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Prions mediated neurodegenerative disorders

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Abstract. – Prions are unprecedented infectious pathogens that are devoid of nucleic acid and cause a group of rare and invariably fatal neurodegenerative disorders, affecting approximately 1 person per 1 million inhabitants annually worldwide. These disorders include Creutzfeld-Jacob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), kuru, fatal insomnia (FI), and variable protease-sensitive prionopathy (VPSPr), all of which involve a conformational change of the normal cellular prion protein (PrPC) into the abnormal scrapie prion protein (PrPSc) through a posttranslational process during which PrPc acquires high β-sheet content. This structural change is accompanied by profound changes in the physicochemical properties of PrPC, rendering the molecule resistant to proteolysis. The conformational change of PrPC can occur due to either spontaneous conversion, dominant mutations in the prion protein (PRNP) gene encoding PrPC, or infection with pathogenic isoform PrPsc from exogenous sources. There is general agreement that PrPC serves as a substrate for conversion to abnormal PrPSc. This latter multiplies exponentially and aggregates in the brain, forming deposits that are associated with the neurodegenerative changes. Although the understanding of the primary causes of prion-induced neurodegeneration is still limited, propagation of PrPSc and neurotoxic signaling seem to interplay in pathogenic process of prions. Here, we review recent findings that have provided fresh insights into this process, and present an overview of incidence, causes and spectrum of related disorders.

Key Words:
Prion, PrPc, PrPsc, Pathogenesis, Propagation, Transmission, Etiology, Disorders, Neurodegeneration, Toxicity.

Abbreviations

BSE = bovine spongiform encephalopathy; FFI = fatal familial insomnia; gCJD = genetic CJD; GSS = Gerstmann-Sträussler-Scheinker syndrome; sCJD = sporadic Creutzfeldt-Jakob disease; sFI = sporadic fatal insomnia; vCJD = variant CJD; VPSPr = variably protease-sensitive prionopathy.

Introduction

Prions are infectious pathogens devoid of nucleic acid that become self-replicating by acquiring an alternative conformation. This transformation is generally accompanied by an increase in β-sheet structure and a propensity to aggregate into oligomers. The most well studied mammalian prion is the scrapie prion protein (PrP^{Sc}) that is formed from the normal cellular prion protein (PrP^{C}) by either spontaneous conversion or dominant mutations in the PRNP gene that encodes PrP^{C}. Both PrP^{C} and PrP^{Sc} have similar amino acid sequence, and PrP^{C} serve as a substrate for conversion to PrP^{Sc}. Several reports argue that PrP^{Sc} multiplies exponentially[1,2], and induces neuronal dysfunction by accumulating in the brain. Conformational variants of PrP^{Sc} due to mutations in the PRNP gene create different regional patterns of prion accumulation and produce different neurodegenerative disease phenotypes that include CJD, GSS, kuru, FI, and VPSPr. Although relatively rare, the lethality of these diseases and the potential for their transmissibility has led to the development of surveillance units worldwide. Furthermore, these diseases have neither reliable preclinical screening tests nor effective treatments. To overcome these limitations, it is absolutely required to understand the basics and details of mechanisms by which prions cause diseases. Therefore, it is important to understand how prions emerge, replicate, and transmit; and particularly how the normal PrP^{C} becomes the disease-associated isoform PrP^{Sc}, and also to understand the mechanisms underlying the neurotoxic activity of this isoform. This review deals with those concerns and presents recent findings that have provided fresh

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insights into the pathogenic mechanisms of prions as well as incidence, causes and spectrum of related disorders.

Mechanisms of Prion Pathogenesis

Prion pathogenesis remains enigmatic. Although prion propagation based on conformational conversion is closely linked with prion pathogenesis, it appears that this phenomenon can occur in the absence of clinical signs[3-5]. Thus, two partially independent pathways seem to interplay in prion pathogenesis, one leading to the propagation of infectious pions and another one mediating neurotoxic signaling[6].

