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MOLECULAR MECHANISMS AND PATHOGENIC CONSEQUENCES OF PRION PROTEIN (PRP) MISFOLDING IN CREUTZFELDT–JAKOB DISEASE: A SYSTEMATIC REVIEW

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ABSTRACT
Creutzfeldt–Jakob disease (CJD) is a rare, rapidly progressive, and fatal neurodegenerative disorder characterized by the accumulation of misfolded prion proteins within the central nervous system. The conversion of the normal cellular prion protein (PrP^{c}) into the pathogenic isoform (PrP^{sc}) represents the fundamental molecular event underlying disease initiation and progression. This systematic review aimed to synthesize peer-reviewed evidence published between 2015 and 2025 regarding the molecular mechanisms of prion protein misfolding and their contribution to the pathogenesis, propagation, and diagnosis of CJD. A systematic literature search was conducted in PubMed, Scopus, Web of Science, and ScienceDirect following PRISMA 2020 guidelines. Studies meeting predefined inclusion and exclusion criteria were screened, evaluated for methodological quality, and synthesized. A total of 34 studies were included in the final review. Evidence from the included studies indicates that CJD pathogenesis is driven by a complex network of molecular and cellular processes initiated by the structural conversion of α-helix-rich PrP^{c} into β-sheet-rich PrP^{sc}, resulting in conformational instability, seeded protein aggregation, and prion propagation. Additional mechanisms identified include genetic susceptibility associated with PRNP mutations and codon 129 polymorphisms, proteostasis dysfunction, oxidative stress, mitochondrial impairment, neuroinflammation, synaptic dysfunction, and neuronal degeneration. Recent advances in cryogenic electron microscopy have provided high-resolution insights into strain-specific prion conformations and mechanisms of propagation, while diagnostic technologies such as real-time quaking-induced conversion (RT-QuIC), protein misfolding cyclic amplification (PMCA), cerebrospinal fluid biomarkers, and diffusion-weighted magnetic resonance imaging have substantially improved ante-mortem diagnostic accuracy. Collectively, the evidence highlights the multifactorial nature of CJD and underscores the importance of integrating molecular, genetic, and cellular perspectives to improve disease understanding, biomarker development, and future therapeutic strategies.

Keywords:
Neurodegeneration, Protein aggregation, Proteostasis, Neuroinflammation, Oxidative stress, Amyloid genesis

INTRODUCTION
Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of rare, progressive, and invariably fatal neurodegenerative disorders characterized by the accumulation of misfolded prion proteins within the central nervous system (Zerr et al., 2024). Unlike conventional infectious diseases caused by viruses, bacteria, or parasites, prion diseases arise through the conformational conversion of the normal cellular prion protein (PrP^{c}) into a pathogenic, aggregation-prone isoform known as PrP^{sc}, which possesses the unique ability to self-propagate and induce further protein misfolding (Sigurdson et al., 2019). Human prion diseases encompass several clinical forms, including sporadic Creutzfeldt–Jakob disease (sCJD), genetic prion diseases associated with mutations in the PRNP gene, acquired forms resulting from iatrogenic transmission, and variant Creutzfeldt–Jakob disease linked to bovine spongiform encephalopathy exposure (Watson et al., 2021; Zerr et al., 2024).

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Among these disorders, sporadic CJD accounts for approximately 85–90% of cases and occurs worldwide with an estimated annual incidence of 1–2 cases per million individuals (Gao et al., 2024; Zerr et al., 2024). Clinically, CJD is characterized by rapidly progressive dementia accompanied by cerebellar dysfunction, visual disturbances, myoclonus, pyramidal and extrapyramidal signs, behavioral changes, and eventual akinetic mutism (Hermann et al., 2021). Disease progression is typically rapid, with most patients surviving less than one year following symptom onset (Zerr et al., 2024). Because of its aggressive clinical course, distinctive molecular pathology, and unique mechanism of transmissibility, CJD remains the most extensively studied human prion disease and serves as an important model for investigating protein misfolding disorders.

The cellular prion protein (PrP^{c}) is a glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein that is highly expressed in neurons and other tissues throughout the body (Castle & Gill, 2017). Structurally, PrP^{c} consists of a flexible N-terminal domain containing octapeptide repeats involved in metal-ion binding and a globular C-terminal domain composed predominantly of α-helical structures (Wulf et al., 2017). Although mice lacking PRNP remain viable, accumulating evidence suggests that PrP^{c} contributes to numerous physiological processes, including synaptic transmission, neuronal survival, cell adhesion, myelin maintenance, stress-response signaling, and regulation of copper and zinc homeostasis (Castle & Gill, 2017; Wulf et al., 2017).

The biological functions of PrP^{c} are particularly relevant to understanding disease pathogenesis because neurodegeneration may result not only from the toxic accumulation of PrP^{sc} but also from disruption of normal PrP^{c}-mediated cellular activities (Castle & Gill, 2017). Alterations in PrP signaling pathways can impair synaptic communication, redox regulation, and neuroprotective mechanisms, thereby increasing neuronal susceptibility to injury. Consequently, understanding the normal structure and physiological roles of PrP^{c} provides a critical foundation for understanding how its conversion into PrP^{sc} initiates disease.

The hallmark molecular event in prion disease is the structural conversion of α-helix-rich PrP^{c} into a β-sheet-rich pathogenic conformer known as PrP^{sc} (Baral et al., 2019). This conformational transition promotes the formation of oligomeric intermediates, amyloid fibrils, and highly ordered protein aggregates capable of acting as templates that recruit and convert additional PrP^{c} molecules into the pathogenic form (Sanz-Hernández et al., 2021). Through this seeded propagation mechanism, PrP^{sc} accumulates progressively within neural tissues, resulting in widespread neurodegeneration and the characteristic spongiform pathology observed in affected brains (Sigurdson et al., 2019).

Current evidence indicates that PrP misfolding is not an isolated molecular event but rather the initiating step of a complex pathogenic cascade involving genetic susceptibility, proteostasis dysfunction, oxidative stress, mitochondrial impairment, neuroinflammation, synaptic injury, and neuronal apoptosis (Sigurdson et al., 2019; Thellung et al., 2022; Spiers et al., 2023). Furthermore, different conformational strains of PrP^{sc} exhibit distinct structural properties that influence disease phenotype, tissue tropism, and progression rates, contributing to the remarkable heterogeneity observed among prion diseases (Kraus et al., 2021; Manka et al., 2023).

Understanding the molecular mechanisms of PrP misfolding is essential for several reasons. First, it provides critical insights into the initiation and propagation of prion diseases, thereby improving understanding of disease pathogenesis. Second, elucidating the structural basis of prion strain diversity may help explain variations in clinical presentation and neuropathological outcomes among patients (Kraus et al., 2021). Third, advances in understanding seeded protein aggregation have directly contributed to the development of highly sensitive diagnostic technologies, including real-time quaking-induced conversion (RT-QuIC) and protein misfolding cyclic amplification (PMCA), which exploit the self-propagating nature of misfolded prion proteins (Green, 2019; Dong & Satoh, 2021).

Moreover, insights into PrP misfolding extend beyond prion diseases and have broader implications for understanding other protein misfolding disorders, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, which share common mechanisms of protein aggregation and neurodegeneration (Mercer & Harris, 2023). Consequently, research on prion biology continues to contribute significantly to the wider field of neurodegenerative disease research.

This systematic review aims to synthesize peer-reviewed evidence published between 2015 and 2025 regarding the molecular mechanisms underlying prion protein (PrP) misfolding in Creutzfeldt–Jakob disease. Particular emphasis is placed on structural conversion, genetic determinants, seeded aggregation and propagation, proteostasis dysfunction, oxidative stress, mitochondrial impairment, neuroinflammation, synaptic dysfunction, neuronal degeneration, advances in structural biology, and the diagnostic implications of PrP misfolding. By integrating findings from molecular, genetic, cellular, and clinical investigations, this review seeks to provide a comprehensive overview of current knowledge and identify key areas that may inform future biomarker development and therapeutic intervention strategies.

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OBJECTIVES

The primary objective of this systematic review was to synthesize current evidence published between 2015 and 2025 regarding the molecular mechanisms underlying prion protein (PrP) misfolding in Creutzfeldt–Jakob disease (CJD). Specifically, this review aimed to: (1) examine the structural conversion of the normal cellular prion protein (PrP^{c}) into the pathogenic isoform (PrP^{sc}); (2) evaluate the role of genetic factors, including **PRNP** mutations and codon 129 polymorphisms, in disease susceptibility and phenotypic variation; (3) assess the mechanisms of seeded protein aggregation and prion propagation; (4) investigate the contribution of proteostasis dysfunction, oxidative stress, mitochondrial impairment, neuroinflammation, synaptic dysfunction, and neuronal degeneration to disease progression; (5) summarize recent advances in structural biology, particularly cryogenic electron microscopy studies that have enhanced understanding of prion architecture and strain diversity; and (6) examine the diagnostic implications of PrP misfolding, including the application of RT-QuIC, PMCA, cerebrospinal fluid biomarkers, and neuroimaging techniques. Through the integration of molecular, genetic, cellular, and clinical evidence, this review seeks to provide a comprehensive understanding of CJD pathogenesis and identify potential directions for future biomarker development and therapeutic intervention.

METHODOLOGY

Study Design

This study employed a systematic review research design to synthesize current evidence regarding the molecular mechanisms underlying prion protein (PrP) misfolding in Creutzfeldt–Jakob disease (CJD). The review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines to ensure a transparent, reproducible, and structured approach to literature identification, screening, eligibility assessment, and study inclusion (Page et al., 2021). The review focused on peer-reviewed studies published between 2015 and 2025 that investigated the structural, genetic, cellular, and diagnostic aspects of PrP misfolding and CJD pathogenesis.

Data Sources

All studies included in this review were identified through a systematic search of major scientific databases to ensure comprehensive coverage of the available literature. The databases searched included PubMed, Scopus, Web of Science, and ScienceDirect. Additional relevant studies were identified through manual screening of reference lists from eligible articles and key review papers. All retrieved publications were evaluated according to PRISMA 2020 guidelines to maintain methodological consistency and reliability throughout the review process.

Literature Search

To ensure a comprehensive and effective search strategy, the selected databases were queried using combinations of keywords and Boolean operators such as AND and OR. Search terms included *"prion protein misfolding," "Creutzfeldt–Jakob disease," "PrPC," "PrPSc," "PRNP mutation," "codon 129 polymorphism," "seeded aggregation," "prion propagation," "proteostasis dysfunction," "oxidative stress," "mitochondrial dysfunction," "neuroinflammation," "cryo-electron microscopy," "RT-QuIC," "PMCA," "CSF biomarkers,"* and *"MRI."* Various combinations of these terms were employed to maximize retrieval of relevant studies.

The search was restricted to peer-reviewed journal articles published between 2015 and 2025 and available in the English language. During the initial screening phase, all retrieved records were assessed based on title, authors, publication year, and source journal. Duplicate records were subsequently removed, and the remaining studies underwent title, abstract, and full-text screening to determine eligibility according to predefined inclusion and exclusion criteria.

