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Aβ, Tau, and α-Synuclein aggregation and integrated role of PARK2 in the regulation and clearance of toxic peptides

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ABSTRACT

Alzheimer's and Parkinson's diseases are one of the world's leading causes of death. > 50 million people throughout the world are suffering with these diseases. They are two distinct progressive neurodegenerative disorders affecting different regions of the brain with diverse symptoms, including memory and motor loss respectively, but with the advancement of diseases, both affect the whole brain and exhibit some common biological symptoms. For instance, > 50% PD patients develop dementia in their later stages, though it is a hallmark of Alzheimer's disease. In fact, latest research has suggested the involvement of some common pathophysiological and genetic links between these diseases, including the deposition of pathological Aβ, Tau, and α-synuclein in both the cases. Therefore, it is pertinent to diagnose the shared biomarkers, their aggregation mechanism, their intricate relationships in the pathophysiology of disease and therapeutic markers to target them. This would enable us to identify novel markers for the early detection of disease and targets for the future therapies. Herein, we investigated molecular aspects of Aβ, Tau, and α-Synuclein aggregation, and characterized their functional partners involved in the pathology of AD and PD. Moreover, we identified the molecular-crosstalk between AD and PD associated with their pathogenic proteins- Aβ, Tau, and α-Synuclein. Furthermore, we characterized their ubiquitinational enzymes and associated interaction network regulating the proteasomal clearance of these pathological proteins.

1. Introduction

The aggregation of certain misfolded proteins is the chief pathogenic event that evokes neurotoxicity in many neurodegenerative disorders like Alzheimer's and Parkinson's disease (Jellinger, 2010). The major structural changes that take place are the rise in β-sheet conformation in misfolded protein that promotes oligomerization and amyloid like fibril formation (Relini et al., 2013). Moreover, the aggregation mechanism involves a crucial step of seed-nucleus formation, where the monomers form a smallest aggregate, termed as 'nucleus' that grows faster by the addition of monomers in comparison to its dissociation back into smaller aggregates and free monomers. The primary nucleation event that triggers the oligomer formation is followed by the secondary nucleation events, where a nucleus formation on the surface of previously existing aggregate, direct a fast increase in the number of oligomers. Then, they attain a fibrillar form known as "fibrillar oligomers" and result into a fibril formation, i.e. seed, which leads to a rapid generation of new fibrils with same morphology and chirality (Linse, 2019). These oligomeric forms are more toxic and can be targeted by certain oligomer eliminating compounds (Dunkelmann et al., 2018). Thus, the seed-nucleation phenomenon governs a series of misfolding, and protein-protein interaction events that exaggerate the protein aggregation process (Breydo and Uversky, 2014). Consequently, aggregated proteins become resistant to proteolysis and cellular clearance that cause chronic endoplasmic reticulum stress, mitochondrial dysfunction, reactive oxygen species formation, intense tissue inflammation and activation of apoptotic pathways leading to the neuronal loss (Rutkowski and Kaufman, 2004; Morimoto, 2008).

Although AD and PD exhibit heterogeneity at the genetic and clinical level which is affecting different regions of the brain, including acetyl-cholinergic neurons in hippocampus and dopaminergic neurons in *substantia nigra pars compacta*. Recent studies revealed significant similarity in their overlapping role of pathological proteins, including amyloid-beta, tau protein, and α-synuclein and suggestive familial link in their pathogenesis (Jellinger, 2012). For instance, above 50% of patients, suffering from AD revealed amyloid like alpha-synuclein peptide aggregates (Marsh and Blurton-Jones, 2012) while PD patients displayed frequent tau deposits (Lei et al., 2010). In addition, it has

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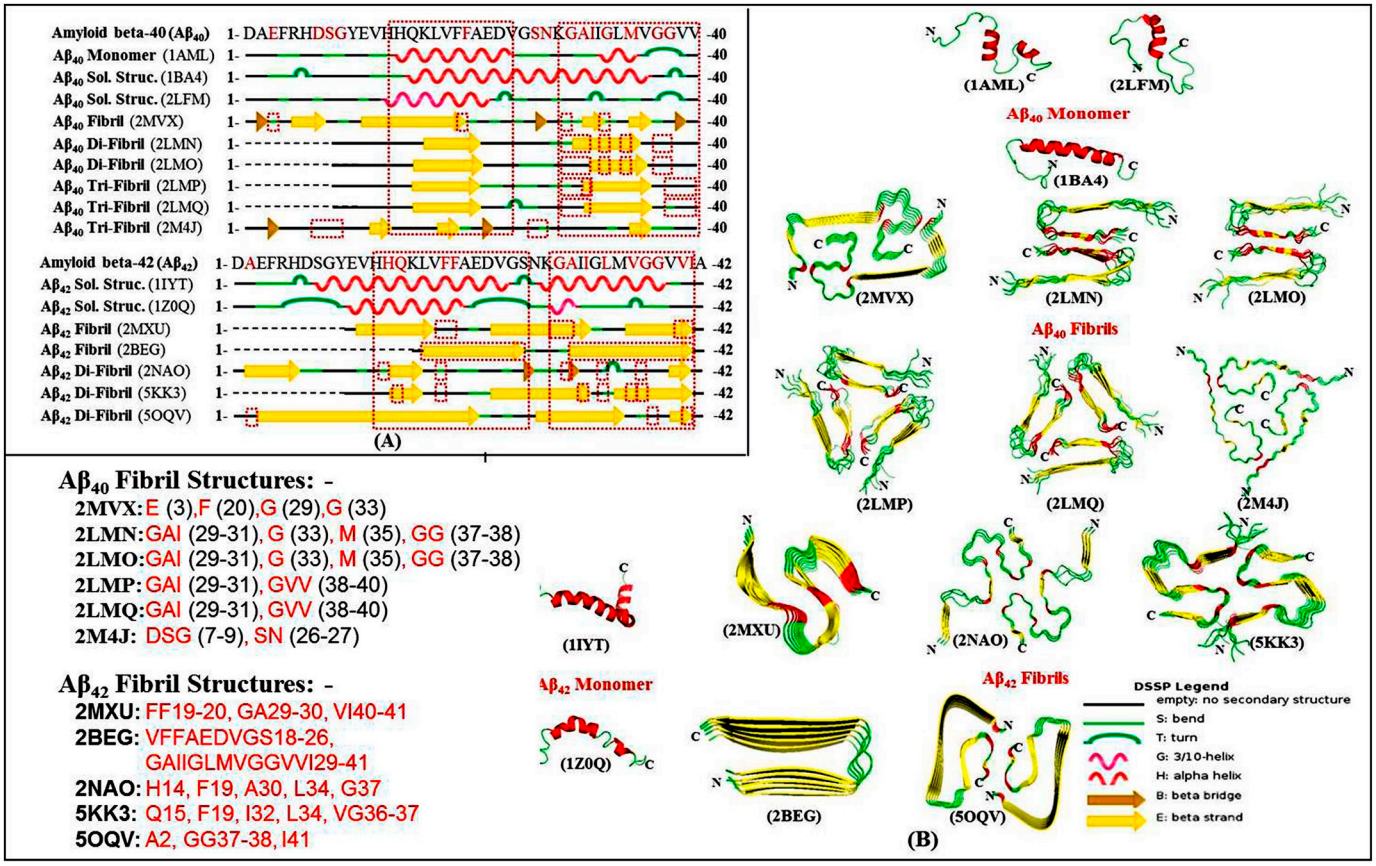


Fig. 1. Molecular basis of Amyloid beta peptide aggregation- (A) Structural changes in $A\beta_{40}/A\beta_{42}$ pathology: Helical conformation of amyloid-beta peptide gets distorted into the fibril structure rich in beta-strands and few beta-bridges. Polypeptide regions, including HQKLVFFAEDV (14-24) and GAIIGLMVGGVV (29-40) are responsible for the intra- and intermolecular fibril formation and consequent aggregation of $A\beta_{40}$ peptides. Similarly, HQKLVFFAEDVGS (14-26) and GAIIGLMVGGVVI (29-41) regions are responsible for intra- and intermolecular fibril formation and consequent aggregation of $A\beta_{42}$ peptides. Moreover, fibril-forming peptides are highlighted in red color, fibril-specific aggregation regions with red rectangles that presented the sequence motif responsible for aggregation of amyloid beta peptide. (B) 3D Structure of $A\beta_{40}/A\beta_{42}$ peptides: Aβ40 monomers (1AML, 2LFM, 1BA4) and Aβ42 monomers (1IYT, 1Z0Q) are shown in its cartoon view with aggregation prone region highlighted in red. The reported $A\beta_{40}/A\beta_{42}$ fibrils are shown in cartoon view with their aggregation prone regions in red color respectively. (2MVX: E3, F20, G29, G33; 2LMN: GAI29-31, G33, M35, GG37-38; 2LMO: GAI29-31, G33, M35, GG37-38; 2LMP: GAI29-31, GVV38-40; 2LMQ: GAI29-31, GVV38-40; 2M4J: DSG7-9, SN26-27/ 2MXU: FF19-20, GA29-30, VI40-41; 2BEG: VFFAEDVGS18-26, GAIIGLMVGGVVI29-41; 2NAO: H14, F19, A30, L34, G37; 5KK3: Q15, F19, I32, L34, VG36-37, 5OQV: A2, GG37-38, I41). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

demonstrated that the accumulation of tau proteins could take place with the help of specific strains of alpha-synuclein (Guo et al., 2013). Another study identified that Aβ Seeding escalates the formation of big sized α-synuclein oligomers that efficiently hampered neuronal SNARE-mediated vesicle fusion at synaptic junctions (Choi et al., 2015). Apart from proteinopathic similarities, some recent breakthroughs suggested a possible genetic link between these diseases in their advanced stages (Bailey et al., 2013; Singleton and Hardy, 2016; Ibanez et al., 2018; Zeng et al., 2018). For instance, LRRK2 mutant G2019S mediated phosphorylation of AβPP at T668, promoted APP intracellular domain's (AICD) nuclear translocation and consequent neurotoxicity in PD (Zeng et al., 2018). Likewise, LRRK2 is reported to directly phosphorylate tau at T149 and T153 signifying their interaction in a disease relevant manner (Bailey et al., 2013). Another study identified pathogenic mutations in AD causing genes- PSEN1 and PSEN2 in sporadic PD patients (Ibanez et al., 2018). Therefore, future research is required to establish well-defined pathological links between them.

However, these pathogenic proteins differ in their structural and functional biology, but they may share their protein-misfolding events and interact together to aggravate the disease symptoms (Barage and Sonawane, 2015; Cuanalo-Contreras et al., 2013). Moreover, they also interact with other ubiquitinational markers to relieve the proteotoxic burden inside neurons, via refolding or targeting proteins to ubiquitin proteasome system for degradation (Ciechanover and Kwon, 2015). Such a crucial ubiquitinational marker is PARK2, which is found to govern the proteasomal clearance of a wide range of substrates belonging to the nuclear proteins, cytoskeleton proteins, cell cycle regulators, heat shock proteins, neurotransmitters, and the cell signaling proteins. Some of these substrates include ataxin, Bcl-2, cyclin E, dopamine transporter, Hsp70, α-synuclein, synphilin-1, and α/β tubulin that regulates a variety of functions in different neurodegenerative disorders (Zhang et al., 2016). For instance, PARK2 regulates mitochondrial trafficking, endosomal sorting, synaptic transmission, programmed necrosis, ER stress, inflammation and cellular homeostasis. (Choong and Mochizuki, 2017; Sassone et al., 2017; Singh et al., 2018; Williams et al., 2018; Sun et al., 2019). Altogether, these findings highlight the need for a clear understanding of precise molecular mechanism behind pathogenic protein aggregation and clearance, and their interaction with other proteins to devise better diagnostic and treatment options for such neurodegenerative disorders.

