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Chapter

Prion Protein Strain Diversity and Disease Pathology

Saima Zafar, Neelam Younas, Mohsin Shafiq and Inga Zerr

Abstract

The infectious agents, prions, are composed mainly of conformational isomers of the cellular prion protein (PrPc) in its abnormal accumulated scrapie forms (PrPSc). The distinct prion isolates or strains have been associated with different PrPSc prion protein conformations and patterns of glycosylation and are associated with disease progression and severity. In humans, sporadic Creutzfeldt-Jakob disease (sCJD) is the most common form and has been divided into six subtypes, based on PrPSc electrophoretic mobility and allelic variation at codon 129, among which sCJD MM1 and sCJD VV2 are the two most commonly occurring subtypes with known clinical manifestations. The strain-specific response of PrPSc suggests both the molecular classification and the pathogenesis of prion diseases along with posttranslational modification of PrP in humans and animals.

Keywords: prion strain, CJD, conformation, dynamics, aggregation

1. Introduction

For the last two decades, scientists have been working on the prion-related diseases, though major features of this transmissible neurodegenerative disease are still not clear. Among some ambiguities, the prion strain phenomenon and the zoonotic potential are the most discussed and enigmatic questions.

Prion diseases are fatal neurodegenerative disorder linked with misfolding of the host-derived protein, named prion protein. The prevalence of the disease in human population is very low (i.e., ~1–2 cases per million) and affect typically aged people. Among this 15% showed genetic concomitant, i.e., point mutation in *PRNP* gene.

Prion diseases are also well-known risk factor for ruminants, including sheep and goats with scrapie, cattle with bovine spongiform encephalopathy, and recently cervids with chronic wasting diseases (CWD). The prion agent was not able to cross the species barriers between humans and ruminant to a high extent, until the new application livestock carcasses recycling into the ruminant alimentary chain. This new implementation resulted in partial inactivation of the BSE prions and cemented the approach with zoonotic potential and spread in humans. This outbreak was famous as the mad cow disease in cattle and the variant CJD (vCJD) in humans. The prion strain diversity, potential to adapt from one host to another, is a mysterious character-impelled scientific community to uncover the concealed story behind.

2. General background

2.1 The prion protein

Cellular form of prion protein PrPc (prion protein) also referred to as CD 230 (cluster of differentiation 230) is coded from *PRNP* gene on the short arm of chromosome 20. The *PRNP* gene of mammals contains three exons. The open reading frame (ORF) lies entirely within exon 3 which transcribes mRNA (2.1–2.5 kb length) with approximately 50 copies/cell in neurons [1, 2]. Physiological involvement of prion protein is diverse, but the active contribution is reflected by the high level of *PRNP* sequence similarity and conservation across the species in mammals. The expression of PrPc is ubiquitous in mammals' bodies, with the highest levels in immune regulatory cells and masses, suggesting a high degree of metabolic involvement in both systems [3].

Cellular prion protein exists in multiple conformations in the cell. In humans, the newly synthesized and unprocessed PrPc is approximately 253 amino acids in length and has a molecular weight of 35–36 kDa. Mature PrPc, after posttranslational modifications, the physiological form of PrP constitutes 208 amino acid residues. PrPc is translocated to the ER lumen due to the presence of N-terminal signal peptide. Glycophosphotidyl (GPI) anchor is added after the removal of C-terminal signal peptide. After the addition of GPI anchor, PrPc is associated to the lipid rafts. Raft association of PrPc is necessary for the proper folding and glycosylation (at two asparagine residues, i.e., Asn 181 and Asn 197) taking place in ER [4] and formation of a disulfide linkage between the two cysteine residues, i.e., 179 and 214, in human PrP in the Golgi apparatus [5]. In addition, mature PrPc contains five octapeptide repeats with a sequence PHGGGWGQ near NH_2-terminal that are encoded by codons 51–91 of the *PRNP* gene [6]. Physiological form of prion protein, PrPc, occurs predominantly along with the truncated, transmembrane COOH–terminal and transmembrane NH_2-terminal forms, namely, PrP^{Ctm} and PrP^{Ntm}, respectively, due to transmembrane insertion of the hydrophobic pocket between aa 110 and 134 [7, 8]. A GPI anchor is attached to PrPc during its life cycle in the cell [9].

In neurons, the cell surface retentivity is very short-lived, like other classical membrane receptors, i.e., a $t_{1/2}$ of 3–5 min. The endocytosis is rather enigmatic. In different cells and different physiological conditions, internalization via both clathrin- and non-clathrin-coated vesicles is reported [10].

