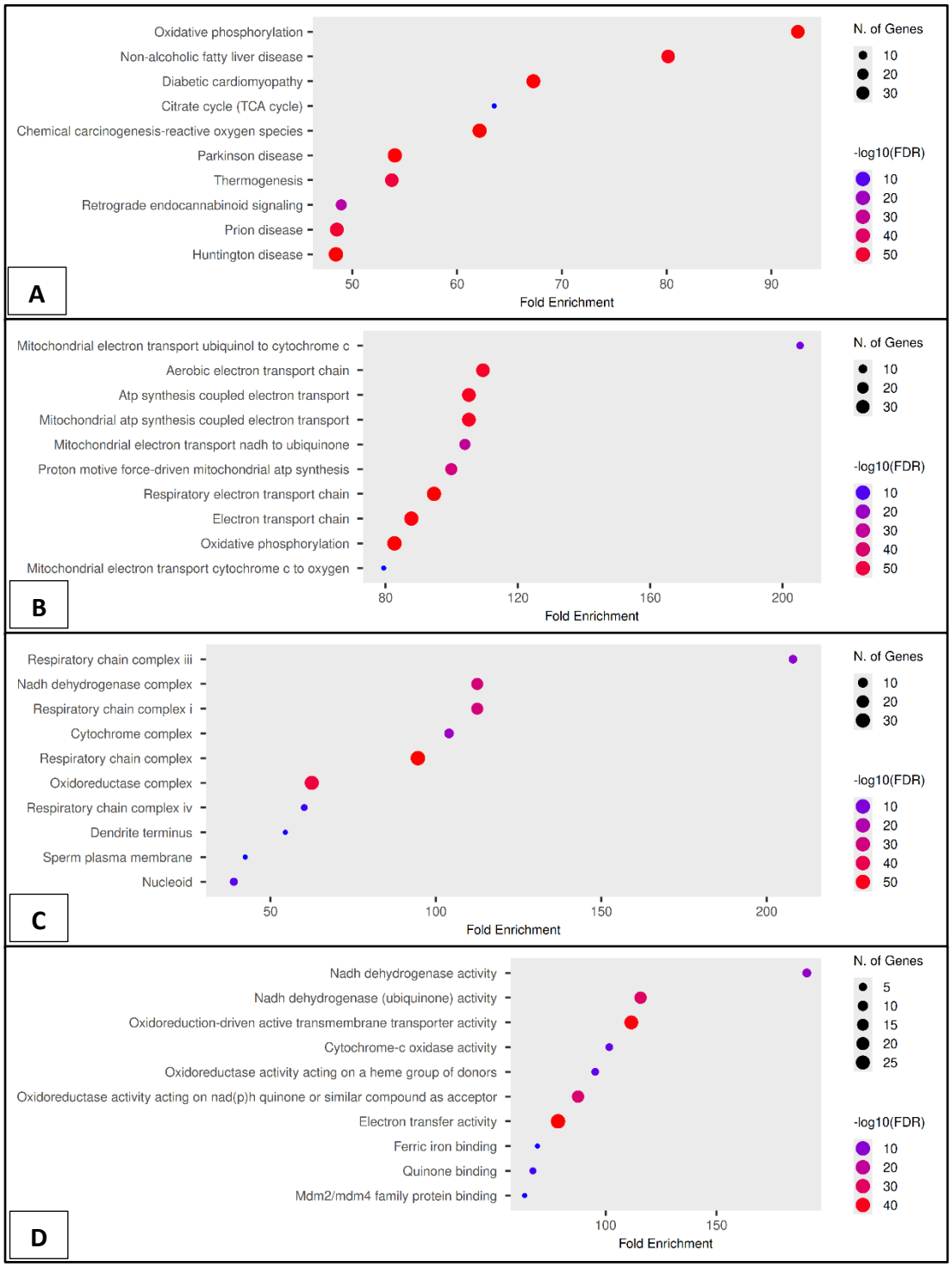

Figure 5. Pathway enrichment analysis of hub proteins. (A) KEGG analysis. (B) GO biological processes. (C) GO cellular components. (D) GO molecular functions.

4. DISCUSSIONS

Alzheimer disease (AD) is the most common type of dementia, characterized by comprehensive dementia, including memory and cognitive disorders.[36] These symptoms can be relieved by care and medication, yet there are no

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specific measures to prevent or cure AD.[37] The inter-correlated pathogenesis of AD enhances its severity which makes therapy targeting various disease pathways promising. The Aβ cascade is the most commonly researched and targeted pathway, however other pathways, such as the ROS pathway, are equally important. As the nervous system consumes a huge amount of energy, mitochondria as the main organelle in energy metabolism plays a central role in neuronal homeostasis.[38] Its function extends to oxidative stress regulation and various signal transduction, further highlighting its importance and detrimental effects shall its functions be impaired. Thus, its dysfunctions are closely associated with various neurodegenerative diseases, including AD.

