

Molecular pathogenesis of sporadic prion diseases in man

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Abbreviations: ALS, amyotrophic lateral sclerosis; CDI, conformation-dependent immunoassay; CJD, Creutzfeldt-Jakob disease; GSS, Gerstmann-Sträussler-Scheinker syndrome; PrP prion protein; PrP^C, normal or cellular prion protein; PrP^{Sc}, pathogenic prion protein; PRNP prion protein gene; rPrP^{Sc}, protease-resistant conformers of pathogenic prion protein; sPrP^{Sc}, protease-sensitive conformers of pathogenic prion protein; sCJD, sporadic Creutzfeldt-Jakob disease; SFI, sporadic fatal insomnia; VPSPr, variable protease-sensitive prionopathy

The yeast, fungal and mammalian prions determine heritable and infectious traits that are encoded in alternative conformations of proteins. They cause lethal sporadic, familial and infectious neurodegenerative conditions in man, including Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), kuru, sporadic fatal insomnia (SFI) and likely variable protease-sensitive prionopathy (VPSPr). The most prevalent of human prion diseases is sporadic (s)CJD. Recent advances in amplification and detection of prions led to considerable optimism that early and possibly preclinical diagnosis and therapy might become a reality. Although several drugs have already been tested in small numbers of sCJD patients, there is no clear evidence of any agent's efficacy. Therefore, it remains crucial to determine the full spectrum of sCJD prion strains and the conformational features in the pathogenic human prion protein governing replication of sCJD prions. Research in this direction is essential for the rational development of diagnostic as well as therapeutic strategies. Moreover, there is growing recognition that fundamental processes involved in human prion propagation—intercellular induction of protein misfolding and seeded aggregation of misfolded host proteins—are of far wider significance. This insight leads to new avenues of research in the ever-widening spectrum of age-related human neurodegenerative diseases that are caused by protein misfolding and that pose a major challenge for healthcare.

Introduction

Prion diseases,¹ originally called transmissible spongiform encephalopathies,² are invariably fatal neurodegenerative diseases that affect humans and animals. The key characteristics of human spongiform encephalopathies are (1) heterogeneity of the clinical and pathologic phenotype,³⁻⁵ (2) a single pathologic process, which may present as a sporadic, genetic or infectious

illness,⁶ and (3) the age dependence of genetic as well as sporadic forms—the annual peak incidence is 3–6 cases per million people between 65 and 79 years of age.^{6,7} Despite their rarity, human prion diseases have gained considerable importance because their unique etiology and pathogenesis challenged basic principles of biology. Furthermore, prion diseases can be transmitted between humans as well as from animals to humans by an agent that is highly resistant to inactivation and which thus poses novel problems to disease control and public health. Finally, because of the marked heterogeneity of their clinical phenotype, prion diseases are difficult to differentiate from other age-related brain neurodegenerations, a feature that has prompted the establishment of specialized prion disease surveillance centers worldwide. Human prion diseases also include inherited forms as well as forms acquired by infection (Table 1). However, this review focuses on the molecular aspects of the pathogenesis of sporadic forms.

From Slow Virus to the Prion Concept

Sporadic Creutzfeldt-Jakob disease (sCJD) is the most common form of human spongiform encephalopathy, accounting for ~85% of all human prion disease.⁸ The term “Creutzfeldt-Jakob disease” (CJD) was introduced by Alfons Maria Jakob in 1921, who referred to a previous case described by Hans Gerhard Creutzfeldt in 1920.^{9,10} These original cases were clinically heterogeneous and neuropathologic review of the historical material with modern tools provided confirmation of the diagnosis of CJD in only two of the original five cases.¹¹ Over the next two decades, the clinicopathological classification of CJD remained uncertain and for example Wilson concluded that CJD is a “dumping ground for several rare cases of presenile dementia.”¹² Although monograph by Kirschbaum¹³ listed the clinical and pathologic characteristics of 150 cases diagnosed before 1965, it included also cases such as Creutzfeldt's original case, which would not fulfill criteria for the diagnosis of CJD today. Importantly, in the 1960s, Nevin and Jones described the typical clinical symptoms, electroencephalogram (EEG) and neuropathologic changes, including spongiform change, which together are now recognized as the paradigm

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Table 1. Etiologic classification of human prion diseases