Inclusion and Exclusion Criteria

This review systematically evaluated the available literature using predefined inclusion and exclusion criteria to ensure the quality, relevance, and reliability of the selected studies. Studies were considered eligible for inclusion if they were peer-reviewed journal articles published between 2015 and 2025, available in full-text English versions, and focused on the molecular mechanisms underlying prion protein (PrP) misfolding in Creutzfeldt–Jakob disease (CJD). Eligible studies investigated topics such as prion propagation, genetic susceptibility, cellular stress responses, neurodegeneration, structural biology, and diagnostic applications associated with CJD. Original experimental studies, clinical investigations, cohort studies, systematic reviews, meta-analyses, and high-quality review articles were included provided that they contributed mechanistic, molecular, structural, genetic, or diagnostic evidence relevant to the objectives of this review.

Studies were excluded if they consisted of editorials, conference abstracts, opinion papers, letters, or commentaries that lacked primary scientific data. Publications focusing exclusively on non-prion

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neurodegenerative disorders without direct relevance to PrP misfolding were also excluded. Additionally, studies that lacked sufficient methodological information, demonstrated limited mechanistic relevance to CJD pathogenesis, were published before 2015, or were unavailable in full-text form were omitted from the review. Duplicate records identified during the screening process were removed prior to eligibility assessment to avoid redundancy and ensure the accuracy of the final synthesis.

Search Results and Study Selection

A total of 121 studies were initially identified through a comprehensive literature search conducted across four major scientific databases, namely PubMed, Scopus, Web of Science, and ScienceDirect. The search strategy was designed to capture the most recent and relevant evidence regarding the molecular mechanisms underlying prion protein (PrP) misfolding in Creutzfeldt–Jakob disease (CJD). To ensure the quality and relevance of the retrieved literature, the search was limited to peer-reviewed articles published in English between 2015 and 2025. This timeframe was selected to incorporate contemporary advances in prion biology, structural studies, genetic investigations, and mechanistic research related to disease pathogenesis.

Following the initial identification stage, all retrieved records were carefully reviewed for duplication. A total of 17 duplicate studies were removed, resulting in 104 unique records that proceeded to the title and abstract screening phase. During this stage, each study was evaluated based on the predefined inclusion and exclusion criteria. Studies that focused on unrelated neurodegenerative disorders, lacked relevance to prion protein misfolding, were non-research articles, or did not specifically address molecular mechanisms associated with CJD were excluded. As a result, 55 studies were removed, leaving 49 articles eligible for further evaluation through full-text review.

The 49 remaining studies underwent a detailed full-text assessment to determine their suitability for inclusion in the review. This evaluation considered the scientific rigor of the methodology, relevance of the findings to PrP structural conversion and aggregation, and the extent to which the studies contributed to understanding the molecular basis of CJD pathogenesis. During this phase, 15 studies were excluded because they provided insufficient mechanistic information, lacked adequate methodological details, focused primarily on diagnostic or clinical aspects without addressing molecular pathways, or demonstrated limited applicability to the objectives of the review. Consequently, 34 studies satisfied all eligibility requirements and were included in the final qualitative synthesis.

The selected studies encompassed a broad range of research approaches, including experimental investigations, molecular and structural analyses, genetic studies, cellular models, animal studies, and comprehensive review articles. Collectively, these publications provided valuable insights into the processes involved in PrP misfolding, conformational instability, amyloid formation, genetic influences, cellular stress responses, and neurodegenerative mechanisms associated with CJD. The complete study selection process, including the stages of identification, screening, eligibility assessment, and final inclusion, is summarized in Figure 1 (PRISMA Flow Diagram) and Table 1 (PRISMA Screening Process).

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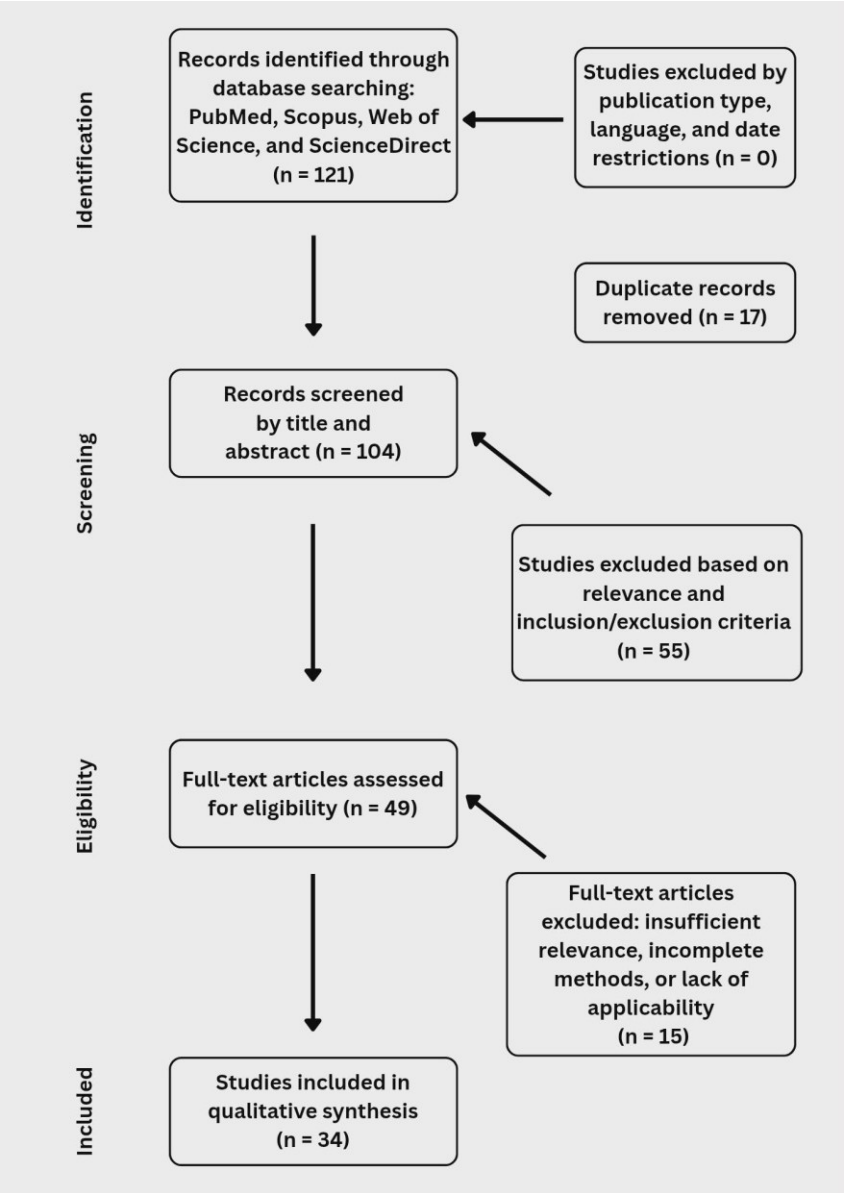

Figure 1. PRISMA 2020 flow diagram illustrating the identification, screening, eligibility assessment, and inclusion of studies investigating the molecular mechanisms of prion protein (PrP) misfolding in Creutzfeldt–Jakob disease.

Table 1. PRISMA Screening Process

Screening Stage	Number of Studies
Records identified	121
Duplicates removed	17
Records screened	104
Full-text articles assessed	49
Excluded articles	15
Included studies	34

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Data Extraction

Data were extracted from all included studies using a standardized data extraction framework developed specifically for this review. Information collected included bibliographic details (authors, publication year, and journal), study design, experimental model or study population, molecular mechanism investigated, analytical methods employed, and principal findings. Additional information was collected regarding structural conversion of PrP^{c} to PrP^{sc}, PRNP mutations, codon 129 polymorphisms, seeded protein aggregation, proteostasis dysfunction, oxidative stress, mitochondrial impairment, neuroinflammation, neuronal degeneration, cryo-electron microscopy findings, and diagnostic applications such as RT-QuIC, PMCA, cerebrospinal fluid biomarkers, and magnetic resonance imaging.

Extracted data were organized into thematic categories and summary tables to facilitate comparison and synthesis across studies. The extracted information formed the basis for the qualitative synthesis presented in the Results and Discussion section.

Risk of Bias Assessment

The methodological quality and risk of bias of included studies were evaluated using criteria adapted from established evidence-appraisal frameworks. Assessment focused on seven domains: (1) clarity of study objectives and methodology; (2) appropriateness of experimental design or clinical cohort selection; (3) adequacy of sample size and study population; (4) validity and reliability of analytical techniques; (5) transparency of data reporting and statistical analysis; (6) consistency of findings with current scientific evidence; and (7) relevance to the molecular mechanisms of CJD pathogenesis.

Each study was categorized as having low, moderate, or high risk of bias based on its overall methodological quality. Across the included literature, the most common limitations included small sample sizes in rare-disease cohorts, heterogeneity of experimental models, variability in biomarker detection methods, and differences in structural characterization techniques. Nevertheless, the majority of included studies demonstrated adequate methodological rigor and provided valuable mechanistic insights into PrP misfolding and CJD pathogenesis.

Risk-of-bias assessments were considered during evidence synthesis to ensure balanced interpretation of findings and to identify areas requiring further investigation. These assessments also helped highlight methodological gaps and opportunities for future research in the field of prion biology and neurodegenerative disease mechanisms.

RESULTS AND DISCUSSION

Characteristics of Included Studies

A total of 34 studies met the eligibility criteria and were included in the final qualitative synthesis. The selected literature encompassed a diverse range of study designs, including review articles, experimental investigations, structural biology studies, clinical cohort studies, diagnostic validation studies, and systematic reviews. The included studies collectively examined multiple aspects of prion biology, including structural conversion of prion proteins, genetic susceptibility, seeded protein aggregation, cellular stress responses, neuroinflammation, neurodegeneration, and diagnostic technologies. Structural biology investigations employing cryogenic electron microscopy provided detailed insights into the architecture of infectious prion fibrils and the structural basis of strain diversity, while genetic studies explored the contribution of PRNP mutations and codon 129 polymorphisms to disease susceptibility and phenotype variation. Clinical and diagnostic studies primarily focused on the performance of RT-QuIC, PMCA, cerebrospinal fluid biomarkers, and magnetic resonance imaging in improving the ante-mortem diagnosis of CJD. Overall, the included literature supports a multifactorial model of disease pathogenesis in which PrP misfolding initiates a cascade of molecular and cellular events that culminate in progressive neurodegeneration.