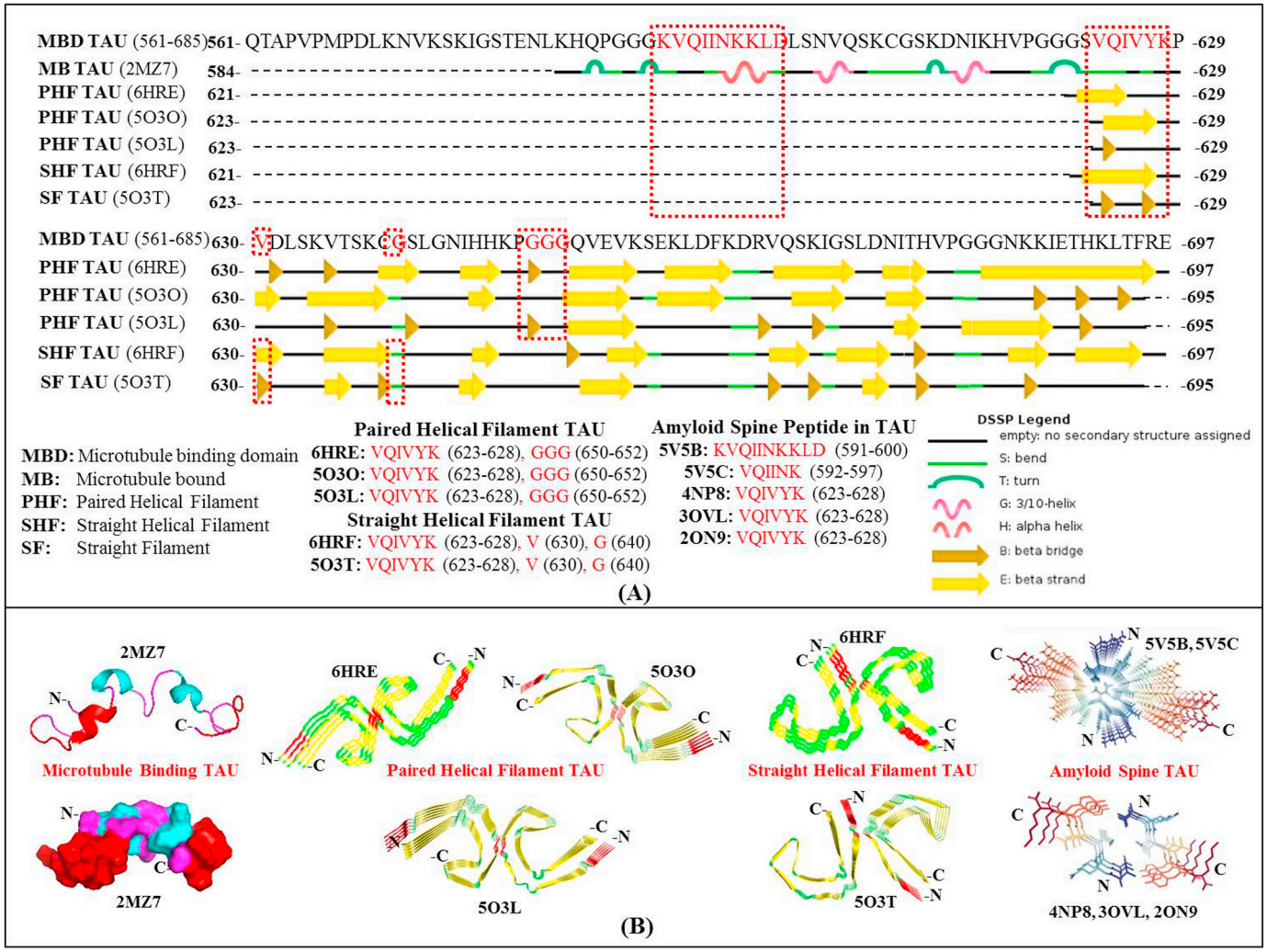


Fig. 2. Molecular basis of TAU protein aggregation- (A) Structural changes in TAU pathology: Helical conformation of microtubule binding domain is distorted into the filament structure (paired helical/ straight helical/ straight) rich in beta-strands and beta-bridges. Tripeptide glycine (650–652; Red) is responsible for paired-helical filament while valine (630; Red) and glycine (640; Red) are accountable for straight helical filament formation and consequent aggregation. Moreover, amyloid spines forming peptides are highlighted in red color that showed the sequence motif VQI(IN)/(VY)K responsible for aggregation of TAU protein in Alzheimer's disease. (B) 3D Structure of TAU protein: Microtubule binding domain of TAU (2MZ7) is shown in its cartoon and surface view with the aggregation-prone region highlighted in red. Paired-helical filaments of TAU (6HRE, 5O3O, 5O3L) reported in AD are shown in ribbon/cartoon view with their aggregation-prone regions in red color. Likewise, Straight-helical filaments of TAU (6HRF, 5O3T) reported in AD are shown in ribbon/cartoon view with their aggregation prone regions in red color. Furthermore, amyloid-spine forming TAU peptide models KVQIINKKLD and VQIVYK are shown in Licorice view that formed amyloid aggregate with help of interactions between valine and Isoleucine. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Material and methods

2.1. Investigation of the molecular basis of Aβ$_{40/42}$, Tau, and α-Synuclein aggregation

The present sequences for the neurotoxic proteins were analysed for their secondary structures, and their available monomer and fibrillar structures were annotated for their hydrophobic residues responsible for aggregation.

2.1.1. Structural determination of Aβ$_{40/42}$, Tau, and α-Synuclein

The peptide sequence of Aβ$_{40/42}$, Tau and α-Synuclein were obtained from protein data bank (RCSB PDB: www.rcsb.org; Berman et al., 2000) and processed for the determination of its secondary structure by Dictionary of protein secondary structure (DSSP) database (Touw et al., 2015).

2.1.2. Macromolecular structure design and hydrophobicity annotation

The macromolecular monomeric and fibrillar structures of Aβ$_{40/42}$, Tau, and α-Synuclein proteins have been analysed for the hydrophobic or aggregation prone residues. These sites were annotated to the available 3D-structures with help of NGL viewer (http://proteinformatics.charite.de/ngl) (Rose and Hildebrand, 2015) and Pymol software (DeLano, 2002).

2.2. Characterization of the functional partners of Aβ$_{40/42}$, Tau, and α-Synuclein involved in the pathology of Alzheimer's and Parkinson's disease

The top interacting partners of the neurotoxic proteins have predicted and analysed for their role in disease pathogenesis.

2.2.1. Prediction of interacting partners of AβPP, Tau, and α-Synuclein

The interacting partners of AβPP, Tau, and α-Synuclein was determined by functional protein association networks tool called STRING, online available at https://string-db.org/. The top interactors

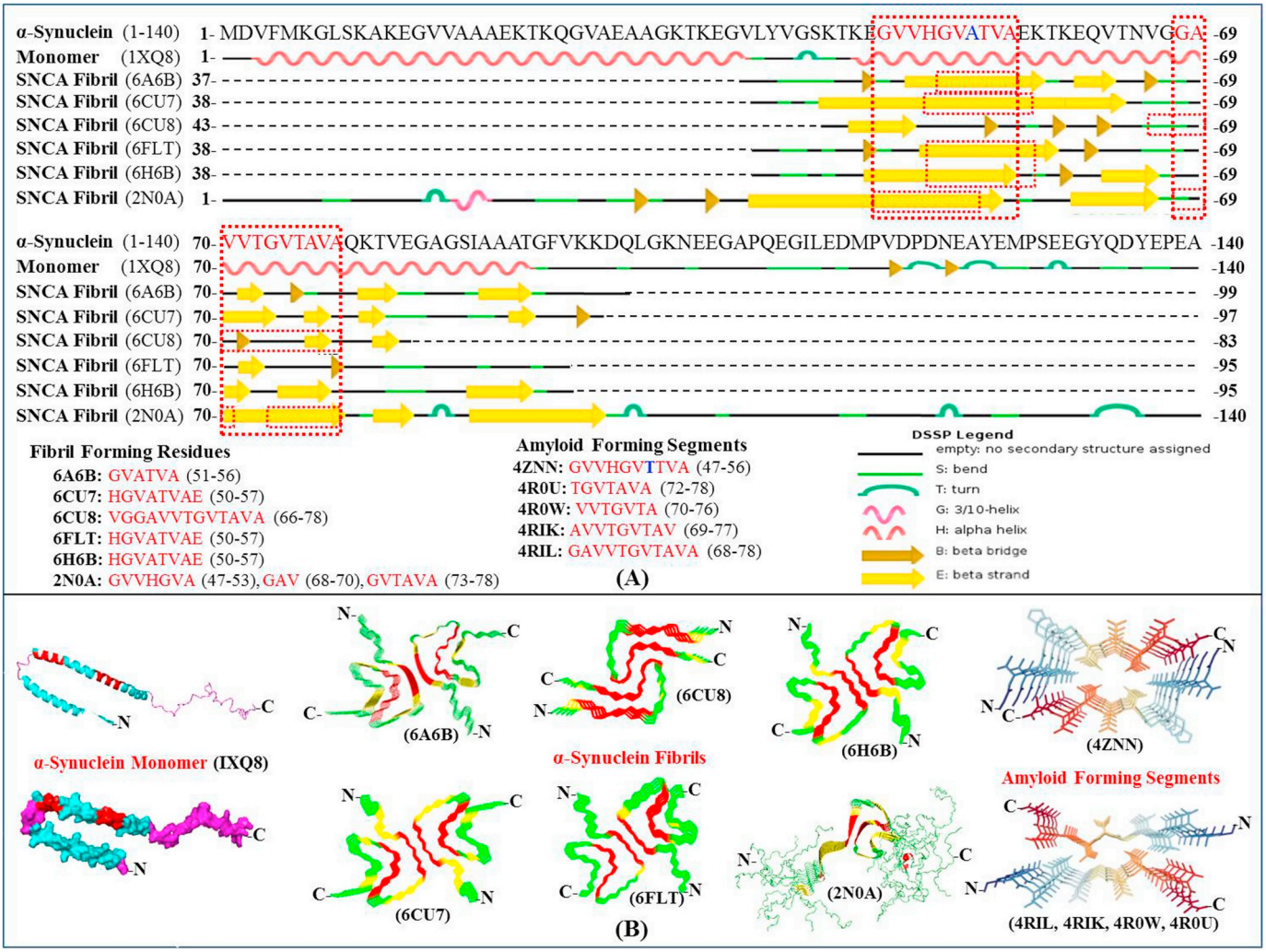


Fig. 3. Molecular basis of α-Synuclein protein aggregation- (A) Structural changes in α-Synuclein pathology: Helical conformations of synuclein monomer are distorted into the fibril structure rich in beta-strands and beta-bridges. Polypeptide regions, including GVVHGVATVAE (47-57) and GAVVTGVTAVA (68-78) are responsible for the intra- and intermolecular fibril formation and consequent aggregation of SNCA protein. Moreover, amyloid forming peptides are highlighted in red color that showed the sequence motif accountable for aggregation of SNCA protein in Alzheimer's disease. Moreover, the fibril-specific aggregation regions are shown with green rectangles. (B) 3D Structure of α-Synuclein protein: SNCA monomer (IXQ8) is shown in its cartoon and surface view with aggregation prone region highlighted in red. Reported alpha synuclein fibrils (6A6B, 6CU7, 6CU8, 6FLT, 6H6B, 2N0A) are shown in ribbon/cartoon view with their aggregation-prone regions in red color. Likewise, Straight helical filaments of TAU (6HRF, 5O3T) reported in AD are shown in ribbon/cartoon view with their aggregation prone regions in red color. Furthermore, amyloid-forming peptide models 4ZNN and 4RIL is shown in Licorice view that formed amyloid aggregate with help of interactions between their intermittent residues, glycine and valine. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

were identified with high confidence at the threshold of interaction score ≥ 0.700, and the network were generated without clustering and evidence based upon text mining, experiments, databases, co-expression, neighborhood, gene fusion and co-occurrence (Jensen et al., 2009).