Etiology	Mechanism	Disease
Sporadic	Somatic mutation or spontaneous conversion of PrP ^C into PrP ^{Sc} ?	Sporadic Creutzfeldt-Jakob disease
	Somatic mutation or spontaneous conversion of PrP ^C into PrP ^{Sc} ?	Sporadic fatal insomnia
Hereditary	Unknown	Variable protease-sensitive prionopathy
	Germ-line mutations in the PRNP gene	Familial Creutzfeldt-Jakob disease
	Germ-line mutations in the PRNP gene	Fatal familial insomnia
	Germ-line mutations in the PRNP gene	Gerstmann-Sträussler-Scheinker disease
Acquired	Infection through ritualistic cannibalism	Kuru
	Zoonotic infection with bovine prions	Variant Creutzfeldt-Jakob disease
	Infection from prion-contaminated human growth hormone, dura mater grafts, etc.	Iatrogenic Creutzfeldt-Jakob disease

features of sporadic CJD.¹⁴ Subsequently, new sporadic forms of the spongiform encephalopathies with unique phenotypic features were described; specifically, sporadic fatal insomnia (SFI) in 1997,^{15,16} and the latest likely new candidate, variable protease-sensitive prionopathy (VPSPr) in 2008.¹⁷

Controversy concerning the cause of human spongiform encephalopathies has polarized the scientific community for decades. The 1950s saw considerable interest in an epidemic of a neurodegenerative disease, kuru, characterized principally by a progressive cerebellar ataxia, among the Fore people of the Eastern Highlands of Papua New Guinea.^{18,19} Fieldwork by Carleton Gajdusek and Vincent Zigas suggested that kuru was transmitted during cannibalistic feasts.²⁰ Importantly, in 1959 Hadlow pointed out the similarities between kuru and scrapie of sheep at the neuropathologic and clinical levels and suggested that human diseases might also be transmissible.²¹ Subsequent transmission of kuru (in 1966) and then CJD (in 1968) by intracerebral inoculation of brain homogenates into chimpanzees, work which was conducted by Gajdusek, Gibbs and colleagues, was a landmark discovery which led to the concept of the “transmissible spongiform encephalopathies.”^{22,23} The transmission of Gerstmann-Sträussler-Scheinker disease (GSS) followed in 1981²⁴ and fatal familial insomnia (FFI) in 1995.²⁵ Interestingly, Jakob, suspecting in his original observations that the condition might be transmissible, inoculated rabbits experimentally, in an attempt to demonstrate this in the 1920s.²⁶ However, thanks to the important role of serendipity in science, his experiment was unsuccessful and we know now that rabbits are uniquely resistant to prion infections.²⁷

The transmission of CJD and of kuru allowed refinement of the diagnostic pathologic criteria for human spongiform encephalopathy and led to the conclusion that all the human conditions share common histopathologic features: spongiform vacuolation (affecting any part of the cerebral gray matter), neuronal loss and astrocytic proliferation that may be accompanied by amyloid plaques.²⁸⁻³¹ One analogy with scrapie in sheep, it was assumed that the causative agent must be some type of atypical “slow virus,” the term Sigurdsson coined in 1954 for scrapie infection.^{2,32} Regrettably, despite extensive efforts in Europe and the US, no non-host DNA or RNA could be found, and a growing body of data pointed to a causative agent having unique characteristics.^{33,34} Most researchers today accept the model according

to which the infectious pathogen responsible for human prion diseases is an abnormal protein, designated PrP^{Sc}.¹

The discovery that proteins could be infectious represented a new paradigm of molecular biology and medicine. Although originally deemed heretical, this protein-only model is now supported by a wealth of biochemical, genetic and animal studies.^{6,35-38} Moreover, the concept of prion diseases has important implications for other neurodegenerative disorders. Recent studies with amyloid β , tau, α -synuclein, huntingtin and superoxide dismutase 1 suggest that molecular and cellular mechanisms that were first discovered in studies of prions are involved in the pathogenesis of other neurodegenerative disorders associated with the accumulation of misfolded proteins, including Parkinsonism, Huntington disease, amyotrophic lateral sclerosis (ALS) and Alzheimer disease.³⁹⁻⁴³

