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Research Article

Ms/Ms Data Analysis to Identify Proteins Common to Alzheimer's and Parkinson's Disorders

Bharti A and Parvesh Bubber*

Biochemistry Discipline, School of Sciences, Indira Gandhi National Open University, New Delhi, India

***Corresponding Author:** Parvesh Bubber, Professor, Biochemistry Discipline, School of Sciences, Indira Gandhi National Open University, New Delhi, India.

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Abstract

Neurodegenerative disorders are characterized by gradual onset and progressive decline of cognitive and physiological functions, including memory, language, reasoning, and decision-making. Alzheimer's disease (AD) and Parkinson's disease (PD) are the most prevalent neurodegenerative disorders affecting millions worldwide. Both diseases have distinct pathological features but share common mechanistic pathways such as neuroinflammation, oxidative stress, and mitochondrial dysfunction. However, the upstream and downstream molecular, cellular and protein network pathways that cause decline in cognition and dementia in these disorders are not well understood. Advances in high-throughput molecular profiling and computational approaches have significantly enhanced the understanding of protein function in disease progression. It enables an integrated approach considering protein-protein interactions, gene ontologies and disease driving pathways. Analysis of protein-level alterations in AD and PD dataset from PRIDE archives was carried out using MaxQuant software platform along with STRING and GeneMANIA to identify and quantify the proteins in AD and PD datasets. Common proteins with UniProt ID that are significantly up-regulated in AD and PD are P08237, P13591, A0A286YF37, O75083 and Q9H4G0. CytoHubba revealed top ten hub proteins namely ACO2, VDAC1, CS, MDH2, PDHA1, ATP5F1A, ATP5F1B, HSPA9, FH and TPI1. These ten hub proteins were ranked based on degree centrality measures and topological algorithms. The most affected pathways mapped with KEGG database are carbon metabolism, biosynthesis of amino acids and TCA cycle with a significant number of interacting proteins. These computational approaches reveal the comprehensive protein network underlying disease diagnosis, prognosis and can be used for drug targeting especially in AD and PD in a scenario where neurodegenerative conditions are extremely vast and complex.

Keywords: *Proteomics; PTMs; PPI; GO; Alzheimer's Disease; Parkinson's Disease*

Abbreviations

AD: Alzheimer's Disease; CSF: Cerebrospinal Fluid; DDA: Data Dependent Acquisition; FDR: False Discovery Rate; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; LFQ: Label-Free Quantification; MANIA: Multiple Association Network Integration Algorithm; MCL: Markov Clustering Algorithm; PD: Parkinson's Disease; PPI: Protein-Protein Interaction; PTM: Post Translational Modification; RAF: Rapidly Accelerated Fibrosarcoma; STRING: Search Tool for the Retrieval of Interacting Genes

Introduction

Neurodegenerative state is associated with the subtle onset and intensified impairment of physiological and cognitive functions. These functions include memory, conception, linguistic, courtesy, intellectual, and decision making. In Alzheimer's disease (AD), progressive

neuronal loss starts pathologically with collective accumulation of β-amyloid protein and hyper-phosphorylated tau protein in the environment [1,2]. Parkinson's Disease is characterized by loss of dopamine producing neurons in substantia nigra part of the brain affecting both the central and peripheral nervous system [3]. The pretentious brain cells of individuals with PD comprise Lewy bodies which is an accumulation of the protein alpha-synuclein with common symptoms like tremor, muscle stiffness, slowing down of the automatic as well as spontaneous movement and overall body balancing. There have been several reports of overlap in clinical presentation and brain neuropathology in AD and PD patients suggesting that these two diseases share common underlying mechanisms [4,5].

The mechanistic pathway in AD and PD encompasses shared biochemical processes such as oxidative stress, amyloid- and -synuclein accumulation, and impaired autophagy, leading to progressive neuronal death. Determination of common protein architecture among both neurodegenerative disorders may help in underlying collective disease mechanisms and simplification of early prognosis. These overlapping pathways are crucial for the understanding and development of potential dual-purpose therapies.

The vast complexities in neurodegenerative conditions have led to the development of several novel approaches to determine the protein processing stages such as post translational modifications, protein interactions and protein misfolding. Nowadays proteomics generated high-throughput data provides valuable population-scale studies capable of identification of thousands of protein biomarkers for disease diagnosis, prognosis, and drug targeting, especially in diseases like neurodegeneration where PPI is huge and extremely difficult for exact precision. MS/MS data analysis using mass spectrometry (MS) makes it possible to determine peptides or whole proteins. High-throughput molecular profiling domain along with superior computational tools applied to large scale datasets transform the way of looking into the function of a protein. Proteomics speaks about unfamiliar arrays that effect both the diagnosis and treatment of neurodegenerative disorders. Protein-level modifications are more directly related to disease pathogenesis. MS/MS analyses of PTMs like oxidation, deamination, phosphorylation and glycosylation are critical for understanding disease progression [6]. Other PTMs, such as glycosylation, citrullination, and acetylation, also modulate the aggregation, clearance, and toxicity of proteins involved in AD, including amyloid precursor protein (APP) and Aβ. The possibility of these modifications to influence the progression of neurodegenerative conditions by altering protein conformations and interactions, thereby impacting cellular signalling pathways and neuronal health cannot be ruled out.

The role of participating proteomic signatures and molecular networks may thus enable early prognosis, precision therapies, and improved outcomes for AD and PD patients. Elucidation of protein modifications and protein-protein interaction using STRING and GeneMANIA, the two leading bioinformatics tools assumes significant importance to unravel protein interaction networks. STRING is a valuable tool to predict physical and functional aspect of the protein-protein interactions. On the other hand, GeneMANIA reveal related genes based on functional data (co-expression, pathways, and co-localization). The combination of these two tools in exploration of functional enrichment and protein networks in AD and PD will help to integrate our understanding of the commonalities between potential biological mechanisms.

Materials and Methods

Proteomics can reveal tissue- or cell-specific proteomes both qualitatively and quantitatively. It is therefore thought to be an ideal technology to elucidate the aberrant protein expression in disease-affected brain. A quantitative proteomics approach label-free data dependent acquisition (DDA) has been used in the analysis of biological samples of AD and PD. MS/MS protein data set was downloaded from a data archive Pride (https://www.ebi.ac.uk/pride/archive/). AD dataset (Pride ID PXD037133) has 8 control and 8 case samples while PD data set (Pride ID PXD022092) has 5 control and 5 case samples of the human brain [7,8].