2.2.2. *Protein interaction network analysis for Alzheimer's and Parkinson's disease*

Top hundred interacting partners of the AβPP, Tau, and α-Synuclein proteins were mapped on the pathways for Alzheimer's and Parkinson's disease from KEGG Pathways database and analysed for their functional association with the disease pathogenesis (Kanehisa et al., 2017).

2.3. *Investigation of the molecular crosstalk between AD and PD*

The combined AD and PD-related top-interactions of AβPP, Tau, and α-Synuclein was iterated for their interactions with another top hundred interacting partner. Those proteins that qualified their AD and PD incidence were selected and identified for their interactions with AβPP, Tau, and α-Synuclein at a high confidence threshold ≥ 0.7 with STRING tool, i.e. a Search Tool for the Retrieval of Interacting Genes/Proteins based on the evidence from text mining, experiments, databases, co-expression, neighborhood, gene fusion and co-occurrence (Szklarczyk et al., 2015). The interacting partner's prediction was followed by the Venn diagram analysis. Thus, the obtained proteins common to both Alzheimer's and Parkinson's disease, and were interacting with AβPP, Tau, and α-Synuclein, were screened as the key marker for AD-PD crosstalk.

2.4. *Identification of ubiquitination enzymes regulating the clearance of $A\beta_{40/42}$, Tau, and α-Synuclein*

The potential ubiquitination enzymes regulating the biology of Aβ, AβPP, Tau, and α-Synuclein proteasomal clearances were identified by determining the interaction among all the ubiquitin E1-activating enzymes, E2-conjugating enzymes, E3-ligating enzymes and deubiquitinating enzymes with AD-PD crosstalk proteins- AβPP, CAPN1, GSK3B, LRRK2, MAPT, PARK2, PLCB2, SNCA, and UBB at different confidences.

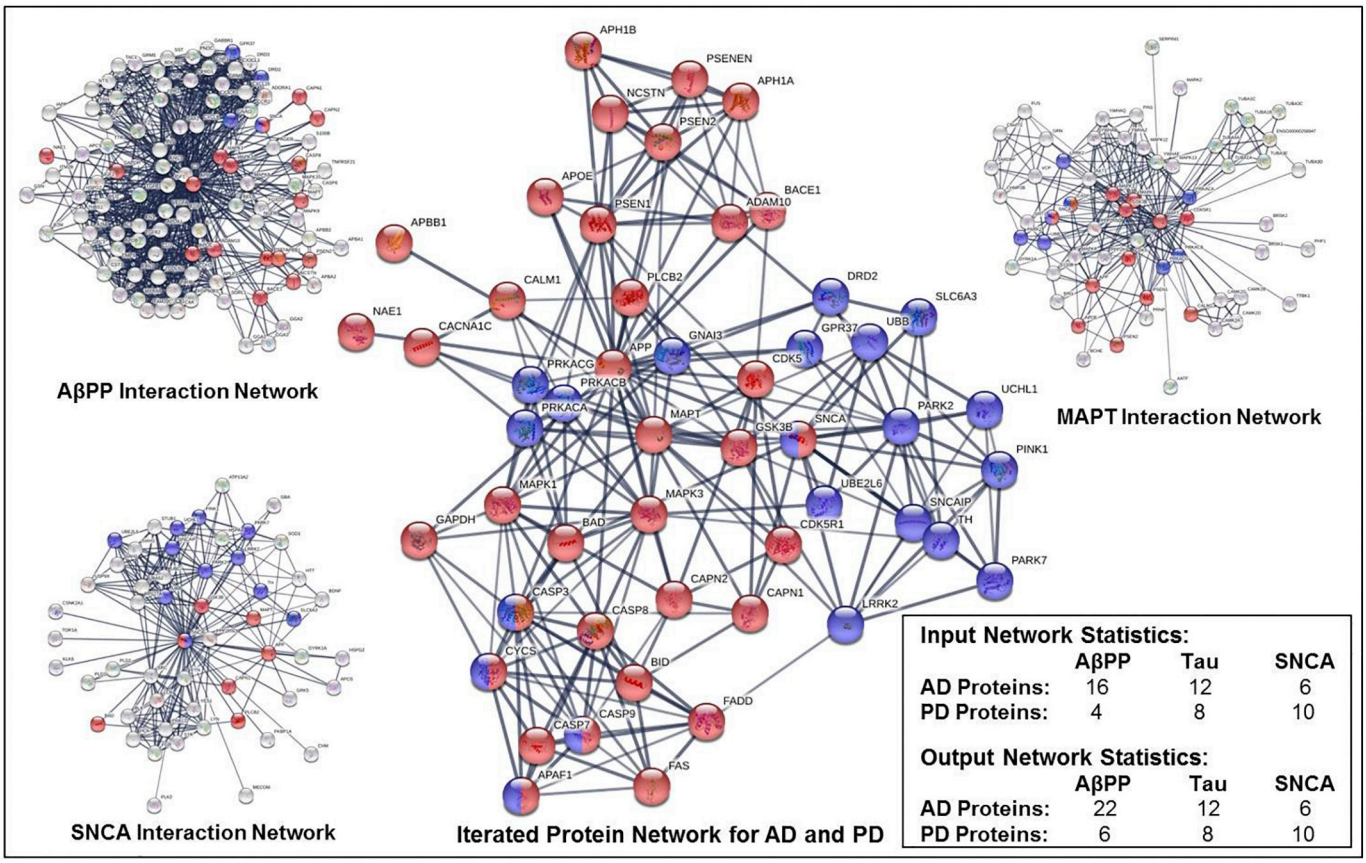


Fig. 4. Functional association network of AβPP, Tau and α-Synuclein interacting-proteins in AD and PD- The prime 100 amyloid-beta precursor interactor input proteins involved 16 AD-related proteins- ADAM10, APBB1, APOE, BACE1, CAPN1, CAPN2, CASP8, GAPDH, MAPK1, MAPK3, MAPT, NAE1, NCSTN, PSEN1, PSEN2, SNCA and 4 PD-related proteins- DRD2, GNAI3, GPR37, SNCA. Similarly, top 100 tau interactor input proteins involved 12 AD-related proteins- APOE, APP, CALM1, CASP3, CDK5, CDK5R1, GSK3B, MAPK1, MAPK3, PSEN1, PSEN2, SNCA and 8 PD-related proteins- CASP3, LRRK2, PRKACA, PRKACB, PRKACG, SNCA, PARK2, UBB. Likewise, leading 100 α-Synuclein interactor input proteins involved 6 AD-related proteins- APP, BAD, CAPN1, GSK3B, MAPT, PLCB2 and 10 PD-related proteins- LRRK2, PARK2, PARK7, PINK1, SLC6A3, SNCAIP, TH, UBB, UBE2L6, UCHL1. Moreover, the top 100 iterated interaction network of AβPP, Tau, and α-Synuclein involved the following proteins. (ADAM10-Disintegrin and metalloproteinase domain-containing protein 10; APBB1-Amyloid-beta A4 precursor protein-binding family B member 1; APH1A-Gamma-secretase subunit APH-1A; APOE-Apolipoprotein E; BACE1-Beta-secretase 1; BAD-Bcl2-associated agonist of cell death; CALM1-Calmodulin-1; CAPN1-Calpain-1 catalytic subunit; CAPN2-Calpain-2 catalytic subunit; CASP3-Caspase-3; CDK5-Cyclin-dependent-like kinase 5; CDK5R1-Cyclin-dependent kinase 5 activator 1; DRD2-D(2) dopamine receptor; GAPDH-Glyceraldehyde-3-phosphate dehydrogenase; GNAI3-Guanine nucleotide-binding protein G(k) subunit alpha; GPR37-Prosaposin receptor GPR37; GSK3B-Glycogen synthase kinase-3 beta; LRRK2-Leucine-rich repeat serine/threonine-protein kinase 2; MAPK1-Mitogen-activated protein kinase 1; MAPK3-Mitogen-activated protein kinase 3; MAPT-Microtubule-associated protein tau; NAE1-NEDD8-activating enzyme E1 regulatory subunit; NCSTN-Nicastrin; PARK2-E3 ubiquitin-protein ligase parkin; PARK7-Protein/nucleic acid deglycase DJ-1; PINK1-Serine/threonine-protein kinase PINK1; PLCB2-1-phosphatidylinositol 4;5-bisphosphate phosphodiesterase beta-2; PRKACA-cAMP-dependent protein kinase catalytic subunit alpha; PRKACB-cAMP-dependent protein kinase catalytic subunit beta; PRKACG-cAMP-dependent protein kinase catalytic subunit gamma; PSEN1-Presenilin-1; PSEN2-Presenilin-2; PSENEN-Gamma-secretase subunit PEN-2; SLC6A3-Sodium-dependent dopamine transporter; SNCAIP-Synphilin-1; TH-Tyrosine 3-monooxygenase; UBB-Polyubiquitin-B; UBE2L6-Ubiquitin/ISG15-conjugating enzyme E2 L6; UCHL1-Ubiquitin carboxyl-terminal hydrolase isozyme L1.)

Furthermore, the protein-protein interactional network among the identified proteins was designed by functional protein-association network prediction STRING tool (Szklarczyk et al., 2015).

2.5. Functional annotation of the AD-PD cross-talk and ubiquitination markers

The analysis of the biological processes, reactome pathways, molecular functions, and the protein domains of the predicted AD-PD cross talk markers and the UPS enzymes- E1s, E2s, E3s, and DUBs were performed with the help of a functional enrichment analysis tool "FunRich" version 3.1.3 (Pathan et al., 2015). It is a tool for the enrichment and interaction network analysis of genes and proteins based on data mining from the available databases, including FunRich, Uniprot, Reactome and Custom. The Unirot and reactome databases have been explored to obtain the best scoring results at very high significant *P* value, i.e. $P < .001$.

3. Results

3.1. Hydrophobic interactions are the basis of neurotoxic protein aggregation

The pathological peptides and proteins, including $A\beta_{40}$, $A\beta_{42}$, Tau, and α-synuclein in AD and PD were studied for their aggregation sites to analyze the protein folding dynamics in its diseased state. The analysis of their secondary structures revealed the transformation of their helical conformations into beta strands from their monomer to fibrillar state respectively. However, their tertiary structures have revealed the presence of crucial hydrophobic sites responsible for their intra- and inter-molecular interactions governing protein aggregation.

3.1.1. Amyloid beta peptide

The amyloid peptide existed in the two common isoforms in the brain, including $A\beta_{40}$ and $A\beta_{42}$ responsible for the senile plaques in

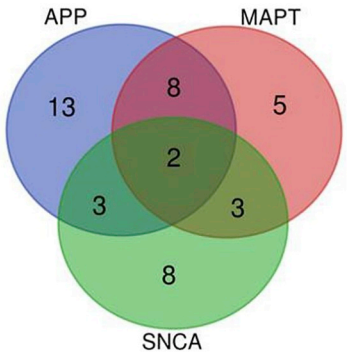
Table 1
Interacting partners of AβPP, Tau and α-Synuclein proteins and their corresponding functions.