Conversion, Replication and Propagation of Prions

The basic event shared by all prion-related disorders is a conformational conversion of PrP^{C} into PrP^{Sc} in the host cells during pathogenesis[7]. The level of PrP^{C} expression required for this process appears to be directly proportional to the rate of PrP^{Sc} formation. The transgenic mouse studies have provided genetic[8] and biochemical[9] evidence that the conversion of PrP^{C} to PrP^{Sc} occurs through the formation of a PrP^{C}/PrP^{Sc} complex. However, it is not clear whether PrP^{C} binds to one or more additional macromolecules during the process of PrP^{C} to PrP^{Sc} conversion. Two different models have been proposed to explain this process (Figure 1). The nuclear polymerization model proposed by Jarrett and Lansbury[10] suggests that PrP^{Sc} and PrP^{C} are in a reversible thermodynamic equilibrium and in the presence of stable oligomeric PrP^{Sc} aggregates, a change from PrP^{C} to PrP^{Sc} is favored. In this model, PrP^{Sc} interacts with other PrP^{Sc} molecules to form oligomeric aggregates that are mounted into an ordered seeds. These seeds recruit monomeric PrP^{Sc}, and eventually go on to higher-order amyloid fibrils, which themselves do not have seeding potential. However, fragmentation of the amyloid fibrils generates new infectious seeds which can recruit further monomeric PrP^{Sc}, and thus results in apparent replication of the agent (Figure 1A). In this model, PrP^{Sc} aggregates are indispensable for prion propagation. The template-assisted conversion model proposed by

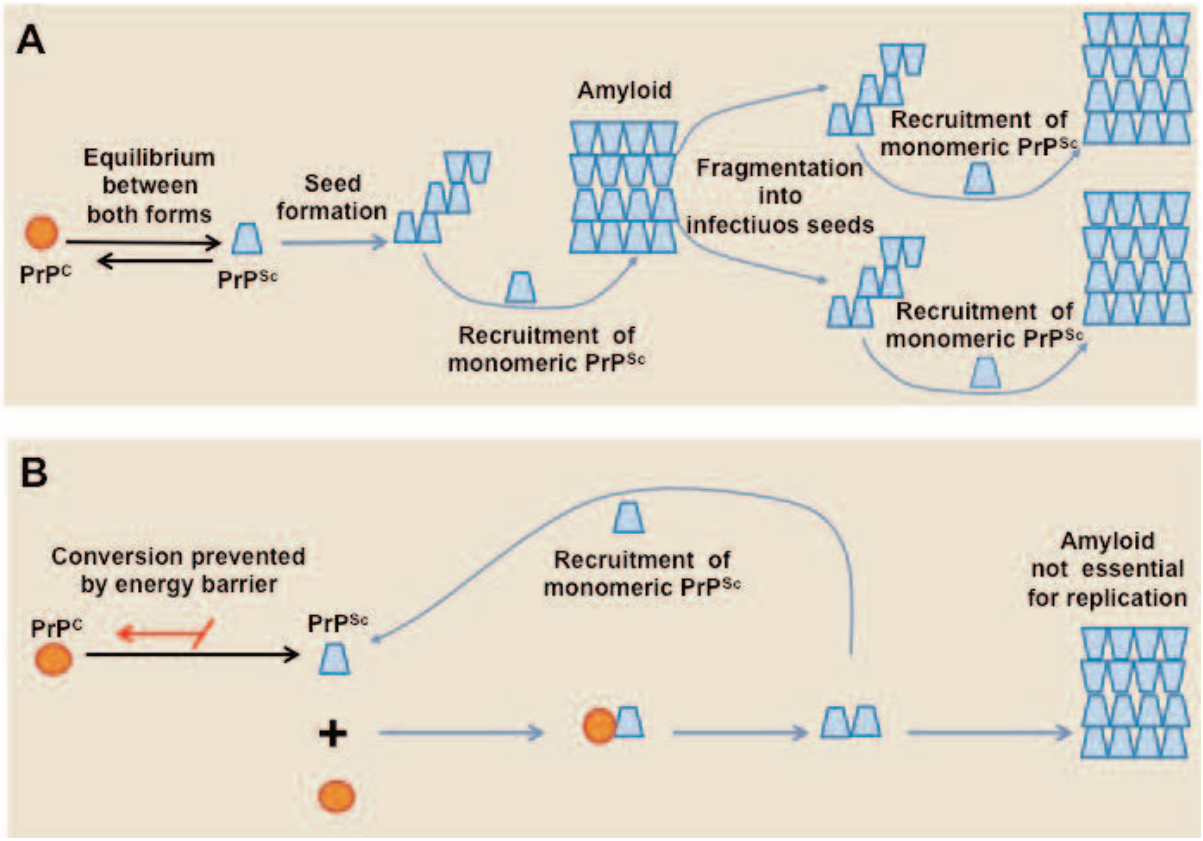

Figure 1. Models for the conformational conversion of PrPC into PrPSc. Please refer to the text for details.