Raw MS/MS data were searched against the UniProt human reference proteome (2024_02_15; 204,052 entries) using MaxQuant platform (Figure 1) [9]. Appropriate enzymes were selected for specific digestion i.e. trypsin, with up to two missed cleavages. Variable modifications were set for "methionine oxidation" and acetylation [protein N-term] whereas "Carbamidomethyl" was set as a fixed

modification. A false discovery rate (FDR) of 0.01 (1%) was used for both peptides and proteins identification. Additionally, 10 ppm was selected as the main search peptide mass tolerance value, and 20 ppm was set for the MS/MS match tolerance. Peptide quantitation was performed using "unique plus razor peptides". The MaxQuant output files were processed and analysed using the Perseus biostatistics platform for statistical analysis. "Contaminants", "reverse", and "only identified by site" proteins were filtered out, and LFQ (label free quantitation) intensity values were Log_2 transformed. As shown in the workflow diagram (Figure 1) all downstream analyses were performed after further data filtering to retain only proteins identified by at least 2 MS/MS counts and detected in minimum valid values in at least one group. Missing values were imputed based on the normal distribution of LFQ values. PTMs was analysed for deamination and oxidation in AD and PD as these non-enzymatic modifications accumulate with age, stimulate protein misfolding, and impair protein degradation network in human brain.

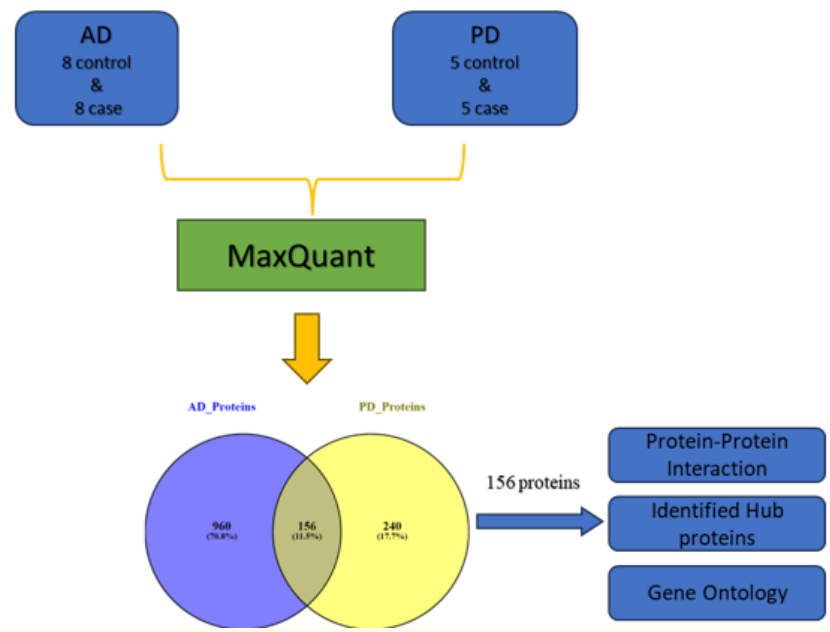

Figure 1: *Pictorial representation of MS-MS data analysis for the identification of pathways common for AD and PD.*

STRING, a biological web resource of a database was applied to predict protein-protein interactions (http://string-db.org/). Common biological processes, molecular functions, cellular components, and KEGG pathways were retrieved from the STRING database [10]. Clustering analysis was done for the PPI using Markov Clustering Algorithm (MCL) [11]. Hub protein that reveals maximum bonding association in the PPI was identified using CytoHubba, again a plugin of Cytoscape [12,13]. The top 10 hub protein that shows maximum association and interactions with other common expressed proteins were identified.

Furthermore, a flexible user-friendly GeneMANIA web interface was applied to predict protein function. The Cytoscape plugin uses a large database of functional interaction network and each related protein is traceable to the source network to make the prediction [14,15] (http://www.GeneMANIA.org/). This tool generates protein networks interactions based on parameters such as physical contacts, co-expression, genetic interactivity, shared protein domains, co-localization and pathways can be retrieved as a form of network. Protein enrichment analysis was done for common proteins in AD and PD for characteristics such as biological processes, molecular functions, cellular components and KEGG pathways were retrieved via STRING database.

Results

MS-MS dataset quatification, common protein pathways analysis and its enrichments

MS-MS data were analyzed using the MaxQuant software platform to identify and quantify the proteins in AD and PD datasets. The analysis enabled high-confidence peptide identification, protein inference, and label-free quantitative comparison between groups (case-control). After running on both AD and PD dataset, 1116 proteins were quantified in AD and 396 proteins in PD. Statistically significant results were obtained from the tool but to increase the quality and accuracy of the analysis, additional filters were applied on the results files. The results were refined i.e. the proteins that have more then 2 unique peptides were selected and 928 and 362 proteins were found in AD and PD respectively (Figure 2). Proteins common in AD and PD were 156 in number and were used for futher studies such as PPI, enrichment anaylsis and identification of hub proteins.

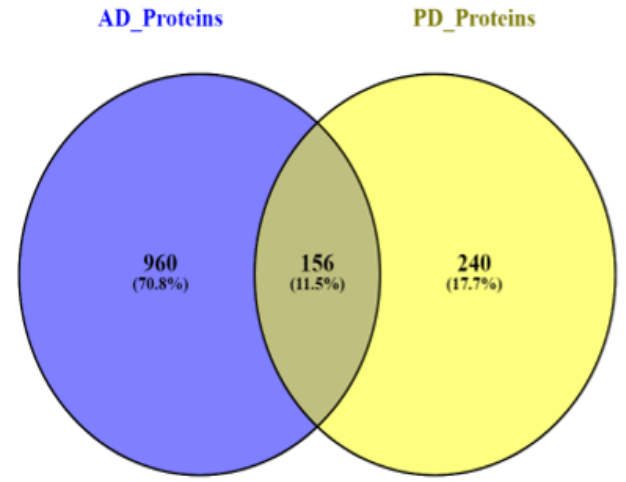

Figure 2: *Venn diagram presenting the unique and common proteins in AD (1116) and PD (396).*

To extend our analysis, modifications such as oxidation and deamination were looked into in dataset of AD and PD. Both deamination and oxidation regularly work in cooperatvie manner, deamidation increases aggregation and oxidation creates reactive aldehyde products that form adducts with proteins in neurodegeneration. Total proteins that have altered peptides with oxidation in AD and PD are 11 and 24 respectively. Total proteins with modified peptides that are found with deaminations in AD and PD are 42 and 22 respectively. Hexokinase-1 (UniProt_ID- P19367) and Brain acid soluble protein 1 (UniProt_ID- P80723) have modified peptide with deamination and intresetingly found to be common in AD and PD datasets specifically in sample of diseased.

Common protein pathways analysis for AD and PD

For studying the metabolic/signaling pathways which are common in both the diseased state, it is important to have prior information on the regulation of proteins i.e. up regulated protiens and down regulated proteins. The value of log2FoldChange indicates up and down regulation of proteins. Any proteins which have greater than 1 value of log2FoldChange were taken as highly up-regulated proteins, ranging between +0.5 to +1 are moderately up-regulated, -0.5 to -1 are moderately down-regulated and less than -1 were highly down-regulated. The common and unique proteins in AD and PD that vary in their nature of regulation, as observed based on Log2FoldChange (Figure 3A) shows higher upregulation and have value ≥+1. Figure 3B shows moderatley up-regulated proteins with value +0.5 to +1; Figure 3C shows moderately down-regulated proteins with value -0.5 to -1 and figure 3D shows highly down regulated proteins with value ≤ -1. So, highly up-regulated proteins common between AD and PD are 5 ATP-dependent 6-phosphofructokinase, Neural cell adhesion

molecule 1, F-box protein 2, Actin-interacting protein 1 and Erythrocyte membrane protein band 4.1-like 1. Proteins with moderate up-regulations were four in number namely Ferritin heavy chain, Neuromodulin, Mitochondrial import receptor subunit TOM22 homolog and Protein AS1. Moderate down-regulated proteins are 3, Importin subunit-Importin subunit beta-1, beta-1 Alpha-actinin-2 and Protein disulfide-isomerase A3. None of the proteins were found common for AD and PD that were highly down-regulated.

