Volume 10, Issue 7, July – 2025 International Journal of Innovative Science and Research Technology
ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1837

Molecular Insights into Prion Degradation in Creutzfeldt Jakob Disease's Challenges and Future Directions: A Review

Mithula Jinde[1]; Dr. Estuti Chandra[2]; Poornima S[3]

B.Sc. Final Year Student[1]; Assistant Professor[2]; Assistant Professor[3]
Core Allied Healthcare, Core Allied Healthcare, Core Allied Healthcare
[1;2;3]School of Allied Healthcare and Sciences JAIN (Deemed to be) University

Publication Date: 2025/08/06

Abstract: Creutzfeldt-Jakob Disease (CJD) is a rare, fatal neurodegenerative disorder characterized by the accumulation of misfolded prion proteins (PrP^Sc) in the central nervous system. This review explores the molecular dynamics of prion misfolding and its implications for disease progression, with a particular focus on cellular degradation pathways. Key proteolytic systems, including the ubiquitin-proteasome system and the autophagy-lysosome pathway, are critically analyzed for their roles in the clearance of PrP^Sc. Special emphasis is placed on lysosomal involvement, where autophagosomes fuse to form autolysosomes, facilitating the breakdown of pathogenic proteins. The interplay between proteases and molecular chaperones in maintaining protein homeostasis is also discussed. Neuropathologically, CJD is marked by spongiform alterations, neuronal loss, and gliosis. Clinically, the disease presents with rapidly progressive dementia, motor impairments, and psychiatric symptoms. The heterogeneity of CJD is addressed by outlining its sporadic, familial, iatrogenic, and variant subtypes. Recent advances in diagnostic techniques, including real-time quaking-induced conversion (RT-QuIC) in peripheral tissues, as well as the integration of machine learning tools and AI-assisted biomarker discovery, are highlighted. Emerging therapeutic strategies targeting proteolytic and lysosomal pathways are also reviewed, offering potential for future intervention in this currently untreatable disease.

Keywords: *Creutzfeldt-Jakob Disease (CJD), Prion Proteins, Neurodegeneration, Sporadic CJD, Variant CJD, Protein Misfolding, Real-Time Quaking-Induced Conversion (RT-QuIC), Biomarkers, Magnetic Resonance Imaging (MRI), Therapeutic Approaches.*

How to Cite: Mithula Jinde; Dr. Estuti Chandra; Poornima S (2025) Molecular Insights into Prion Degradation in Creutzfeldt Jakob Disease's Challenges and Future Directions: A Review. *International Journal of Innovative Science and Research Technology*, 10(7), 3001-3013. https://doi.org/10.38124/ijisrt/25jul1837

I. INTRODUCTION

Prion Proteins, found primarily on the surface of neurons and minor quantity in other tissues, have an incompletely understood physiological function. These proteins typically facilitate normal brain functions. However, prions are prone to abnormal folding, leading to misfolded prion proteins that cause diseases like Creutzfeldt-Jakob Disease (CJD), a neurodegenerative disorder affecting the brain.(Prusiner, 1998) (Sigurdson et al., 2019) This review focuses on prion misfolding, the normal folding of prions, and the degradation mechanisms of healthy prion proteins versus the persistence of misfolded prions. Prion diseases are characterized by the accumulation of misfolded proteins that lead to neurodegenerative conditions in humans. When cells fail to regulate large proteins, these proteins accumulate in the extracellular matrix, causing severe cell damage and potentially triggering apoptosis. The buildup of these misfolded proteins is a primary cause of neurodegenerative diseases. Transmissible spongiform encephalopathies (TSE), which include CJD, are rare but fatal neurological disorders occurring in sporadic, familial, and environmentally acquired forms. Historically, iatrogenic cases (acquired through medical procedures) have caused nearly 200 deaths over the past two decades.(*Enzymatic Degradation of Prion Protein in Brain Stem from Infected Cattle and Sheep | The Journal of Infectious Diseases | Oxford Academic*, n.d.) Contaminated human growth hormone from cadavers, dura mater grafts, and, in isolated cases, contaminated neurosurgical instruments and corneal grafts, have been significant sources. There is growing concern about the potential risk of acquiring TSE through blood or blood products, though no epidemiological evidence currently supports this. Experiments are ongoing to determine which blood components or plasma derivatives might pose the highest risk.(Prusiner, 1998) Globally, CJD affects approximately 1 to 2 people per million annually, making it one of the rarest neurodegenerative disorders with no cure or appropriate

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1837

treatment. The incidence of CJD can vary widely by region due to factors like genetic predisposition, surveillance practices, and demographics. In the United States, the incidence is about 1 case per million people per year. The United Kingdom saw an increase in CJD cases due to variant CJD (vCJD) linked to bovine spongiform encephalopathy (BSE or "mad cow disease"), though vCJD cases have significantly decreased in recent years.(Gao et al., 2024) Japan reports one of the highest incidences of sporadic CJD worldwide, while the incidence varies across European countries. Surveillance and reporting practices also differ between countries, impacting the accuracy and comparability of incidence data. For detailed statistics on CJD incidence by region and country, it is recommended to refer to public health authorities or scientific publications.

CJD can occur naturally (sporadic), due to genetic mutations (familial), or through the spread of prion protein from contaminated tissues (acquired). The cause of sporadic CJD is unknown, but familial cases are caused by mutations in the PRNP gene. Acquired CJD can result from medical procedures, contaminated meat, or contact with infected tissue. At UCSF, CJD is sometimes called the "great mimicker" because it causes symptoms similar to many other neurological diseases. Symptoms of CJD, which progress rapidly, vary based on the type of CJD and the affected brain regions. Early symptoms include behavioral and personality changes, confusion, memory problems, depression, insomnia, lack of coordination, and vision problems. Cognitive symptoms include progressive memory impairment, confusion, disorientation, difficulty concentrating or reasoning, and decline in executive function. Behavioral and psychological symptoms include personality changes, social withdrawal, psychotic symptoms, and mood symptoms such as depression and anxiety. Physical symptoms include muscle stiffness, myoclonus, ataxia, weakness, and speech disorders.(Kojima et al., 2013) Neurological symptoms include rapidly progressive dementia, seizures, visual disturbances, and sensory disturbances. Symptoms vary from person to person, and not all symptoms appear in every case of CJD. Early diagnosis and supportive measures are crucial for treating CJD and optimizing the quality of life for affected individuals and their families. (*Creutzfeldt-Jakob Disease | National Institute of Neurological Disorders and Stroke*, n.d.)

II. TYPES OF CREUTZFELDT-JAKOB DISEASE

- *Sporadic Creutzfeldt-Jakob Disease (sCJD)*

Sporadic CJD, the most common form of this fatal neurodegenerative disorder, occurs spontaneously without genetic mutations or environmental triggers. It involves the misfolding of normal prion proteins (PrPC) into the infectious form (PrPSc), leading to brain prion aggregates. the clinical manifestations include rapidly progressive dementia, cognitive decline, memory loss, and impaired executive function, along with muscle stiffness, myoclonus, coordination difficulties, gait abnormalities, and visual disturbances. The disease escalates at faster pace, resulting in severe disability and significantly impacting the patient's quality of life. (*Classification of Sporadic Creutzfeldt-Jakob Disease Based on Molecular and Phenotypic Analysis of 300 Subjects - Parchi - 1999 - Annals of Neurology - Wiley Online Library*, n.d.)

- *Genetic or Familial Creutzfeldt-Jakob Disease (gCJD)*

Genetic or Familial CJD is caused by mutations in the PRNP gene on chromosome 20, which encodes the normal prion protein (PrPC). These mutations lead to an inherited risk of prion protein misfolding. Scientists have discovered over 50 mutations, the clinical presentation varies widely within affected families, including rapidly progressive dementia, myoclonus, ataxia, and psychiatric symptoms. The age of onset, disease duration, and predominant symptoms differ significantly among individuals and families due to the diversity of PRNP mutations.(Guentchev et al., 1999)

- *Iatrogenic Creutzfeldt-Jakob Disease (iCJD)*

Iatrogenic CJD results from medical interventions, such as exposure to contaminated surgical instruments, corneal grafts, dura mater grafts, or pituitary-derived growth hormone. Infectious prions come from individuals unknowingly carrying the abnormal protein. Symptoms, similar to sporadic CJD, include rapidly progressive dementia, muscle stiffness, myoclonus, and neurological deficits. Symptoms manifest according to exposure timing, and the incubation periods are variable(*Iatrogenic Creutzfeldt-Jakob Disease, Final Assessment - PMC*, n.d.)