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Table 2. Summary of the Characteristics and Major Findings of Included Studies

Author	Year	Study Type	Key Mechanism Investigated	Major Findings
Castle & Gill	2017	Review	Physiological function of PrP^c	PrP^c is involved in neuronal signaling, protection, adhesion, and cellular homeostasis.
Wulf et al.	2017	Review	Cellular role of PrP^c	PrP^c contributes to synaptic function, neuroprotection, and stress response.
Bagyinszky et al.	2018	Review	PRNP mutations	Summarized pathogenic PRNP variants linked to inherited prion diseases.
Penke et al.	2018	Review	Heat shock proteins and autophagy	Chaperones and autophagy pathways are potential neuroprotective mechanisms.
Bernardi et al.	2019	Review	Mutation-related mechanisms	PRNP mutations promote abnormal folding and inherited neurodegeneration.
Sigurdson et al.	2019	Review	Molecular pathogenesis	Prion disease involves PrP misfolding, aggregation, strain variation, and neurotoxicity.
Carroll & Chesebro	2019	Review	Microglia and inflammation	Neuroinflammatory responses influence disease progression and cell damage.
Green	2019	Review	RT-QuIC diagnosis	RT-QuIC provides a highly specific diagnostic test for sporadic CJD.
Glynn et al.	2020	Cryo-EM study	Human prion fibril core	Described hydrophobic, protease-resistant core features of human prion fibrils.
Giaccone & Moda	2020	Review	PMCA detection	PMCA can detect PrP^{sc} in peripheral tissues, especially in variant CJD.
López-Pérez et al.	2020	Review	Autophagy impairment	Autophagy dysfunction may worsen PrP clearance and disease progression.
Baiardi et al.	2021	Clinicopathologic study	Genetic CJD classification	Mutation type, PrP^{sc} type, and codon 129 influence clinical diversity.
Dong & Satoh	2021	Literature review	RT-QuIC assay	RT-QuIC improves detection of prion seeding activity in diagnostic samples.
Hermann et al.	2021	Review/Guideline	Biomarkers and diagnosis	Established modern diagnostic guidelines using RT-QuIC, CSF markers, and MRI.
Kraus et al.	2021	Cryo-EM study	Prion fibril structure	Revealed high-resolution infectious mammalian prion structures and strain differences.
Li et al.	2021	Review	Neuroinflammation	Microglia and astrocytes contribute to prion propagation and neuronal injury.
Park et al.	2021	Systematic Review/Meta-analysis	MRI diagnostic value	Diffusion-weighted MRI has high diagnostic value in sporadic CJD.
Altuna et al.	2022	Narrative Review	Diagnostic biomarkers	CSF biomarkers, RT-QuIC, and imaging improve diagnostic confidence.
Appleby et al.	2022	Review	Genetic susceptibility	PRNP mutations and polymorphisms influence disease risk and phenotype.
Caughey et al.	2022	Review	High-resolution prion structures	Discussed how structural data explain prion strains and infectivity.
Hallinan et al.	2022	Cryo-EM study	Human prion filament structure	Identified distinct filament structures in Gerstmann–Sträussler–Scheinker disease.
Kim et al.	2022	Experimental study	Mitochondrial dysfunction	Prion toxicity disrupts mitochondrial quality control and promotes apoptosis.
Manka et al.	2022	Cryo-EM study	Ex vivo prion fibrils	Provided near-atomic structure of infectious RML prion fibrils.
Thellung et al.	2022	Review	Proteostasis dysfunction	Proteasome and autophagy impairment contribute to PrP accumulation and neurodegeneration.

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Hermann et al.	2023	Surveillance study	RT-QuIC performance	Confirmed high diagnostic accuracy of RT-QuIC in CJD surveillance.
Hermann & Zerr	2023	Review	Biochemical biomarkers	Highlighted unmet needs in biomarker development for human prion diseases.
Manka et al.	2023	Structural study	Strain diversity	Showed that prion strain variation is encoded in fibril architecture.
Mercer & Harris	2023	Review	Prion-induced toxicity	Synaptic dysfunction, calcium imbalance, and apoptosis contribute to neuronal death.
Senesi et al.	2023	Cohort study	CSF biomarker performance	RT-QuIC showed strong diagnostic value compared with traditional CSF markers.
Spiers et al.	2023	Review	Oxidative stress	Redox imbalance contributes to neuronal injury and disease progression.
Eraña et al.	2024	Experimental study	Prion generation and amplification	Demonstrated spontaneous generation of bona fide prions using misfolding amplification.
Hermann & Zerr	2024	Review	Biochemical biomarkers	Highlighted emerging biomarker strategies and diagnostic challenges in prion diseases.
Makarava et al.	2024	Review	Reactive astrocytes	Astrocytes may contribute to neuronal and endothelial damage in prion disease.
Sanz-Hernández et al.	2024	Experimental study	Misfolding and amyloid formation	Human PrP misfolds through monomer, oligomer, and amyloid stages.

As shown in Table 2, the included studies collectively demonstrate that CJD pathogenesis involves a complex interaction of structural, genetic, cellular, and diagnostic factors. Research published between 2017 and 2024 increasingly emphasized the integration of molecular mechanisms with advanced structural and diagnostic technologies. Earlier studies primarily focused on the physiological functions of PrP^{c} and genetic susceptibility, whereas more recent investigations employed Cryo-EM, RT-QuIC, PMCA, and biomarker analyses to elucidate disease mechanisms and improve diagnostic accuracy. This progression reflects a growing understanding of prion biology and highlights the multidisciplinary nature of contemporary CJD research.

Structural Conversion of PrP^{c} to PrP^{sc}

The structural conversion of the normal cellular prion protein (PrP^{c}) into the pathogenic scrapie isoform (PrP^{sc}) is widely recognized as the fundamental molecular event underlying the initiation and progression of Creutzfeldt–Jakob disease (CJD). Under physiological conditions, PrP^{c} exists as a glycosylphosphatidylinositol-anchored membrane glycoprotein that is highly expressed in neurons and other tissues. The protein is composed predominantly of α-helical structures and participates in various physiological processes, including neuronal signaling, synaptic maintenance, cellular protection, and metal-ion homeostasis (Castle & Gill, 2017; Wulf et al., 2017). Despite its normal biological functions, PrP^{c} possesses an intrinsic capacity to adopt alternative conformations. When specific molecular conditions favor structural destabilization, the protein undergoes a conformational transition into PrP^{sc}, a pathogenic isoform characterized by increased β-sheet content, aggregation propensity, and resistance to proteolytic degradation (Sigurdson et al., 2019). This conversion initiates a cascade of molecular events that ultimately leads to neuronal dysfunction and neurodegeneration.

A defining characteristic of PrP misfolding is the transition from an α-helix-rich structure to a β-sheet-rich conformation. This structural rearrangement fundamentally alters the physicochemical properties of the protein, promoting intermolecular interactions that facilitate aggregation and self-propagation. Unlike PrP^{c}, which remains soluble and functionally active, PrP^{sc} exhibits a greater tendency to form insoluble aggregates capable of accumulating within neural tissues. Sigurdson et al. (2019) reported that the increased β-sheet content of PrP^{sc} contributes to its remarkable structural stability and enables it to serve as a template for the conversion of additional PrP^{c} molecules. Through this templated conversion mechanism, a relatively small quantity of misfolded protein can trigger extensive propagation of pathogenic conformations, resulting in progressive accumulation of PrP^{sc} throughout the brain. Consequently, the α-helix to β-sheet transition represents the critical molecular switch that transforms a physiologically functional protein into a self-replicating pathogenic agent.

Conformational instability plays a crucial role in facilitating this structural transition. Evidence suggests that the native conformation of PrP^{c} exists in a delicate thermodynamic balance that can be disrupted by genetic mutations, post-translational modifications, environmental factors, or interactions with pre-existing PrP^{sc}

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aggregates (Bagyinszky et al., 2018; Bernardi et al., 2019). Destabilization of the native structure increases the probability of adopting intermediate conformations that are more susceptible to pathogenic folding pathways. Studies investigating inherited prion diseases have demonstrated that several pathogenic **PRNP** mutations reduce structural stability and increase the likelihood of spontaneous misfolding, thereby enhancing disease susceptibility (Bernardi et al., 2019). These findings support the concept that conformational instability serves as an important predisposing factor in prion pathogenesis by lowering the energetic barriers that normally maintain the native protein structure.

Recent experimental evidence has further demonstrated that PrP misfolding occurs through a series of progressive conformational stages rather than a single abrupt event. Sanz-Hernández et al. (2024) reported that human prion proteins undergo sequential transitions from monomeric forms to soluble oligomeric intermediates and eventually mature amyloid fibrils. This observation suggests that oligomers may represent important pathogenic intermediates that contribute both to protein propagation and cellular toxicity. Similarly, Eraña et al. (2024) successfully demonstrated the spontaneous generation and amplification of infectious prions through protein misfolding amplification techniques, providing strong experimental support for the protein-only hypothesis. These findings indicate that once misfolding is initiated, the conversion process can become self-sustaining and progressively amplify pathogenic protein species even in the absence of nucleic acid involvement.

The ultimate consequence of PrP misfolding is the formation of amyloid fibrils and highly ordered protein aggregates that accumulate within the central nervous system. Amyloid formation occurs when misfolded proteins self-assemble into elongated fibrillar structures enriched in β-sheets, resulting in the development of insoluble deposits that are characteristic of prion diseases. These fibrils not only serve as reservoirs of infectious material but also contribute to cellular dysfunction by disrupting membrane integrity, impairing intracellular trafficking, and overwhelming protein quality-control systems (Sigurdson et al., 2019). The accumulation of amyloid structures has been strongly associated with neuronal injury, synaptic dysfunction, and progressive neurodegeneration, highlighting their importance in disease progression.

Advances in cryogenic electron microscopy (Cryo-EM) have significantly enhanced understanding of the structural basis of amyloid formation and prion infectivity. Glynn et al. (2020) described the hydrophobic and protease-resistant core regions of human prion fibrils, providing insight into the molecular features responsible for their exceptional stability. Subsequent studies by Kraus et al. (2021), Manka et al. (2022), and Hallinan et al. (2022) resolved high-resolution structures of infectious mammalian prions and disease-associated fibrils, revealing highly ordered β-sheet-rich architectures that differ substantially from the native conformation of PrP^{c}. These investigations demonstrated that distinct fibrillar structures can encode strain-specific biological properties, thereby explaining the clinical and pathological heterogeneity observed among prion diseases. More recently, Manka et al. (2023) further showed that strain diversity is directly encoded within fibril architecture, reinforcing the relationship between structural conformation and disease phenotype.

Table 3. Structural Changes Associated with PrP Misfolding

Molecular Event	Description	Pathological Consequence
α-Helix to β-Sheet Conversion	Structural transition of PrP^{c} into β-sheet-rich PrP^{sc}	Increased aggregation propensity and protease resistance
Conformational Instability	Destabilization of native PrP structure due to mutations or molecular interactions	Enhanced susceptibility to misfolding
Oligomer Formation	Assembly of misfolded PrP into soluble oligomeric intermediates	Increased cellular toxicity and propagation potential
Amyloid Fibril Formation	Progressive aggregation into highly ordered fibrillar structures	Prion accumulation and neurodegeneration
Strain-Specific Conformations	Distinct PrP^{sc} structural architectures maintained during propagation	Phenotypic diversity and variable disease progression

As summarized in Table 3, the conversion of PrP^{c} to PrP^{sc} involves a series of interconnected structural changes that collectively drive disease initiation and progression. The transition from α-helical to β-sheet-rich conformations promotes aggregation, while conformational instability increases susceptibility to pathogenic

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folding. The formation of oligomers and amyloid fibrils enables self-propagation and accumulation of infectious prions, whereas strain-specific structural variations contribute to differences in clinical presentation and neuropathological outcomes. Collectively, the evidence reviewed in this section demonstrates that structural conversion represents the primary molecular event responsible for the emergence, propagation, and pathological consequences of prion diseases, making it a central focus for both diagnostic and therapeutic research.

Genetic Factors Contributing to PrP Misfolding

Genetic susceptibility represents one of the most important determinants of prion disease development and provides compelling evidence that alterations in protein structure alone can initiate neurodegeneration. The PRNP gene, located on chromosome 20 (20p13), encodes the cellular prion protein (PrP^{c}), a glycosylphosphatidylinositol-anchored glycoprotein that is predominantly expressed in neurons and plays essential roles in synaptic transmission, neuronal survival, cellular signaling, and protection against oxidative stress (Castle & Gill, 2017; Wulf et al., 2017). Under physiological conditions, PrP^{c} adopts a stable α-helical conformation that maintains its normal biological functions. However, inherited genetic alterations within the PRNP gene can destabilize this native structure, increasing the likelihood of spontaneous conformational conversion into the pathogenic β-sheet-rich isoform, PrP^{sc}. Although approximately 85–90% of CJD cases occur sporadically, nearly 10–15% are inherited and arise from pathogenic PRNP mutations, demonstrating that genetic abnormalities alone are sufficient to initiate prion disease without exposure to infectious prions (Bagyinszky et al., 2018; Appleby et al., 2022).