Interacting proteins		Molecular functions
ADAM10	Disintegrin and metalloproteinase domain-containing protein 10	Responsible for the proteolytic release of TNF-alpha and several other cell-surface proteins, including heparin-binding epidermal growth-like factor, ephrin-A2, CD44, CDH2 and for constitutive and regulated alpha-secretase cleavage of amyloid precursor protein (APP)
APBB1	Amyloid-beta A4 precursor protein-binding family B member 1	Transcription coregulator with both coactivator and corepressor functions that forms a transcriptionally active complex with the gamma-secretase-derived amyloid precursor protein (APP) intracellular domain play a role in DNA damage response
APH1A	Gamma-secretase subunit APH-1A	Endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors and APP
APOE	Apolipoprotein E	Mediates the binding, internalization, and catabolism of lipoprotein particles
BACE1	Beta-secretase 1	Responsible for the proteolytic processing of the amyloid precursor protein (APP)
BAD	Bcl2-associated agonist of cell death	Promotes cell death and appears to act as a link between growth factor receptor signaling and the apoptotic pathways
CALM1	Calmodulin-1	Mediates the control of a large number of enzymes, ion channels, aquaporins and other proteins through calcium-binding
CAPN1	Calpain-1 catalytic subunit	Catalyzes limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction
CAPN2	Calpain-2 catalytic subunit	Catalyzes limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction
CASP3	Caspase-3	Involved in the activation cascade of caspases responsible for apoptosis execution
CDK5	Cyclin-dependent-like kinase 5	Essential for neuronal cell cycle arrest and differentiation and may be involved in apoptotic cell death in neuronal diseases by triggering abortive cell cycle re-entry
CDK5R1	Cyclin-dependent kinase 5 activator 1	Neuron specific activator of CDK5 involved in dendritic spine morphogenesis and required for neurite outgrowth and cortical lamination
DRD2	D(2) dopamine receptor	Dopamine receptor whose activity is mediated by G proteins which inhibit adenylyl cyclase and promote cell proliferation
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Play a role in glycolysis and nuclear functions via participating in transcription, RNA transport, DNA replication and apoptosis
GNAI3	Guanine nucleotide-binding protein G(k) subunit alpha	Function as transducers downstream of G protein-coupled receptors (GPCRs) in numerous signaling cascades
GPR37	Prosaposin receptor GPR37	Receptor for the neuroprotective and glioprotective factor prosaposin where ligand binding induces endocytosis, followed by an ERK phosphorylation cascade
GSK3B	Glycogen synthase kinase-3 beta	Acts as a negative regulator in the hormonal control of glucose homeostasis, Wnt signaling and regulation of transcription factors and microtubules
LRRK2	Leucine-rich repeat serine/threonine-protein kinase 2	Positively regulates autophagy through a calcium- dependent activation of the CaMKK/AMPK signaling pathway
MAPK1	Mitogen-activated protein kinase 1	Acts as an essential component of the MAP kinase signal transduction pathway and mediates diverse biological functions such as cell growth, adhesion, survival and differentiation through the regulation of transcription, translation, cytoskeletal rearrangements
MAPK3	Mitogen-activated protein kinase 3	Acts as an essential component of the MAP kinase signal transduction pathway and mediates diverse biological functions such as cell growth, adhesion, survival and differentiation through the regulation of transcription, translation, cytoskeletal rearrangements
NAE1	NEDD8-activating enzyme E1 regulatory subunit	Regulatory subunit of the dimeric UBA3-NAE1 E1 enzyme
NCSTN	Nicastrin	Catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors and APP (amyloid-beta precursor protein)
PARK2	E3 ubiquitin-protein ligase parkin	Functions within a multiprotein E3 ubiquitin ligase complex, catalyzing the covalent attachment of ubiquitin moieties onto substrate proteins
PARK7	Protein/nucleic acid deglycase DJ-1	Catalyzes the deglycation of the Maillard adducts formed between amino groups of proteins or nucleotides and reactive carbonyl groups of glyoxals and functions as a protein deglycase that repairs methylglyoxal- and glyoxal-glycated proteins, and releases repaired proteins and lactate or glycolate, respectively
PINK1	Serine/threonine-protein kinase PINK1	Protects against mitochondrial dysfunction during cellular stress by phosphorylating mitochondrial proteins and triggering selective autophagy (mitophagy) by mediating activation and translocation of Parkin
PLCB2	1-phosphatidylinositol 4;5-bisphosphate phosphodiesterase beta-2	Involved in the production of the second messenger molecules diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3)
PRKACA	cAMP-dependent protein kinase catalytic subunit alpha	Phosphorylates a large number of substrates in the cytoplasm and the nucleus and regulates the abundance of compartmentalized pools of its regulatory subunits
PRKACB	cAMP-dependent protein kinase catalytic subunit beta	Mediates cAMP-dependent signaling triggered by receptor binding to GPCRs that regulates diverse cellular processes such as cell proliferation, the cell cycle, differentiation and regulation of microtubule dynamics, chromatin condensation and decondensation, nuclear envelope disassembly and reassembly
PRKACG	cAMP-dependent protein kinase catalytic subunit gamma	Phosphorylates a large number of substrates in the cytoplasm and the nucleus
PSEN1	Presenilin-1	Presenilin-2; Probable catalytic subunit of the gamma-secretase complex, an endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors and APP (amyloid-beta precursor protein) and may play a role in intracellular signaling and gene expression or in linking chromatin to the nuclear membrane
PSEN2	Presenilin-2	Presenilin-2; Probable catalytic subunit of the gamma-secretase complex, an endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors and APP (amyloid-beta precursor protein) and may play a role in intracellular signaling and gene expression or in linking chromatin to the nuclear membrane
PSENEN	Gamma-secretase subunit PEN-2	Catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors and APP (amyloid-beta precursor protein) and modulates both endoproteolysis of presenilin and gamma-secretase activity
SLC6A3	Sodium-dependent dopamine transporter	Terminates the action of dopamine by its high affinity sodium-dependent reuptake into presynaptic terminals
SNCAIP	Synphilin-1	Isoform 2 inhibits the ubiquitin ligase activity of SIAH1 and inhibits proteasomal degradation of target proteins
TH	Tyrosine 3-monooxygenase	Plays an important role in the physiology of adrenergic neurons

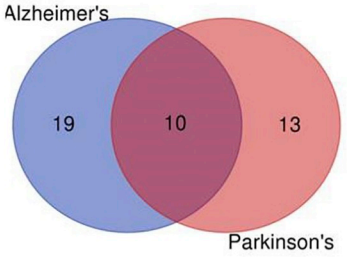
(*continued on next page*)

Table 1 (*continued*)

Interacting proteins		Molecular functions
UBB	Polyubiquitin-B	Form polyubiquitin chains on target proteins and regulate different functions depending on the Lys residue of the ubiquitin that is linked
UBE2L6	Ubiquitin/ISG15-conjugating enzyme E2 L6	Catalyzes the covalent attachment of ubiquitin or ISG15 to other proteins
UCHL1	Ubiquitin carboxyl-terminal hydrolase isozyme L1	Ubiquitin-protein hydrolase involved both in the processing of ubiquitin precursors and of ubiquitinated proteins



Names	total	elements
APP MAPT SNCA	2	GSK3B PARK2
APP MAPT	8	PSEN2 MAPK1 CDK5 SNCA CASP3 MAPK3 APOE PSEN1
APP SNCA	3	PLCB2 CAPN1 MAPT
MAPT SNCA	3	UBB APP LRRK2
APP	13	PSENEN BACE1 ADAM10 APH1A GNAI3 APBB1 NCSTN DRD2 CAPN2 GPR37 CASP8 GAPDH NAE1
MAPT	5	CDK5R1 PRKACA PRKACB PRKACG CALM1
SNCA	8	PINK1 SLC6A3 BAD PARK7 UBE2L6 SNCAIP TH UCHL1



Names	total	elements
Alzheimer's Parkinson's	10	PLCB2 UBB APP GSK3B SNCA CAPN1 CASP3 PARK2 LRRK2 MAPT
Alzheimer's	19	PSENEN BACE1 ADAM10 CASSP8 APH1A APBB1 PSEN2 MAPK1 NCSTN BAD CDK5 CDK5R1 CAPN2 MAPK3 APOE CALM1 GAPDH PSEN1 NAE1
Parkinson's	13	PINK1 GNAI3 SLC6A3 DRD2 PARK7 PRKACA PRKACB UBE2L6 PRKACG GPR37 SNCAIP TH UCHL1

Key markers for AD and PD crosstalk:

APP- Amyloid beta precursor protein
GSK3B- Glycogen synthase kinase 3 beta
MAPT- Microtubule associated protein Tau
PLCB2- Phospholipase C Beta 2
CAPN1- Calpain-1 catalytic subunit
LRRK2- Leucine-rich repeat kinase 2
PARK2- Parkin
SNCA- Synuclein Alpha

Fig. 5. Crosstalk markers involved in the pathology of Alzheimer's and Parkinson's disease- The first Venn diagram analysis highlighted the common interacting partners of Aβ, Tau and α-Synuclein while the second Venn diagram analysis showed the disease incidences of their interacting proteins into Alzheimer's and Parkinson's disease.

Alzheimer's disease. We reported high content of alpha helical structure in $A\beta_{42}$ isoforms than $A\beta_{40}$. However, $A\beta_{42}$ found to exist in mono-fibril to di-fibril state while $A\beta_{40}$ reported up to tri fibrillar state in terms of their complexity. Moreover, they shared their aggregation regions, rich in hydrophobic residues with some additional amino acids. For instance, the aggregation sites responsible for intra- and intermolecular fibril formation in $A\beta_{40}$ and $A\beta_{42}$ were HQKLVFFAEDV (14-24)/GAIIGLMVGGVV (29-40) and HQKLVFFAEDVGS (14-26)/GAIIGLMVGGVVI (29-41) respectively, which were almost similar with only few residue differences. However, the sequence specific aggregation sites for individual fibrillar peptides have shown in Fig. 1 that was populated with glycine, alanine, valine, leucine, isoleucine and phenylalanine.

3.1.2. Microtubule associated Tau protein

The MAPT is a 758 amino acid residue long protein whose microtubule-binding domain spans from 561 to 697 amino acids. It is hydrophilic, unstructured and dynamic in its aggregation. Therefore, it binds to microtubule in a random coil like fashion. The amyloid spine forming TAU (5V5B, 5V5C, 4NP8, 3OVL, 2ON9) revealed the KVQIINKKLD (591-600), VQIINK (592-597) and VQIVYK (623-628) sequence motif crucial for its aggregation. In addition, Tau's fibrillar form (6HRE, 5O3O, 5O3L) identified two types of helical filaments, including paired-helical and straight-helical form. The paired-helical filaments reported intra- and inter-molecular interactions with help of VQIVYK (623-628) and GGG (650-652) residues while straight-helical filaments with help of VQIVYK (623-628) and Valine (630), and Glycine (640) residues. Moreover, the protein-specific aggregation sites and the structural changes in their secondary-structures have shown in Fig. 2.

3.1.3. Alpha-Synuclein

The α-Synuclein is a small protein of 140 amino acids chief among other isoforms, i.e. β- and γ-synuclein. The conformational changes in its native structure take place to form pre-fibrillar oligomers and consequent fibril formation. It is also reported to attain amyloid like conformations (4R0U, 4R0W, 4RIK, 4RIL) that are identified to be formed by GVVHGVTTVA (47-56), TGVTAVA (72-78), VVTGVTA (70-76), AVVTGVTAV (69-77), and GAVVTGVTAVA (68-78) segments. Furthermore, the polypeptide regions- GVVHGVATVAE (47-57) and

Table 2
The key ubiquitin E3 ligases regulating the clearance of AD-PD crosstalk markers.