Pathogenetic Mechanisms of Human Sporadic Prion Diseases

The basic event shared by all three forms of prion diseases—sporadic, inherited and acquired by infection—is a change in conformation of the normal or cellular PrP (PrP^C) which is converted into a pathogenic PrP isoform commonly identified as PrP^{Sc} for prototypic scrapie (Sc) PrP.¹ Human PrP^C is encoded by the PrP gene (*PRNP*) on chromosome 20 and expressed at different levels in most mammalian cells. It comprises 209 amino acids (23–231), two sites of N-linked glycosylation, a disulfide bond and a glycolipid anchor.⁴⁴⁻⁴⁹ The variable degree of glycosylation is responsible for the presence of di-, mono- and un-glycosylated forms of PrP^C. Because of the glycolipid anchor, most of the PrP^C is extracellularly linked in specialized cholesterol-rich domains (caveoli) of the cell plasma membrane.^{48,50} In normal conformation, human PrP^C comprises a C-terminal globular domain (involving residues 127–231), which consists of three α -helices and two short β -sheets.⁵¹ In contrast, the N-terminus is unstructured.⁵¹

The PrP^{Sc}, in contrast, is pathogenic, infectious and displays different biophysical features, forming insoluble aggregates of different sizes with predominantly β -sheet secondary structure; the C-terminal region is relatively resistant to proteolytic degradation.⁵²⁻⁵⁴ The conformational transition that underlies these pronounced changes is believed to involve refolding of the

C-terminal region whereby the α -helical structures of PrP^C are replaced by β -sheet to different extents and with variable patterns.⁵⁵ Although insolubility and protease resistance were the original defining features of PrP^{Sc}, the finding of protease-sensitive small oligomers of pathogenic PrP^{Sc}^{56,57} significantly broadened the spectrum of pathogenic conformers.

Although some authors believe that the toxicity in prion disease can be explained as a loss of function of PrP^C due to the conformational transformation,⁵⁸ others argue that a gain of toxic function is more likely.^{59,60} Nevertheless, there is general agreement that PrP^C serves as a substrate for conversion to PrP^{Sc} and as a major receptor for toxic effects of PrP^{Sc}. Thus the neuroprotective function physiologically provided by PrP^C could be lost following its conversion to PrP^{Sc}. The prevailing presence of PrP^{Sc} on neuronal plasma membrane also at the synapse might explain the widespread spongiform degeneration, which can be viewed as intracellular edema. All these changes are pathognomonic of sCJD. Apoptosis and oxidative stress are reported to occur when PrP^{Sc} aggregates form at the cell surface and may well contribute to the neuronal cell loss that is prominent in prion diseases of long duration and in prion diseases like SFI, which manifest no or minimal spongiosis as well as severe neuronal loss.⁶¹ Astrogliosis, a common reaction to injury, is considered a secondary response.

The Origin and Phenotypic Heterogeneity of sCJD

Several explanations have been proposed for the etiology of sporadic prion diseases. These include spontaneous somatic mutations in the PRNP gene or rare stochastic conformational changes in the structure of PrP^C.⁶² These explanations presuppose that the mutant PrP^{Sc} would have to be capable of recruiting wild-type PrP^C; however, this process which might occur with some mutations or conformations but is unlikely with others.⁶³ According to a second explanation, low amounts of PrP^{Sc}-like isoforms are normally present in brain, and possibly bound to other proteins such as heat shock proteins, however, this protective mechanism fails with aging.^{64,65} Finally, it has been suggested that at least some cases of apparent sCJD result from covert, low-level exposure to a “common external factor.”⁶⁶

With more cases investigated after the original reports by Jakob, it became clear that the clinical as well as histopathologic features of sCJD are remarkably diverse, perhaps making the human prion diseases the most heterogeneous of all neurodegenerative disease. On the basis of predominant clinical and pathologic features, the following phenotypic subtypes of sCJD were proposed in the 1980s: (1) myoclonic or cortico-striatal-spinal, (2) amaurotic or Heidenhain, (3) classical or diffuse, (4) thalamic, (5) ataxic or cerebellar and (6) amyotrophic.⁶⁷⁻⁶⁹ Researchers today generally agree that the genotype at codon 129 of the chromosomal gene PRNP, and to some degree the phenotypes of these diseases, underlie susceptibility to prion diseases.⁵ Additionally, many lines of evidence from experiments with laboratory prion strains support the view that the phenotype of sCJD—its distinctive incubation time, clinical features and brain pathology—is enciphered in the strain-specific conformation of PrP^{Sc}.^{56,70-73} However, in contrast to the experiments with

laboratory rodent prion strains, in which the digestion of brain PrP^{Sc} with proteolytic enzyme proteinase K (PK) consistently results in a single protease-resistant domain with mass ~19 kDa, the outcome in sCJD is more complex. Distinctive glycosylation patterns and up to four PK-resistant fragments of the pathogenic prion protein (rPrP^{Sc}) found in sCJD brains are easily distinguishable on western blot (WB).^{5,71,74-77}