Prusiner[11] postulates on interaction between exogenously introduced PrP^{Sc} and endogeneous PrP^{C} that is induced to transform itself into further PrP^{Sc}. A high energy barrier may prevent spontaneous conversion of PrP^{C} into PrP^{Sc}. Specifically, PrP^{Sc} recruits and converts PrP^{C} to form nascent PrP^{Sc}. The resulting PrP^{Sc}-PrP^{Sc} homodimer then dissociates to allow the formation of a new heterodimer and the generation of further PrP^{Sc} molecules. Over the time, PrP^{Sc} molecules assemble into the higher-order myloid fibrils. In this model, the PrP^{Sc} aggregates are not considered essential for the prion conversion process (Figure 1 B).

Prion Toxicity

Although PrP^{Sc} generated as a result of PrP^{C} conversion, is progressively accumulated, and deposited as amyloid in the brain[12], it is not clear whether the gain of toxic PrP^{Sc} function is responsible for the downstream events that cause neurodegeneration. The depletion of PrP^{C} due to its conversion into PrP^{Sc} is an unlikely cause, in view of the finding that abrogation of PrP does not cause scrapie-like neuropathological changes[13]. Moreover, it was observed that depletion of PrP^{C} in mice with established prion infection reversed early spongiform degeneration and prevented neuronal loss and progression to clinical disease[5]. This occurred despite the accumulation of extraneuronal PrP^{Sc} to levels similar to those observed in terminally ill, wild-type infected animals. These data suggest that PrP^{Sc} might not be directly responsible for neurodegeneration. It is more likely that the toxicity of PrP^{Sc} depends on some PrP^{C}-dependent process culminating in neuronal dysfunction and death[3].

Experimental evidence in support of this hypothesis was provided when neuronal tissue overexpressing PrP^{C} was grafted into the brain of PrP-deficient mice[14]. Following scrapie inoculation, these mice remained free of symptoms for at least 70 weeks, exceeding the survival time of scrapie-infected donor mice by at least sevenfold[3]. Therefore, the presence of a continuous source of PrP^{Sc} does not exert any clinically detectable adverse effects on a mouse devoid of PrP^{C}. On the other hand, the grafted tissue developed characteristic histopathological features of scrapie after inoculation. The course of the disease in the graft was very similar to that observed in the brain of scrapie-inoculated wild-type mice[15].

These findings suggest that the expression of PrP^{C} by an infected cell, rather than the extracellular deposition of PrP^{Sc}, is one of the critical prerequisites for the development of scrapie pathology. Furthermore, what is important is not only the expression of PrP^{C}, but also its location at the plasma membrane. Indeed, scrapie-infected transgenic mice expressing PrP lacking the glycosylphosphatidylinositol (GPI) membrane anchor, failed to develop disease despite PrP^{Sc} plaque formation in their brains[4]. Moreover, removal of the GPI anchor abolishes the activation of downstream pathways and susceptibility to clinical disease while preserving the competence of soluble PrP^{C} to support prion replication. This observation is consistent with the idea that PrP^{C} may function as a signaling molecule.

It has been suggested that the binding of PrP^{Sc} to PrP^{C} triggers a signal transduction pathway, leading to neuronal damage. Accordingly, injection of antibodies that recognize and cross-link PrP^{C} in the mouse hippocampus results in rapid and extensive neuronal apoptosis[16]. However, it is still possible that abnormal topology or altered trafficking of PrP^{C} might also underlie toxicity.