More than 50 pathogenic mutations have been identified in the PRNP gene, with missense mutations accounting for the majority of inherited prion diseases (Bagyinszky et al., 2018). These mutations alter amino acid residues that are essential for maintaining the tertiary structure of PrP^{c}, thereby reducing conformational stability and facilitating spontaneous misfolding. Bernardi et al. (2019) reported that mutation-induced structural destabilization lowers the energetic barrier separating the native and pathogenic conformations, allowing PrP^{c} to adopt abnormal folding pathways more readily. Once the pathogenic conformation is established, PrP^{sc} serves as a template that converts additional PrP^{c} molecules into the same abnormal structure, initiating the self-propagating cycle characteristic of prion diseases. Unlike sporadic CJD, in which the initial trigger remains poorly understood, inherited prion diseases clearly demonstrate that alterations in the primary amino acid sequence are sufficient to initiate the molecular cascade leading to neurodegeneration.

Several PRNP mutations have been extensively investigated because of their strong association with distinct inherited prion disorders. The E200K mutation is the most frequently reported pathogenic variant worldwide and is considered the principal cause of familial Creutzfeldt–Jakob disease (fCJD). Individuals carrying this mutation typically develop rapidly progressive dementia, cerebellar dysfunction, and neuropathological changes that closely resemble sporadic CJD (Bagyinszky et al., 2018). In contrast, the D178N mutation exhibits remarkable phenotypic heterogeneity because its clinical manifestation is determined by the polymorphic residue present at codon 129. When methionine occupies codon 129, affected individuals develop Fatal Familial Insomnia (FFI), a disorder characterized by progressive insomnia, autonomic dysfunction, and selective thalamic degeneration. Conversely, when valine is present at codon 129, the same D178N mutation results in familial Creutzfeldt–Jakob disease, illustrating how a single amino acid polymorphism can profoundly alter disease phenotype (Appleby et al., 2022). The V210I mutation has likewise been associated with genetic CJD and produces clinical manifestations closely resembling sporadic disease, whereas the P102L mutation is primarily linked to Gerstmann–Sträussler–Scheinker syndrome (GSS), which is characterized by slowly progressive cerebellar ataxia, amyloid plaque formation, and a longer disease duration than classical CJD (Bernardi et al., 2019).

Table 4. Major PRNP Mutations Associated with Human Prion Diseases

Mutation	Associated Disease	Molecular Effect
E200K	Familial Creutzfeldt–Jakob Disease (fCJD)	Destabilizes the native PrP^{c} structure, increasing spontaneous conversion to PrP^{sc} and accelerating seeded aggregation.
D178N	Fatal Familial Insomnia (FFI)	Alters PrP folding; phenotype depends on codon 129 polymorphism, demonstrating genotype–phenotype interaction.
V210I	Genetic Creutzfeldt–Jakob Disease	Reduces structural stability of PrP^{c} and increases susceptibility to spontaneous misfolding.
P102L	Gerstmann–Sträussler–Scheinker Syndrome (GSS)	Promotes amyloid fibril formation, progressive aggregation, and prolonged disease progression.

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As shown in Table 4, despite producing different clinical syndromes, these mutations share a common pathogenic mechanism: disruption of the structural integrity of PrP^{c}, resulting in increased conformational instability and enhanced susceptibility to pathogenic conversion. The variability in disease phenotype reflects differences in the location of each mutation, its influence on protein folding, and its interaction with additional genetic modifiers. These observations demonstrate that relatively small alterations in the amino acid sequence of PrP can profoundly influence protein stability, aggregation kinetics, strain formation, and disease progression.

Beyond pathogenic mutations, the codon 129 polymorphism represents the most important genetic modifier influencing susceptibility to human prion diseases. This polymorphism results in the incorporation of either methionine (M) or valine (V) at amino acid position 129, producing three possible genotypes: MM, MV, and VV. Numerous epidemiological and molecular studies have consistently demonstrated that codon 129 significantly influences disease susceptibility, incubation period, neuropathological characteristics, and clinical phenotype (Baiardi et al., 2021; Appleby et al., 2022). Approximately 70% of patients with sporadic CJD possess the MM genotype, indicating a substantially greater susceptibility compared with heterozygous individuals. In contrast, the MV genotype appears to confer partial protection by reducing the efficiency of PrP conversion and delaying disease onset, whereas the VV genotype has been associated with specific molecular subtypes of sporadic CJD. These findings emphasize that host genetic background plays a decisive role not only in determining whether disease develops but also in shaping its biological behavior.

The interaction between pathogenic mutations, codon 129 polymorphism, and prion strain characteristics has further expanded current understanding of disease heterogeneity. Baiardi et al. (2021) proposed a histomolecular classification system demonstrating that combinations of mutation type, codon 129 genotype, and PrP^{sc} subtype explain much of the variability observed in clinical presentation, neuropathological distribution, disease duration, and progression rate. Appleby et al. (2022) similarly reported that genetic background influences age at onset, disease severity, biomarker profiles, and diagnostic characteristics. These observations indicate that disease phenotype cannot be explained solely by the presence of a pathogenic mutation but instead results from the interaction between inherited genetic factors and the structural properties of misfolded prion proteins.

Recent advances in structural biology have strengthened the relationship between genetics and protein misfolding. High-resolution Cryo-EM studies have demonstrated that mutations capable of destabilizing PrP^{c} increase the probability of forming intermediate conformations that subsequently propagate through seeded aggregation mechanisms (Sigurdson et al., 2019; Manka et al., 2023). Once pathogenic conformers are established, they preserve strain-specific structural characteristics that influence infectivity, tissue tropism, and clinical phenotype. This evidence indicates that genetic alterations affect not only the initiation of PrP misfolding but also the structural evolution and biological behavior of infectious prion assemblies throughout disease progression.

The evidence synthesized in this section demonstrates that genetic factors constitute a fundamental component of the molecular pathogenesis of CJD. Pathogenic PRNP mutations directly destabilize the native structure of PrP^{c}, while codon 129 polymorphisms modify susceptibility, phenotype expression, and disease progression. The interaction between genetic variation, structural instability, and prion propagation provides a comprehensive explanation for the remarkable heterogeneity observed among human prion diseases. These findings also highlight the clinical importance of genetic screening and molecular characterization in improving disease classification, prognostic assessment, and the future development of genotype-specific therapeutic strategies.

Seeded Protein Aggregation and Prion Propagation

A defining characteristic of prion diseases is the ability of misfolded prion proteins to propagate through a self-perpetuating process known as seeded protein aggregation. Unlike conventional infectious agents that rely on nucleic acids for replication, prions propagate through a protein-only mechanism in which the pathogenic isoform, PrP^{sc}, serves as a structural template that induces the conversion of normal cellular prion protein (PrP^{c}) into additional copies of the misfolded form. This concept forms the basis of the protein-only hypothesis, which remains the most widely accepted explanation for prion infectivity and disease transmission (Sigurdson et al., 2019). Once a pathogenic conformer is generated, either spontaneously, genetically, or through acquired exposure, it can recruit normal PrP^{c} molecules and initiate a cycle of continuous protein misfolding and accumulation.

The mechanism of templated misfolding is central to understanding how prion diseases spread within the nervous system. During this process, PrP^{sc} interacts directly with PrP^{c} and induces structural rearrangements that convert the normal protein into the pathogenic conformation. The newly formed PrP^{sc} molecules subsequently

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become capable of converting additional PrP^{c} molecules, resulting in exponential amplification of misfolded proteins. Sigurdson et al. (2019) emphasized that this process enables disease propagation without the involvement of genetic material, distinguishing prions from all other known infectious agents. Experimental evidence supporting this mechanism has strengthened considerably in recent years. Eraña et al. (2024) demonstrated the spontaneous generation and amplification of infectious prions using protein misfolding amplification techniques, providing direct evidence that misfolded proteins alone are sufficient to initiate and sustain infectivity. These findings further validate the protein-only hypothesis and reinforce the central role of seeded aggregation in disease pathogenesis.

The propagation process is closely linked to the formation of oligomers and amyloid fibrils. Following initial misfolding, PrP molecules assemble into small oligomeric aggregates that can serve as seeds for further aggregation. These seeds subsequently grow through the recruitment of additional misfolded proteins, ultimately forming larger fibrillar structures that accumulate within neural tissues. Sanz-Hernández et al. (2024) demonstrated that human prion proteins undergo progressive transitions from monomeric forms to oligomers and mature amyloid fibrils, highlighting the dynamic nature of the aggregation process. Oligomeric intermediates are of particular importance because they are believed to contribute substantially to cellular toxicity while simultaneously promoting the formation of larger aggregates. As the concentration of pathogenic seeds increases, the efficiency of conversion and propagation also increases, resulting in accelerated disease progression.

Recent advances in structural biology have provided valuable insights into the molecular basis of prion replication and propagation. Cryo-electron microscopy studies have revealed that infectious prion fibrils possess highly ordered and stable architectures that allow the faithful transmission of structural information during replication (Glynn et al., 2020; Kraus et al., 2021). These structural templates preserve specific conformational characteristics, enabling the formation of distinct prion strains that maintain their biological properties across successive rounds of propagation. Caughey et al. (2022) explained that differences in fibril architecture contribute to variations in infectivity, tissue tropism, incubation periods, and disease phenotype. Similarly, Manka et al. (2023) demonstrated that prion strain diversity is encoded directly within fibrillar structures, providing a structural explanation for the heterogeneity observed among human and animal prion diseases.

An important consequence of seeded aggregation is the progressive spread of pathology throughout the central nervous system. As misfolded proteins accumulate, they can disseminate between interconnected neuronal networks, facilitating the transmission of pathogenic conformers from one brain region to another. This process contributes to the characteristic progression of neurological dysfunction observed in CJD, where cognitive impairment, motor abnormalities, and behavioral disturbances worsen rapidly over time. Li et al. (2021) suggested that glial cells may also contribute to the dissemination of pathogenic proteins by participating in inflammatory responses and influencing the local cellular environment. Consequently, prion propagation is not limited to protein-protein interactions alone but involves complex interactions between misfolded proteins and surrounding neural tissues.

Collectively, the evidence reviewed indicates that seeded protein aggregation and prion propagation are fundamental mechanisms driving the progression of CJD. Through templated misfolding, PrP^{sc} acts as a self-replicating pathogenic entity capable of continuously converting normal PrP^{c} into additional misfolded proteins. The formation of oligomers and amyloid fibrils facilitates both infectivity and neurotoxicity, while strain-specific conformations influence disease phenotype and progression. These findings demonstrate that prion propagation represents a unique biological phenomenon in which protein structure itself functions as the carrier of pathogenic information, making seeded aggregation a central process in the molecular pathogenesis of prion diseases.