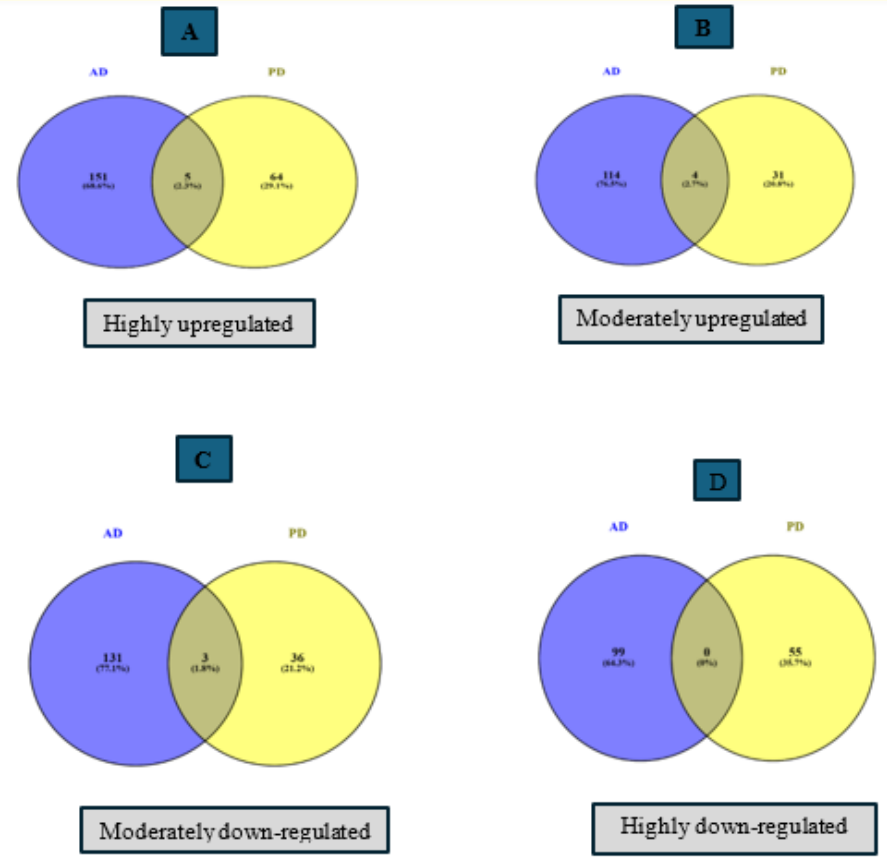

Figure 3: *Venn diagram: (A)Log2FoldCHange value ≥+1, B- Log2FoldCHange value +0.5 to +1, C- Log2FoldCHange value -0.5 to -1, D-Log2FoldCHange value ≤ -1.*

Network-based PPI (Protein-Protein Interaction) method is a computational practice that helps in identifying the configurational structure of protein interaction networks that can predicts new interactions too, identify protein that play central role in the network, or can identify hub proteins involved in diseases. The interactions among common proteins were done using two applications i.e., STRING and GeneMANIA as plugins in Cytoscape software. STRING works on the phylogeny to predict the functional interaction (direct interactions as well as indirect interactions) whereas the GeneMANIA working principle is based on functional proteomics data.

Protein-protein interaction network was generated from 156 common proteins as input in STRING software [for confidence cut off of 40 and maximum additional interaction was set at 20]. The generated network (Figure 4A) has been found to be dense with 111 nodes and 892 edges, nodes representing proteins and edges reflect the connections between the proteins. Further, protein-protein interaction has been observed within the compartments. Figure 4B, represents the distribution of proteins across different cellular compartments. The x-axis lists various cellular compartments, such as cellular anatomical entity, organelle, cytoplasm, extracellular region, vesicle, etc.

The y-axis shows the number of proteins associated with each compartment. The data reveals that the highest number of proteins (32) are linked to the mitochondrial matrix, followed by melanosome (14), mitochondrial protein-containing complex (16) and Oxidoreductase complex (11). The compartment with lesser numbers of proteins are: ficolin-1-rich granule lumen (10), ficolin-1-rich granule (11), Mitochondrial nucleoid (7), Inner mitochondrial membrane protein complex (10), Main axon (6) and Tricarboxylic acid cycle enzyme complex (4), Proteins were found to be unevenly distributed among cellular compartments, with certain structures like organelles and cytoplasmic regions being more protein-rich than others.