Causes of Prion-Related Disorders

The source of prions responsible for prion-related disorders can be either external or internal (Figure 2). Prion disorders are categorized into sporadic, inherited and acquired groups, depending on the cause. Acquired prion disorders are caused by ingestion of, or exposure to external prion material derived from either homologous or heterologous source[17]. Specifically, infection of the externally derived abnormal PrP^{Sc} induces conformational changes of the natively expressed normal PrP^{C}, leading to the development of different diseases such as variant CJD (vCJD), iatrogenic CJD (iCJD), and kuru. Inherited and sporadic prion disorders are caused by internal factors such as germ-line or somatic mutations on the PRNP gene and a spontaneous change in the conformation of PrP^{C} to PrP^{Sc}[18]. When there are mutations in the PRNP gene, the mutated normal PrP^{C} is more prone to be refolded into PrP^{Sc}, leading to development of genetic CJD (gCJD), GSS, and familial FI (FFI). The spontaneous internal change implies a random protein-misfolding event, leading to the development of sporadic CJD (sCJD), sporadic FI (sFI) and VPSPr.

Risk in all etiological groups is highly associated with a methionine (M)/valine (V) polymorphism at codon 129 of the PRNP gene[19].

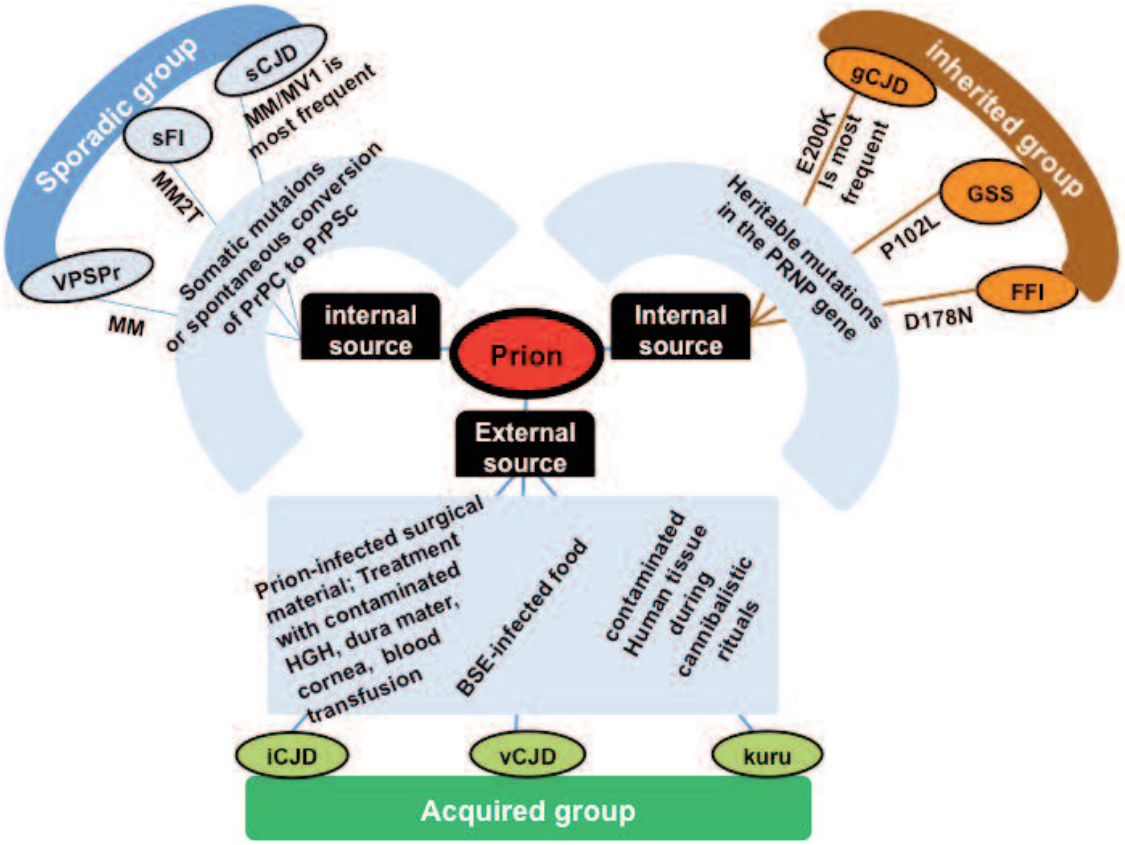

Figure 2. Prion source responsible for different etiological groups of prion diseases. sCJD (sporadic Creutzfelt-Jakob disease), sFI (sporadic fatal insomnia), VPSPr (variably protease-sensitive prionopathy), vCJD (variant Creutzfelt-Jakob disease), iCJD (iatrogenic Creutzfelt-Jakob disease), FFI (fatal familial insomnia), GSS (Gersmann-Straussler-Scheinker syndrome), gCJD (genetic Creutzfelt-Jakob disease), BSE (bovine spongiform encephalopathy). Please refer to the text for details.