Cellular Stress and Proteostasis Dysfunction

The maintenance of cellular protein homeostasis, commonly referred to as proteostasis, is essential for normal neuronal function and survival. Proteostasis is regulated through a complex network of molecular chaperones, protein degradation pathways, and quality-control mechanisms that ensure proteins are correctly folded, repaired, or removed when damaged. In prion diseases, the continuous accumulation of misfolded prion proteins places substantial stress on these protective systems, ultimately disrupting their ability to maintain cellular homeostasis. As PrP^{sc} aggregates progressively accumulate, neurons experience increasing difficulty in managing abnormal protein species, resulting in widespread proteostasis dysfunction and enhanced susceptibility to neurodegeneration (Thellung et al., 2022).

One of the earliest cellular responses to protein misfolding involves the activation of molecular chaperones, particularly heat shock proteins (HSPs). These proteins assist in proper protein folding, prevent aggregation, and

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facilitate the refolding or degradation of damaged proteins. Penke et al. (2018) reported that heat shock proteins play a critical neuroprotective role by limiting the accumulation of misfolded proteins and supporting cellular stress responses. Increased expression of HSPs has been observed in various neurodegenerative disorders and is generally considered a protective mechanism against protein aggregation. However, the persistent production and accumulation of PrP^{sc} may eventually overwhelm chaperone capacity, reducing their effectiveness and allowing toxic protein aggregates to accumulate within neurons. As a result, protective cellular responses become insufficient to counteract ongoing prion propagation and protein misfolding.

The endoplasmic reticulum (ER) is another major site affected by proteostasis disruption. Because the ER is responsible for protein synthesis, folding, and quality control, the accumulation of abnormal proteins can trigger a condition known as ER stress. When misfolded proteins accumulate beyond the capacity of ER quality-control systems, cells activate the unfolded protein response (UPR) to restore homeostasis. While initially protective, prolonged activation of ER stress pathways may contribute to cellular dysfunction and apoptosis. Thellung et al. (2022) noted that chronic ER stress has been implicated in several neurodegenerative disorders, including prion diseases, where persistent accumulation of PrP^{sc} places continuous pressure on intracellular protein-folding machinery. Consequently, ER stress may represent an important link between protein misfolding and neuronal degeneration.

The ubiquitin–proteasome system (UPS) serves as one of the primary mechanisms responsible for eliminating damaged and misfolded proteins. Under normal conditions, proteins targeted for degradation are tagged with ubiquitin molecules and subsequently degraded by the proteasome. However, increasing evidence suggests that PrP aggregates interfere with proteasomal activity, impairing the cell's ability to remove toxic proteins effectively (Thellung et al., 2022). Dysfunction of the UPS results in further accumulation of misfolded proteins, creating a self-reinforcing cycle in which aggregate formation progressively impairs cellular clearance mechanisms. This cycle promotes cellular stress, disrupts normal neuronal function, and accelerates disease progression.

Autophagy represents another critical pathway involved in maintaining proteostasis. Unlike the proteasome, which primarily degrades individual proteins, autophagy is capable of removing larger protein aggregates and damaged organelles through lysosomal degradation. López-Pérez et al. (2020) demonstrated that impairment of autophagic pathways can significantly reduce the clearance of PrP aggregates, leading to enhanced accumulation of pathogenic proteins within neural tissues. Similarly, Penke et al. (2018) emphasized that stimulation of autophagy may represent a promising therapeutic strategy for reducing aggregate burden and protecting neurons from degeneration. However, persistent prion accumulation can disrupt autophagic function, limiting the effectiveness of this protective mechanism and contributing to disease progression.

The combined dysfunction of heat shock proteins, ER quality-control systems, the ubiquitin–proteasome system, and autophagy ultimately creates a state of chronic cellular stress. Under these conditions, neurons become increasingly vulnerable to oxidative damage, mitochondrial dysfunction, inflammatory signaling, and apoptosis. Thellung et al. (2022) proposed that proteostasis failure should be considered a central pathogenic mechanism in prion diseases because it amplifies the toxic effects of protein misfolding and accelerates neuronal injury. Rather than serving solely as a consequence of PrP accumulation, proteostasis dysfunction actively contributes to disease progression by reducing the cell's capacity to eliminate pathogenic proteins and maintain intracellular homeostasis.

Overall, the evidence reviewed demonstrates that cellular stress and proteostasis dysfunction are integral components of CJD pathogenesis. The accumulation of misfolded PrP proteins overwhelms cellular quality-control systems, resulting in impaired protein folding, reduced degradation capacity, and progressive aggregate accumulation. Dysregulation of heat shock proteins, ER stress responses, the ubiquitin–proteasome system, and autophagy collectively contributes to neuronal vulnerability and disease progression. These findings highlight the importance of proteostasis pathways as potential therapeutic targets aimed at restoring cellular homeostasis and limiting the pathological consequences of PrP misfolding.

Oxidative Stress and Mitochondrial Dysfunction

Oxidative stress is recognized as a major contributor to neuronal damage in Creutzfeldt–Jakob disease (CJD) and represents one of the key downstream consequences of prion protein misfolding. Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the capacity of cellular antioxidant defense systems, resulting in damage to proteins, lipids, nucleic acids, and other cellular components. In prion diseases, the accumulation of pathogenic PrP^{sc} disrupts normal cellular homeostasis and promotes excessive ROS generation, creating a toxic intracellular environment that contributes to neuronal injury and disease progression (Spiers et al., 2023). Because neurons possess high metabolic demands and limited regenerative capacity, they

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are particularly vulnerable to oxidative damage, making oxidative stress an important factor in the pathogenesis of CJD.

Several mechanisms have been proposed to explain the relationship between PrP misfolding and oxidative stress. Under normal physiological conditions, PrP^{c} contributes to maintaining redox balance and metal-ion homeostasis, particularly through interactions with copper ions and antioxidant pathways (Castle & Gill, 2017; Wulf et al., 2017). These functions help protect neurons from oxidative injury and support cellular survival. However, conversion of PrP^{c} into PrP^{sc} disrupts these protective activities, reducing the cell's ability to regulate reactive oxygen species effectively. As pathogenic proteins accumulate, oxidative stress intensifies, resulting in damage to cellular membranes, proteins, and nucleic acids. Spiers et al. (2023) emphasized that persistent redox imbalance contributes significantly to neuronal dysfunction and may accelerate disease progression by amplifying the toxic effects of protein aggregation.

One of the most damaging consequences of oxidative stress is lipid peroxidation, a process in which reactive oxygen species attack membrane lipids and compromise membrane integrity. Neuronal membranes are especially susceptible because of their high concentration of polyunsaturated fatty acids. Oxidative damage to membrane components can impair signal transduction, alter ion transport, and disrupt communication between neurons. In addition, oxidative modification of proteins may reduce enzymatic activity and promote the formation of further protein aggregates, creating a cycle in which oxidative stress and protein misfolding reinforce one another. These events contribute to progressive deterioration of neuronal structure and function and may accelerate the development of neurodegenerative changes associated with prion diseases.

Mitochondria are among the primary targets of oxidative damage and play a central role in the progression of neurodegeneration. As the principal site of cellular energy production, mitochondria generate adenosine triphosphate (ATP) through oxidative phosphorylation, providing the energy required for neuronal survival and function. Experimental evidence presented by Kim et al. (2022) demonstrated that prion toxicity disrupts mitochondrial quality-control mechanisms, leading to impaired mitochondrial dynamics, reduced bioenergetic efficiency, and activation of apoptotic signaling pathways. Dysfunctional mitochondria exhibit decreased ATP production and increased ROS generation, further exacerbating oxidative stress and cellular injury. Given the high energy requirements of neurons, even moderate mitochondrial impairment can significantly affect neuronal viability and contribute to disease progression.

Disruption of cellular energy metabolism represents another important consequence of mitochondrial dysfunction in CJD. Reduced ATP availability compromises essential neuronal processes, including maintenance of membrane potential, neurotransmitter release, protein synthesis, and intracellular transport. Energy deficits may also impair cellular repair mechanisms and reduce the efficiency of protein degradation pathways responsible for eliminating misfolded proteins. Consequently, mitochondrial dysfunction not only causes direct cellular injury but also indirectly promotes the accumulation of pathogenic protein aggregates. Kim et al. (2022) further reported that impaired mitochondrial quality control increases susceptibility to apoptosis, highlighting the close relationship between mitochondrial dysfunction and neuronal loss in prion diseases.

The relationship between oxidative stress and mitochondrial dysfunction is highly interconnected and self-perpetuating. Increased ROS production damages mitochondrial proteins, lipids, and DNA, impairing mitochondrial performance and reducing energy production. In turn, dysfunctional mitochondria generate additional reactive oxygen species, creating a vicious cycle of oxidative damage and metabolic impairment (Spiers et al., 2023). This cycle contributes to progressive neuronal dysfunction and provides an important mechanistic link between PrP misfolding and widespread neurodegeneration. The findings discussed in this section indicate that oxidative stress and mitochondrial dysfunction are not merely secondary consequences of prion accumulation but active participants in disease progression. Their combined effects on redox homeostasis, cellular metabolism, and neuronal survival underscore their importance as potential targets for future therapeutic strategies aimed at slowing neurodegeneration in CJD.

Neuroinflammation and Glial Activation

Neuroinflammation is increasingly recognized as a major contributor to the progression of Creutzfeldt–Jakob disease (CJD) and other prion disorders. Although prion diseases are initiated by the misfolding and accumulation of pathogenic prion proteins, the resulting cellular damage triggers a complex inflammatory response within the central nervous system. This response is primarily mediated by glial cells, particularly microglia and astrocytes, which serve as the principal immune regulators of the brain. While neuroinflammation initially functions as a protective mechanism aimed at limiting tissue damage and promoting the clearance of

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abnormal proteins, persistent activation of glial cells can contribute to neuronal dysfunction and accelerate neurodegeneration (Carroll & Chesebro, 2019; Li et al., 2021).

Microglia are the resident immune cells of the central nervous system and represent one of the earliest cellular populations to respond to prion accumulation. Under normal conditions, microglia continuously monitor the neural environment and remove cellular debris through phagocytosis. However, the progressive accumulation of PrP^{sc} activates microglia and induces a sustained inflammatory response characterized by morphological changes, increased phagocytic activity, and production of inflammatory mediators (Carroll & Chesebro, 2019). Although activated microglia may initially contribute to the clearance of pathogenic proteins, prolonged activation can lead to excessive production of pro-inflammatory molecules that damage surrounding neurons.

This dual role suggests that microglia can exert both protective and detrimental effects depending on the stage and severity of disease progression.

Astrocytes also play an important role in the neuroinflammatory processes associated with prion diseases. These cells are responsible for maintaining neuronal homeostasis, regulating neurotransmitter concentrations, supporting metabolic activity, and preserving blood–brain barrier integrity. In response to prion accumulation, astrocytes undergo a process known as reactive astrogliosis, characterized by structural and functional changes that alter their normal physiological roles. Li et al. (2021) reported that activated astrocytes contribute to inflammatory signaling and may facilitate the progression of neuronal injury through the release of cytokines and other inflammatory mediators. More recently, Makarava et al. (2024) suggested that reactive astrocytes may directly contribute to neuronal and endothelial damage, highlighting their potential role as active participants in disease pathogenesis rather than merely secondary responders to neuronal injury.

A hallmark of neuroinflammation in prion diseases is the increased production of cytokines and other inflammatory signaling molecules. Activated microglia and astrocytes release pro-inflammatory cytokines, chemokines, and reactive oxygen species that can alter neuronal function and exacerbate tissue damage. Carroll and Chesebro (2019) emphasized that persistent inflammatory signaling contributes to disease progression by promoting a toxic neural environment that amplifies the effects of protein aggregation and cellular stress. Furthermore, inflammatory mediators may influence synaptic function, calcium homeostasis, and mitochondrial activity, thereby linking neuroinflammation to other pathogenic mechanisms involved in CJD. These observations suggest that inflammatory responses are closely integrated with multiple aspects of prion-induced neurodegeneration.