The WB findings together with human PRNP gene polymorphism led Parchi, Gambetti and colleagues to posit a clinicopathological classification of sCJD into five or six subtypes; notably, the WB characteristics of PrP^{Sc} breed true upon transmission to susceptible transgenic mice and Guinea pigs (*Cavia porcellus*).^{5,71,74,78} An alternative classification of the PrP^{Sc} types and their pairing with CJD phenotypes has been proposed by Collinge and collaborators.^{37,75,76,79} This classification differs from the previous one in two major aspects: first, it recognizes three (not two) PrP^{Sc} electrophoretic mobilities and second, it also identifies PrP^{Sc} isoforms with different ratios of the three PrP glycoforms.³⁷ Although the disease phenotypes of patients with sCJD are remarkably heterogeneous, 21 kDa fragments of unglycosylated PrP^{Sc} (Type 1) frequently differ from the disease duration and phenotypes associated with the 19 kDa fragments of unglycosylated PrP^{Sc} (Type 2).^{3,5,71,74}

Cumulatively, these findings argue that the PrP^{Sc} type represents yet an additional major modifier of the phenotype in human prion diseases; accordingly, WB-based clinicopathologic classifications became an important tool in studies of prion pathogenesis in human brains and in transgenic mice models.^{37,71} Because two distinct PK cleavage sites in PrP^{Sc} Types 1 and 2 most likely stem from distinct conformations, some investigators contend that PrP^{Sc} Types 1 and 2 code distinct prion strains.^{3,71,75,80} However, the heterogeneity of sCJD, along with a growing number of studies including bioassays, all suggest that the range of prions causing sCJD exceeds the number of categories recognized within the current WB-based clinicopathologic schemes.⁸¹⁻⁸³ Additionally, recent findings of the co-occurrence of PrP^{Sc} Types 1 and 2 in 40% or more sCJD cases created a conundrum and suggested that the originally observed differences were quantitative rather than qualitative.⁸⁴⁻⁸⁹ Finally, up to 90% of brain PrP^{Sc} in sCJD eludes WB analysis because it is destroyed by proteinase-K treatment, which is necessary to eliminate PrP^C.⁸¹ Consequently, the conformation or role of this major protease-sensitive (s) fraction of PrP^{Sc} in the pathogenesis of the disease is a subject of speculation.^{64,81,90} Thus, no direct structural data are available for sCJD brain PrP^{Sc} beyond the evidence that it is resistant to proteolytic digestion. Nevertheless, to determine the full spectrum of sCJD prion strains, and the conformational features in the pathogenic human prion protein governing replication of sCJD prions is fundamental for the rational development of diagnostic as well as therapeutic strategies.

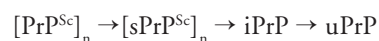
Novel Conformational Methods Derived from a Conformation-dependent Immunoassay (CDI)

Three obstacles have slowed progress in the research of human prions: phenotypic variability of sCJD on complex genetic

background, biosafety constraints, and lack of suitable tools for studying molecular characteristics beyond WB typing. Aiming to advance our understanding of the molecular pathogenesis of human prion diseases, we developed the conformation-dependent immunoassay (CDI),^{56,81,91} to determine the conformational range and strain-dependent molecular features of sCJD PrP^{Sc}, first in patients who were homozygous for codon 129 of the PRNP gene.⁹² Even relatively minute variations in a soluble protein structure can be determined by measuring conformational stability in a denaturant such as Gdn HCl.⁹³ Utilizing this concept, we designed a procedure in which PrP^{Sc} is first exposed to denaturant Gdn HCl and then exposed to europium-labeled mAb against the epitopes hidden in the native conformation.⁵⁶ As the concentration of Gdn HCl increases, PrP^{Sc} dissociates and unfolds from native β -sheet-structured aggregates, and more epitopes become available to antibody binding. These experiments involve insoluble oligomeric forms of PrP^{Sc}, and denaturation of this protein is irreversible in vitro; consequently the Gibbs free energy change (ΔG) of PrP^{Sc} cannot be calculated.⁹⁴ Therefore we chose instead to use the Gdn HCl value found at the half-maximal denaturation ($[GdnHCl]_{1/2}$) as a measure of the relative conformational stability of PrP^{Sc}. The differences in stability reveal evidence of distinct conformations of PrP^{Sc}.^{56,93,94}