Incidence of Prion-Related Disorders

The incidence of prion-related disorders is relatively stable worldwide and has been estimated at one case per 1 million inhabitants annually[20]. Most common forms are sporadic, accounting for 85% of all cases, whereas approximately 10-15% of instances are inherited forms, and less than 2% of cases are acquired forms. The frequency of codon 129 polymorphism for M allele is more than 0.9 in Asians and 0.55 in Africans[21]. In Caucasian populations, about 51% are MV heterozygous, 38% are MM homozygous, and 11% are VV homozygous[22-24].

Among sporadic forms, sCJD is by far the most frequent and also the most intensively studied. It occurs in all ethnicities with no gender prediction, affecting individuals between the age of 55 and 75 years[25]. Prevalence, annual incidence, and yearly mortality rate are approximately of 0.5 to 1 per million. However, in countries with active surveillance programs, the reported values are often higher. In Switzerland, the annual incidence has reached $3.0/10^6$ per year[26,27], and in France the mean annual mortality rate is higher with a value of 1.44 compared with Germany, Italy, and UK where the mortality rate is 1.23, 1.28, and 1.04, respectively[28]. Among 300 cases of sCJD from Europe and the USA, 71.6% were MM homozygous, 11.7% were MV heterozygous, and 16.7 were VV heterozygous[29].

Among inherited forms, gCJD is the most frequent. At present, more than 20 distinct mutations associated with gCJD are known[30]. Some of them have been noted worldwide in many patients, while others are rare. The age of gCJD onset is variable and depends on the specific mutation, but individuals generally are affected before 55 years of age. The E200K mutation is often the most present with an average age at onset of 61 years[1]. In the majority of European countries, this mutation is the most frequent except in Italy where V210I mutation is the most frequent. In

Slovakia, exceptionally a cluster of E200K mutation is observed with near 70% of cases linked to this mutation.

Among acquired forms, vCJD has received considerable attention during the last decade with the probable transmission of bovine spongiform encephalopathy (BSE) to human. Commonly called mad cow disease, BSE was found to be a prion disease upon the discovery of protease-resistant PrP in the brain of ill cow[31]. Although the exact mechanism of BSE transmission to human remains enigmatic, epidemiological evidence suggested that vCJD was caused by BSE prion, through consumption of meat from cattle infected with mad cow disease. Slightly more than 200 cases of vCJD were reported worldwide, vCJD[32]. Among these cases, only four probable cases have been identified after transmission of red blood cells from asymptomatic donors that late developed. A high number of cases have occurred in the UK and France, with smaller numbers in 10 other countries. To date, 176 cases have been diagnosed in the UK, 27 in France, 5 in Spain, 4 in Ireland, 3 in the United States, 3 in the Netherlands, 2 in Portugal, 2 in Italy, 2 in Canada and one each in Japan, Saudi Arabia, and Taiwan[28,33]. The epidemic in the UK reached a peak in 1999 with 28 deaths, and has declined to a current incidence of about 1 to 2 diagnoses/death per year. In France, the peak of deaths was observed in 2006, then the number of incident cases declined and only 2 cases were reported since 2009[28]. All definite vCJD cases that have been tested were codon 129 MM homozygous.

iCJD is a rare form of prion diseases, with only more than 400 confirmed cases worldwide[34]. The most affected country for duramater-related cases is Japan, while France is the most affected country for growth hormone-related cases.

Kuru was once of the most common cause of death among New Guinea woman in the Fore region of the Highlands, affecting persons primarily between the age of 5 and 60 years, with an equal gender ratio among preadolescents but a striking excess in female adults[35,36]. The number of deaths per year related to this disease has steadily declined since the 1950s. Fewer than 15 deaths from kuru per year have been reported since 1985. This decline in kuru incidence is attributable to cessation of ritual cannibalism[37]. Field surveillance among the Fore tributes has documented 11 cases of kuru occurring between 1996 and 2004, but all the victims were born before the late 1950s, and 8 of 10 were MV heterozygous at the codon 129[38].