- *Variant Creutzfeldt-Jakob Disease (vCJD)*

Variant CJD is linked to consuming meat contaminated with the prion causing bovine spongiform encephalopathy (BSE or "mad cow disease"). It generally targets younger individuals, with onset in the late teens to early 30s. Early symptoms include psychiatric issues like depression, anxiety, and hallucinations, along with sensory abnormalities such as pain or numbness. As the disease progresses, ataxia, myoclonus, and rapidly evolving dementia become prominent.(*A New Variant of Creutzfeldt-Jakob Disease in the UK - The Lancet*, n.d.), (*Variant Creutzfeldt–Jakob Disease: Risk of Transmission by Blood Transfusion and Blood Therapies - IRONSIDE - 2006 - Haemophilia - Wiley Online Library*, n.d.)6

- *Current Diagnosis and Advancements in Diagnostics*

Early diagnosis of sporadic Creutzfeldt-Jakob Disease (sCJD) remains challenging due to the heterogeneous and often subtle onset of symptoms. Magnetic resonance imaging (MRI) and electroencephalography (EEG) are key non-invasive tools in the clinical workup. MRI typically reveals hyperintensities in cortical regions (especially parietal and occipital lobes), basal ganglia (caudate, putamen), and thalamus using diffusion-weighted imaging (DWI) and fluid-attenuated inversion recovery (FLAIR) sequences. In a study involving 436 sCJD patients and 141 controls, involvement of at least two cortical regions or both the caudate and putamen significantly improved diagnostic sensitivity and specificity (Zerr et al., 2009). EEG findings such as periodic sharp wave complexes (PSWCs) may also support diagnosis, though these can be absent early in the disease course (Mader, 2013).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1837

Molecular assays further enhance diagnostic precision. The Real-Time Quaking-Induced Conversion (RT-QuIC) assay detects prion seeding activity with high sensitivity and specificity using cerebrospinal fluid, olfactory mucosa, or skin biopsies (Orrù et al., 2023; Mammana et al., 2020). RT-QuIC is more effective in sporadic forms of CJD than in variant forms.

The Protein Misfolding Cyclic Amplification (PMCA) assay mimics the natural conversion of PrP^C to PrP^Sc and amplifies misfolded prions in vitro. Though technically demanding and reliant on fresh brain tissue, it remains a valuable research tool and complements RT-QuIC in diagnostic pipelines (Wang et al., 2023; Ferreira & Caughey, 2020).

Additionally, bioinformatics tools such as PLAAC, PrionScan, and PAPA predict prion-like domains across species, facilitating the study of structural mutations (e.g., 1E1S, 1FKC, 2K1D) in the PRNP gene that promote misfolding via altered cation–π interactions and structural instability (Gil-Garcia et al., 2021; George Priya Doss et al., 2013).

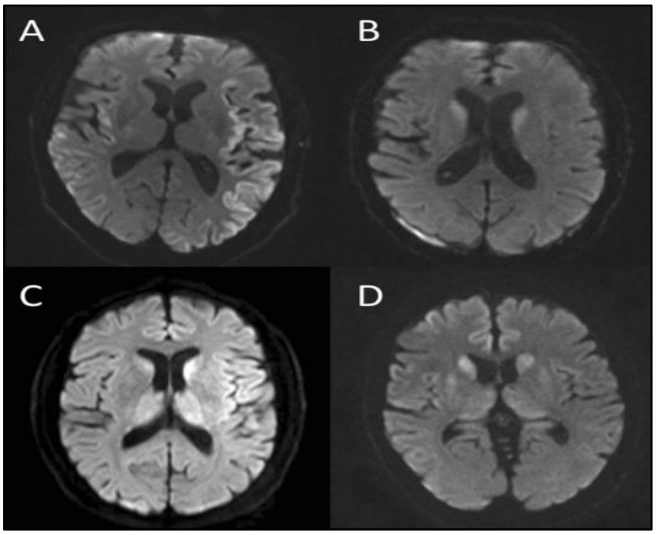

Fig 1 Adapted from (Jesuthasan et al., 2022) Article

Pictorial representation in which the MRI scan of 4 cases which are different from one another, Image A shows significant cortical involvement, which the radiologist noted with differential diagnoses of seizure or hypoxia. Image B exhibits high-intensity changes in the striatum that were not documented in the report. Images C and D demonstrate high-intensity changes in all three regions typically affected in CJD: the cortex, thalamus, and striatum. The report for Image C missed these changes entirely, while the report for Image D only mentioned abnormal signals in the basal ganglia and suggested differentials such as hypoglycemia, hypoxia, and mitochondrial or metabolic causes.

- *Structural Insights into the Prion Protein (PrP) and its Misfolding*

The human prion protein (PrP) comprises two main domains: a flexible, intrinsically disordered N-terminal region (residues 23–124) and a structured C-terminal globular domain (residues 125–231). The N-terminal region includes functionally relevant motifs such as a polybasic segment, histidine-rich octapeptide repeats (residues 59–90), and a hydrophobic stretch implicated in membrane interactions. The C-terminal domain adopts a conserved structure composed of three α-helices (residues 144–154, 175–193, and 200–219) and two short antiparallel β-strands (residues 128–131 and 161–164), forming the core of the prion fold. (Shafiq et al., 2022)

In prion diseases, PrPC misfolds into PrPS^c, a β-sheet-rich, aggregation-prone isoform resistant to proteolysis and chemical disinfection. This structural transition remains incompletely understood but involves significant energy barriers, unfolding events, and complex oligomerization kinetics. Cryo-EM and biophysical studies suggest that PrPS^c exists in multiple conformers, including oligomers, protofibrils, and fibrils, with varying infectivity and stability.

Computational studies using well-tempered meta dynamics reveal multiple unfolding pathways, suggesting that PrP misfolding does not necessarily correlate with increased β-sheet content, challenging previous assumptions. Instead, the metastability of PrPCC, particularly in H2 and H3, allows environmental factors like temperature, pH, and pressure to induce unfolding.

The PRNP gene encodes PrPC, which adopts its native structure during translation and co-translational processing in the endoplasmic reticulum lumen. While genetic mutations or infectious agents can drive PrPS^c formation, the mechanisms underlying sporadic misfolding remain unclear. Understanding these transitions is crucial for elucidating prion pathogenesis and developing targeted therapies.

III. GENETICAL REPRESENTATION OF PRNP

- *Prion Mutations and the Pathogenesis of Creutzfeldt-Jakob Disease (CJD)*

Prion diseases arise from mutations in the PRNP gene, including single base pair substitutions and insertions of 24-base pair repeats, leading to the misfolding of the normal prion protein (PrPCC) into its pathogenic isoform (PrPS^c). Codon 129 polymorphism significantly influences the risk and phenotype of sporadic and genetic prion diseases.

CJD was first described in the 1920s by Creutzfeldt and Jakob as a distinct spongiform encephalopathy. The disease is characterized by progressive neurodegeneration, cognitive decline, and motor dysfunction due to the accumulation of PrPS^c, which is resistant to protease degradation.

Prion diseases in animals include scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle, which led to a variant form of CJD (vCJD) in humans through the consumption of contaminated beef in the 1980s–1990s. The BSE outbreak resulted in massive economic losses and public health crises, prompting stringent regulations on animal product handling.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1837

CJD remains incurable, with treatment focused on symptom management. Diagnosis involves clinical evaluation, brain imaging, and laboratory tests, with definitive confirmation requiring post-mortem analysis of prion accumulation.

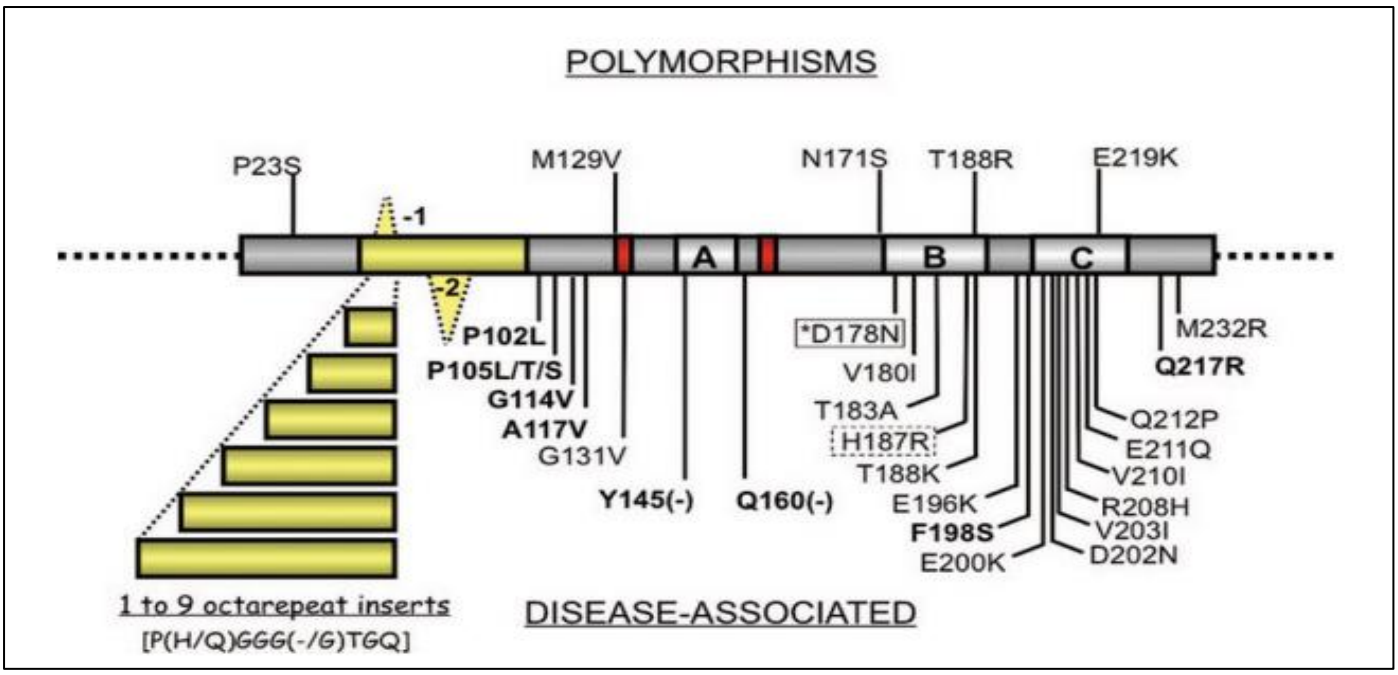

Fig 2 Mastrianni, J. A. (2010).