Structural studies of recombinant human PrPc reveal that the protein consists of three α-helices at aa residues 144–154, 175–193, and 200–219 and two small antiparallel β-sheets between aa residues 128–131 and 161–164 [11]. PrPc contains a flexible domain at N-terminal between amino acid positions 23–120, whereas a folded domain at C-terminal between amino acids 121–231.

The presence of the PrPc on cell surface suggests its role as a cell receptor. Many studies relate PrPc to diverse signaling pathways. The N-terminal domain containing the octapeptide repetitive motif is reported to exhibit a high affinity for copper ions (Cu^{2+}), suggesting the involvement of PrPc in copper metabolism [12, 13]. PrP is also reported to regulate the influx of Zn^{2+} into the neuronal cells via α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors, by acting as a zinc sensor to the AMPA receptor acting as transporter for Zn^{2+}. These results also suggest that PrP-mediated zinc uptake may contribute to neurodegeneration in prion and other neurodegenerative diseases [14, 15]. PrPc also promotes cellular Ca^{2+} influx via VGCC [16, 17]. Likewise, the activation of Ras GTPases after interaction of PrPc leading to Erk activation is also reported [18]. Activation of protein kinase C and PI3 kinase/Akt signaling is also reported to be associated to PrP, but the mechanism of activation is poorly understood [19, 20].

Derivatives resulting from the various PrPc-proteolytic cleavages are associated to the alteration of PrPc physiology. An α-cleavage at aa residues 110/111 results in N1 and C1 fragments, whereas a β-cleavage event at aa residue 90 results in N2 and C2 fragments. On the cell surface, some proportion of PrPc also undergoes an ADAM10-driven cleavage at GPI anchor called as shedding, resulting in the release of full-length PrPc molecule in extracellular milieu [21].

2.2 Prion diseases

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are rare progressive, incurable fatal neurodegenerative diseases that have the property of transmissibility [2, 22, 23]. Prion diseases affect humans and animals. Human prion diseases include Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI), Gerstmann-Sträussler-Scheinker syndrome, variably protease-sensitive prionopathy (VPSPr), vCJD, and Iatrogenic CJD (iCJD) [24]. Animal prion diseases include bovine spongiform encephalopathy (BSE) in cattle [25], chronic wasting disease (CWD) in deer and elk [26], and scrapie in sheep, goats and experimentally infected rodents [12].

Human prion diseases occur at a rate of one to two cases per million per year. Among human prion diseases, 80–95% are sporadic Creutzfeldt-Jakob disease (sCJD), 10–15% are genetic (often familial), and less than 1% are acquired. In sCJD, the conversion of PrPc to PrPSc is thought to occur spontaneously (or possibly through a somatic mutation of *PRNP*). In genetic prion diseases, it is thought that mutations in the prion protein gene, *PRNP*, make the PrPc more susceptible to changing conformation (misfolding) into PrPSc. In acquired forms, PrPSc is accidentally transmitted to a person, causing their endogenous PrPc to misfold [27].

Prion diseases belong to a growing family of protein misfolding diseases that are attributed to misfolding (conformational alterations) and aggregation of proteins in specific brain regions, including Alzheimer's disease, Parkinson's disease, and systemic amyloidosis [28, 29]. Some characteristic features of prion diseases are their wide phenotypic heterogeneity and their multiple modes of occurrence (sporadic, genetic, or acquired) [30, 31]. Central hypothesis in prion diseases is the conversion of an endogenous protease-sensitive cellular prion protein, PrPc, into a conformationally altered self-replicating protease-resistant pathological isoform, PrPSc [32], in the central and lymphoreticular systems. PrPSc binds to cellular PrPc and catalyzes its conversion to an infectious form by nucleation and fragmentation cycle [33]. Prions are resistant to proteases, heat, and decontamination treatments, which is a major challenge for the prevention of prion diseases. Although protease-resistant prions correlate only slightly with infectivity, infectivity is linked to protease-sensitive oligomers [34]. PrPc-to-PrPSc conversion brings in neurotoxicity to the attributes of PrPSc [35]. Diseases arising due to prion misfolding are enlisted in **Table 1**.

Human prion diseases are characterized by a range of clinical symptoms and are classified by both clinico-pathological symptoms and etiology, with subclassifications according to the molecular features. Clinical manifestations include spongiform degeneration, motor and cognitive impairments, neuronal loss, gliosis, astrocytosis, and neuronal dysfunction [23]. Prion diseases have long incubation periods; once clinical symptoms appear, disease progresses very rapidly with lethality in all cases.