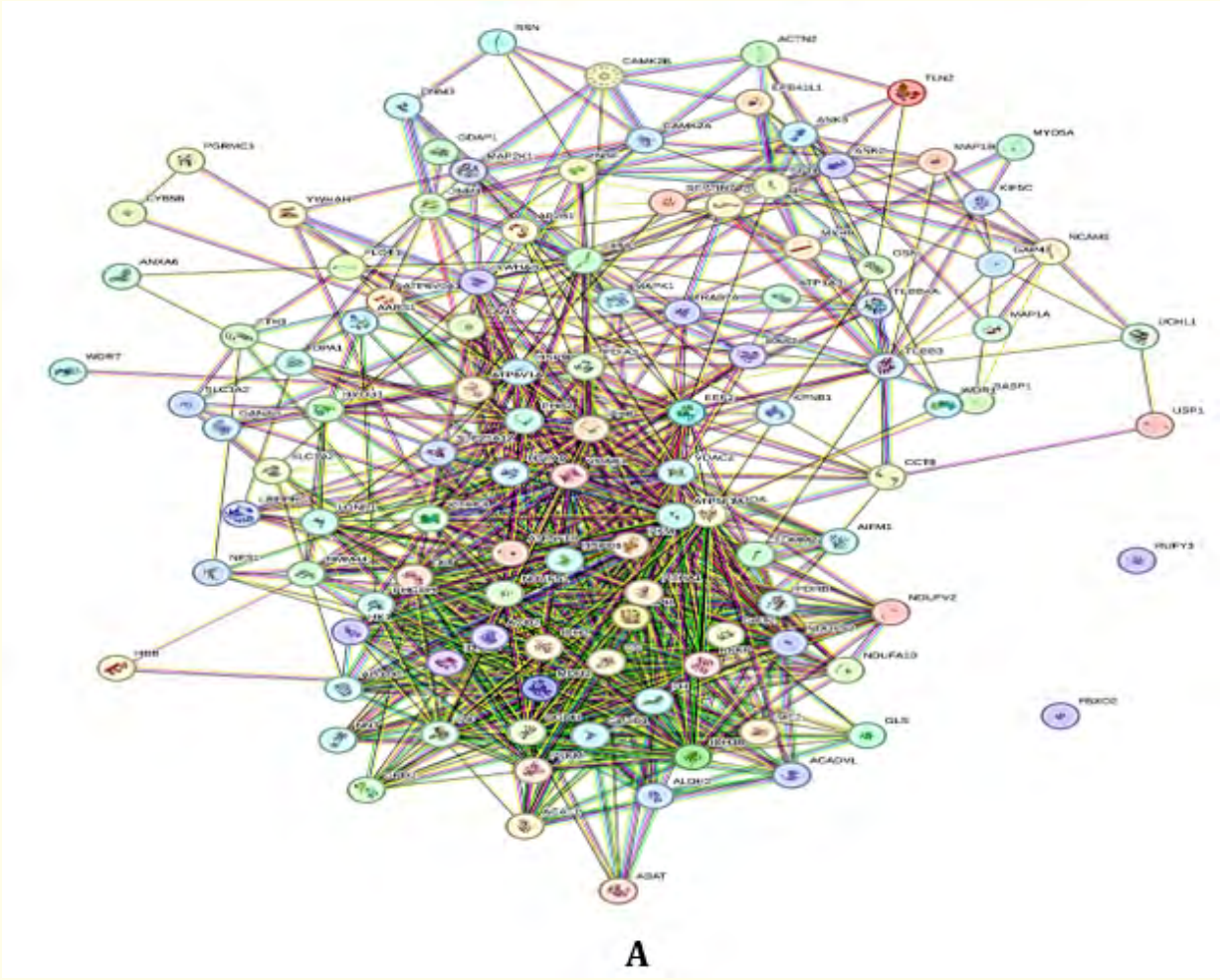

Figure 4A: *Protein-Protein Interaction (PPI) network for the common proteins in AD and PD. The circle is the node representing protein and the line between them is the edge representing the interaction. The interaction among proteins shows very dense interaction at the centre with proteins like ACO2, ATP5F1A, TPI1, HSPA9. The coloured edges with pink and green are known interaction which can be seen prominently in the network. Purple- and orange-coloured edges are predicted interactions which are very few and distant from the dense part of the network.*

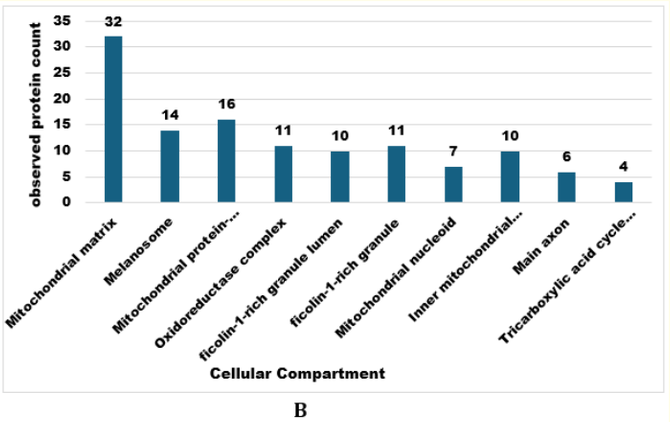

Figure 4B: *Distribution of proteins across different cellular compartments.*

The Markov Clustering Algorithm (MCL) is a fast, extensible and user-friendly unsupervised graph clustering algorithm. It is widely used for identifying protein families and functional modules. The PPI network was then subjected for identification of clusters using MCL, a functionality of STRING. The inflation parameters which define the extent of the strengthening or weakening of the interaction, was set to 1.7. The network formed was found to be highly dense with the inflation which revealed that these clusters have strengthening interactions with each other (Figure 5). It is evidently seen that there are 4 clusters out of which one cluster is much more packed with interacting proteins followed by lighter cluster with 23 proteins and other two clusters with only 15 and 5 proteins respectively. In the big cluster, proteins that have role in carbon metabolism are ACO2, VDAC1, CS, MDH2, PDHA1, ATP5F1A, ATP5F1B, HSPA9, FH and TPI1. Cluster 2 proteins have been found to be mainly involved in COPI-mediated anterograde transport, regulation of protein depolymerization and structural constituent of cytoskeleton. Clusters with lesser proteins have a protein family for synaptic vesicle cycle. Cluster with least number of proteins (MAP2K1, CAMK2B, CAMK2A, YWHAB and YWHAH) have been involved in RAF activation pathways.

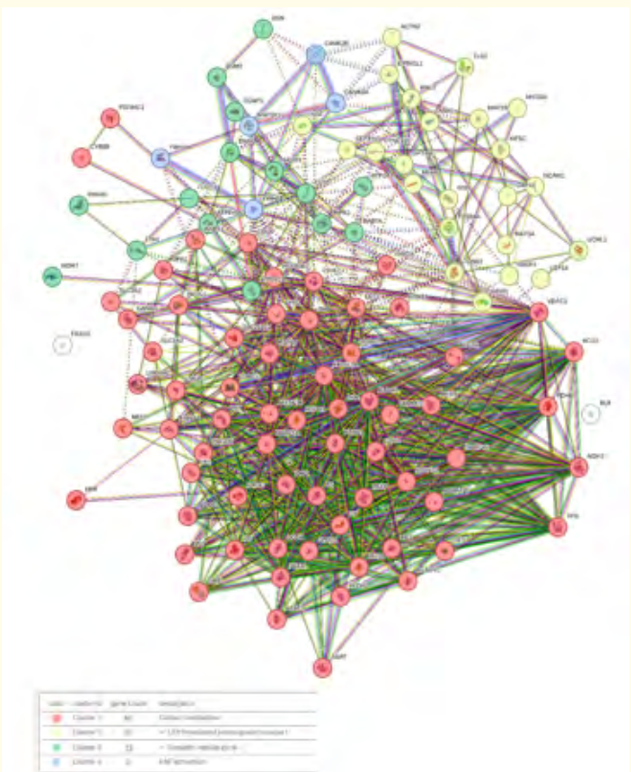

Figure 5: *Protein-Protein Network generated with MCL clustering on DEGs common for AD and PD. Three major clusters have been identified: cluster 1 nodes are coloured in red (66 proteins). Cluster 2 nodes are of yellow in colour (23 proteins). Cluster 3 nodes in green color have least number of proteins (15 proteins) and cluster 4 has 5 proteins.*

Protein-protein interaction network was then subjected to hub protein identification. CytoHubba, a plugin of Cytoscape was used to find the hub proteins (Figure 6). The parameters adopted was degree centrality. Networks revealed top 10 hub proteins (ACO2, CS, VDAC1, ATP5F1A, MDH2, HSPA9, PDHA1, TPI1, FH and PDHB). Protein ACO2 is the hub node of this network with reference to degree centrality measure. Maximum interacting proteins are ACO2 and CS that interact with 51 other proteins in the network, VDAC1 interact with 48 proteins followed by ATP5F1A, MDH2 interact with 47 proteins, HSPA9, PDHA1, TPI1 interact with 44 proteins, FH and PDHB interact with 43 and 42 respectively.