The above picture represents the overview of PRNP gene polymorphisms and mutations. The schematic displays the PRNP gene, highlighting major polymorphisms and mutations related to prion diseases. All mutations are associated with a CJD phenotype, except those in bold (GSS), in a solid box (FFI or CJD, depending on codon 129 genotype), and in a dotted box (CJD phenotype but variable pathology). which was mentioned in (Mastrianni, J. A. (2010)'s) Paper.

- *Synthesis of Prion Protein and Translational Modifications:*

The mechanism of cell death in prion diseases is unknown but is involved in the generation of misfolded conformers of the prion protein. We report that the disease-associated prion protein specifically inhibits the proteolytic β-subunit of the 26S proteasome. (Kristiansen et al., 2007) Using reporter substrates, fluorogenic peptides and β-subunit activity probes, this inhibitory effect was demonstrated in pure 26S proteasomes and three different cell lines. Taken together, these data suggest a mechanism for intracellular neurotoxicity mediated by misfolded prion protein oligomers.(*Molecular Mechanism of the Misfolding and Oligomerization of the Prion Protein: Current Understanding and Its Implications | Biochemistry*, n.d.) Prions are a unique class of infectious agents responsible for a group of devastating prion diseases. These diseases are characterized by the transformation of the normal cellular prion protein (PrPC) into its disease-related form, known as PrPSc. PrPSc has a distinct structural configuration compared to PrPC and is notably resistant to degradation.

The exact molecular mechanisms behind the formation of PrPSc and its role in infectivity and neurodegeneration remain enigmatic. In recent research, there is a growing understanding that posttranslational modifications, or the absence thereof, may have a pivotal role in the conversion of PrPC to PrPSc. Human PrP possesses two consensus sites for N-linked glycosylation, at specific amino acid positions. Glycosylation, the addition of sugar molecules to the protein, can potentially influence the conformation of PrPC or the stability of PrPSc. This, in turn, could impact the rate at which PrPSc is cleared from the body. However, it's important to note that the structural details of glycosylated PrP remain elusive, as only the NMR structures of recombinant, non-glycosylated prions have been determined. Native amino acid mutations in PrP are frequently found near these glycosylation sites, further underscoring the potential significance of posttranslational modifications in prion pathogenesis. Moreover, normal PrPC has been revealed to have copper-binding properties, and emerging evidence suggests a link between the level of PrP expression and the organism's tolerance to oxidative stress. (Roucou et al., 2004)

Oxidative stress is characterized by an imbalance between the production of harmful reactive oxygen species (ROS) and the body's ability to detoxify them. Additionally, histological studies employing markers like nitrotyrosine have been employed to detect neuronal labelling in prion-infected mouse brains, indicating peroxynitrite-mediated neuronal degradation and nitrative stress. PrPC undergoes proteolytic cleavage and shedding of PrPc occurs. It represents two important cleavage events αcleavage occurs at the middle PrPc which releases an unstructured N- N-terminal protein fragment that leaves the globular C- terminal which is attached to the membrane. This cleavage takes place during vesicular trafficking of PrPC within the secretory pathway.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1837

Initial reports identified the serine protease plasmin or ADAMS as a potential protease, yet recent data do not support this observation and the exact nature of the responsible protease remains unclear. The PrPc protein has misfolded and formed as PrPsc. (7 rupalAdmin, "About CJD and Prion Disease.") Prion protein malfunction: Mechanisms of neurodegeneration in prion disease Loss of function of PrPC vs toxic gain of function of PrPSc. A key event in the pathophysiology of prion diseases is the PrPSc template-directed misfolding of PrPC into a pathogenic, conformationally altered, β-sheet-rich version of itself. This conversion process lies at the root of the now widely accepted prion hypothesis, which states that the infectious agent for prion diseases (the "prion") is entirely made up of proteins and devoid of specific nucleic acids.

➢ *Physiological Roles of Prion Protein (PrPCC) and its Implications in Disease*

Prion protein (PrPCC) plays a critical role in neural development, synapse formation, neuroprotection, myelin maintenance, and immune regulation (Sigurdson et al., 2019). It is involved in T-cell activation and antigen-presenting cell interactions, with its absence leading to dysregulated immune responses, as observed in experimental autoimmune encephalomyelitis (EAE). While PrPCC's precise physiological functions remain unclear, it participates in cell adhesion, metal ion homeostasis, and neuroprotection. Its conversion into the misfolded, pathogenic $PrPS^c$ isoform disrupts normal cellular processes, triggering neurodegeneration, microglial activation, and pro-inflammatory responses (Onodera, 2014). PrPScS^c self-propagates by inducing misfolding of native PrPCC, leading to its accumulation and progressive neuronal dysfunction. PrPCC exhibits antioxidant and anti-apoptotic properties, mitigating oxidative stress and inhibiting Bax-mediated apoptosis, thus enhancing cellular resilience (Roucou et al., 2004). The absence of PrP renders cells more susceptible to oxidative damage and programmed cell death, underscoring its neuroprotective role. Disruptions in PrPCC function due to $PrPS^c$ conversion contribute to the pathology of prion diseases, emphasizing the need for targeted therapeutic interventions.

➢ *Schematic Overview of Prion Protein Misfolding*

- Conformational Shift: Normal prion proteins undergo a structural change forming cross-β filaments stable, protease-resistant aggregates seen in neurodegenerative diseases.
- Self-Propagation: Misfolded PrP* induces conformational changes in nearby normal PrP, making it infectious unlike other aggregation-prone proteins which are not transmissible.
- Cross-β Filament: Common in prion and other proteinopathies, these structures are stabilized by hydrogen bonding along the peptide backbone.
- Structural Model: One proposed model shows two α-helices in PrP converting into four β-strands in PrP*, though the exact structure of PrP* remains unresolved due to aggregation.