Sporadic Creutzfeldt-Jakob disease (sCJD) has average survival of about 6 months, with 85–90% of patients dying within 1 year. The peak age of onset is 55–75 years of age, with median age of onset of about 67 years and mean of 64 years [36]. Sporadic Creutzfeldt-Jakob disease has been classified based on combination of two features: a *PRNP* polymorphism at codon M129V [37] and the size of PK-digested PrPSc on Western blot giving two main types: type 1, with a more distal

	Phenotypes
Familial (inherited)	Familial Creutzfeldt-Jakob-disease (fCJD)
	Fatal familial insomnia (FFI)
	Gerstmann-Sträussler-Scheinker disease (GSS)
	Mixed or undefined forms
Sporadic	CJD (sporadic)
	Typical (MM1 and MV1)
	Early onset (VV1)
	Long duration (MM2)
	Kuru plaques (MV2)
	Ataxic (VV2)
	Sporadic familial insomnia (sFI)
Acquired	Kuru
	Iatrogenic CJD (iCJD)
	variant CJD (vCJD)

Modified [93].

Table 1.
Classification of human prion disease.

cleavage site, are 21 kDa and type 2, with a more proximal cleavage site, are 19 kDa. These factors result in six possible combinations (MM1, MV1, VV1, MM2, MV2, and VV2) [36]. Codon 129 M/M homozygosity is reported to be associated with an early-onset and aggressive dementia in the CJD patients, whereas V/V homozygosity correlates to a more prolonged pathology with ataxic onset [38]. Apart from codon 129, two other polymorphisms have been reported, i.e., N171S and E219K [39, 40]. Disease-specific PrP mutations have been reviewed in detail by [41]. GSS associated *PRNP* mutations include P102L, P105L, A117V, F198S, D202N, Q212P, and Q217R. *PRNP* mutations associated to fCJD include P102L, P105L, A117V, F198S, D202N, Q212P, and Q217R, whereas a single missense mutation (D178N) has been reported for FFI. This vast structural diversity and switching to disease causing PrPSc make prion protein and its derivatives interesting subject of study.

Although many laboratories are working on therapeutic strategies for prion disease, still they are incurable although some of the symptoms can be temporarily treated [27]. Three randomized double-blinded placebo-controlled trials have failed to alter disease outcome [27, 42].

3. Prion strains and impact on biological parameters

3.1 Prion strain diversity

Prion diseases affect a range of mammalian species and are caused by misfolding of normal cellular PrPc to self-propagating pathological isoform (PrPSc) [43]. Prions can form several distinct self-templating conformers, called prion strains (or variants), which confer dramatic variation in disease pathology and transmission [44]. Diverse strains of prions [45] exist and are operationally defined by differences in a heritable phenotype under controlled experimental transmission setups. Prion strains can differ in tissue tropism, incubation period, clinical signs of disease, and host range.

Prion disorders remain a challenge to modern science in the twenty-first century because of their strain diversity and interspecies transmission properties. Different clinicopathological properties of prion ailments are associated to biochemical heterogeneity in pathogenic protein. Unfortunately, little is known about the mechanisms that drive these differences in biochemical properties.

The mechanism by which a protein pathogen can encode strain diversity is only beginning to be understood. The identification of strain-specific cellular cofactors persuading the generation of new prion strains or the selection, from a conformationally heterogeneous population of PrPSc, of the most suitable prion conformation in a specific environment, denotes an important milestone toward the understanding of the mechanisms of prion strain diversity, which can have vital clinical and therapeutic implications. Adaptation to a new host is the basis of interspecies transmission of prion infections. In some cases, no abnormally folded PrP is found, reflecting a molecular species barrier to disease transmission [46, 47].

Although significant advancements have been made in comprehending the phenomenon of prion strains, many pieces of information are still missing, most important among them is the definitive evidence for the structural differences between prion strains and the relationship between the strain-specific properties of PrPSc and the resulting phenotype of disease [48, 49].