Direct regulators for AβPP, Tau, and α-Synuclein clearance

Names	Total	Elements
APP, LRRK2, MAPT, PARK2, SNCA	1	STUB1
APP, LRRK2, MAPT, SNCA	1	PARK2
GSK3B, PARK2, SNCA	2	FBXW7, SIAH1
LRRK2, PARK2, SNCA	1	FBXO7
APP, CAPN1	1	WDTC1
APP, MAPT	1	SYVN1
GSK3B, SNCA	1	TRAF6
PARK2, SNCA	4	RNF19A, SIAH2, TRIM32, NEDD4
APP	1	FBXL2
MAPT	1	MARCH7
SNCA	1	FBXO45

Indirect regulators for AβPP, Tau, and α-Synuclein clearance

Names	Total	Elements
GSK3B, LRRK2, PARK2	1	TRAF2
CAPN1, GSK3B	1	CDH1
CAPN1, PARK2	1	TRIM63
GSK3B, PARK2	5	CUL3, SKP1, RBX1, CUL1, UBE3A
LRRK2, PARK2	4	WSB1, RANBP2, HERC2, HACE1
PARK2, PLCB2	1	RNF41
GSK3B	9	UBR5, TRIM29, BIRC2, MAP3K1, PIAS1, XIAP, NHLRC1, MDM2, APC2
LRRK2	4	ERCC8, TRIM23, RHOBTB1, RHOBTB3
PARK2	149	TRAF7, MUL1, RNF114, SPSB2, RNF217, HEWE1, DET1, ASB7, HERC1, BTBD1, VPRBP, TRIM69, HECW2, FBXL18, RNF115, ASB14, HERC3, ASB11, LRRC41, FBXW4, RNF182, KLHL2, UBR1, SKP2, ASB17, RNF25, TCEB1, FBXL16, UBE3C, ASB4, RNF34, FBXL5, FBXO4, FBXL12, FBXO21, FBXO6, CDC20, MYLIP, TRIM9, TRIM11, FBXL22, KBTBD6, UBOX5, KBTBD8, ASB16, NEDD4L, ASB6, KLHL22, CDC23, RNF14, HERC4, RBCK1, ASB1, KLHL21, TCEB2, HECTD3, ASB9, FBXL3, SOCS3, ASB12, FBXW8, TRIP12, FBXL15, CUL2, ZBTB16, MGRN1, FBXL4, RNF7, CUL7, RNF19B, SPSB1, FBXO41, RNF123, RNF31, KLHL25, ITCH, HECTD1, RNF138, ARIH1, FBXO2, RFWD2, AMFR, TRIM36, UBR4, FBXL13, LRR1, MARCH5, TRIM39, KLHL20, CCNF, CUL5, FBXL19, TRIM71, SOCS1, WWP1, FBXL8, LNX1, ASB13, FBXO27, RNF111, RCHY1, TRIM21, PJA2, FBXO22, PJA1, TRIM4, FBXO17, LRSAM1, FBXO10, UBE3B, ASB8, FBXO30, SH3RF1, FBXO44, FBXW10, ASB18, RNF130, VHL, FBXO15, DZIP3, FBXW2, TRIM37, ASB5, ZNF645, FBXO9, FBXW9, ZNRF1, UBE4A, UBE3D, CDC26, ASB10, UBR2, ASB2, FBXW5, FBXL7, ASB15, FBXL14, TRIM50, ARIH2, HECTD2, FBXO11, FBXO31, FBXO40, KLHL9, KLHL11, RBBP6, TRIM41, RLIM, TRAIP

Table 3
Ubiquitinational markers for AD-PD crosstalk proteins.

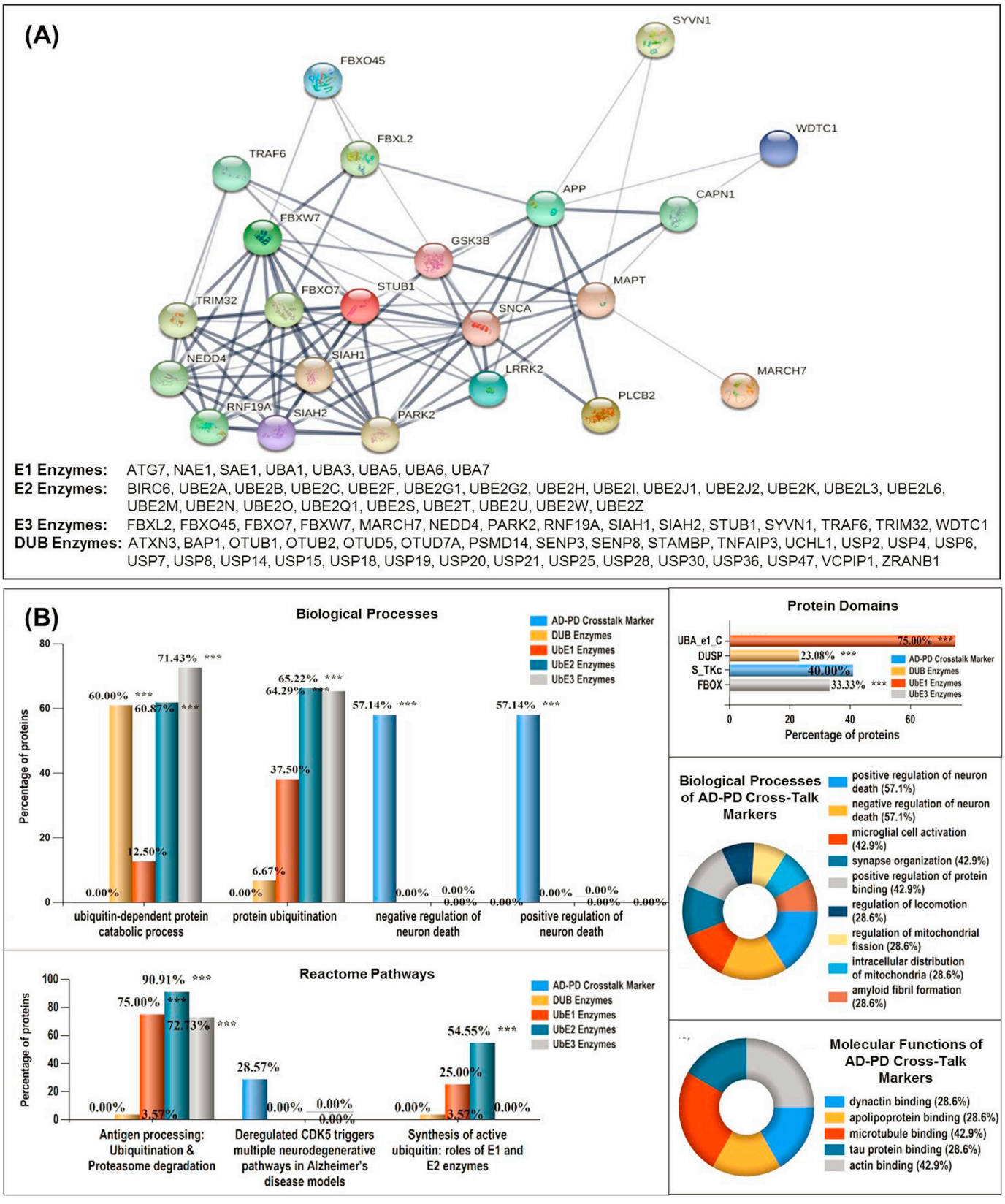
Pathological Targets	Key Ubiquitin E3 ligases	Ubiquitinating E2 Conjugating Enzymes	Ubiquitin E1 Activating Enzymes	Deubiquitinating Enzymes
AβPP, Tau, α-Synuclein, LRRK2, PARK2	**STUB1**	UBE2A, UBE2B, UBE2C, UBE2F, UBE2G1, UBE2G2, UBE2H, UBE2J1, UBE2J2, UBE2K, UBE2L6, UBE2M, UBE2N, UBE2O, UBE2Q1, UBE2S, UBE2U, UBE2W, UBE2Z, UBE2L3, BIRC6, UBE2I, UBE2T	ATG7, NAE1, SAE1, UBA1, UBA3, UBA5, UBA6, UBA7	ATXN3, USP8, USP7, USP19, PSMD14, SENP3
AβPP, Tau, α-Synuclein, LRRK2	**PARK2**	UBE2A, UBE2B, UBE2C, UBE2F, UBE2G1, UBE2G2, UBE2H, UBE2J1, UBE2J2, UBE2K, UBE2L6, UBE2M, UBE2N, UBE2O, UBE2Q1, UBE2S, UBE2U, UBE2W, UBE2Z, UBE2L3	ATG7, NAE1, SAE1, UBA1, UBA3, UBA5, UBA6, UBA7	ATXN3, USP8, USP30, UCHL1, BAP1, PSMD14, USP15
AβPP, α-Synuclein, LRRK2, PARK2	**FBXO7 (FBXL2, FBXO45)**	UBE2A, UBE2B, UBE2C, UBE2F, UBE2G1, UBE2G2, UBE2H, UBE2J1, UBE2J2, UBE2K, UBE2L6, UBE2M, UBE2N, UBE2O, UBE2Q1, UBE2S, UBE2U, UBE2W, UBE2Z	ATG7, NAE1, SAE1, UBA1, UBA3, UBA5, UBA6, UBA7	USP47,UCHL1
α-Synuclein, GSK3B, PARK2	**FBXW7**	UBE2A, UBE2B, UBE2C, UBE2F, UBE2G1, UBE2G2, UBE2H, UBE2J1, UBE2J2, UBE2K, UBE2L6, UBE2M, UBE2N, UBE2O, UBE2Q1, UBE2S, UBE2U, UBE2W, UBE2Z	ATG7, NAE1, SAE1, UBA1, UBA3, UBA5, UBA6, UBA7	USP47, USP28, USP7, USP36
α-Synuclein, GSK3B, PARK2	**SIAH1**	UBE2A, UBE2B, UBE2C, UBE2F, UBE2G1, UBE2G2, UBE2H,UBE2J1, UBE2J2, UBE2K, UBE2L6, UBE2M, UBE2N, UBE2O, UBE2Q1, UBE2S, UBE2U, UBE2W, UBE2Z, UBE2I	ATG7, NAE1, SAE1, UBA1, UBA3, UBA5, UBA6, UBA7	USP19, USP4, USP6, USP15, USP20
AβPP, Tau	**SYVN1**	UBE2G2, UBE2J1, ATG3, UBE2J2, UBE2K, UBE2G1, UBE2S	ATG7, NAE1, SAE1, UBA1, UBA3, UBA5, UBA6, UBA7	ATXN3, PSMD14, USP19, VCPIP1
α-Synuclein, PARK2	**NEDD4**	UBE2A, UBE2B, UBE2C, UBE2F, UBE2G1, UBE2G2, UBE2H, UBE2I, UBE2J1, UBE2J2, UBE2K, UBE2L3,	ATG7, NAE1, SAE1, UBA1, UBA3, UBA5,	USP2, USP7, USP8, USP14, USP18, USP20, USP25,
		UBE2L6, UBE2M, UBE2N, UBE2O, UBE2Q1, UBE2S, UBE2T, UBE2U, UBE2W, UBE2Z	UBA6, UBA7	USP28, STAMBP
-Synuclein, PARK2	**RNF19A**	UBE2A, UBE2B, UBE2C, UBE2F, UBE2G1, UBE2G2, UBE2H, UBE2J1, UBE2J2, UBE2K, UBE2L6, UBE2M, UBE2N, UBE2O, UBE2Q1, UBE2S, UBE2U, UBE2W, UBE2Z, UBE2L3	ATG7, NAE1, SAE1, UBA1, UBA3, UBA5, UBA6, UBA7	–
α-Synuclein, PARK2	**SIAH2**	UBE2A, UBE2B, UBE2C, UBE2F, UBE2G1, UBE2G2, UBE2H, UBE2J1, UBE2J2, UBE2K, UBE2L6, UBE2M, UBE2N, UBE2O, UBE2Q1, UBE2S, UBE2U, UBE2W, UBE2Z, UBE2I	ATG7, NAE1, SAE1, UBA1, UBA3, UBA5, UBA6, UBA7	USP19, USP4, USP6, USP15, USP20
α-Synuclein, PARK2	**TRIM32**	UBE2A, UBE2B, UBE2C, UBE2F, UBE2G1, UBE2G2, UBE2H, UBE2J1, UBE2J2, UBE2K, UBE2L6, UBE2M, UBE2N, UBE2O, UBE2Q1, UBE2S, UBE2U, UBE2W, UBE2Z	ATG7, NAE1, SAE1, UBA1, UBA3, UBA5, UBA6, UBA7	–
AβPP, CAPN1	**WDTC1**	UBE2M	ATG7, NAE1, SAE1, UBA1, UBA3, UBA5, UBA6, UBA7	SENP8
α-Synuclein, GSK3β	**TRAF6**	UBE2N, UBE2I, UBE2K, UBE2L3, UBE2O, UBE2S	ATG7, UBA1, UBA3, UBA5, UBA6, UBA7	USP4, USP7, OTUB1, OTUB2, TNFAIP3, ZRANB1, OTUD5, OTUD7A, STAMBP, UCHL1, USP2, USP15, USP20, USP21, USP25
Tau	**MARCH7**	UBE2G2, UBE2K, UBE2N	ATG7, UBA1, UBA3, UBA5, UBA6, UBA7	USP7