Emerging evidence indicates that neuroinflammation may also influence the propagation of pathogenic prion proteins. Li et al. (2021) proposed that activated glial cells can affect the local cellular environment in ways that facilitate the spread and accumulation of PrP^{sc}. Inflammatory processes may alter protein clearance mechanisms, increase oxidative stress, and promote neuronal vulnerability, thereby creating conditions that favor disease progression. Consequently, neuroinflammation should not be viewed solely as a response to prion accumulation but also as a factor capable of modulating the course and severity of disease.

The studies reviewed demonstrate that neuroinflammation and glial activation are integral components of CJD pathogenesis. Microglia and astrocytes respond to the accumulation of misfolded prion proteins through activation of inflammatory pathways that initially serve protective functions but may become detrimental when sustained over time. The release of cytokines and other inflammatory mediators contributes to neuronal dysfunction, enhances cellular stress, and accelerates neurodegeneration. These findings highlight the complex relationship between prion propagation and neuroimmune responses and suggest that modulation of neuroinflammatory pathways may represent a promising strategy for reducing neuronal damage and slowing disease progression in prion disorders.

Synaptic Dysfunction and Neuronal Death

Synaptic dysfunction and neuronal death represent the final pathological consequences of the molecular and cellular abnormalities initiated by prion protein misfolding. Although the accumulation of PrP^{sc} is the hallmark of Creutzfeldt–Jakob disease (CJD), increasing evidence suggests that neuronal degeneration results from a complex cascade of events involving synaptic impairment, disrupted intracellular signaling, calcium imbalance, mitochondrial dysfunction, oxidative stress, and neuroinflammation. These processes progressively compromise neuronal communication and viability, ultimately leading to the widespread neurodegeneration characteristic of prion diseases (Mercer & Harris, 2023). The rapid clinical progression of CJD is largely attributed to the extensive loss of neuronal function and the inability of the nervous system to compensate for ongoing cellular damage.

Synapses are particularly vulnerable to the toxic effects of misfolded prion proteins because they serve as

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critical sites for neuronal communication and information processing. Under normal physiological conditions, PrP^{c} contributes to synaptic maintenance, neuronal signaling, and neuroprotective functions (Castle & Gill, 2017; Wulf et al., 2017). However, the conversion of PrP^{c} into PrP^{sc} disrupts these physiological roles and interferes with normal synaptic activity. Mercer and Harris (2023) reported that misfolded prion proteins impair synaptic transmission and alter signaling pathways essential for neuronal function. These disturbances may occur before substantial neuronal loss becomes evident, suggesting that synaptic dysfunction represents an early event in disease progression. As synaptic integrity deteriorates, cognitive deficits, memory impairment, and behavioral abnormalities become increasingly apparent in affected individuals.

Calcium dysregulation is another important mechanism contributing to neuronal injury in prion diseases. Intracellular calcium ions play essential roles in neurotransmitter release, signal transduction, gene expression, and neuronal survival. However, excessive calcium influx or impaired calcium homeostasis can activate destructive cellular pathways that promote neuronal damage. Mercer and Harris (2023) emphasized that prion-induced alterations in calcium signaling contribute significantly to neurotoxicity by activating proteases, disrupting mitochondrial function, and triggering apoptotic pathways. Sustained calcium imbalance places considerable stress on neurons and may accelerate the progression from functional impairment to irreversible cellular injury.

Apoptosis, or programmed cell death, is considered one of the primary mechanisms responsible for neuronal loss in CJD. Unlike necrosis, which results from acute cellular injury, apoptosis is a regulated process involving activation of intracellular signaling pathways that lead to controlled cellular destruction. Multiple pathogenic mechanisms associated with prion diseases, including oxidative stress, mitochondrial dysfunction, ER stress, and calcium dysregulation, can activate apoptotic pathways (Kim et al., 2022; Mercer & Harris, 2023). Mitochondrial damage, in particular, promotes the release of pro-apoptotic factors that initiate a cascade of molecular events culminating in neuronal death. As increasing numbers of neurons undergo apoptosis, neural circuits become progressively disrupted, leading to the severe neurological deficits observed in advanced stages of CJD.

The cumulative effects of synaptic dysfunction, calcium imbalance, and apoptosis ultimately result in widespread neurodegeneration throughout the brain. Histopathologically, this degeneration is characterized by neuronal loss, spongiform changes, gliosis, and the accumulation of misfolded prion proteins within affected tissues (Sigurdson et al., 2019). Different brain regions may exhibit varying degrees of vulnerability depending on prion strain characteristics, genetic factors, and patterns of protein propagation. Nevertheless, the progressive destruction of neuronal networks remains the principal cause of the rapidly deteriorating neurological function associated with CJD. The severity of neuronal loss correlates closely with clinical manifestations such as dementia, ataxia, myoclonus, visual disturbances, and eventual akinetic mutism.

The evidence reviewed in this section indicates that synaptic dysfunction and neuronal death represent the culmination of multiple interconnected pathogenic processes initiated by PrP misfolding. Disruption of synaptic signaling, loss of calcium homeostasis, activation of apoptotic pathways, and progressive neurodegeneration collectively contribute to the rapid clinical decline characteristic of CJD. These findings underscore the importance of preserving neuronal function and preventing synaptic injury as potential therapeutic strategies aimed at slowing disease progression and mitigating the devastating neurological consequences of prion disorders.

Recent Advances in Structural Biology

Recent advances in structural biology have significantly enhanced the understanding of prion protein architecture and the molecular mechanisms underlying prion propagation. For many years, detailed characterization of infectious prion structures remained challenging because of their insolubility, heterogeneity, and resistance to conventional structural analysis techniques. The emergence of cryogenic electron microscopy (Cryo-EM) has transformed this field by enabling the visualization of prion fibrils at near-atomic resolution. These technological developments have provided unprecedented insights into the structural organization of misfolded prion proteins, revealing how specific conformations contribute to infectivity, strain diversity, and disease progression (Kraus et al., 2021; Caughey et al., 2022).

One of the most important contributions of Cryo-EM has been the detailed characterization of the structural core of infectious prion fibrils. Glynn et al. (2020) examined human prion fibrils and identified highly ordered hydrophobic and protease-resistant core regions that contribute to the remarkable stability of PrP^{sc} aggregates. These structural features help explain why pathogenic prion proteins are resistant to degradation and capable of persisting within neural tissues for extended periods. The identification of these stable fibrillar cores has strengthened the understanding of how specific molecular conformations facilitate infectivity and propagation.

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Furthermore, these findings provide a structural basis for the long-recognized observation that PrP^{sc} exhibits biochemical properties distinct from those of the normal cellular isoform.

Subsequent Cryo-EM investigations have revealed increasingly detailed structural models of infectious prion fibrils. Kraus et al. (2021) reported high-resolution structures of mammalian prions, demonstrating that infectious fibrils possess highly organized β-sheet-rich architectures that differ substantially from the native conformation of PrP^{c}. Similarly, Manka et al. (2022) resolved near-atomic structures of ex vivo infectious prion fibrils, providing further evidence that specific fibrillar arrangements are associated with biological infectivity. These studies confirmed that pathogenic prions are not random protein aggregates but rather highly ordered molecular assemblies capable of preserving structural information during propagation. Such findings have greatly improved understanding of the relationship between protein structure and disease transmission.

Structural biology has also provided important insights into the phenomenon of prion strain diversity. One of the most intriguing features of prion diseases is the existence of distinct strains that produce different clinical manifestations, neuropathological patterns, and incubation periods despite being composed of the same protein. Recent structural studies suggest that this diversity is encoded by differences in fibril architecture rather than variations in amino acid sequence. Caughey et al. (2022) emphasized that distinct conformational arrangements within prion fibrils can influence infectivity, tissue tropism, and disease phenotype. Supporting this concept, Manka et al. (2023) demonstrated that strain-specific biological properties are directly associated with unique fibrillar structures. These observations provide compelling evidence that protein conformation itself serves as a carrier of biological information in prion diseases.

Further evidence for structural heterogeneity has been reported in studies of inherited prion disorders. Hallinan et al. (2022) identified distinct filament structures in Gerstmann–Sträussler–Scheinker (GSS) disease, demonstrating that different prion diseases may possess unique fibrillar architectures despite sharing common pathogenic mechanisms. These findings suggest that structural variations contribute not only to strain diversity within a single disease but also to differences among various human prion disorders. Understanding these structural distinctions is essential for explaining disease heterogeneity and may facilitate the development of more precise diagnostic and therapeutic approaches.

The growing body of structural evidence has important implications for both research and clinical practice. High-resolution structural models provide a foundation for investigating how pathogenic conformations arise, how they interact with normal PrP^{c} molecules, and how they evade cellular clearance mechanisms. In addition, detailed structural information may aid in the design of therapeutic strategies aimed at stabilizing native PrP^{c}, preventing conformational conversion, or disrupting fibril propagation. Structural insights may also improve diagnostic technologies by identifying conformation-specific biomarkers capable of distinguishing between different prion strains and disease subtypes.

The findings discussed in this section demonstrate that advances in Cryo-EM and related structural biology techniques have fundamentally transformed the understanding of prion diseases. High-resolution visualization of infectious prion fibrils has revealed the molecular basis of prion stability, infectivity, and strain diversity, providing critical insights into the mechanisms that drive disease progression. These discoveries have established structural biology as a cornerstone of contemporary prion research and continue to guide efforts aimed at improving diagnosis, understanding disease heterogeneity, and developing targeted therapeutic interventions.

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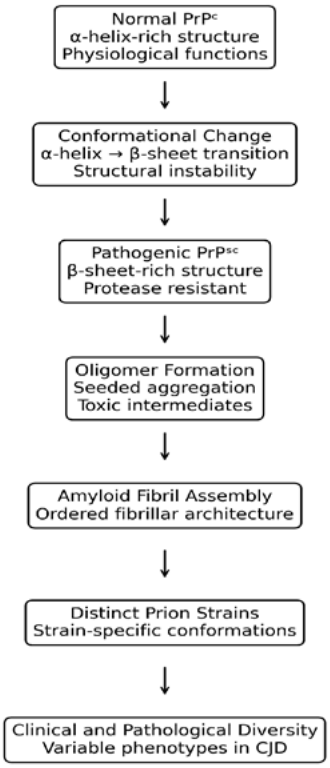

Figure 2. Structural conversion of the cellular prion protein (PrP^{c}) into the pathogenic isoform (PrP^{sc}), illustrating the progression from conformational instability and β-sheet formation to oligomerization, amyloid fibril assembly, and the development of strain-specific prion architectures. Recent Cryo-EM studies have demonstrated that distinct fibrillar conformations encode biological properties responsible for variations in infectivity, neuropathology, and clinical phenotype (Glynn et al., 2020; Kraus et al., 2021; Manka et al., 2022; Manka et al., 2023).