The aim of this study is to explore the proteins and mechanisms of AD related to mitochondria using network pharmacology with protein-protein interaction (PPI) network analysis. We visualized the interaction of 580 proteins related to both AD and mitochondria before analyzing both the main network and its clusters based on four centrality parameters: betweenness centrality (BC), closeness centrality (CC), degree centrality (DC) and eigenvector centrality (EC). Additionally, KEGG and GO pathway enrichment analyses were performed to gain insight into the disease pathways, biological processes, cellular components, and molecular functions of the proteins placed within the top 5% of each centrality parameters, which we defined as hub proteins.

Cluster analysis of protein interaction networks and grouping of proteins based on function reveal several pathways associated with AD, including OXPHOS machinery, mitochondrial translation system, mitochondrial protein translation, fatty acid β-oxidation, mitochondrial central carbon metabolism, and electron transfer pathway. These findings are supported by the results of pathway enrichment analysis, which identifies energy metabolism, particularly OXPHOS and ETC, as the top affected functions in AD. These findings are consistent with the current understanding of AD's pathophysiology.

The OXPHOS system, consisting of the ETC complex (Complex I–IV), ATP synthase (Complex V), and associated assembly factors, plays a central role in ATP production. Dysfunction of the OXPHOS system is often linked with primary mitochondrial diseases

affecting high-energy tissues, such as the brain.[39] Dysfunctional OXPHOS leads to elevated production of ROS and reactive nitrogen species (RNS). In AD brains, oxidative stress markers are elevated, leading to damage of lipids, proteins, and mitochondrial DNA (mtDNA).[40] Our study finds OXPHOS proteins, particularly those of the ETC I complex, such as NDUFS1 and NDUFA9, as the central hub nodes with the highest centrality values. Similarly, UQCRC2, as part of the ETC complex III has also shown top scores among the centrality metrics and subsequent enrichment analysis. These findings suggest that impaired oxidative phosphorylation may be the key driver of neuronal dysfunction in AD. Additionally, previous analysis by Sonsungsan et al., (2024) similarly identified mitochondrial ETC enzymes as central hub proteins in AD which is consistent with our results.[41]

Other identified proteins in cluster 2 and cluster 3 account for mitochondrial protein synthesis, which comprises of the mitochondrial translation system and nuclear-encoded aminoacyl-tRNA synthetases (mt-aaRSs). The mitochondrial translation system regulates protein production, while mt-aaRSs catalyze the accurate charging of mitochondrial tRNAs with cognate amino acids to initiate translation, directly supporting cluster 2 processes.[42,43] Previous studies have proven that mitochondrial translation alterations, such as reduced mtDNA-encoded protein synthesis, RNA modification, translation inhibition experiments, contributes to mitochondrial dysfunction in AD.[44,45]

Aside from proteins related to energy metabolism and mitochondrial protein production, cluster analysis finds proteins related to lipid metabolism as part of the statistically significant clusters. Lipid metabolism has been tied to the pathophysiology of AD as fatty-acid β-oxidation (FAO) normally provides acetyl-CoA and reducing equivalents for the TCA cycle and OXPHOS, especially under conditions where glucose is limited.[46] If FAO is impaired, mitochondria lose a key energy substrate, potentially contributing to the energy deficits observed in AD neurons and glia.[47] Moreover, dysfunction of FAO could lead to the accumulation of LDs in astrocytes, which in turn promote oxidative stress and neuroinflammation.[48,49]

5. CONCLUSIONS

The present study provides a foundation for evaluating several hub proteins as targets of therapeutic interventions for AD. PPI network and enrichment analysis performed in this study revealed that mitochondrial proteins exert a crucial role in AD through several pathways, mainly energy metabolism and oxidative phosphorylation. Our findings suggest that proteins making up the electron transport chain, mainly complex I and III (e.g. NDUFS3, UQCRC2, CYC1), and proteins involved in signal transduction, especially stress response and cell death regulations (e.g. TP53, AKT1, BCL2), are key therapeutic targets. Modulating these genes could help prevent AD and alleviate its symptoms. This research paves the way for further exploration into potential therapeutic interventions targeting these proteins.

This study is an exploratory analysis based on data-mining approaches. Therefore, further studies employing in silico, in vitro and in vivo experimental designs are required to validate the results of this study. Furthermore, the findings are derived solely from proteomic data, which may not fully capture the broader molecular landscape. A multi-omic approach should be considered for future research to enable cross-validation and strengthen the biological relevance of the conclusions.

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