Clinical and Pathological Features of Prion-related Disorders

Sporadic Prion-Related Disorders

sCJD cases are currently sub-classified according to the methionine/valine polymorphism at codon 129 of the PRNP gene[18]. To date, six pure and 2 mixed types of sCJD have been well characterized with regard to their molecular composition as well as their pathological and clinical phenotypes in the following way: (1) The MM/MV1 type, which represent almost three-quarters of the sCJD cases, shows small vacuoles, synaptic PrP^{Sc} deposition, and is clinically characterized by rapid cognitive decline, (2) The VV2 type shows plaque-like deposits and perineural staining in the cortex as well as plaque-like deposits in the cerebellar cortex and white matter. Clinically, gait ataxia is prominent in early stages. (3) The MV2K type shows kuru plaques on histological examination. Clinically, dementia and ataxia are characteristic features. (4) The MM2 C type shows confluent vacuoles in the cerebral cortex with perivacuolar and coarse focal PrP^{Sc} staining. (5) The MM2T type, also known as sFI, is indistinguishable from FFI which is caused by a D178N mutation. Clinically, insomnia is prominent, while pathologically there is atrophy of the thalamus and the inferior olive. (6) The VV1 type shows severe spongiform change in corticostriatal regions, but only very faint punctate PrP^{Sc} staining on immunohistochemistry. Type MM/MV1+2C shows large confluent vacuoles in some areas, in addition to the small vacuolar change typical of MM/MV1. Type VV2+1 shows the histological appearance very similar to the pure VV2 type.

VPSPr is clinically characterized with behavioral and mood changes, aphasia, cognitive impairment, ataxia and parkinsonism. Histopathology shows relatively large vacuoles, and immunohistochemistry reveals microplaques in the molecular layer of the cerebellum as well as target-like rounded formations of clusters of granules that increase in size toward the center.

Inherited Prion-Related Disorders

gCJD shows stripe-like, coarse, granular PrP deposits in the molecular layer of the cerebellum, patchy PrP deposits in the cerebellum in cases

with additional octarepeats, and others[30]. Clinically, gCJD is characterized by a rapid progressive dementia, with myoclonus and pseudoperiodic discharges on electroencephalogram.

FFI differs from gCJD in a number of ways[39]. It is characterized by refractory insomnia, hallucinations, dysautonomia, autonomic activation, and cognitive and motor signs that are associated with thalamic atrophy. The pathology is dominated by a severe atrophy of the anterior ventral, mediodorsal and pulvinar thalamic nuclei as well as atrophy of the inferior olives. Spongiform degeneration is limited to the entorhinal cortex.

GSS is clinically characterized by ataxia and/or dementia; the pathology shows large, PrP-positive plaques and a variable degree of spongiform change.

Acquired Prion-Related Disorders

vCJD starts at a relatively earlier age, with prominent early psychiatric symptoms, often depression and anxiety, dys- or paresthesia, ataxia, myoclonus, chorea and dystonia. Pathologically, vCJD displays a distinct feature within the brain characterized by abundant florid plaques, widespread accumulation of PrP^{Sc} and thalamic gliosis.

In iCJD, histopathology showed florid plaque, but at a much lower frequency than in vCJD. Clinically, iCJD presents with progressive ataxia and later dementia.

Kuru produces rapidly progressive cerebellar dysfunction with cortical and brainstem symptoms after an insidious onset. The ataxia is associated with a shivering tremor that affects head, trunk, and legs more than arms. Extrapyramidal and cerebellar signs worsen gradually until patients are unable to ambulate or even move without disabling ataxic tremors; this is accompanied by decline in mentation and behavior, ultimately producing severe dementia and dysarthria.

Conclusions

The study of prion has taken several unexpected turns over the years. The development and appropriate use of tools and technologies have allowed answering some long-standing key questions. Nevertheless, many questions remain unanswered. Indeed, despite the identification of PRNP gene encoding PrP^{C}, the function of the protein is shrouded in mystery. Elucidation of the physiological function of PrP^{C} has the potential to help researchers understand the mechanisms involved in prion-induced pathogenesis and can result in a major impact on identifying suitable targets for efficient antiprion therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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