Diagnostic Implications of PrP Misfolding

Advances in the understanding of prion protein misfolding have significantly improved the diagnosis of Creutzfeldt–Jakob disease (CJD). Historically, definitive diagnosis relied primarily on postmortem neuropathological examination, which limited opportunities for early clinical intervention and accurate disease surveillance. The discovery that misfolded prion proteins possess self-propagating properties has led to the development of highly sensitive diagnostic techniques capable of detecting prion seeding activity in biological samples. Modern diagnostic approaches now integrate molecular assays, cerebrospinal fluid (CSF) biomarkers, and neuroimaging findings to improve diagnostic accuracy and facilitate earlier recognition of disease (Hermann et al., 2021; Altuna et al., 2022).

One of the most important advances in prion diagnostics is the development of Real-Time Quaking-Induced Conversion (RT-QuIC). This assay exploits the seeded aggregation properties of PrP^{sc} by amplifying minute amounts of misfolded protein present in clinical samples. During the assay, pathogenic prion seeds induce the conversion of recombinant prion protein substrates into aggregated forms that can be detected through fluorescence signals. Green (2019) highlighted RT-QuIC as a highly specific diagnostic tool for sporadic CJD, while Dong and Satoh (2021) reported that the technique substantially improves the detection of prion seeding activity in cerebrospinal fluid and other diagnostic specimens. More recently, surveillance data from Hermann et al. (2023) confirmed the high diagnostic sensitivity and specificity of RT-QuIC, establishing it as one of the most reliable ante-mortem diagnostic methods currently available. The widespread adoption of RT-QuIC has transformed clinical practice by enabling earlier and more accurate diagnosis of prion diseases.

Another important diagnostic technology is Protein Misfolding Cyclic Amplification (PMCA), which similarly utilizes the self-replicating nature of pathogenic prion proteins. PMCA amplifies trace amounts of PrP^{sc} through repeated cycles of incubation and sonication, allowing detection of prions even when present at extremely low

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concentrations. Giaccone and Moda (2020) demonstrated that PMCA can detect pathogenic prions in peripheral tissues and biological fluids, particularly in cases of variant CJD. Although PMCA is used less frequently in routine clinical practice than RT-QuIC, it remains a valuable research and diagnostic tool because of its exceptional analytical sensitivity. Together, RT-QuIC and PMCA illustrate how knowledge of prion propagation mechanisms has directly contributed to the development of innovative diagnostic technologies.

In addition to seeding assays, cerebrospinal fluid biomarkers continue to play an important role in the diagnostic evaluation of CJD. Traditional biomarkers such as 14-3-3 protein and total tau protein have long been used as supportive indicators of rapid neuronal damage. However, these markers lack specificity because they may also be elevated in other neurological disorders. Recent studies have focused on improving diagnostic accuracy through the integration of multiple biomarker approaches. Altuna et al. (2022) reported that combining CSF biomarkers with RT-QuIC significantly improves diagnostic confidence, while Senesi et al. (2023) demonstrated that RT-QuIC outperforms conventional CSF biomarkers in identifying prion disease. Furthermore, Hermann and Zerr (2023) highlighted the need for additional biochemical markers capable of improving early disease detection and distinguishing between different prion disease subtypes. These findings indicate that biomarker development remains an active area of research with considerable clinical importance.

Magnetic resonance imaging (MRI) has also become an essential component of contemporary CJD diagnosis. Characteristic abnormalities observed on diffusion-weighted imaging (DWI) and fluid-attenuated inversion recovery (FLAIR) sequences can provide valuable evidence supporting a diagnosis of prion disease. Typical findings include cortical ribboning and hyperintensities within the basal ganglia, thalamus, and other affected brain regions. Hermann et al. (2021) incorporated MRI findings into modern diagnostic guidelines because of their strong diagnostic utility. Supporting this recommendation, Park et al. (2021) conducted a systematic review and meta-analysis demonstrating that diffusion-weighted MRI possesses high sensitivity and specificity for the diagnosis of sporadic CJD. These imaging findings are particularly valuable when interpreted alongside clinical features and laboratory results, providing a non-invasive method for assessing disease involvement.

Recent research has increasingly emphasized the importance of integrating multiple diagnostic modalities rather than relying on a single test. Modern diagnostic criteria combine clinical assessment, RT-QuIC results, CSF biomarkers, and neuroimaging findings to maximize diagnostic accuracy and reduce the likelihood of misdiagnosis (Hermann et al., 2021; Altuna et al., 2022). This multimodal approach reflects the complex biology of prion diseases and acknowledges that no single diagnostic method is sufficient to capture all aspects of disease pathology. Advances in molecular diagnostics have therefore shifted CJD diagnosis from a predominantly exclusion-based process toward a more precise and evidence-driven framework.

Table 5. Major Diagnostic Approaches for Creutzfeldt–Jakob Disease

Diagnostic Method	Biological Principle	Clinical Specimen	Advantages	Limitations	Clinical Application
RT-QuIC (Real-Time Quaking-Induced Conversion)	Amplifies prion seeding activity by converting recombinant PrP into detectable aggregates	Cerebrospinal fluid (CSF), olfactory mucosa	Very high sensitivity and specificity (>90–95%); detects prion seeding activity during life	Requires specialized laboratory equipment and technical expertise	Current gold standard molecular assay for ante-mortem diagnosis
PMCA (Protein Misfolding Cyclic Amplification)	Amplifies minute amounts of PrP^{sc} through repeated incubation and sonication cycles	CSF, blood, peripheral tissues	Extremely sensitive; capable of detecting very low prion concentrations	Limited availability; primarily used in research laboratories	Detection of variant CJD and studies on prion infectivity
CSF Biomarkers (14-3-3, Total Tau, Neurofilament Light Chain)	Measures proteins released during neuronal injury and neurodegeneration	Cerebrospinal fluid	Rapid, widely available, useful as supportive biomarkers	Lower specificity because biomarkers are elevated in other neurological disorders	Adjunctive diagnostic testing
MRI (Diffusion-Weighted Imaging/FLAIR)	Detects characteristic cortical and deep gray matter	Brain imaging	Non-invasive; high diagnostic sensitivity for sporadic CJD	Imaging findings may overlap with other neurological	Supports early clinical diagnosis and differential

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	abnormalities caused by prion pathology			disorders and vary with disease stage	diagnosis
Multimodal Diagnostic Approach	Integrates molecular assays, biomarkers, neuroimaging, and clinical findings	Multiple clinical sources	Highest overall diagnostic accuracy; improves diagnostic confidence	Requires multidisciplinary evaluation and access to multiple diagnostic modalities	Recommended in current international diagnostic guidelines

The rapid progression of Creutzfeldt–Jakob disease has historically made accurate diagnosis particularly challenging because definitive confirmation traditionally relied on postmortem neuropathological examination. Advances in the understanding of prion protein misfolding have fundamentally transformed this diagnostic landscape by enabling the direct detection of pathogenic prion activity during life. The studies included in this review demonstrate that modern diagnostic strategies are now based on identifying the molecular consequences of PrP misfolding rather than relying solely on clinical manifestations, which often overlap with other rapidly progressive neurodegenerative disorders (Green, 2019; Hermann et al., 2021).

As summarized in Table 5, current diagnostic approaches differ in their underlying biological principles but collectively target key features of prion disease pathology. Molecular amplification techniques such as Real-Time Quaking-Induced Conversion (RT-QuIC) and Protein Misfolding Cyclic Amplification (PMCA) directly exploit the self-propagating nature of misfolded prion proteins. RT-QuIC has emerged as the preferred molecular assay because it detects prion seeding activity with reported sensitivities and specificities exceeding 90–95%, allowing reliable ante-mortem diagnosis using cerebrospinal fluid or olfactory mucosa samples (Green, 2019; Dong & Satoh, 2021; Hermann et al., 2023). PMCA applies a similar amplification principle but relies on repeated cycles of incubation and sonication to replicate pathogenic prions. Although PMCA demonstrates exceptional analytical sensitivity and has proven valuable for detecting variant CJD and studying peripheral tissue infectivity, its application remains largely confined to specialized research laboratories because of technical complexity and limited clinical availability (Giaccone & Moda, 2020).

Conventional cerebrospinal fluid biomarkers continue to play an important complementary role in the diagnostic evaluation of CJD. Biomarkers such as 14-3-3 protein, total tau, and more recently neurofilament light chain (NfL) reflect the extensive neuronal injury that accompanies rapidly progressive neurodegeneration. However, unlike RT-QuIC, these biomarkers do not directly detect pathogenic prion proteins and may also be elevated in other neurological disorders, including Alzheimer's disease, autoimmune encephalitis, and acute stroke. Consequently, their diagnostic specificity is lower despite their widespread clinical availability (Altuna et al., 2022; Hermann & Zerr, 2023). The evidence reviewed indicates that these biomarkers are most valuable when interpreted alongside molecular assays and clinical findings rather than being used as standalone diagnostic tests.

Neuroimaging has likewise become an indispensable component of contemporary CJD diagnosis. Diffusion-weighted imaging (DWI) and fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging frequently demonstrate characteristic abnormalities, including cortical ribboning and hyperintensity within the basal ganglia, caudate nucleus, putamen, and thalamus. These radiological features reflect the underlying distribution of spongiform degeneration and neuronal loss and often appear during the early stages of disease. Park et al. (2021) reported that diffusion-weighted MRI possesses excellent diagnostic performance for sporadic CJD, while Hermann et al. (2021) incorporated MRI findings into contemporary international diagnostic criteria because of their substantial clinical value. The combination of molecular and radiological evidence therefore provides greater diagnostic certainty than either modality alone.

The reviewed literature consistently supports the adoption of a multimodal diagnostic approach, integrating molecular amplification assays, cerebrospinal fluid biomarkers, neuroimaging findings, and comprehensive clinical evaluation. This strategy reflects the multifactorial nature of prion disease pathogenesis and acknowledges that no single diagnostic modality can fully characterize disease biology. The integration of these complementary methods has substantially improved diagnostic sensitivity, specificity, and overall clinical confidence while reducing the likelihood of misdiagnosis (Altuna et al., 2022; Hermann et al., 2021). Moreover, improved ante-mortem diagnosis has strengthened national surveillance programs and facilitated earlier identification of patients eligible for clinical trials investigating experimental therapies.

The findings synthesized in this section demonstrate that advances in understanding PrP misfolding have fundamentally reshaped the diagnosis of CJD. The development of highly sensitive molecular amplification

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assays, together with improvements in biomarker discovery and advanced neuroimaging, has shifted diagnosis from a predominantly exclusion-based process to one supported by direct molecular evidence of prion propagation. Continued refinement of diagnostic technologies and the discovery of novel biomarkers are expected to further improve early disease detection, disease subtype classification, and monitoring of future therapeutic interventions, thereby enhancing both clinical management and prion disease surveillance.

Integrated Model of CJD Pathogenesis

The evidence synthesized in this review supports a multifactorial model of Creutzfeldt–Jakob disease (CJD) pathogenesis in which prion protein misfolding serves as the initiating event that triggers a cascade of interconnected molecular and cellular abnormalities. Although individual mechanisms such as genetic susceptibility, protein aggregation, oxidative stress, and neuroinflammation have traditionally been studied separately, current evidence indicates that these processes function as components of an integrated pathogenic network. The conversion of the normal cellular prion protein (PrP^{c}) into the pathogenic isoform (PrP^{sc}) initiates a self-propagating cycle of protein misfolding that progressively disrupts cellular homeostasis and ultimately culminates in widespread neurodegeneration (Sigurdson et al., 2019).