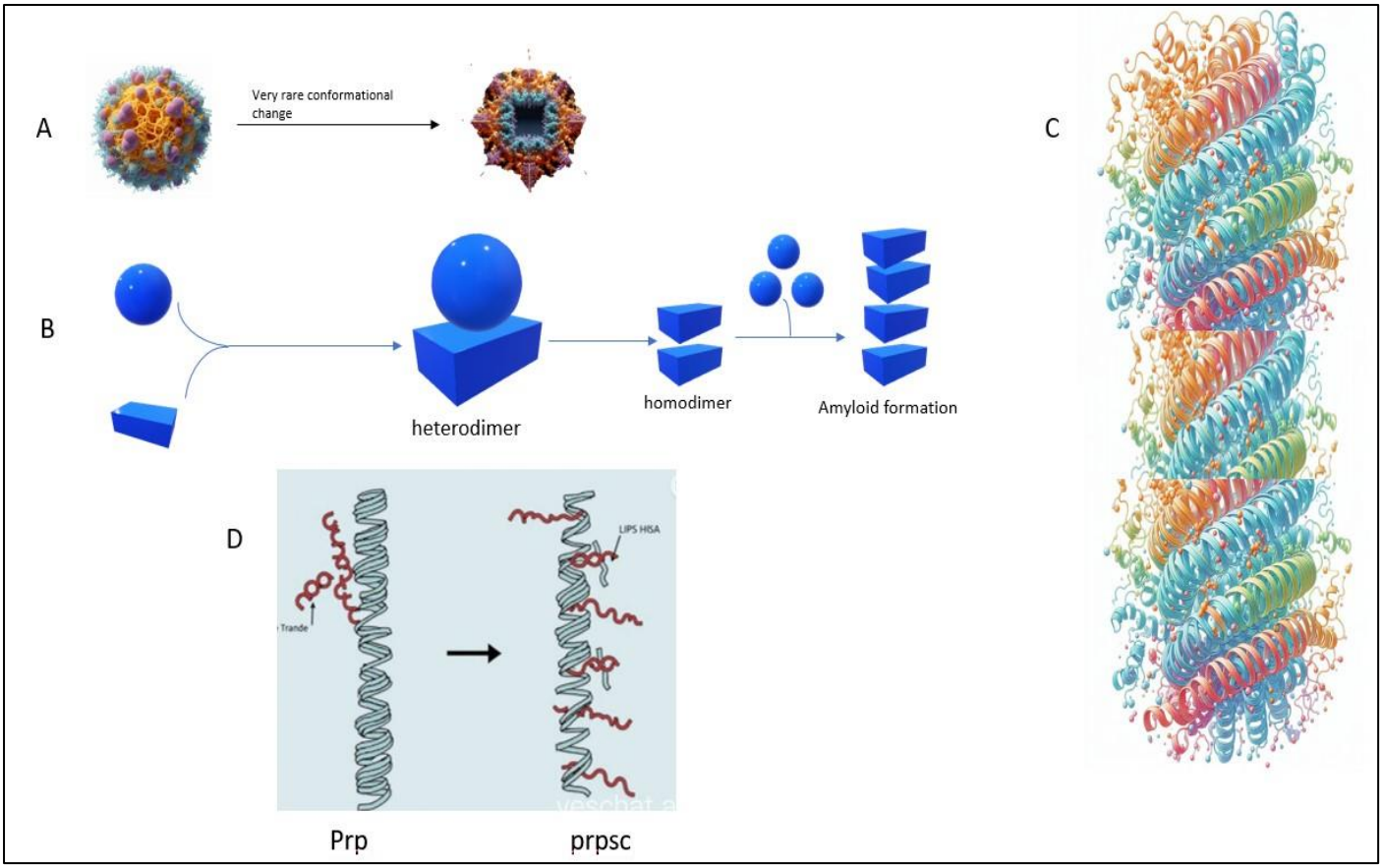

Fig 3 (Adapted from Alberts B, Garland Science; 2002)

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1837

➢ *Mechanism of Prions*

Histopathology and Molecular Mechanisms of Prion Diseases Prion diseases are characterized by spongiform encephalopathy, neuronal loss, gliosis, and prion protein (PrP) aggregation into oligomers and fibrils, with structural variations depending on the disease type (ScienceDirect, n.d.). The misfolded prion protein (PrPS^S) influences its own folding, aggregation, and propagation, suggesting that the definition of prions may extend to other pathogenic proteins (Nature Medicine, n.d.).

Recent studies reveal that PrP misfolding can occur even in the absence of the amyloidogenic segment 106–126, with the C-terminal domain forming amyloids under physiological conditions. Targeting structural fluctuations with monoclonal antibodies has shown promise in preventing PrP aggregation (Biochemistry, n.d.). Prion diseases share molecular similarities with other neurodegenerative proteinopathies, all marked by β-sheet-rich protein aggregation in the CNS. Currently, prion diseases remain incurable, with treatments limited to symptom management. Emerging evidence suggests that misfolded PrP oligomers drive neurovirulence and infectivity, making their molecular characterization crucial for developing anti-prion therapeutics. Understanding these mechanisms may also offer insights into broader neurodegenerative disorders.

➢ *Mechanism of Prion Protein Misfolding and Aggregation*

Prion diseases are caused by the misfolding of the normal cellular prion protein (PrPC^C) into its infectious, self-propagating isoform (PrPS^S), following the seeding-nucleation model (Guo et al., 2015). This misfolded form is highly resistant to protease degradation and chemical disinfection, leading to toxic aggregation and neurodegeneration.

Protein misfolding can be studied using protein misfolding cyclic amplification (PMCA), a technique analogous to PCR that amplifies misfolded proteins in vitro (Moreno-Gonzalez & Soto, 2011). Simulations suggest that PrPS^c is stable at neutral pH, but low pH conditions can induce a conformational shift toward PrPS^S-like structures.

Structurally, PrPS^S differs significantly from PrPC^C, adopting a β-sheet-rich conformation that promotes aggregation. Biochemically, PrPS^S induces the misfolding of PrPC^C, enabling its self-replication and disease progression. Despite advances, the precise molecular mechanisms underlying prion conversion remain incompletely understood, highlighting the need for further research (Hackl et al., 2019).

IV. DEGRADATION OF NORMAL PRION PROTEIN

➢ *Cellular Pathways for Prion Protein Degradation Mechanisms and Therapeutic Potential*

Protein degradation occurs primarily through two distinct pathways: the ubiquitin-proteasome system (UPS) and the lysosomal pathway (Prion Degradation Pathways: Potential for Therapeutic Intervention - ScienceDirect, n.d.). The lysosomal pathway serves as a fundamental cellular mechanism for degrading proteins, including misfolded and abnormal proteins such as prions. Prions are infectious, misfolded proteins implicated in various neurodegenerative disorders. Lysosomal proteases play a crucial role in the degradation of these misfolded proteins.(Goold et al., 2015)

➢ *Lysosomal Pathway in Prion Degradation*

The lysosomal degradation process begins with the recognition of target proteins, followed by their transport into lysosomes, which contain enzymes capable of breaking down various macromolecules. Studies indicate that cellular prion protein (PrPC) undergoes endocytosis and is transferred to lysosomes for degradation. This endocytic process likely occurs via clathrin-coated vesicles, leading to the packaging of internalized prion proteins into early endosomes before their degradation in lysosomes (Prusiner, 1998; Full Article: The Prion Protein and Lipid Rafts (Review), n.d.).

➢ *Ubiquitin-Proteasome System and Prion Degradation*

The ubiquitin-proteasome system (UPS) is responsible for degrading most short-lived proteins, particularly those involved in cell cycle regulation and signalling pathways. This pathway involves the covalent attachment of ubiquitin molecules to target proteins, marking them for proteasomal degradation. In contrast, the lysosomal system primarily degrades long-lived proteins, damaged organelles, and cellular debris. Both pathways contribute to the degradation of normal prion proteins (PrP), though the precise mechanisms underlying PrPSc recognition and degradation remain incompletely understood.(Homma et al., 2015)

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1837

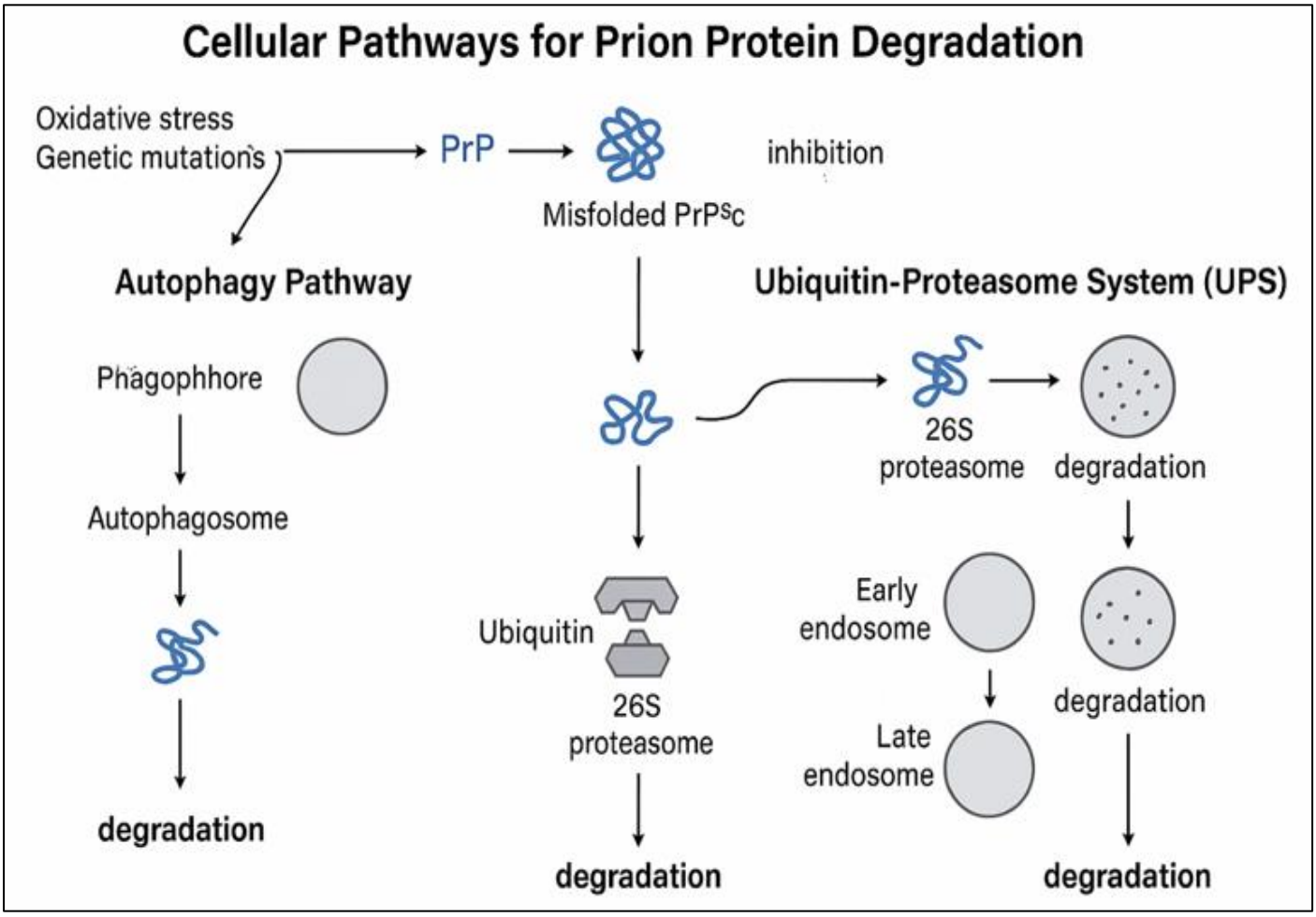

Fig 4 Adapted from López-Pérez et al. (2020), Kristiansen et al. (2007), Goold et al. (2015), Roucou et al. (2004), Prusiner (1998)

➢ *Autophagy Degradation and its Molecular Mechanism*

Autophagy is a highly conserved cellular process responsible for the degradation and recycling of intracellular components, including damaged organelles and misfolded proteins. This process is crucial for maintaining cellular homeostasis, responding to stress conditions, and preventing neurodegenerative diseases. The molecular mechanism of autophagy consists of several key stages: initiation, nucleation, elongation, and degradation.