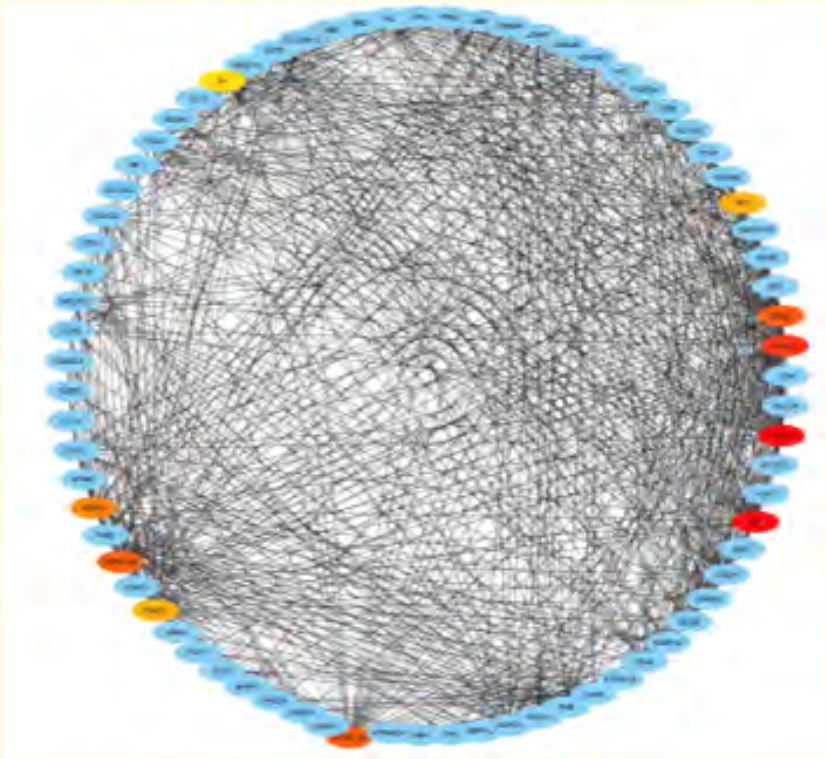

Figure 6: *Interaction of hub proteins (common in AD and PD) with other proteins using CytoHubba. The proteins have been highlighted (node in oval shaped).*

The interaction based on the proteomics function of a protein network was predicted using GeneMANIA for the determination of functionally associated proteins in protein-protein networks. 156 proteins were used as input to generate network via GeneMANIA. Protein-protein interaction network shows a dense interaction based on various parameters (Figure 7). Based on physical interactions parameters, proteins that scored 33.50 have been marked in pink color while those showing co-expression-based interaction have been marked in light purple colour (score 40.95), proteins with co-localization have been marked in blue (8.65), genetic interactions have been marked in light green color (score 3.86). The rest of the proteins were not interacting significantly. The protein displayed in the network at the centre represents maximum interacting genes that show maximal interactions. All the hub proteins have been highlighted yellow in colour and have significant physical interactions, co-expression and co-localization.

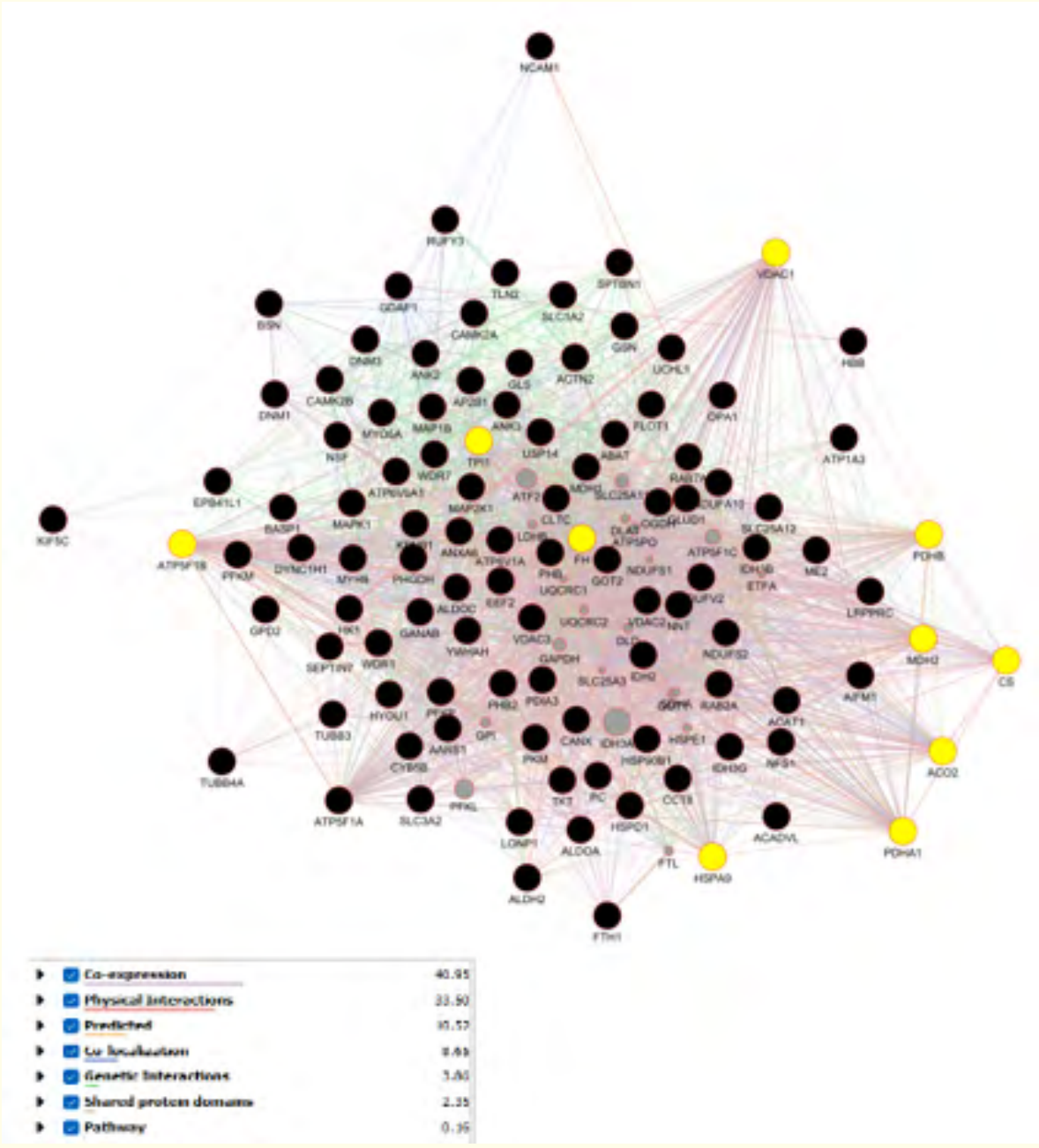

Figure 7: *GeneMANIA on Cytoscape application. The colours represent the various types of relations between expressed genes. Circle in yellow colour are hub proteins. Pink color genes are showing co-expression with the value of 40.95% which has high density in the network. Other interactions include co-localization (color of blue) with value of 8.65% followed by physical interaction (color of orange) with value of 33.50%, shared protein domains in color of brown shows value of only 2.35% and interactions shown in green color are genes which has pathways with values of 3.86% whereas genetic interaction are least with 0.16%.*