Red- Highest Confidence; Blue- High Confidence; Green- Medium Confidence

GAVVTGVTAVA (68-78) found to interact and form intra- and intermolecular fibril that have shown in Fig. 3. These α-synuclein fibrils are rich in beta-strands and beta-bridges with varied structural conformations that initiate the lewy body formation in Parkinson's disease.

3.2. Functional partners of Aβ, Tau, and α-Synuclein involved in Alzheimer's and Parkinson's disease pathology

The top-hundred interacting partners among Aβ, Tau, and α-Synuclein



(caption on next page)

Fig. 6. (A) Ubiquitination enzyme interaction network for the clearance of AD-PD cross talk markers including Aβ, Tau and α-synuclein: - E1-activating enzymes: ATG7- Ubiquitin-like modifier-activating enzyme ATG7; NAE1- NEDD8-activating enzyme E1 regulatory subunit; SAE1- SUMO-activating enzyme subunit 1; UBA1/3/5/6/7- Ubiquitin-like modifier-activating enzyme 1/3/5/6/7. E2 conjugating enzymes: BIRC6- Baculoviral IAP repeat-containing protein 6; **UBE2A/B/C/F/G1/G2/H/I/J1/J2/K/L3/L6/M/N/O/Q1/S/T/U/W/Z-** Ubiquitin Conjugating Enzyme E2 A/B/C/F/G1/G2/H/I/J1/J2/K/L3/L6/M/N/O/Q1/S/T/U/W/Z. E3-ligases: FBXL2- F-box/LRR-repeat protein 2; FBXO45- F-box/SPRY domain-containing protein 1; FBXO7- F-box only protein 7; FBXW7- F-box/WD repeat-containing protein 7; MARCH7- Membrane Associated Ring-CH-Type Finger 7; NEDD4- Neural precursor cell expressed developmentally down-regulated protein 4; PARK2- Parkin; RNF19A- Ring finger protein19A; SIAH1/2- Siah E3 Ubiquitin Protein Ligase 1/2; STUB1- Ubiquitin-protein ligase CHIP; SYVN1- Synoviolin; TRAF6- TNF receptor-associated factor 6; TRIM32- Tripartite Motif Containing 32. Deubiquitinases: ATXN3- Ataxin-3; BAP1- Ubiquitin carboxyl-terminal hydrolase BAP1; OTUB1/2- OTU Deubiquitinase 1/2; OTUD5/7A- OTU domain-containing protein 5/7A; PSMD14- 26S proteasome non-ATPase regulatory subunit 14; SENP3/8- Sentrin-specific protease 3/8; STAMBP- STAM-binding protein; TNFAIP3- Tumor necrosis factor alpha-induced protein 3; UCHL1- Ubiquitin carboxyl-terminal hydrolase isozyme L1; USP2/4/6/7/8/14/15/18/19/20/21/25/28/30/36/47- Ubiquitin carboxyl-terminal hydrolase 2/4/6/7/8/14/15/18/19/20/21/25/28/30/36/47; VCPIP1- Valosin Containing Protein Interacting Protein 1; WDTC1- WD and tetratricopeptide repeats protein 1; ZRANB1- Zinc Finger RANBP2-Type Containing-1. (B) Functional enrichment analysis of AD-PD cross talk markers and the ubiquitination enzymes: - It has analysed the most important biological processes, reactome pathways, molecular functions, and protein domains at high significance *P*-values i.e. $P < .001$. The bar graph compares the percentage of input proteins with their associated top scoring biological processes, reactome pathways and protein domains. Biological processes: Ubiquitin-dependent protein catalytic process (UbE1s- UBA6; UbE2s- BIRC6, UBE2A/B/C/G1/G2/H/I/K/L3/L6/N/S/Z; UbE3s- FBXL2, FBXO7/45, NEDD4, RNF19A, SIAH1/2, STUB1, SYVN1, TRIM32; DUBs- ATXN3, BAP1, PSMD14, USP2/4/6/7/8/14/15/18/20/21/25/28/30/36/47), Protein ubiquitination (UbE1s- SAE1, UBA1/6; UbE2s- BIRC6, UBE2A/B/C/G1/G2/H/J2/K/L3/N/S/T/W/Z; UbE3s- FBXL2, FBXO7/45, FBXW7, NEDD4, PARK2, STUB1, SYVN1, TRIM32, WDTC1; DUBs- USP7, VCPIP1), Negative regulation of neuron death (AD-PD cross-talk markers- APP, GSK3B, LRRK2, SNCA), Positive regulation of neuron death (AD-PD cross-talk markers- APP, GSK3B, MAPT, SNCA). Reactome pathways: Antigen processing: Ubiquitination and Proteasome degradation (UbE1s- ATG7, UBA1/3/5/6/7; UbE2s- UBE2A/B/2C/F/G1/G2/H/J1/J2/K/L3/L6/M/N/O/Q1/S/U/W/Z; UbE3s- FBXO7, FBXW7, NEDD4, RNF19A, SIAH1/2, STUB1, TRIM32; DUBs- PSMD14), Deregulated CDK5 triggers multiple neurodegenerative pathways in Alzheimer's disease models (AD-PD cross-talk markers- APP, CAPN1), Synthesis of active ubiquitin: roles of E1 and E2 enzymes (UbE1s- UBA1/6; UbE2s- UBE2A/B/C/G1/G2/H/K/L3/S/T/W/Z; DUBs- USP7). Protein domains: UBA_e1_C (UbE1s- UBA1/6/7), DUSP (DUBs- USP4/15/20), S_TKc (AD-PD cross-talk markers- GSK3B, LRRK2), FBOX (UbE3s- FBXL2, FBXO7/45, FBXW7). The doughnut chart depicted the biological processes and molecular functions associated with the AD-PD cross talk markers. Biological processes: Positive regulation of neuron death (APP, GSK3B, MAPT, SNCA), Negative regulation of neuron death (APP, GSK3B, LRRK2, SNCA), Microglial cell activation (APP, MAPT, SNCA), Synapse organization (APP, MAPT, SNCA), Positive regulation of protein binding (APP, GSK3B, LRRK2), Regulation of locomotion (LRRK2, SNCA), Regulation of mitochondrial fission (MAPT, LRRK2), Intracellular distribution of mitochondria (MAPT, LRRK2), Amyloid fibril formation (APP, MAPT). Molecular functions: Dynactin binding (MAPT, GSK3B), Apolipoprotein binding (APP, MAPT), Microtubule binding (LRRK2, MAPT, SNCA), Tau protein binding (GSK3B, SNCA), Actin binding (LRRK2, MAPT, SNCA).

proteins were deduced and further iterated with another leading 100 interactors at high confidence, which revealed a set of 22 AβPP interacting proteins in AD while 6 in PD. Similarly, it identified 12 Tau interacting proteins in AD and 8 in PD. Likewise, six α-Synuclein interacting proteins were reported in AD and 10 in PD. In summary, the network identified 26 AD-related proteins, including ADAM10, APBB1, APH1A, APOE, APP, BACE1, BAD, CALM1, CAPN1, CAPN2, CASP3, CASSP8, CDK5, CDK5R1, GAPDH, GSK3B, MAPK1, MAPK3, MAPT, NAE1, NCSTN, PLCB2, PSEN1, PSEN2, PSENEN, and SNCA. However, it revealed 18 PD-related proteins, including PRKACB, PRKACG, PRKACA, GNAI3, TH, DRD2, GPR37, LRRK2, SNCA, SLC6A3, SNCAIP, PARK7, PARK2, CASP3, UBE2L6, UCHL1, PINK1, and UBB. The network maps of the functional partners are shown in Fig. 4, and their corresponding functions are summarized in Table 1. Altogether, these proteins were involved in the regulation of protein catalytic activity, amyloid fiber formation and associated signaling by receptor tyrosine kinases.

3.3. GSK3B and PARK2 are key markers for AD-PD crosstalk commonly interacting with Aβ, Tau, and α-Synuclein

The Venn diagram analysis of the top interacting partners reported only two proteins, including GSK3B and PARK2 to be commonly interacting with Aβ, Tau, and α-Synuclein. Instead other markers, found to interact either any of the two or any one of the Aβ, Tau, and α-Synuclein proteins. For instance, PSEN2, MAPK1, CDK5, SNCA, CASP3, MAPK3, APOE, and PSEN1 found to interact with AβPP and Tau, while PLCB2, CAPN1, and MAPT interacting with AβPP and α-Synuclein respectively. Likewise, UBB, APP, and LRRK2 observed to interact with Tau and α-Synuclein proteins. Moreover, their disease incidence analysis reported these eight key markers, including APP, CAPN1, GSK3B, LRRK2, MAPT, PARK2, PLCB2, and SNCA involved in the crosstalk of Alzheimer's and Parkinson's disease at the molecular level. While other markers, specific for AD and PD interacting with any of their pathological partners- Aβ, Tau and α-Synuclein is shown in Fig. 5. Here, only PARK2 and GSK3B found to interact commonly with all diseased proteins, including Aβ, Tau and α-synuclein. Since, PARK2 is a ubiquitin E3 ligase. Therefore, its interaction with Aβ, Tau and α-synuclein would certainly regulate their levels in our body.