- *Induction:*

TORC1 inhibits autophagy under normal conditions. Nutrient scarcity/stress inactivates TORC1, activating the Atg1 kinase complex to initiate autophagy.

- *Nucleation:*

Phagophore formation begins. Atg6 (BECN1) forms a complex with Atg14, Vps34, and Vps15 to recruit membrane components. Bcl-2 inhibits Atg6, while Atg9 supports membrane transport.

- *Elongation & Maturation:*

Two conjugation systems assist autophagosome formation:

Atg12-Atg5-Atg16 complex (via Atg7, Atg10). Atg8 (LC3) binds PE (via Atg4, Atg7, Atg3) for membrane expansion.

- *Prion-Induced Dysregulation:*

Atg6 & Atg9 downregulated in prion-infected brains. Atg5 expression varies: Up in some prion-infected sheep, down in scrapie-infected mice & sCJD patients Atg8-PE (LC3-II) dysregulated: Up in early disease, down in advanced prion disease. Autophagy disruption contributes to neurodegeneration in prion diseases. Targeting autophagy could offer therapeutic potential. (López-Pérez et al., 2020)

➢ *Factors Influencing Prion Degradation*

Several factors influence PrPC degradation, including genetic mutations and environmental stressors. Mutations such as T83A in the prion protein gene may alter protein structure and stability, potentially impacting recognition and degradation pathways (Mechanism of Misfolding of the Human Prion Protein Revealed by a Pathological Mutation | PNAS, n.d.). Additionally, environmental factors such as toxin exposure and cellular stress can impair protein degradation, contributing to the accumulation of misfolded proteins. Aging further exacerbates these processes, reducing cellular efficiency in protein folding and degradation, thereby increasing the risk of prion aggregation and disease progression

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1837

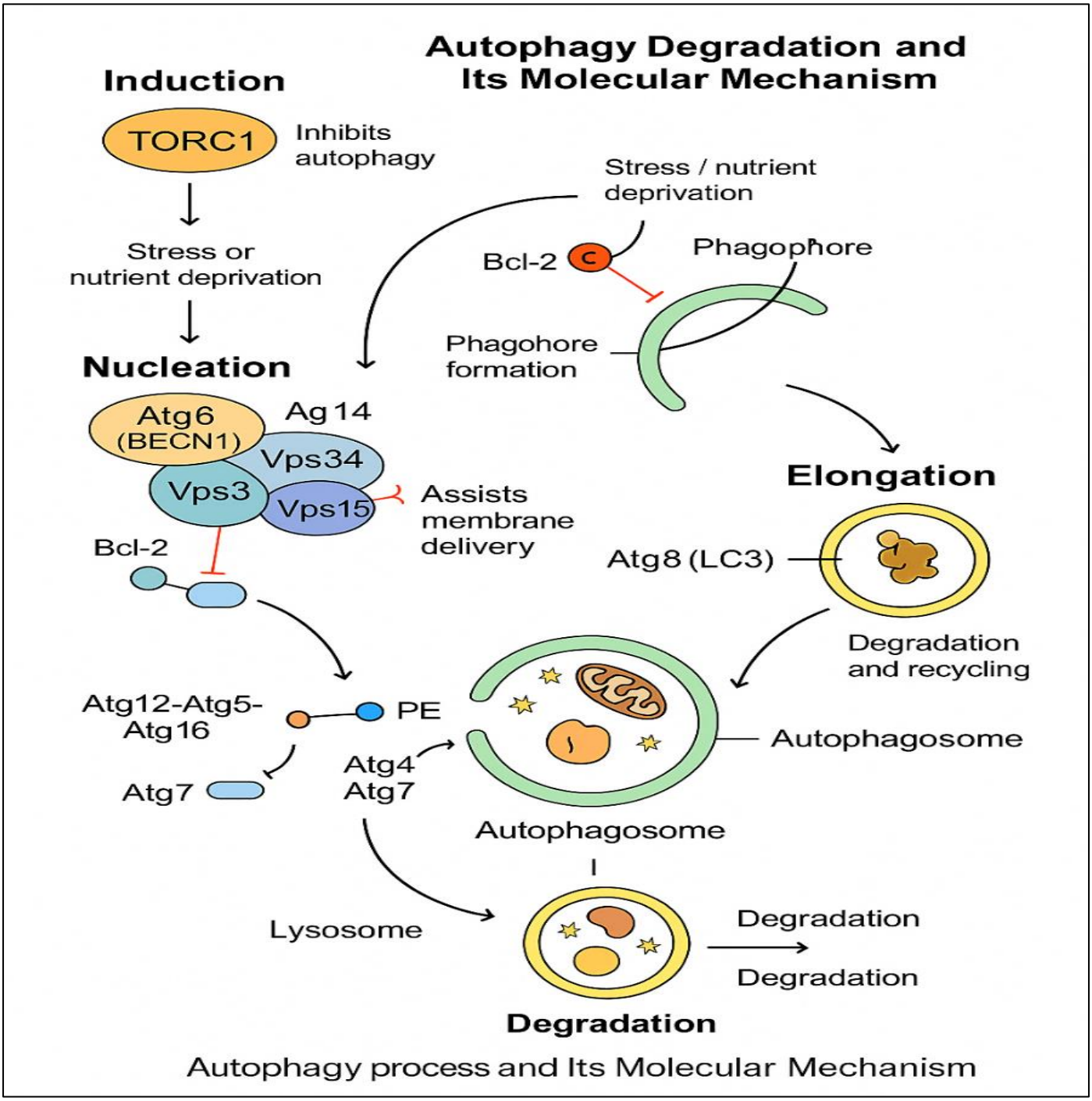

Fig 5 (Chen et al., 2023) (Yang & Klionsky, 2009)

➢ *Therapeutic Approaches to Prion Degradation*

Emerging therapeutic strategies for prion diseases focus on enhancing the degradation and clearance of the misfolded prion protein (PrP^Sc). Recent studies on prion degradation in cattle and sheep suggest enzymatic methods, particularly keratinase treatment combined with heat denaturation, as effective strategies for eliminating PrPSc from contaminated equipment. Western blot and immunochemical assays have confirmed the reduction of PrPSc to undetectable levels. Additionally, the regulatory role of eukaryotic initiation factor 2-alpha (eIF2α) in protein synthesis modulation under cellular stress conditions has been highlighted as a potential therapeutic target (Walter & Ron, 2011).

Autophagy Induction via Lysosome-Activating Compounds Trehalose: This natural disaccharide induces autophagy through an mTOR-independent pathway. Studies have shown that trehalose treatment reduces PrPS^c levels in prion-infected neuronal cells by promoting autophagic clearance (López-Pérez et al., 2020) Rapamycin: An mTOR inhibitor, rapamycin stimulates autophagy, facilitating the degradation of aggregated proteins, including PrP^Sc. Combined treatment with trehalose and rapamycin has demonstrated additive effects in enhancing autophagic activity. (Sarkar et al., 2007).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1837