Enrichment analysis using GO processes and KEGG pathways of common proteins in AD and PD

Common GO enriched biological processes

Proteins common for AD and PD i.e. 156 remarkably enriched for 175 different GO biological processes were retrieved using STRING. The FDR of 0.05 along with fold enrichment with higher values were considered. Biological processes such as generation of precursor metabolites and energy (GO:0006091) have 32 proteins and 8 hub proteins (ACO2, TPI1, PDHB, MDH2, CS, FH, PDHA1 and ATP5F1A) that have been found to be associated in these processes (Figure 8A). Cellular respiration (GO:0045333) and energy derivation by oxidation of organic compounds (GO:0015980) contained 19 proteins and 21 proteins respectively with the association of 7 hub proteins (ACO2, ATP5F1B, PDHB, MDH2, CS, FH and ATP5F1A). Aerobic respiration (GO:0009060) have 17 proteins with 7 hub protein (ACO2, ATP5F1B, PDHB, MDH2, CS, FH, ATP5F1A). Tricarboxylic acid cycle (GO:0006099) have 11 proteins with 5 hub proteins (ACO2, PDHB, MDH2, CS, FH) and Pyruvate metabolic process (GO:0006090) have 13 proteins with 3 hub proteins (TPI1, PDHB, VDAC1).

Common GO enriched molecular function

In GO for molecular processes, small molecule binding (GO:0036094) have been found with 58 proteins with FDR value of 1.35e-19. These includes four hub proteins (ATP5F1B, HSPA9, VDAC1, ATP5F1A). Similarly, nucleotide binding (GO:0000166) and anion binding (GO:0043168) have been found to be associated with 50 and 49 proteins respectively (Figure 8B) with same hub proteins (ATP5F1B, HSPA9). Purine ribonucleotide binding (GO:0032555) molecular process have been found to be related to 40 proteins including 3 hub proteins (ATP5F1B, HSPA9, ATP1A3). Several processes with least protein count were also found. However, protein binding (GO:0005488), organic cyclic compound binding (GO:0097159) function have been observed with minimum signal value. They have 71 and 76 proteins respectively with 7 hub proteins (TPI1, ATP5F1B, HSPA9, MDH2, FH, VDAC1, ATP5F1A).

Common GO enriched cellular components

In total 112 common GO enrichment particularly for cellular components have been identified for AD and PD. Mitochondrion (GO:0005739) has least FDR value of 9.67e-25 with protein count of 54 (Figure 8C) and include 9 hub proteins (ACO2, ATP5F1B, HSPA9, PDHB, MDH2, CS, FH, VDAC1, ATP5F1A). The extracellular vesicle (GO:1903561) with protein count 57 proteins have 4 hub proteins (TPI1, ATP5F1B, HSPA9, ATP5F1A) and showed the FDR value of 5.24e-23. Both extracellular exosome (GO:0070062) and mitochondrial matrix (GO:0005759) with protein count of 56 and 32 have FDR values of 1.01e-22 and 5.36e-22 respectively. Extracellular exosome includes 5 hub proteins (TPI1, ATP5F1B, HSPA9, MDH2, VDAC1) and Mitochondrial matrix have 9 hub protein (ACO2, ATP5F1B, HSPA9, PDHB, MDH2, CS, FH, VDAC1, ATP5F1A). Overall extracellular region, mitochondrial membrane system, secretory vesicle, melanosome have been found to be the leading GO enriched cellular components.

Common KEGG pathways

72 KEGG pathways were found to be common in AD and PD. Carbon metabolism pathway, Biosynthesis of amino acids and Citrate cycle (TCA) were found to be the significant pathways (Figure 8D). Carbon metabolism pathway (Pathway ID: hsa01200) has 23 proteins with 6 hub proteins (ACO2, TPI1, PDHB, MDH2, CS, FH). Biosynthesis of amino acids (pathway ID: hsa01230) included 15 proteins with 3 hub proteins (ACO2, TPI1, CS). Citrate cycle (TCA cycle) (pathway ID: hsa00020) have 10 protein with 5 hub proteins (ACO2, PDHB, MDH2, CS, FH).

Discussion

The availability of high-throughput mass spectrometry dataset has led to an increasing number of proteomics research for neurodegenerative disorders like AD and PD. This approach proved as a one of the best practices because of its unbiased identification and quantification of thousands of proteins. Protein post-translational modifications (PTM) are covalent, transitional and enzymatic in