3.4. PARK2 and STUB1 are the key ubiquitin E3 ligases regulating the clearance of pathological markers in AD and PD

The ubiquitin E3 ligases were identified against all the AD-PD cross talk markers and were classified as direct- and indirect-regulators depending on their potential interaction with Aβ, Tau and α-Synuclein. Those E3 ligases that were involved in the ubiquitination of Aβ, Tau and α-Synuclein is classified as direct-regulators, while those involved with other pathological markers classified as indirect-regulators. We reported only PARK2 and STUB1 to be commonly interacting with most of the AD-PD cross talk markers, including AβPP, MAPT, SNCA, and LRRK2. Instead, other E3 ligases found to regulate AβPP, Tau, and α-Synuclein ubiquitination individually or in different combinations. Likewise, we reported indirect-regulators that were involved with the ubiquitination of markers other than AβPP, Tau, and α-Synuclein, such as CAPN1, GSK3B, LRRK2, PARK2, and PLCB2. Among them, TRAF2 found to regulate GSK3B, LRRK2, and PARK2 markers commonly. Similarly, we defined a spectrum of E3 ligases involved in the ubiquitination of AD-PD cross talk markers that are summarized in Table 2. The comprehensive study reported 149 regulatory ubiquitin E3 ligases for the ubiquitination of PARK2 ubiquitin E3-ligase. It suggested the involvement of PARK2 in both the pathology and clearance biology, i.e. negative and positive role in neurodegenerative disorders like AD and PD.

3.5. Ubiquitination biology of toxic Aβ, Tau, and α-synuclein protein clearance

The ubiquitination reaction of Aβ, Tau, and α-synuclein clearance is a complex biology of interactions among a series of E1-activating, E2-conjugating, E3-ligating and deubiquitinating enzymes. Here, we reported the important ubiquitination markers, including the E3 ligases- PARK2, STUB1, FBXW7 SIAH1, FBXO7, WDTC1, SYVN1, TRAF6, RNF19A, SIAH2, TRIM32, NEDD4, FBXL2, MARCH7, and FBXO45, and

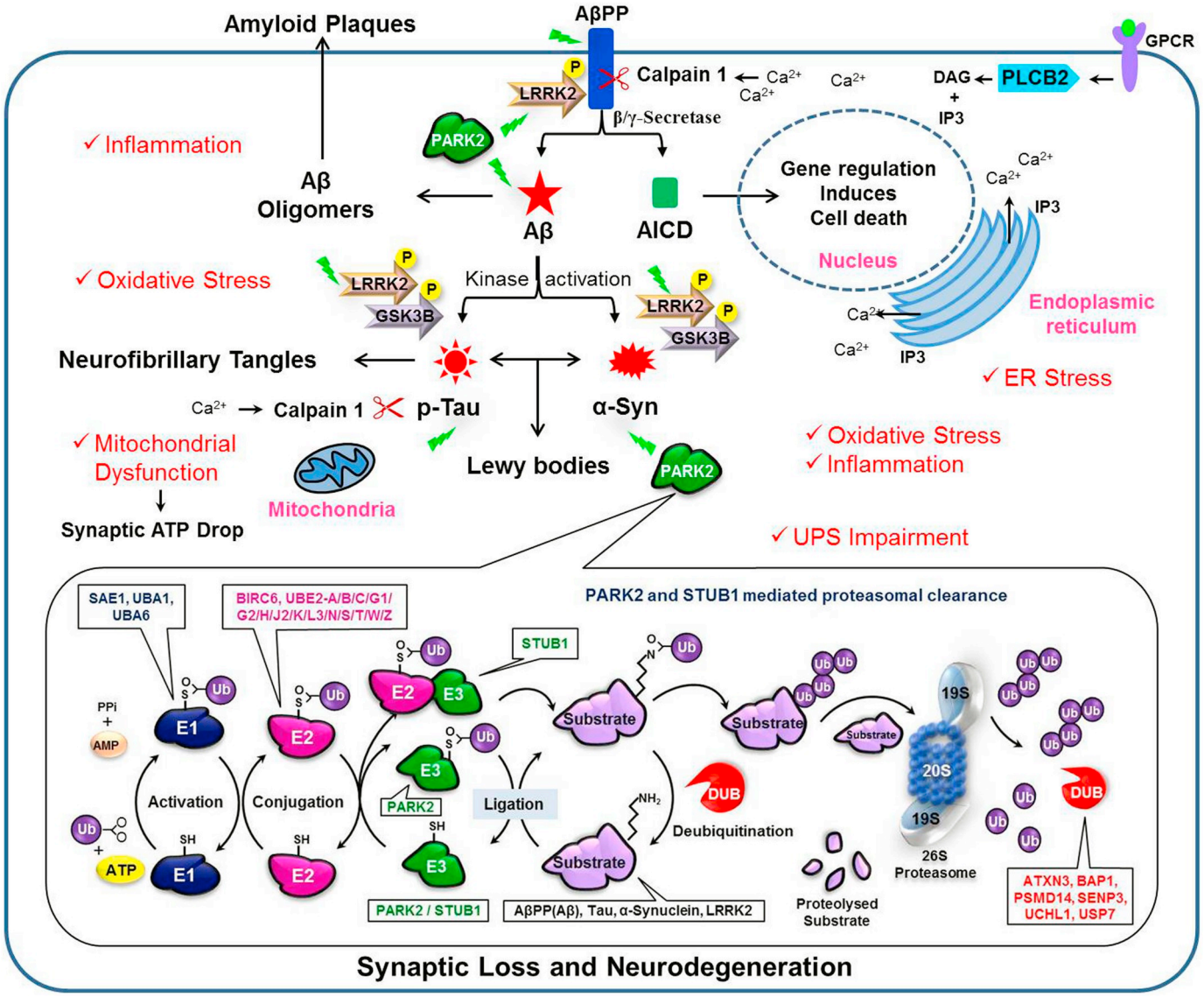


Fig. 7. Molecular mechanism of AD-PD crosstalk marker's associated neurodegeneration and their proteasomal clearance- In diseased state, proteolytic processing of AβPP leads to the production of Aβ and amyloid intracellular domain. Here, amyloid production activates the kinases like LRRK2 and GSK3B, which in turn accelerate the phosphorylation of Tau and α-Synuclein protein thereby resulting neurofibrillary tangles and lewy bodies. On the other side, AICD triggers the apoptotic gene regulation and induces cell death. Furthermore, G-protein coupled receptor activates phospholipase B (PLCB2) that triggers the calcium release from ER and consequent action of calpain 1 on their substrates, including AβPP and Tau. Here, calapin 1 mediated tau cleavage products, induces mitochondrial dysfunction and thus ATP loss. On the other side, Parkin (PARK2) as an E3 ligase acts on the pathogenic proteins- AβPP(Aβ), Tau, α-Synuclein, and LRRK2 to mark them for proteasomal degradation with the help of diverse ubiquitin activating enzymes, conjugating enzymes, and deubiquitinases. Altogether, these toxic proteins are associated with the ER stress, oxidative stress, mitochondrial dysfunction, inflammation, and UPS impairment in neurons thereby leading to synaptic loss and neurodegeneration. AICD- Amyloid intracellular domain; DAG- Diacylglycerol; PLCB2- Phospholipase B.

the deubiquitinases- ATXN3, USP8, PSMD14, and UCHL1 directly regulating the clearance of Aβ, Tau and α-synuclein. Furthermore, identified their corresponding ubiquitin E2-conjugating enzymes- UBE2A/B/C/F/G1/G2/H/J1/J2/K/L6/M/N/O/Q1/S/U/W/Z and ubiquitin E1-activating enzymes- ATG7, NAE1, SAE1, UBA1/3/5/6/7 involved in the ubiquitination biology of all these protein aggregates. In addition, we reported the majority with a similar set of E2s and E1s with crucial differences in their deubiquitinases. The important ubiquitination enzymes for the clearance of AD-PD cross talk markers are summarized in Table 3. Moreover, the enzymatic regulation of ubiquitination reaction for Aβ, Tau, and α-synuclein with their specific ubiquitin E3-ligases are mapped in Fig. 6A. These UPS enzymes along with their target AD-PD cross-talk markers were further investigated for their functional annotations, including the biological processes, reactome pathways, protein domains, and molecular functions (Fig. 6B). The biological process analysis revealed only the UPS enzymes including **UbE1s-***SAE1, UBA1/6;***UbE2s-***BIRC6, UBE2A/B/C/G1/G2/H/J2/K/L3/N/S/T/W/Z;***UbE3s-***FBXL2, FBXO7/45, FBXW7, NEDD4, PARK2, STUB1, SYVN1, TRIM32, WDTC1;* and **DUBs-***USP7, VCPIP1* to be associated with protein ubiquitination, while **UbE1s-***UBA6;***UbE2s-***BIRC6, UBE2A/B/C/G1/G2/H/I/K/L3/L6/N/S/Z;***UbE3s-***FBXL2, FBXO7/45, NEDD4, RNF19A, SIAH1/2, STUB1, SYVN1, TRIM32,* and **DUBs-***ATXN3, BAP1, PSMD14, USP2/4/6/7/8/14/15/18/20/21/25/28/30/36/47* to be linked with the ubiquitin dependent protein catabolic process. On the other hand, AD-PD cross-talk markers were reported to be linked with both positive (APP, GSK3B, MAPT, SNCA) and negative regulation (APP, GSK3B, LRRK2, SNCA) of neuronal death. The analysis revealed the peculiarity of LRRK2 in the pathogenesis of disease. Furthermore, the reactome pathway analysis identified three pathways with most significant scores, including i) UPS associated antigen processing (**UbE1s-***ATG7,*

*UBA1/3/5/6/7;***UbE2s-***UBE2A/B/2C/F/G1/G2/H/J1/J2/K/L3/L6/M/N/O/Q1/S/U/W/Z;***UbE3s-***FBXO7, FBXW7, NEDD4, RNF19A, SIAH1/2, STUB1, TRIM32; DUBs- PSMD14*), ii) CDK5 linked neurodegeneration (AD-PD cross-talk markers- APP, CAPN1), and iii) synthesis of active ubiquitin protein (**UbE1s-***UBA1/6*; **UbE2s-***UBE2A/B/C/G1/G2/H/K/L3/S/T/W/Z;***DUBs-***USP7*). In addition, the Protein domain analysis revealed the majority of ubiquitin E1 activating enzymes with UBA_e1_C domain, while ubiquitin E3 ligases with FBOX domain. Likewise, it reported DUSP domain in most of the deubiquitinases, while S_TKc domain in the majority of the AD-PD crosstalk markers. Moreover, the molecular functions and biological processes analysis of AD-PD crosstalk markers identified their roles in microglial activation, synapse organization, mitochondrial fission, amyloid fibril formation and binding with various cellular proteins, including actin, dynactin, apolipoprotein, tau and microtubule. Altogether, these findings have provided new insights into the molecular mechanisms for neurotoxic protein clearance in AD and PD.

3.6. Molecular cross talk among neurotoxic proteins and their clearance in Alzheimer's and Parkinson's disease

The functional enrichment analysis of the UPS enzymes has provided valuable insights for the choice of potential ubiquitination enzymes important for the proteasomal clearance of pathogenic proteins, while that of AD-PD cross talk markers revealed their role in protein aggregation. However, ubiquitination is a complex process of targeted protein degradation with the help of an array of ubiquitinational enzymes, especially the ubiquitin E3 ligases that are crucial for imparting the substrate specificity, but the obtained results have revealed the facts to a greater extent. Based on the evidence from our findings, we can hypothesize the molecular mechanism of the toxic protein aggregation and their clearance through the ubiquitination process for better understanding the medical state of Alzheimer's and Parkinson's disease (Fig. 7). The clinical reports suggest that the identified AD-PD cross-talk markers have their pathogenic role in both the diseases, but CAPN1 and PLCB2 are two novel markers whose roles need to be investigated in both diseases, although they are known to play some role in Alzheimer's disease. Moreover, future research is required to translate these findings to devise better diagnostic and therapeutic avenues for life management and care for the patients suffering with Alzheimer's and Parkinson's disease.