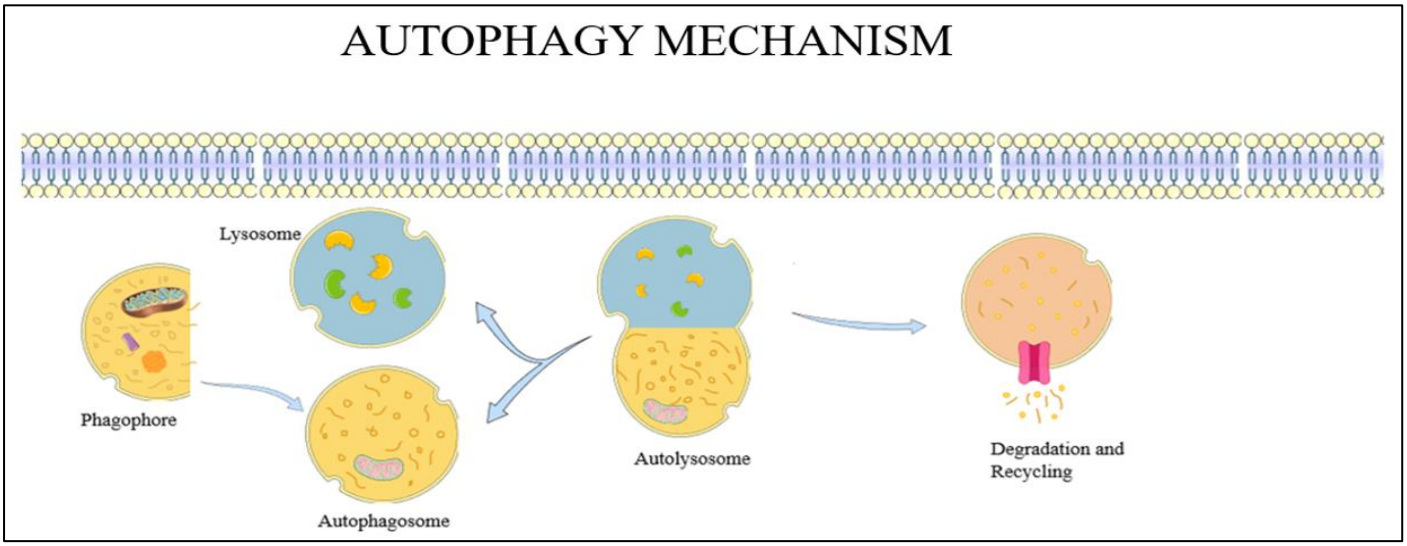

Fig 6 The Reference was Collected from (Jalali et al., 2025), Image was Created using (*Bioicons - High Quality Science Illustrations*, n.d.)

➢ *Advances in Gene Silencing for Prion Disease Treatment*

Prion diseases, such as Creutzfeldt-Jakob disease, result from misfolded prion proteins (PrPSc) that trigger neurodegeneration. Since prion protein is not essential for survival, reducing its levels presents a promising therapeutic strategy.

A recent study by Vallabh et al. (2024) introduced CHARM (Coupled Histone tail for Autoinhibition Release of Methyltransferase), a novel gene-silencing tool. Unlike CRISPR off, CHARM utilizes zinc finger proteins for targeted DNA methylation, allowing efficient prion gene suppression without excessive toxicity. Delivered via adeno-associated virus (AAV), CHARM reduced prion protein levels by up to 80% in mouse brains.(*Developing Treatments for Prion Diseases*, 2024)

➢ *Emerging Diagnostics and AI Technology*

The diagnosis of Creutzfeldt-Jakob Disease (CJD) has evolved significantly with advancements in molecular assays and artificial intelligence (AI)-driven tools.

➢ *Real-Time Quaking-Induced Conversion (RT-QuIC)*

RT-QuIC is a highly sensitive and specific assay that detects misfolded prion proteins (PrP^Sc) by amplifying their seeding activity. Initially validated for cerebrospinal fluid (CSF) samples, RT-QuIC has been successfully applied to peripheral tissues. (Poleggi et al., 2022 For instance, a study demonstrated that RT-QuIC applied to skin punch biopsies achieved 89% sensitivity and 100% specificity in diagnosing CJD, supporting its utility in clinical practice.(Mammana et al., 2020) Similarly, RT-QuIC testing of olfactory epithelium samples obtained from nasal brushings showed high accuracy in diagnosing CJD, indicating substantial prion seeding activity in the nasal vault.(Orrú et al., 2014)

➢ *Protein Misfolding Cyclic Amplification (PMCA)*

PMCA is another ultrasensitive technique that amplifies minute amounts of PrP^Sc by mimicking the natural conversion process of prion proteins. It has been optimized for detecting prions in various biological samples, including blood, urine, nasal brushings, lymph nodes, and CSF. (Ferreira & Caughey, 2020) A pilot study demonstrated that PMCA achieved 89% sensitivity and 100% specificity in detecting PrPSc in blood samples from experimentally infected and diseased hamsters. (Wang et al., 2023) Furthermore, PMCA has been applied to olfactory mucosa samples from sCJD patients, enhancing diagnostic capabilities. (Cazzaniga et al., 2022)

➢ *AI and ML in Diagnostics of CJD*

AI and machine learning (ML) are increasingly employed to enhance the accuracy and efficiency of CJD diagnosis. Recent studies have developed AI-based frameworks that utilize both ML and deep learning methods to accurately classify whole-slide images (WSIs) as positive or negative for prion disease and quantify the distribution of prion proteins across entire tissue sections. Additionally, AI-driven analysis of neuroimaging data, such as diffusion MRI, has improved the sensitivity of detecting prion diseases, aiding in early diagnosis.(Salvi et al., 2023). It detects minute quantities of PrPSc by amplifying its misfolded structure in cerebrospinal fluid and now, more recently, in olfactory mucosa and skin punch biopsies. Sensitivities over 95% and specificities above 98% have been reported, marking it as a non-invasive, accessible biomarker-based diagnostic (Orrù et al., 2023). Additionally, computational biology tools such as PrionScan, PLAAC (Prion-Like Amino Acid Composition), and PAPA (Prion Aggregation Prediction Algorithm) allow for the prediction of prion-like domains (PrLDs) in proteins across species. These AI-driven analysis of neuroimaging data, such as diffusion MRI, has improved the sensitivity of detecting prion diseases, aiding in early diagnosis.(Bizzi et al., 2020)

V. CONCLUSION

The degradation of prion proteins involves complex cellular mechanisms, primarily through the lysosomal and ubiquitin-proteasome systems. Disruptions in these pathways may contribute to prion disease pathogenesis. Further research is essential to elucidate the detailed mechanisms of

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1837

prion degradation and explore therapeutic interventions targeting these pathways.

Misfolded prion proteins drive the progression of Creutzfeldt-Jakob Disease (CJD) through PrPSc aggregation, leading to neuronal damage. The lysosomal pathway, autophagy pathway, ubiquitin- protein degradation pathways are crucial role for clearing these toxic proteins, presenting a potential therapeutic target. Enhancing lysosomal degradation could slow disease progression, but further research is needed to fully understand the molecular mechanisms involved. Advancing knowledge in this area may pave the way for novel therapeutic strategies against CJD and related neurodegenerative disorders.

FUNDING

This is Self-funding a review paper for publication represents a commitment to scholarly excellence and intellectual autonomy. By taking on the financial responsibility, researchers gain full control over the publication process, ensuring the integrity and quality of the work, while it demands personal investment, self-funding empowers researchers to pursue their academic interests without external constraints, resulting in a publication that reflects their genuine passion and expertise. Ultimately, self-funding for publication exemplifies a dedication to advancing knowledge and making meaningful contributions to the academic community.

ACKNOWLEDGEMENT

I gratefully acknowledge to the University for their support and resources that have contributed to the completion of this review paper. Their commitment to academic excellence and research development has been instrumental in shaping our understanding of the subject matter. We extend our sincere appreciation to the faculty, staff, and administration for their guidance and encouragement throughout this endeavour. University's dedication to fostering a conducive environment for scholarly pursuits has been invaluable in the realization of this publication.

ABBREVIATIONS

- **CJD** – Creutzfeldt-Jakob Disease
- **sCJD** – Sporadic Creutzfeldt-Jakob Disease
- **fCJD** – Familial Creutzfeldt-Jakob Disease
- **vCJD** – Variant Creutzfeldt-Jakob Disease
- **iCJD** – Iatrogenic Creutzfeldt-Jakob Disease
- **PrP** – Prion Protein
- **PrP^Sc** – Scrapie-associated Prion Protein
- **CNS** – Central Nervous System
- **RT-QuIC** – Real-Time Quaking-Induced Conversion
- **MRI** – Magnetic Resonance Imaging
- **CSF** – Cerebrospinal Fluid
- **EEG** – Electroencephalogram
- **BSE** – Bovine Spongiform Encephalopathy
- **WHO** – World Health Organization
- **PET** – Positron Emission Tomography
- **FDG** – Fluorodeoxyglucose
- **AI** – Artificial Intelligence
- **CRISPR** – Clustered Regularly Interspaced Short Palindromic Repeats
- **Cas9** – CRISPR-associated protein 9
- **NGS** – Next-Generation Sequencing

REFERENCES

[1]. Sigurdson, C. J., Bartz, J. C., & Glatzel, M. (2019). Cellular and molecular mechanisms of prion disease. *Annual Review of Pathology: Mechanisms of Disease*, *14*, 497-516.

[2]. Prusiner, S. B. (1998). Prions. Proceedings of the National Academy of Sciences, 95(23), 13363-13383.

[3]. Langeveld, J. P., Wang, J. J., Van de Wiel, D. F., Shih, G. C., Garssen, G. J., Bossers, A., & Shih, J. C. (2003). Enzymatic degradation of prion protein in brain stem from infected cattle and sheep. *The Journal of Infectious Diseases*, *188*(11), 1782-1789.

[4]. Soto, C., & Castilla, J. (2004). The controversial protein-only hypothesis of prion propagation. Nature Medicine, 10(Suppl), S63-S67.