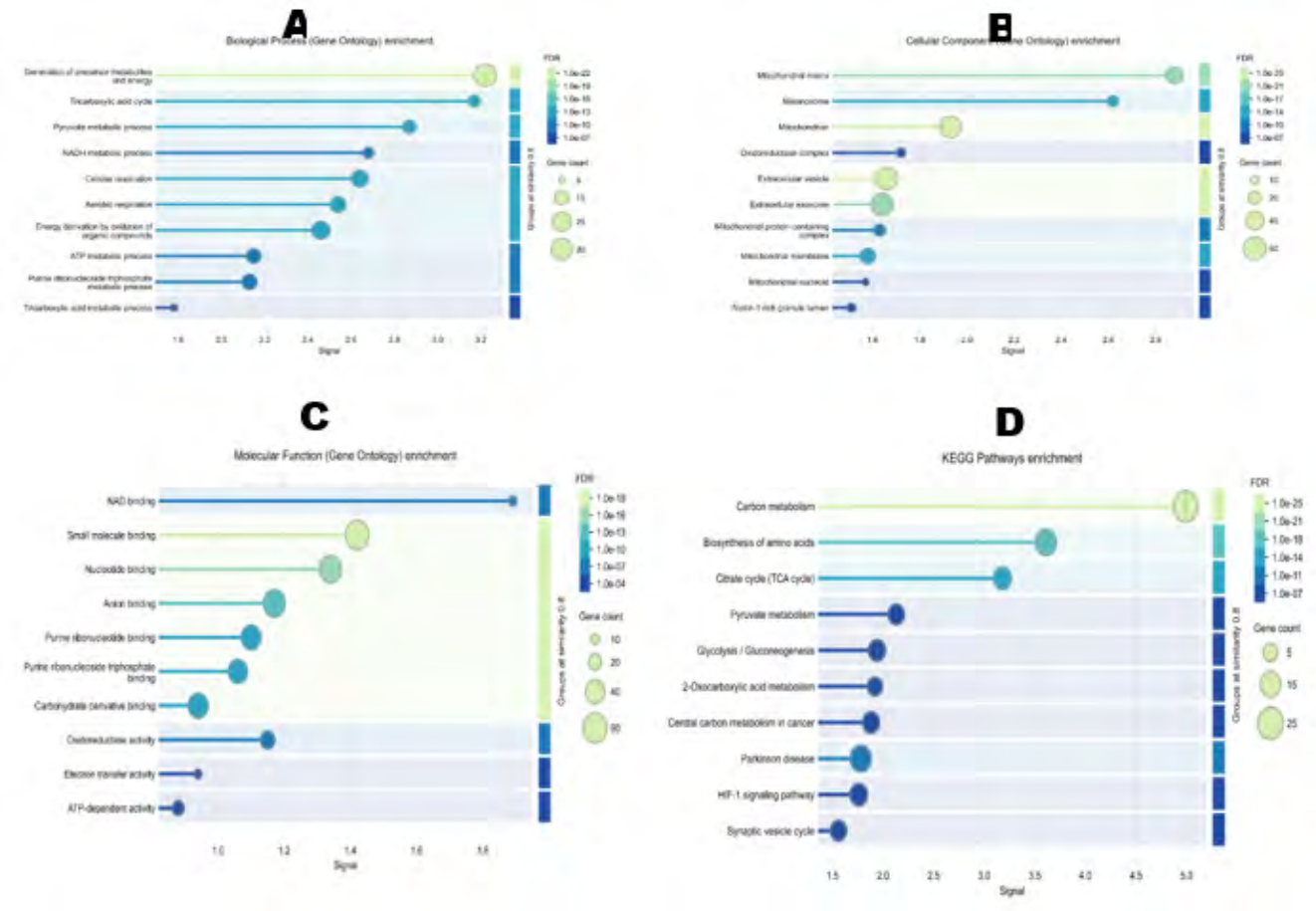

Figure 8: *Protein enrichment analysis of the identified proteins common in AD and PD. (A) Biological processes, (B) Cellular components, (C) Molecular functions and ((D) KEGG pathways.*

nature and increases the functional diversity of the whole proteome. PTMs involve addition of functional groups, proteolytic cleavage, or degradation thereby influencing cell signaling, localization, and activity. Variable modification in the dataset includes oxidation and deamination. Deamination, specifically protein deamidation (the translation of asparagine/glutamine into aspartic/glutamic acid) is spontaneous and widely considered for its role in cellular aging, disease progression, and the efficacy of therapeutic proteins. It acts as a "molecular clock" that encourages protein misfolding, aggregation, and the formation of neurotoxic plaques. In our study, total of 42 and 22 deaminated proteins from AD and PD respectively were identified and out of that two deaminated proteins were found to be common in AD and PD. These includes hexokinase-1 and brain acid soluble protein 1. Hexokinase-1 (HK1) is a critical glycolytic enzyme whose dysfunction drives metabolic collapse and neuroinflammation in AD and PD. In AD, reduced mitochondrial HK1 in astrocytes impairs metabolism, while in PD, pathology, such as-synuclein, disrupts the HK-VDAC1 axis, hindering energy production. In a study by Wang., *et al*. 2022 [16] impaired isoAsp restoration triggers deamidation buildup before AD progression. So, deamidation-based biomarkers have been suggested to be highly sensitive regarding early detection of the diseases. Adav (2025) reported an elevation in levels of isoaspartyl residues (a product of deamidation) found in the blood of patients with cognitive decline, signifying their potential as a diagnostic marker for AD, FTD, and PD [17]. An interesting PTM found in our study is the oxidation of methionine in AD (11 in number) and PD (24 in

number) respectively but showed no common modified protein. Oxidative stress, which is elevated in both conditions, induces covalent modifications on proteins including protein carbonylation, nitration (3-NT), methionine oxidation, and S-glutathionylation. These modifications lead to protein misfolding, aggregation, and loss of function, ultimately driving neuronal death [18].

Regulation of a protein based on Log2foldchange is a statistical way to determine if a protein's abundance has increased or decreased or its regulation has altered in biological system, thereby allowing researchers to quantify biological significance in the diseased condition [19]. This approach is essentially required for high-throughput proteomic studies (e.g. using LC-MS/MS) to visualize and filter thousands of proteins for those that are truly regulated and to increase the accuracy and precision in the result [20]. A large number of upregulated proteins have been found to be common for AD and PD whereas only 3 proteins were commonly down-regulated (Figure 3). ATP-dependent 6-phosphofructokinase (ATP-PFK), a key rate-limiting enzyme in glycolysis that plays a critical role in brain energy metabolism and was significantly altered in both AD and PD. Although activity of ATP-PFK in the brain decreased frequently due to continuously glucose utilization, but there have been reports of its increased activity in certain brain areas in AD, particularly associated with reactive astrocytosis [21]. Second common protein with upregulation is neural cell adhesion molecule 1 (NCAM1). It is a glycoprotein critical for synaptic plasticity and structural renovation, whose dysfunction is implicated in AD and PD. Protein NCAM1 is particularly crucial during brain development where expression of cell adhesion molecules maintains the balance between stabilization and elimination of synapses. The shape of the neuronal network during development is involved in cognitive functions and memory. It is mainly localized at synaptic junctions where it contributes to the modification of neuronal action by altering the morphology and strength of synaptic networks [22]. Another upregulated protein is F-box protein 2. It is a neuron-enriched ubiquitin ligase adaptor, that plays a significant role in neurodegenerative diseases like AD and PD by regulating the degradation of glycoproteins. In AD, Fbox2 mediates the degradation of β-site APP-cleaving enzyme (BACE1), the key enzyme in generating amyloid-beta. Overexpression of Fbox2 decreases BACE1 levels and its depletion accelerates amyloid processing [23]. In PD, Fbox2 variants have been observed as risk modifiers and plays important roles in the ubiquitin-proteasome protein-degradation pathway and mitochondrial maintenance, both of which are robustly associated with PD pathogenesis [24]. Actin-interacting protein 1 (AIP1), is a critical regulator of actin filament dynamics. Its asscoiation with ADF/cofilin holds key to promote the disassembly of actin filaments. In neurodegenerative diseases like AD and PD, actin dynamics are impaired, regularly leading to the development of stable, pathological cofilin-actin rods. AIP1, as a key partner of cofilin plays a direct role in regulating the formation and turnover of these rods. The deficiency in AIP1 causes severe, often fatal, abnormalities in many tissues, indicating its crucial role in cellular homeostasis. Another protein erythrocyte membrane protein band 4.1-like 1 (EPB41L1), is a neuronal cytoskeletal protein who's main role is to stabilize receptors at the plama membrane which is critical for synaptic plasticity, making it relevant to neuronal health and neurodegenerative processes. It has been explored in neurodevelopmental disorders due to its role in dopamine receptor signaling.

So far down-regulation of the proteins in both AD and PD includes Importin subunit-Importin subunit beta-1, beta-1 Alpha-actinin-2 and Protein disulfide-isomerase A3. Importin subunit-Importin subunit beta-1 is a nuclear transport receptor that facilitates the import of proteins into the nucleus i.e. major protein that maintain neuronal homeostasis. Several studies have pointed out that importin-α and importin-β decrease their nuclear but increase their cytoplasmic localization in neurodegenerative conditions eventually leading to a failure in transport efficiency. Any imparirment of these protiens have huge impact in causation of neurodegerneative disorders [25].