There are two main theories about possible interspecies transmission and adaptive properties of prion infections: the first one considers that strains are present as a single clone in inoculum, and if a new strain arises, it can be assumed that a stain shift has occurred. The second one considers that strains exist as a pool of different molecular species with a dominant type of PrPSc that is preferentially propagated in a given host, but in a different host, a minor PrPSc type can be favored, causing a shift in the strain. The second theory seems to better explain the high level of strain diversity that is reported from experimental data, although the likelihood that prion strains can infect the host as a single clone cannot be excluded. Plausible explanation for the second theory can be that from a pool of different conformations of PrPSc, only a specific fraction is able to replicate in a certain host species, in a manner that is dependent on the sequence and conformation of the PrPc, on the natural clearance capacity of the infected cells [50–53] and on the presence of cofactors [54–56]. In such a model, a prion strain behaves as a quasi-species and represents a pool of molecules that are kept under control by the host [57]. Hence, in a given host, a strain will be constituted of a principal molecular component and a minor one.

Accordingly, interspecies transmission depends on compatibility between the conformation of pathological PrPSc and of the PrPc of the new host, on cell and tissue environment and cofactors [58, 59]. When a prion strain of one species infects an animal of a different species, there are two possible outcomes. The first is that the pathological PrPSc has no conformation compatibility with the host PrPc, resulting in non-conversion; in this case, the species barrier is defined as absolute. The second possibility is that the PrPSc conformation is compatible with the PrPc host conformation, allowing conversion and, ultimately, infection. In this case the proliferated strain can be identical to the infecting unit [60] or can change into a conformationally different strain due to cellular environment, polymorphisms, and cofactors [58, 59]. So, this type of transmission can facilitate the replication of the minor molecular component, if it is favored in the new host, or the generation of a new PrPSc different from the one of the inoculum [61, 62].

Many studies have been performed to reveal the nature of the cofactors that may be involved. It has been demonstrated that RNA molecules; protein chaperones, such as Hsp104 and GroEL; and others have been shown to change strain properties of prions highlighting the role of different cofactors in determining prion strain' propagation properties.

3.2 Transmissibility, heritable phenotype, and species barrier

In the early 1900s, the intraspecies transmission of the TSE agent was first documented with sheep scrapie [63]. The intraspecies transmission (i.e., sheep-sheep) showed marked attack rate as compared to the cross species transmission (i.e., sheep-mice) which showed incomplete attack rate and longer incubation periods. In cross species transmissions, the main hindrance was the adaptation of prion to its new host that leads to the vitiated prions after few subpassages, i.e., 2–3 passages. Previously, this phenomenon hindered the development of rodent models. Later, it has been reported that distinct prion strains, upon serial adaptation of sheep or goat scrapie isolates, could be raised and propagate in different lines of mice. The incubation time, disease severity, and vacuolation distribution in the brain of the mice-adapted strains showed marked signature of the specific disease [64]. However, the major goal at that time was to establish disease-specific end-stage response with clinical symptomatic phase leading to the anatomic distribution with significant lesion score profile. The first experiments reported inoculation of sheep scrapie to goats [65–67]. By that time, prion transmission from one species to the other, i.e., mink to small ruminants, was reported [68], and the bank vole showed maximum transmission capability and turned out as the universal prion strain acceptor [69–71]. In contrast, few studies also reported partial species barriers to pass prions from one species to another, i.e., scrapie isolates to cattle [72].

The emerging field of engineered transgenic mouse models, in combination with endogenous mouse PrP expression (presence or absence), significantly enhanced the possibilities for studying the zoonotic potential of prions [73–76]. In many cases, these experimental setups made emerged the idea that almost every prion could adapt to almost every PrP substrate, provided that some critical parameters have been set up in order to adapt the strain to its new host PrP [77–79]. The transmission efficacy of vCJD strain to wild-type mice also showed conserved and uniform characteristic BSE strain phenotype. The incubation period, glycoform analysis, and lesion profile did not show differential alterations in brain regions and in lymphoreticular tissue [80].

4. Prion strains and disease response

4.1 Phenotypic variants of PrP and human prion strains

The cellular prion protein is a product of *PRNP* gene-residing the chromosome 20 in human. The conformational variations of PrPc in transmissible spongiform encephalopathies (TSEs) give rise to multiple phenotypic variants of PrP-scrapie form (PrPSc), referred to as prion strains. A pure strain refers to a molecular population of PrPSc with characteristic features such as incubation time, PrPSc distribution patterns, resultant spongiosis, and relative severity of the spongiform changes in the brain, when inoculated into distinct host species. In a given prion pathology, a strain species predominantly exists along with minimal concentrations of strains. Classically prion strains are classified based on abovementioned features. Characteristic pattern of prion strains on the western blotting has also been used for the strain classification. The differences of western blotting patterns occur due to the variability of proteinase k cleavage sites in prion protein and abundance of differential PrP glycoforms (i.e., di-glycosylated, mono-glycosylated, and un-glycosylated isoforms). Rather recently, nontrivial approaches such as seeding potential of prion variants and differential strain-specific oligomeric populations have expanded the spectrum of strain classification [81].