[5]. Kojima, G., Tatsuno, B. K., Inaba, M., Velligas, S., Masaki, K., & Liow, K. K. (2013). Creutzfeldt-Jakob disease: a case report and differential diagnoses. *Hawai'i journal of medicine & public health: a journal of Asia Pacific Medicine & Public Health*, *72*(4), 136–139

[6]. Aguzzi, A., & Lakkaraju, A. K. (2016). Cell biology of prions and prionoids: a status report. Trends in Cell Biology, 26(1), 40-51.

[7]. Soto, C., Estrada, L., & Castilla, J. (2006). Amyloids, prions and the inherent infectious nature of misfolded protein aggregates. *Trends in biochemical sciences*, *31*(3), 150-155.

[8]. Cappai, R., & Collins, S. J. (2004). Structural biology of prions. *Prions*, *11*, 14-32.

[9]. Parchi, P., Giese, A., Capellari, S., Brown, P., Schulz-Schaeffer, W., Windl, O., ... & Kretzschmar, H. (1999). Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Annals of Neurology, 46(2), 224-233.

[10]. [1] Geschwind, M. D. (2015). Prion Diseases. Continuum: Lifelong Learning in Neurology, 21(6, Neuroinfectious Disease), 1612–1638. https://doi.org/10.1212/CON.0000000000000230

[11]. Brown, P., Brandel, J. P., Sato, T., Nakamura, Y., MacKenzie, J., Will, R. G., ... & Schonberger, L. B.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1837

(2012). Iatrogenic Creutzfeldt-Jakob disease, final assessment. *Emerging infectious diseases*, *18*(6), 901.

[12]. Ironside, J. W. (2006). Variant Creutzfeldt–Jakob disease: risk of transmission by blood transfusion and blood therapies. *Haemophilia*, *12*, 8-15.

[13]. Brown, P., Preece, M., Brandel, J. P., Sato, T., McShane, L., Zerr, I., ... & Collins, S. J. (2000). Iatrogenic Creutzfeldt–Jakob disease at the millennium. *Neurology*, *55*(8), 1075-1081.

[14]. Will, R. G., Ironside, J. W., Zeidler, M., Estibeiro, K., Cousens, S. N., Smith, P. G., ... & Hofman, A. (1996). A new variant of Creutzfeldt-Jakob disease in the UK. *The Lancet*, *347*(9006), 921-925.

[15]. Collinge, J., Sidle, K. C., Meads, J., Ironside, J., & Hill, A. F. (1996). Molecular analysis of prion strain variation and the aetiology of'new variant'CJD. *Nature*, *383*(6602), 685-690.

[16]. Heath, C. A., Cooper, S. A., Murray, K., Lowman, A., Henry, C., MacLeod, M. A., ... & Will, R. G. (2010). Validation of diagnostic criteria for variant Creutzfeldt–Jakob disease. *Annals of neurology*, *67*(6), 761-7

[17]. Zerr, I., Kallenberg, K., Summers, D. M., Romero, C., Taratuto, A., Heinemann, U., ... & Collins, S. J. (2009). Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Brain, 132(10), 2659-2668.

[18]. Kovács, G. G., Puopolo, M., Ladogana, A., Pocchiari, M., Budka, H., Van Duijn, C., ... & Zerr, I. (2005). Genetic prion disease: the EUROCJD experience. Human genetics, 118(2), 166-174.

[19]. Parchi, P., & Gambetti, P. (2011). Human prion diseases. Cold Spring Harbor Perspectives in Biology, 3(1), a006833. https://doi.org/10.1101/cshperspect.a006833

[20]. Pankevich, D. E., Wizemann, T. M., Altevogt, B. M., & Institute of Medicine (US). (2011)

[21]. Collinge, J., & Clarke, A. R. (2007). A general model of prion strains and their pathogenicity. Science, 318(5852), 930-936.

[22]. Prusiner, S. B. (2013). Biology and genetics of prions causing neurodegeneration. Annual Review of Genetics, 47, 601-623.

[23]. Aguzzi, A., & Zhu, C. (2017). Prions: A century of scientific puzzles and a Nobel prize. Cell, 171(2), 229-244. Prusiner, S. B. (2013). Biology and genetics of prions causing neurodegeneration. Annual review of genetics, 47, 601-623.

[24]. Wille, H., & Prusiner, S. B. (2016). Prion protein structure and biology. Handbook of Clinical Neurology, 153, 21-37.

[25]. Guo, B. B., Bellingham, S. A., & Hill, A. F. (2015). The neutral sphingomyelinase pathway regulates the packaging of the prion protein into exosomes. *Journal of Biological Chemistry*, *290*(6), 3455-3467.

[26]. Hanson, S., Nadig, L., & Altevogt, B. (2010). Forum on Neuroscience and Nervous System Disorders Board on Health Sciences Policy.

[27]. Sanz-Hernández, M., Barritt, J. D., Sobek, J., Hornemann, S., Aguzzi, A., & De Simone, A. (2021). Mechanism of misfolding of the human prion protein revealed by a pathological mutation. *Proceedings of the National Academy of Sciences*, *118*(12), e2019631118.

[28]. Moreno-Gonzalez, I., & Soto, C. (2011, July). Misfolded protein aggregates: mechanisms, structures, and potential for disease transmission. In *Seminars in cell & developmental biology* (Vol. 22, No. 5, pp. 482-487). Academic Press.

[29]. Ugalde, C. L., Finkelstein, D. I., Lawson, V. A., & Hill, A. F. (2016). Pathogenic mechanisms of the prion protein, amyloid-β, and α-synuclein misfolding: The prion concept and neurotoxicity of protein oligomers. *Journal of Neurochemistry*, *139*(2), 162-180.

[30]. Mastrianni, J. A. (2010). The genetics of prion diseases. *Genetics in Medicine*, *12*(4), 187–195. https://doi.org/10.1097/GIM.0b013e3181cd7374

[31]. Kristiansen, M., Deriziotis, P., Dimcheff, D. E., Jackson, G. S., Ovaa, H., Naumann, H., ... & Tabrizi, S. J. (2007). Disease-associated prion protein oligomers inhibit the 26S proteasome. *Molecular cell*, *26*(2), 175-188.

[32]. Singh, J., & Udgaonkar, J. B. (2015). Molecular mechanism of the misfolding and oligomerization of the prion protein: current understanding and its implications. *Biochemistry*, *54*(29), 4431-4442.

[33]. (https://www.ucsfhealth.org/conditions/creutzfeldt-jakob-disease/symptoms)

[34]. Wechselberger, C., Wurm, S., Pfarr, W., & Höglinger, O. (2002). The physiological functions of prion protein. *Experimental cell research*, *281*(1), 1-8.

[35]. Onodera, T., Sakudo, A., Tsubone, H., & Itohara, S. (2014). Review of studies that have used knockout mice to assess the normal function of prion protein under immunological or pathophysiological stress. *Microbiology and immunology*, *58*(7), 361-374.

[36]. Roucou, X., Gains, M., & LeBlanc, A. C. (2004). Neuroprotective functions of prion protein. *Journal of Neuroscience Research*, *75*(2), 153-161.

[37]. Otvos Jr, L., & Cudic, M. (2002). Post-translational modifications in prion proteins. *Current protein and peptide science*, *3*(6), 643-652.

[38]. Benetti, F., Biarnés, X., Attanasio, F., Giachin, G., Rizzarelli, E., & Legname, G. (2014). Structural determinants in prion protein folding and stability. *Journal of molecular biology*, *426*(22), 3796-3810.

[39]. Singh, R. K., Chamachi, N. G., Chakrabarty, S., & Mukherjee, A. (2017). Mechanism of unfolding of human prion protein. *The Journal of Physical Chemistry B*, *121*(3), 550-564.

[40]. Hackl, S., & Becker, C. F. (2019). Prion protein—Semisynthetic prion protein (PrP) variants with posttranslational modifications. *Journal of Peptide Science*, *25*(10), e3216.

[41]. Basler, K., Oesch, B., Scott, M., Westaway, D., Wälchli, M., Groth, D. F., ... & Weissmann, C. (1986). Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. *Cell*, *46*(3), 417-428.

[42]. López-Pérez, Ó., Badiola, J. J., Bolea, R., Ferrer, I., Llorens, F., & Martín-Burriel, I. (2020). An Update on

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25jul1837

Autophagy in Prion Diseases. *Frontiers in bioengineering and biotechnology*, *8*, 975. https://doi.org/10.3389/fbioe.2020.00975 López-Pérez, Ó., Badiola, J. J., Bolea, R., Ferrer, I., Llorens, F., & Martín-Burriel, I. (2020). An Update on Autophagy in Prion Diseases. *Frontiers in bioengineering and biotechnology*, *8*, 975. https://doi.org/10.3389/fbioe.2020.00975

[43]. Walter, P., & Ron, D. (2011). The unfolded protein response: from stress pathway to homeostatic regulation. *science*, *334*(6059), 1081-1086.

[44]. Goold, R., McKinnon, C., & Tabrizi, S. J. (2015). Prion degradation pathways: Potential for therapeutic intervention. *Molecular and Cellular Neuroscience*, *66*, 12-20.