Loss of synaptic integrity and neuroinflammation are two important hallmarks that are common in both AD and PD. The dysfunction accompanied by alpha-actinin-2 protein have been observed that causes modification in the actin regulatory proteins like cofilin and drebrin, which are critical contributor to synaptic destabilization and cognitive decline in AD whereas in PD it blocks the cytoskeleton impainirng axonal transprot and synapase function [26,27]. Protein disulfide-isomerase A3 (PDIA3) is a chaperone protein that plays a complex role in response to stress in endoplasmic reticulum. PDIA3 expression is altered in the limbic brain regions of Alzheimer's patients. Its pathophysiological effects continues in later stages on account of its interaction with the mammalian target of rapamycin (mTOR) pathway [28].

Proteins that are normally bound to aggregation-prone proteins can become sequestered and mislocalized in protein inclusions, leading to their loss of function. Protein-protein interaction network in figure 4A, illustrates a highly interconnected web of proteins with very strong functional association. In our dataset, interacting proteins includes ACO2, TPI1, MDH2, ATP5F1A, LRPRRC, HSP90B1, VDAC1, CS and FH where as FBXO2, RUFY3 are the proteins that doesn't have interaction directly with other proteins. Protein expressions in different cellular components and their relative distribution across various part of the cell (Figure 4B) suggests their specific cellular functions. Extensive protein expression in cell structures reflect significance of these regions for example mitochondrial matrix, melanosome and mitochondrial protein-containing complex. Our studies revealed that an enhanced protein expression is associated with the mitochondrial matrix (Figure 4B). This fact has been corroborated by several previous studies that have highlighted that mitochondrial matrix dysfunction plays a central feature in AD and PD. Since mitochondrial is the power house of the cell as adenosine triphosphate (ATP), is the main energy currency of the cell; any malfunctioning in the powerhouse will drive the bioenergetic failure and oxidative stress gradually leading to neuronal death.

Protein function is a very complex web and therefore to perform any molecular function certain proteins work together forming a protein family or protein cluster. Our result (Figure 5) revealed that carbon metabolism is one of biggest clusters. There are about 66 proteins in same protein family that shows interaction strongly via various molecular function such as NAD binding, oxidoreductase activity, electron transfer activity, iron-sulfur cluster binding and small molecule binding. Maximum number of proteins from all these molecular functions is happening in the mitochondrial matrix. Recent study by Dong., *et al*. 2025 have identified fourteen key mitochondria related genes that demonstrated significant dysregulation in the postmortem brain tissues from AD patients. These genes have strong connections to oxidative stress, indicating that mitochondrial dysfunction plays a crucial role in Alzheimer's disease pathology [29]. Another cluster with 23 proteins namely COP1 (Coat Protein Complex I) is fundamentally known for facilitating reversing transport (Golgi-to-ER) and intra-Golgi trafficking. In the context of AD and PD, disturbances in COPI-dependent transport contribute to dysfunctional protein sorting and ER stress [30].

Proteins such as ACO2, CS, VDAC1, MDH2, ATP5F1A, HSPA9, PDHA1, TPI1, FH and PDHB are mainly involved in carbon metabolism which clearly define their role in formation of energy in the brain cells (Figure 6, a circular layout of the PPI). In figure 7 PPI via GeneMANIA revealed that all these hub proteins are having co-expression, co-localization and conduct physical interactions too.

CS (Citrate synthase) and ACO2 (aconitase 2) protein, are key enzymes in the mitochondrial tricarboxylic acid (TCA) cycle, are significantly linked to neurodegenerative diseases AD and PD as it direct affects energy production within neurons. Binding of alpha-synuclein to ACO2 is increased in the mitochondria of PD patients, promoting aconitase degradation and reducing its activity. It also results in mitochondrial complex I dysfunction., where reduced activity of CS can lead to decreased energy production thereby contributing to the progression of neurodegeneration in PD [31].

VDAC1 (Voltage-Dependent Anion Channel 1), a key regulator of mitochondrial permeability, control the exchange of ions and metabolites between the mitochondria and the cell cytoplasm. An extensive overexpression in AD brains, cause the release of cytochrome via oligomerization, that eventually triggers apoptosis (cell death) [32]. MDH2 (Malate dehydrogenase 2), mitochondrial protein also gets highly elevated in neurodegenerative conditions. Oxidative stress, a common early event in neurodegeneration, can increase MDH2 activity and contributes to disease progression [33]. There have been very few reports on the role of MDH2, although some studies have shown mixed results regarding MDH2 levels in AD brains, with some reports signifying higher abundance, while others suggest a lower abundance of this enzyme in the neurodegenerative brain. Our study showed its upregulation in AD and PD. Altered MDH2 expression, along with other TCA cycle enzymes (e.g. PDHA1), have been associated with mitochondrial dysfunction in the neurodegenerative disorders.

TPI1 (Triosephosphate isomerase 1) protein is being used as a potential biomarker for AD. An altered expression of this protein during disease progression and its co-localization with neurofibrillary tangles showed precipitation of tau protein in AD. The down regulation expression of TPI1 in PD suggest modulation in the glycolytic pathways influencing the progression of disease [34].

HSPA9, also known as Mortalin/GPR75, is a mitochondrial chaperone protein that's downregulated in the brains of AD and PD patients. Reduced expression in the HSPA9 led to decreased respiration that can lead to oxidative stress, and eventually increased peroxisomal degradation. HSPA9 reduction results to extreme mitochondrial fragmentation because of oxidative stress, which later on causes damage to dopaminergic neurons to accelerate the process of neurodegeneration. Furthermore, HSPA9 interacts with multiple PD-associated proteins, including Pink1, DJ-1, and α-synuclein [35].

Conclusion

The previous traditional methods for the identification of PTMs include techniques such as western blotting, immunoprecipitation, and radiolabeling which. offers the advantage of being straightforward suffer from several limitations such as dependency on high-quality, modification-specific antibodies, which are not always available for less studied or novel PTMs. These techniques provide limited information on the exact sites of modification and often lack the sensitivity. Their capacity for large-scale or high-throughput analysis is also restricted, making them less suitable for comprehensive and comparative proteomic studies.

The computational tools-such as search engines MaxQuant match MS spectra overcome these restrictions for high throughput analysis of databases for protein identification and functional insights. The combination of proteomics with STRING data analysis enables the mapping of protein-protein interaction (PPI) networks, simplifying the functional understanding of complex experimental datasets. Another tool such as GeneMANIA helps to creates a composite network from multiple data sources such as co-expression, co-localization, physical interaction etc. It leads to the identification of the key functional modules, signalling pathways, or central regulators (hubs) that might not be immediately obvious. A well detailed data set analysis enables precise, high-throughput insight at cellular, molecular, and system levels of neurodegenerative conditions underlying AD and PD. It increases the chances of common or specific protein biomarkers for diseases along with post-translational modification that adds to our understanding of the molecular mechanism of pathological pathways, disease conditions and mapping. The further applications of tools also lie in the disease diagnosis, prognosis and drug targeting especially in AD and PD where neurodegenerative conditions are extremely vast and complex.

Ethics Approval and Consent to Participate

Not applicable.

Human and Animal Rights

No Animals/Humans were used for studies that are base of this research**.**

Consent for Publication

Not applicable.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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