In human prion strains, variation is determined by proteinase K (PK) resistance. PK-resistant PrP occurs in two forms based on the migration on western blots, i.e., PrPSc type 1 migrates at 21 kDa, whereas type 2 PrPSc migrates at 19 kDa (resultant of two distinct PK digestion at amino acids 96 and 85, respectively) [82]. Atypical cases of variably protease-sensitive prionopathy (VPSPr) exhibit a different sensitivity profile to the Proteinase K. Some cases have been reported to exhibit no PK resistance (viz., protease-sensitive prionopathy, PSPr), whereas some other VPSPr cases present less PK resistance resulting in a ladder-like pattern on western blot ranging from 27 to 7 kDa. Details of human prion strains in combination with codon 129 M/V polymorphism are listed in **Table 2** [83].

4.2 Templating activity

Prion templating activity coupled with the structure studies is also used as an index for strain classification. Baskakov and colleagues have been able to differentiate the hamster recombinant PrP strains based on the structure profiles formed under different conditions, i.e., R and S fibrils, result of polymerization while rotating and shaking the monomers, respectively [84]. Structural validations of the prion protein polymers are challenging due to overly high hydrophobic nature of the polymers. For robust templating activity-based classification of prion strains, two methods have been established, namely, protein misfolding cyclic amplification (PMCA) and real-time quacking-induced cyclic amplification (RT-QuIC), where prion strains are utilized as templates for the recombinant prion protein. Templating in PMCA is usually validated with downstream Western blotting, where RT-QuIC is a fluorometry-based method and provides the real-time information, utilizing thioflavin-T binding to polymers. Lag phase and final fluorescence signals could be used for discrimination between different prion strains [71]. RT-QuIC

Strain type	Histological characteristics	Disease subtype, % age occurrence of all prion pathologies
MM/MV 1	Diffuse synaptic deposits	sCJD, 40%
VV 2	Perineuronal and cerebellar plaque-like deposits	sCJD, 15%
MV 2K	Kuru plaques	sCJD, 8%
MM 2C	Cortical confluent vacuoles	sCJD, 1%
MM 2T (sFI)	Thalamo-olivary atrophy	sCJD
VV 1	Corticostriatal synaptic deposits	sCJD
MM 2V (vCJD)	Florid plaques	sCJD
MM/MV1+2C	Mixed diffuse synaptic deposits and cortical confluent vacuoles	sCJD, 30%
MV 2K+C	Mixed Kuru plaques and cortical confluent vacuoles	sCJD
MM-VPSPr	Large vacuoles, PrPSc 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	VPSPr
MV-VPSPr		
VV-VPSPr		

Modified from [83].
Abbreviations: BSE, bovine spongiform encephalopathy; sCJD, sporadic Creutzfeldt-Jakob disease; sFI, sporadic fatal insomnia; VPSPr, variably protease-sensitive prionopathy; gCJD, genetic CJD; GSS, Gerstmann-Sträussler-Scheinker disease; FFI, fatal familial insomnia; vCJD, variant CJD.

Table 2.
Human prion strain histopathological profiles, influenced by codon 129 polymorphism determined in different backgrounds of human transmissible spongiform encephalopathies (TSEs).

proves to be a highly specific and sensitive method and has been utilized to establish strain differences of typical and atypical prionopathies, e.g., the L-type BSE and classical BSEs [85].

A recent report showed oligo-/poly-thiophene derivate as a potent fluorescent approach to discriminate between prion strains [86]. The excitation/emission spectra were obtained from the CWD and scrapie strains, and the interactive association between thiophene and different aggregates were used in combination with conformational restriction to characterize different strains.

4.3 Distribution of density variants

Prion strain polymerization is a sequential process where single molecules are converted to polymers via a multitude of conformational variants. Different prion strains have been identified in animal and human cases based upon differential population densities of these quaternary structures [87]. Quaternary structure conformers of PrP have been isolated and studied using sucrose density gradient by many groups [88–91]. Differential prion strains have been also identified for the rapidly progressive forms of Alzheimer's disease with distinct population of high-density PrP oligomeric species [92].