[45]. Taylor, D. R., & Hooper, N. M. (2006). The prion protein and lipid rafts. *Molecular membrane biology*, *23*(1), 89-99

[46]. Benetti, F., Biarnés, X., Attanasio, F., Giachin, G., Rizzarelli, E., & Legname, G. (2014). Structural determinants in prion protein folding and stability. *Journal of molecular biology*, *426*(22), 3796-3810.

[47]. Soto, C., & Castilla, J. (2004). The controversial protein-only hypothesis of prion propagation. Nature Medicine, 10(Suppl), S63-S67

[48]. Zerr, I., Kallenberg, K., Summers, D. M., Romero, C., Taratuto, A., Heinemann, U., Breithaupt, M., Varges, D., Meissner, B., Ladogana, A., Schuur, M., Haik, S., Collins, S. J., Jansen, G. H., Stokin, G. B., Pimentel, J., Hewer, E., Collie, D., Smith, P., Roberts, H., … Sanchez-Juan, P. (2009). Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. *Brain: a journal of neurology*, *132*(Pt 10), 2659–2668. https://doi.org/10.1093/brain/awp191

[49]. Gil-Garcia, M., Iglesias, V., Pallarès, I., & Ventura, S. (2021). Prion-like proteins: from computational approaches to proteome-wide analysis. *FEBS open bio*, *11*(9), 2400-2417.

[50]. George Priya Doss, C., Rajith, B., Rajasekaran, R., Srajan, J., Nagasundaram, N., & Debajyoti, C. (2013). In silico analysis of prion protein mutants: A comparative study by molecular dynamics approach. *Cell biochemistry and biophysics*, *67*, 1307-1318.

[51]. Peden, A. H., McGuire, L. I., Appleford, N. E., Mallinson, G., Wilham, J. M., Orru, C. D., ... & Head, M. W. (2012). Sensitive and specific detection of sporadic Creutzfeldt–Jakob disease brain prion protein using real-time quaking-induced conversion. *Journal of general virology*, *93*(2), 438-449.

[52]. Jesuthasan, A., Sequeira, D., Hyare, H. *et al.* Assessing initial MRI reports for suspected CJD patients. *J Neurol* **269**, 4452–4458 (2022). https://doi.org/10.1007/s00415-022-11087-x

[53]. Mastrianni, J. A. (2010). The genetics of prion diseases. *Genetics in Medicine*, *12*(4), 187-195.

[54]. Alberts, B., Johnson, A., & Lewis, J. (2002). Molecular biology of the cell. New York: Garland Science. The Lipid Bilayer.

[55]. Neumann, E. N., Bertozzi, T. M., Wu, E., Serack, F., Harvey, J. W., Brauer, P. P., Pirtle, C. P., Coffey, A., Howard, M., Kamath, N., Lenz, K., Guzman, K., Raymond, M. H., Khalil, A. S., Deverman, B. E., Minikel, E. V., Vallabh, S. M., & Weissman, J. S. (2024). Brainwide silencing of prion protein by AAV-mediated delivery of an engineered compact epigenetic editor. *Science, 384*(6703), ado7082. https://doi.org/10.1126/science.ado7082

[56]. Biró, A., Cuesta-Vargas, A. I., & Szilágyi, L. (2023). Precognition of mental health and neurogenerative disorders using AI-parsed text and sentiment analysis. *Acta Universitatis Sapientiae*, *15*(2), 359-403.

[57]. Hossain, K. M., Islam, M. A., Hossain, S., Nijholt, A., & Ahad, M. A. R. (2023). Status of deep learning for EEG-based brain–computer interface applications. *Frontiers in Computational Neuroscience, 16*. https://doi.org/10.3389/fncom.2022.1006763

[58]. Poleggi, A., Baiardi, S., Ladogana, A., & Parchi, P. (2022). The use of real-time quaking-induced conversion for the diagnosis of human prion diseases. *Frontiers in Aging Neuroscience*, *14*, 874734.

[59]. Mammana, A., Baiardi, S., Rossi, M., Franceschini, A., Donadio, V., Capellari, S., Caughey, B., & Parchi, P. (2020). Detection of prions in skin punch biopsies of Creutzfeldt-Jakob disease patients. *Annals of clinical and translational neurology*, *7*(4), 559–564. https://doi.org/10.1002/acn3.51000

[60]. Orrú, C. D., Bongianni, M., Tonoli, G., Ferrari, S., Hughson, A. G., Groveman, B. R., ... & Zanusso, G. (2014). A test for Creutzfeldt–Jakob disease using nasal brushings. *New England Journal of Medicine*, *371*(6), 519-529.

[61]. do Carmo Ferreira, N., & Caughey, B. (2020). Proteopathic seed amplification assays for neurodegenerative disorders. *Clinics in laboratory medicine*, *40*(3), 257-270.

[62]. Wang, F., Pritzkow, S., & Soto, C. (2023). PMCA for ultrasensitive detection of prions and to study disease biology. *Cell and tissue research*, *392*(1), 307–321. https://doi.org/10.1007/s00441-022-03727-5

[63]. Cazzaniga, F. A., Bistaffa, E., De Luca, C. M. G., Portaleone, S. M., Catania, M., Redaelli, V., Tramacere, I., Bufano, G., Rossi, M., Caroppo, P., Giovagnoli, A. R., Tiraboschi, P., Di Fede, G., Eleopra, R., Devigili, G., Elia, A. E., Cilia, R., Fiorini, M., Bongianni, M., Salzano, G., … Moda, F. (2022). PMCA-Based Detection of Prions in the Olfactory Mucosa of Patients With Sporadic Creutzfeldt-Jakob Disease. *Frontiers in aging neuroscience*, *14*, 848991. https://doi.org/10.3389/fnagi.2022.848991

[64]. Salvi, M., Molinari, F., Ciccarelli, M., & others. (2023). Quantitative analysis of prion disease using an AI-powered digital pathology framework. *Scientific Reports, 13*, 17759. https://doi.org/10.1038/s41598-023-44782-4

[65]. Bizzi, A., Pascuzzo, R., Blevins, J., et al. (2020). Evaluation of a new criterion for detecting prion disease with diffusion magnetic resonance imaging. *JAMA Neurology, 77*(9), 1141–1149.

https://doi.org/10.1001/jamaneurol.2020.1319

[66]. Aguib, Y., Heiseke, A., Gilch, S., Riemer, C., Baier, M., Schätzl, H. M., & Ertmer, A. (2009). Autophagy induction by trehalose counteracts cellular prion infection. *Autophagy*, *5*(3), 361–369. https://doi.org/10.4161/auto.5.3.7662

[67]. Sarkar, S., Davies, J. E., Huang, Z., Tunnacliffe, A., & Rubinsztein, D. C. (2007). Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. *The Journal of biological chemistry*, *282*(8), 5641–5652. https://doi.org/10.1074/jbc.M609532200

[68]. Jalali, P., Shahmoradi, A., Samii, A., Mazloomnejad, R., Hatamnejad, M. R., Saeed, A., ... & Salehi, Z. (2025). The role of autophagy in cancer: from molecular mechanism to therapeutic window. *Frontiers in Immunology*, *16*, 1528230.

[69]. López-Pérez, Ó., Badiola, J. J., Bolea, R., Ferrer, I., Llorens, F., & Martín-Burriel, I. (2020). An update on autophagy in prion diseases. Frontiers in Bioengineering and Biotechnology, 8, 975. https://doi.org/10.3389/fbioe.2020.00975

[70]. Kristiansen, M., Deriziotis, P., Dimcheff, D. E., Jackson, G. S., Ovaa, H., Naumann, H., ... & Tabrizi, S. J. (2007). Disease-associated prion protein oligomers inhibit the 26S proteasome. Molecular Cell, 26(2), 175–188. https://doi.org/10.1016/j.molcel.2007.03.015

[71]. Goold, R., McKinnon, C., & Tabrizi, S. J. (2015). Prion degradation pathways: Potential for therapeutic intervention. Molecular and Cellular Neuroscience, 66, 12–20. https://doi.org/10.1016/j.mcn.2015.03.010

[72]. Roucou, X., Gains, M., & LeBlanc, A. C. (2004). Neuroprotective functions of prion protein. Journal of Neuroscience Research, 75(2), 153–161. https://doi.org/10.1002/jnr.10843

[73]. Yang, Z., & Klionsky, D. J. (2009). An overview of the molecular mechanism of autophagy. *Current topics in microbiology and immunology*, *335*, 1–32. https://doi.org/10.1007/978-3-642-00302-8_1

[74]. Chen, L., Yang, L., Li, Y., Liu, T., Yang, B., Liu, L., & Wu, R. (2023). Autophagy and Inflammation: Regulatory Roles in Viral Infections. *Biomolecules*, *13*(10), 1454. https://doi.org/10.3390/biom13101454

[75]. Shafiq, M., Da Vela, S., Amin, L., Younas, N., Harris, D. A., Zerr, I., ... & Glatzel, M. (2022). The prion protein and its ligands: Insights into structure-function relationships. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1869*(6), 119240.