The pathogenic cascade begins with structural destabilization of PrP^{c}, which may occur spontaneously, through inherited PRNP mutations, or following exposure to infectious prions. Genetic studies have demonstrated that pathogenic mutations and codon 129 polymorphisms significantly influence susceptibility to misfolding and disease phenotype (Bagyinszky et al., 2018; Baiardi et al., 2021; Appleby et al., 2022). Once PrP^{sc} is formed, it acts as a conformational template that recruits additional PrP^{c} molecules and promotes their conversion into the pathogenic state. This process of seeded aggregation results in the formation of oligomers, amyloid fibrils, and highly ordered prion aggregates that accumulate progressively within neural tissues (Sanz-Hernández et al., 2024; Eraña et al., 2024). Structural studies have further shown that distinct fibrillar conformations encode strain-specific biological properties that contribute to clinical and pathological heterogeneity (Kraus et al., 2021; Manka et al., 2023).

As pathogenic prion proteins accumulate, cellular protein quality-control systems become increasingly overwhelmed. Impairment of molecular chaperones, the ubiquitin–proteasome system, autophagy pathways, and endoplasmic reticulum stress responses contribute to the progressive failure of proteostasis mechanisms responsible for maintaining protein homeostasis (Penke et al., 2018; López-Pérez et al., 2020; Thellung et al., 2022). Reduced clearance of misfolded proteins further accelerates aggregate accumulation and amplifies cellular stress. Consequently, neurons become increasingly vulnerable to secondary pathological processes that contribute to disease progression.

Proteostasis dysfunction is closely linked to oxidative stress and mitochondrial impairment, which represent major drivers of neuronal injury in prion diseases. The accumulation of PrP^{sc} disrupts redox homeostasis and promotes excessive production of reactive oxygen species, resulting in oxidative damage to proteins, lipids, and nucleic acids (Spiers et al., 2023). Simultaneously, mitochondrial quality-control mechanisms become impaired, leading to reduced ATP production, altered calcium regulation, and activation of apoptotic pathways (Kim et al., 2022). The interaction between oxidative stress and mitochondrial dysfunction creates a self-reinforcing cycle of cellular injury that further compromises neuronal viability and accelerates neurodegeneration.

Neuroinflammation represents another critical component of the pathogenic network. Activated microglia and astrocytes respond to the accumulation of pathogenic prion proteins through the release of cytokines, chemokines, and reactive oxygen species that contribute to inflammatory signaling within the brain (Carroll & Chesebro, 2019; Li et al., 2021). Although these responses may initially serve protective functions, chronic glial activation can exacerbate neuronal injury and promote disease progression. Recent evidence further suggests that reactive astrocytes may directly contribute to neuronal and endothelial damage, highlighting their active role in prion pathogenesis (Makarava et al., 2024).

The cumulative effects of protein aggregation, proteostasis failure, oxidative stress, mitochondrial dysfunction, and neuroinflammation ultimately converge at the level of synaptic dysfunction and neuronal death. Misfolded prion proteins disrupt neuronal signaling pathways, impair calcium homeostasis, and activate apoptotic mechanisms that progressively compromise neural network integrity (Mercer & Harris, 2023). As neuronal loss becomes increasingly widespread, patients develop the characteristic clinical manifestations of CJD, including rapidly progressive dementia, ataxia, myoclonus, visual disturbances, and eventual akinetic mutism. The rapid progression and uniformly fatal nature of the disease reflect the extensive and irreversible destruction of neuronal circuits throughout the central nervous system.

The integrated model presented in this review highlights the interconnected nature of the molecular mechanisms underlying CJD. Rather than acting independently, structural conversion, genetic susceptibility, seeded

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aggregation, proteostasis dysfunction, oxidative stress, mitochondrial impairment, neuroinflammation, and neuronal degeneration function as components of a continuous pathogenic cascade. Understanding how these mechanisms interact provides a more comprehensive framework for explaining disease progression and identifying potential therapeutic targets. Interventions aimed at stabilizing PrP^{c}, inhibiting prion propagation, enhancing protein clearance pathways, reducing oxidative stress, preserving mitochondrial function, or modulating neuroinflammatory responses may offer promising strategies for slowing disease progression and improving outcomes in future clinical applications.

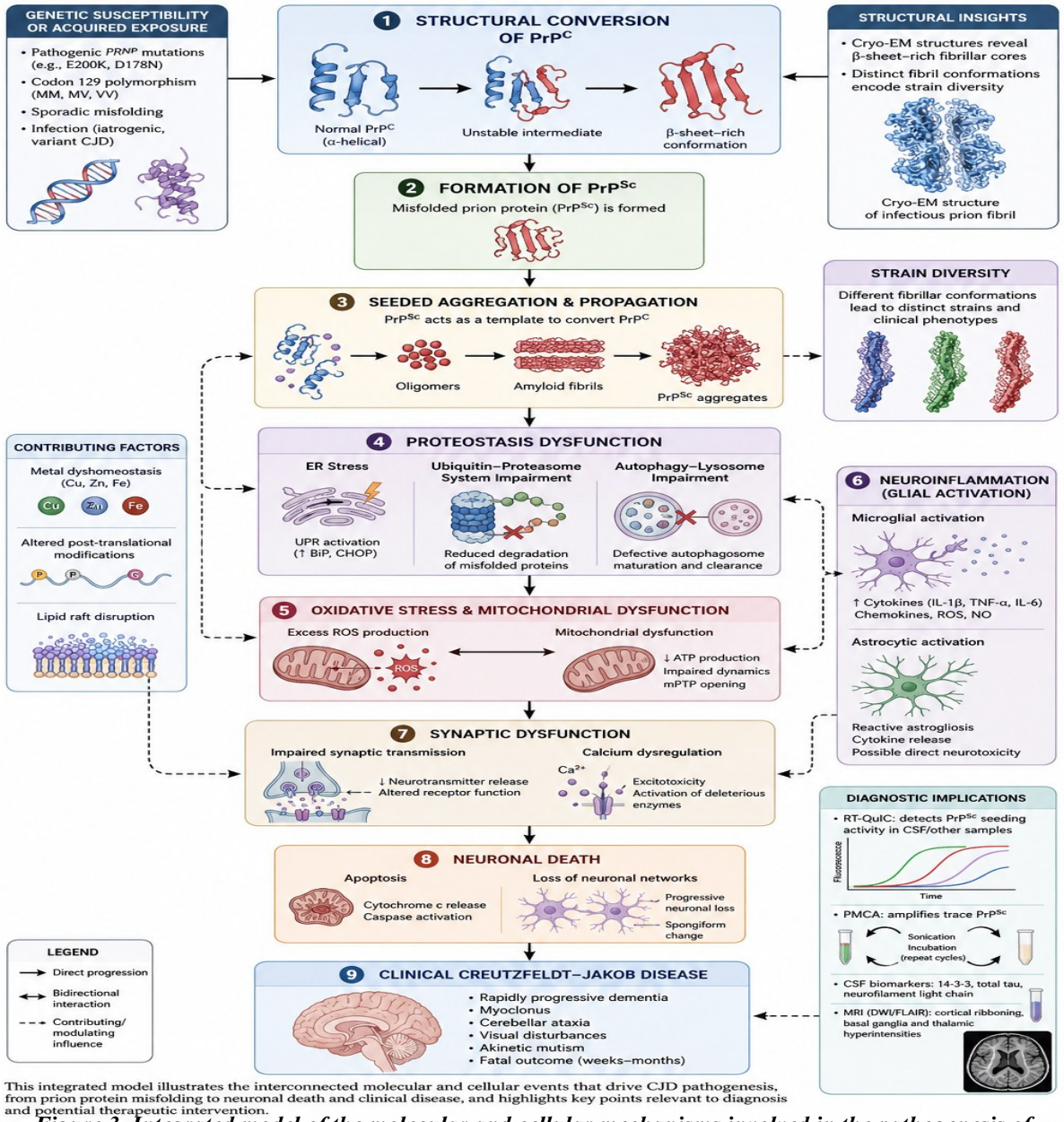

Figure 3. Integrated model of the molecular and cellular mechanisms involved in the pathogenesis of Creutzfeldt–Jakob disease. The diagram illustrates the progression from genetic susceptibility and prion protein misfolding to seeded aggregation, cellular dysfunction, neuroinflammation, neuronal loss, and clinical disease manifestation.

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CONCLUSION

Creutzfeldt–Jakob disease (CJD) remains one of the most aggressive and fatal neurodegenerative disorders, distinguished by its rapid clinical progression, extensive neuronal loss, and lack of effective disease-modifying treatments. The unique capacity of prion proteins to function as self-propagating infectious agents has made CJD a subject of considerable scientific interest and an important model for understanding protein misfolding disorders. This systematic review synthesized evidence published between 2015 and 2025 to examine the molecular mechanisms underlying prion protein (PrP) misfolding and their role in disease pathogenesis, progression, and diagnosis.

The findings consistently identify the conversion of the normal cellular prion protein (PrP^{c}) into the pathogenic isoform (PrP^{sc}) as the central molecular event responsible for disease initiation. This conformational transition, characterized by the transformation of α-helical structures into β-sheet-rich aggregates, promotes seeded protein aggregation, amyloid fibril formation, and the propagation of infectious prion conformations throughout the central nervous system. Recent advances in structural biology, particularly through cryogenic electron microscopy, have substantially improved understanding of prion architecture and have demonstrated that strain-specific biological properties are encoded by distinct fibrillar conformations. These discoveries have provided important insights into the molecular basis of disease heterogeneity and prion infectivity.

Evidence reviewed in this study further demonstrates that PrP misfolding initiates a complex network of downstream pathological processes involving proteostasis dysfunction, endoplasmic reticulum stress, impaired autophagy, ubiquitin–proteasome system failure, oxidative stress, mitochondrial dysfunction, neuroinflammation, synaptic impairment, and neuronal death. These mechanisms interact in a highly interconnected manner, creating a progressive cycle of cellular injury that ultimately culminates in widespread neurodegeneration. Genetic factors, particularly pathogenic **PRNP** mutations and codon 129 polymorphisms, were also shown to influence disease susceptibility, clinical phenotype, neuropathological characteristics, and disease progression, highlighting the importance of genetic variability in shaping the manifestations of prion disease.

Substantial progress has also been achieved in the diagnosis of CJD as a result of advances in the understanding of PrP misfolding and propagation. Techniques such as Real-Time Quaking-Induced Conversion (RT-QuIC) and Protein Misfolding Cyclic Amplification (PMCA) have significantly improved the detection of pathogenic prion proteins, while cerebrospinal fluid biomarkers and advanced neuroimaging methods have enhanced diagnostic accuracy and clinical decision-making. The integration of molecular, biochemical, and radiological approaches has transformed the diagnostic framework for prion diseases and has facilitated more reliable ante-mortem identification of affected individuals.

The evidence synthesized in this review demonstrates that CJD pathogenesis is driven by the interaction of structural, genetic, molecular, and cellular mechanisms centered on the misfolding and propagation of prion proteins. Continued investigation of these pathways remains essential for advancing knowledge of disease biology and identifying potential therapeutic targets. Future research should focus on elucidating the earliest molecular events involved in PrP conversion, characterizing strain-specific structural variations, developing more sensitive and disease-specific biomarkers, and identifying interventions capable of preventing prion propagation or enhancing the clearance of pathogenic aggregates. Advancements in these areas may contribute to the development of effective diagnostic and therapeutic strategies and provide broader insights into the mechanisms underlying protein misfolding and neurodegeneration in both prion and non-prion disorders.

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