4. Discussion

The soluble forms of amyloid beta peptides- $A\beta_{40}$ and $A\beta_{42,}$ microtubule associated tau protein, and the α-synuclein protein is well evident to cause neurotoxicity in both Alzheimer's and Parkinson's disease pathology. Here, we studied their aggregation sites to analyze the protein folding dynamics in its diseased state. We found that these proteins transform their secondary structural helical conformations into beta strands upon their transition from monomer to fibrillar state. Recently, Balupuri et al. (2019) has also shown the occurrence of α-strand in the monomer to drive sheet formation in the oligomers that initiates and promotes α-synuclein aggregation and fibrillation (Balupuri et al., 2019). Moreover, the tertiary structures of amyloid beta have revealed the hydrobhobic sites HQKLVFFAEDV (14-24)/ GAIIGLMVGGVV (29-40) and HQKLVFFAEDVGS (14-26)/ GAIIGLMVGGVVI (29-41) in $A\beta_{40}$ and $A\beta_{42}$ respectively that are responsible for the intra- and inter-molecular interactions during protein aggregation. The hydrophobic residues at these sites rearrange their non-covalent interactions via disrupting their previous secondary structures and resulting in a seed-nucleus formation. It provides a surface for a series of misfolding events and molecular interactions with other proteins to form oligomers and consequent protofibrils (Marinko et al., 2019). Interestingly, Cox et al. (2018) also confirmed that protein aggregation is a biological driven process that evades out the intrinsic hydrophobic regions due to mutations or protein misfolding events (Cox et al., 2018). Moreover, Hao et al. (2010) demonstrated that $A\beta_{20–29}$ peptide blocks apoE-Aβ interaction through competitive binding at c-terminal domain of apoE and consequently, reduces full-length $A\beta_{40/42}$ fibril formation and cytotoxicity (Hao et al., 2010). This indicates that the proximal aggregation sites HQKLVFFAEDV (14-24) in $A\beta_{40}$ and HQKLVFFAEDVGS (14-26) in $A\beta_{42}$ as shown in our study has unique ability for self-aggregation as well as interaction with other proteins like apoE to facilitate its own aggregation.

Furthermore, the aggregation sites in amyloid beta peptides have revealed the spanning lysine residues that was also reported by Sinha et al. (2012) who have shown the role of lysine residues (K16) in amyloid beta folding, assembly and toxicity (Sinha et al., 2012). Moreover, the aggregation sequences in Aβ, Tau, and α-synuclein protein was highly populated with glycine and hydrophobic residues predominating alanine, valine, isoleucine and phenylalanine. Matsui et al. (2017) has also demonstrated the α-helix rules i.e. hydrophobicity of amino acids in the α-helix structure as a potential rationale for aggregation hotspot prediction (Matsui et al., 2017). Moreover, the arrangement of monomer in antiparallel fashion led to cooperative formation of β-sheet conformation (Lovas et al., 2013), and they attained different topologies based on the diversity of their intra- and inter-chain interactions. In fact, NMR studies have also shown the contribution of hydrophobic interaction and salt bridges in imparting the stability to beta-sheets and turns in protein folding and assembly (Petkova et al., 2002, 2006). However, their reverse transition from β-sheets to random coils is the governing principle for neuro-protection adopted by small peptides like NAP (NAPVSIPQ) and SAL (SALLRSIPA) in tau protein (Mokhtari et al., 2016). Interestingly, we identified VQIVYK (623–628) sequence in Tau protein responsible for its fibrillar state indicating their potential role in causing neurotoxicity that can be prevented by disrupting their specific interactions with help of certain short peptides. Moreove, the presence of glycine residue is also important for imparting flexibility to the protein structure, which can fit into both hydrophilic and hydrophobic environments due to its minimal side chain (Scott et al., 2007). Therefore, it sterically allows the protein aggregate to attain different conformations. In addition, hydrophobic regions are also responsible for the hydrophobic interactions among themselves that allow the protein to attain a variety of fibrillar forms with a common hydrophobic core (Kalinowska et al., 2017). On the other side, hydrophobic interactions with other proteins also trigger neuroinfammation. For instance, hydrophobic interactions of glycine zipper fragments of amyloid beta peptides with nitric oxide synthase facilitate the nitric oxide formation and consequent inflammation in neurons (Padayachee and Whiteley, 2013). Altogether, these findings enforce that the surface hydrophobicity guides the process of neurotoxic protein aggregation and consequent inflammation in different neurodegenerative disorders.

Alzheimer's and Parkinson's disease are two distinct neurodegenerative disorders with some pathological similarities. Therefore, numerous studies have investigated the links between them at protein level and found some protein aggregates /CSF peptides in common (Jolkkonen et al., 1991; Moskvina et al., 2013). The primary objective of this study was to investigate the shared pathogenic markers associated with Alzheimer's and Parkinson's disease. Since, the reported pathological proteins (Aβ, Tau and α-synuclein) interact synergistically to accelerate the neuropathology (Clinton et al., 2010); thus, we deduced the AD-PD cross-talk markers by identifying their functional interacting partners involved in the pathology of AD and PD. Altogether, we reported five markers apart from Aβ or AβPP, MAPT, and SNCA, including GSK3B, PARK2, LRRK2, PLCB2, and CAPN1 that are expressed in common during the diseased state. However, these markers have shown their involvement in either of the disease previously, but recent findings have shown their role in both diseases. For instance, LRRK2 variant R1628P increased the risk of AD in the population, and in-vitro findings suggested its predisposition to apoptosis (Zhao et al., 2011). On the other hand, LRRK2 mutation,

G2019S reported to promote AβPP phosphorylation and consequent AICD activity mediated neurotoxicity in PD (Chen et al., 2017). Likewise, apart from tau hyper-phosphorylation, it increased β-amyloid production and inflammatory responses in AD (Hooper et al., 2008). Moreover, GSK3B dysregulations, also contributed towards Parkinson's disease like pathology with induced phosphorylation and aggregation of Tau and α-synuclein (Credle et al., 2015), while it acts positively for neuronal growth in his health state (Yang et al., 2015). Similarly, PARK2 has shown their role in AD, since its enhancement has compensated mitophagic alterations in their pathology (Martin-Maestro et al., 2016). Here, CAPN1 and PLCB2 are two novel markers for AD-PD crosstalk whose roles are still illusive in both diseases, although they are known to play some role in Alzheimer's disease.

Interestingly, our study has reported GSK3B and PARK2 among the crosstalk markers commonly interacting with Aβ, Tau, and α-Synuclein signifying them as the potential candidates for regulating the pathophysiology of Alzheimer's and Parkinson's disease. Another objective of our study was to investigate the UPS markers crucial for the proteasomal clearance of toxic peptides from neurons. Here, we reported fifteen critical ubiquitin E3 ligases for the clearance of AD-PD crosstalk markers, including STUB1, PARK2, FBXW7, SIAH1, FBXO7, WDTC1, SYVN1, TRAF6, RNF19A, SIAH2, TRIM32, NEDD4, FBXL2, MARCH7, and FBXO45. Interestingly, only PARK2 and STUB1 are found to interact with all the key toxic proteins- Aβ, Tau, and α-Synuclein. It suggested the involvement of PARK2 in both the pathology and clearance biology, i.e. negative and positive role in neurodegenerative disorders like AD and PD. For instance, in the healthy state, HSPs, STUB1 and Parkin are known to play a critical role in refolding or targeting of these neurotoxic proteins to ubiquitin proteasome system for degradation (Yao, 2010), but the expression varies. However, in case of AD, Parkin's level is elevated along with HSPs and STUB1, but in case of PD, loss of parkin has been observed with high HSP and STUB1 level resulting in altered mitophagy and consequent pathologies (Kumar et al., 2012). Altogether, these findings reinstate that Parkin has a dual role, i.e. itself a molecular marker for AD-PD cross talk, and its role in the ubiquitination biology of toxic aggregates. Furthermore, AD-PD cross talk markers and their ubiquitination enzymes were extensively investigated for the precise molecular mechanism of Aβ, Tau, and α-Synuclein ubiquitination. It proposed the members from different classes of ubiquitination enzymes associated with the protein clearance in humans, including UbE1s- SAE1, UBA1/6; UbE2s- BIRC6, UBE2A/B/C/G1/G2/H/J2/K/L3/N/S/T/W/Z; UbE3s- FBXL2, FBXO7/45, FBXW7, NEDD4, PARK2, STUB1, SYVN1, TRIM32, WDTC1; and DUBs- USP7, VCPIP1. Moreover, the AD-PD cross talk markers are mapped on the reactome pathways and analysed for their biological functions in neurons. Based on the current findings, we hypothesized the molecular mechanism of neurotoxic protein aggregation and their proteasomal clearance in AD and PD. Overall, our study findings suggest a crucial role of PARK2 in the pathogenesis and therapeutics of neurodegenerative disorders like Alzheimer's and Parkinson's disease.

5. Conclusion

The formation of misfolded protein aggregates is the key hallmark of many neurodegenerative diseases that trigger the neurotoxicity and consequent proteostatic collapse. In addition, active research is going on to unravel the mechanism of protein folding and aggregation. Here, the distortion of helical conformation into beta-strands/bridges containing fibrils is the active principle for aggregation in amyloid-beta, Tau, and α-synuclein proteins. Moreover, aggregation sequences in $Aβ_{40/42}$: HQKLVFFAEDV (14-24), GAIIGLMVGGVV (29-40)/ HQKLVFFAEDVGS (14-26), GAIIGLMVGGVVI (29-41); Tau: GGG(650-652) and V630/G640 in paired and straight helical filaments, and VQI(I/V)(N/Y)K in amyloid-spines; α-Synuclein: GVVHGVATVAE (47-57) and GAVVTGVTAVA (68-78) was rich in glycine and hydrophobic residues-alanine, valine, isoleucine and phenylalanine. These hydrophobic residues were involved in the intra-chain and inter-chain interactions and reported to interact with other proteins involved in the pathogenesis of AD and PD. Therefore, the elucidation of aggregation sites in these pathological proteins and identification of their interacting partners would enable us to identify novel therapies for multiple disease states. Furthermore, the interactome analysis identified AβPP, CAPN1, GSK3B, LRRK2, MAPT, PARK2, PLCB2, and SNCA as key markers for AD-PD cross talk with GSK3B and PARK2 as common interactor of amyloid-beta, tau, and alpha synuclein. In addition, the identification of ubiquitinational markers, including E3 ligases- PARK2, STUB1, FBXW7, SIAH1, FBXO7, WDTC1, SYVN1, TRAF6, RNF19A, SIAH2, TRIM32, NEDD4, FBXL2, MARCH7, and FBXO45; and the deubiquitinases- ATXN3, USP8, PSMD14, and UCHL1 as direct-regulators of Aβ, Tau and α-synuclein ubiquitination, would help us to devise better therapeutic options for targeting misfolded proteins and large-scale rebalancing of proteostatic network. Moreover, the ubiquitination reaction is a complex biology of interactions among a series of E1-activating, E2-conjugating, E3-ligating and deubiquitinating enzymes that are addressed here for the clearance of AD-PD cross talk markers. Altogether, these key findings can help the scientists to accelerate the identification of novel therapeutic modalities for such age related incurable neurodegenerative pathologies, including AD and PD.

Declaration of Competing Interest

There is no conflict or competing interest declared by the authors.

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