5. Conclusions and future outlook

Prion strains and the interspecies barriers are still enigmatic phenomena. One of the surprising things about prion protein is that this single protein can fold up in

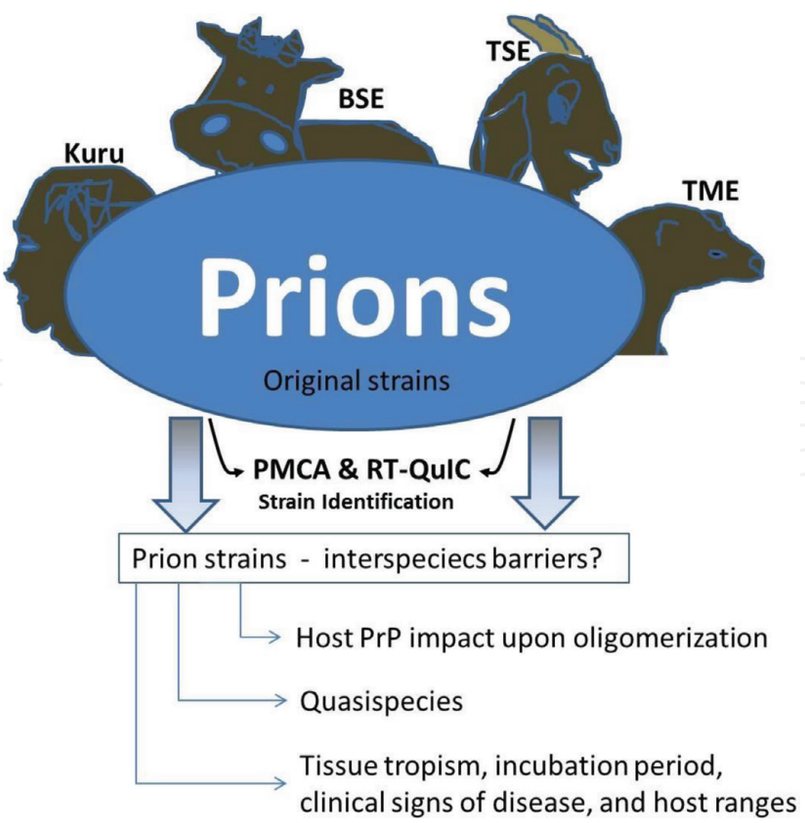

Figure 1.
Prion strain emergence and interspecies transmission. The original prion strains were named as Kuru in human, BSE in cows, TSE in goat and sheep, and TME in minks. PMCA and RT-QuiC mobilized the prion strain characterization. The interspecies transmission of prions linked with host PrP oligomerization role, appearance of subassemblies named as quasispecies, tissue tropism, incubation period of prions, symptomatic stages of the diseases, and host range.

so many different ways that are toxic and cause disease. Recent advances in PrPSc amplification methods, i.e., PMCA and RT-QuIC, might lead to clear improvements in the characterization of the prion strain.

From last many years, prion protein strain characterization and impact on disease are under debate. The use of prion transgenic models has been influential for studying and clarifying the molecular mechanisms in which the protein is involved. The ability to cross species barrier may be a result of either quasispecies theory or host PrP impact on progressive templating deformation upon oligomerization theory (**Figure 1**). These phenomena are mostly time dependent. By learning the structural variation and potential interspecies transmissions, we may progress toward the understanding of disease pathology and subsequently development of novel therapeutic approaches to such devastating disorders.

Acknowledgements

The study was performed within the recently established Clinical Dementia Center at the University Medical Hospital Goettingen and was partly supported by grants from the EU Joint Program Neurodegenerative Disease Research (JPND ± DEMTEST) (Biomarker-based diagnosis of rapid progressive dementias-optimization of diagnostic protocols, 01ED1201A). This work was supported by a grant from Helmholtz-Alberta Initiative-Infectious Diseases Research (HAI-IDR) and APRI-Human prions distinguishing sporadic from familial forms via structure and function as well as from the DZNE clinical project (Helmholtz).

Conflict of interest

We have no conflict of interest to declare.

Author details

Saima Zafar[1,2]*, Neelam Younas[1,2], Mohsin Shafiq[1,2] and Inga Zerr[1,2]*

1 Department of Neurology, Clinical Dementia Center, University Medical Center Goettingen (UMG), Georg-August University, Goettingen, Germany

2 German Center for Neurodegenerative Diseases (DZNE), Goettingen, Germany

*Address all correspondence to: sz_awaan@yahoo.com
and ingazerr@med.uni-goettingen.de

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