To measure the concentration of different forms of PrP^{Sc} and follow the unfolding, we used europium-labeled mAb 3F4⁹⁵ (epitope residues 107–112) for detection and 8H4 mAb (epitope residues 175–185),⁹⁶ to capture human PrP^{Sc} in a sandwich CDI format.^{81,97} The analytical sensitivity and specificity of the optimized CDI for detection of PrP^{Sc} was previously reported by us and others in numerous publications^{56,81,91,98–100} and has been shown to be as low as ~500 fg (~20 attomoles) of PrP^{Sc} which is similar to the sensitivity of human prion bioassay in Tg(MHu2M)5378/Prnp^{0/0} mice.⁸¹ Because CDI is not dependent on protease treatment, it allowed us to address fundamental questions concerning the concentration and conformation of different isoforms of sCJD PrP^{Sc}, including protease-sensitive (s) and protease-resistant (r)PrP^{Sc}.⁹²

The dissociation and unfolding of PrP^{Sc} in the presence of increasing concentration of Gdn HCl can be described as follows:



where $[PrP^{Sc}]_n$ are native aggregates of PrP^{Sc}, $[sPrP^{Sc}]_n$ are soluble protease-sensitive oligomers of PrP^{Sc}, iPrP is an intermediate, and uPrP is completely unfolded (denatured) PrP.^{53,57,94} The CDI monitors the global transition from native aggregates to fully denatured monomers of PrP^{Sc}. In contrast, the WB-based techniques monitor either the partial solubilization of PrP^{Sc}¹⁰¹ or conversion of rPrP^{Sc} to protease-sensitive conformers⁷² after exposure to denaturant. As a result, the stability data on soluble

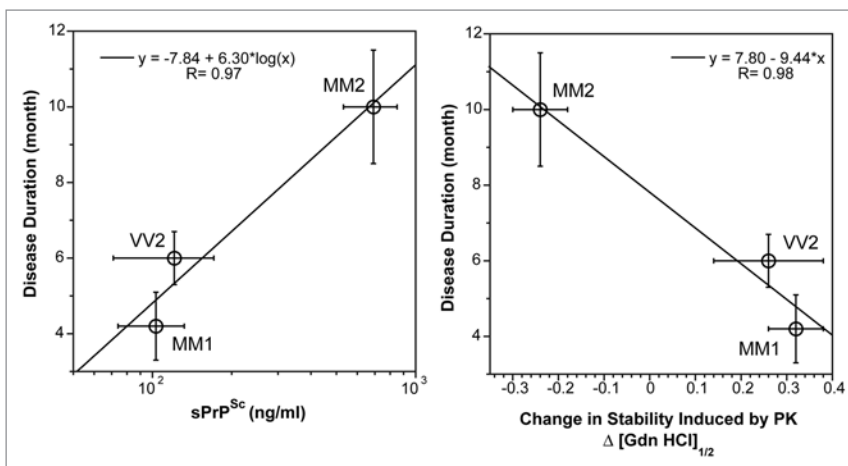


Figure 1. The relationship between duration of the disease, (left part) concentration and (right part) conformational stability of sPrP^{Sc} conformers in sCJD patients with different WB patterns and PRNP gene polymorphisms.⁹² The stability of PrP^{Sc} was determined with CDI before and after protease treatment and the change is expressed as $\Delta [Gdn HCl]_{1/2}$.⁹² The symbols are mean \pm SEM in each sCJD group.

protease-sensitive oligomers and intermediates of PrP^{Sc} cannot be obtained with WB techniques and may explain some markedly different values.¹⁰²

Structural Heterogeneity of sCJD PrP^{Sc} and the Role of sPrP^{Sc} in Pathogenesis

Our recent finding of 6-fold difference in concentrations of PrP^{Sc} between Type 1 and Type 2 PrP^{Sc} (129M) in the frontal cortex was surprising, even though some variability was to be expected due to differences in the predominantly affected areas in distinct sCJD phenotypes.^{81,92} Moreover, the average levels of PrP^{Sc} were up to 100-fold lower than those in standard laboratory prion models such as Syrian hamsters infected with Sc237 prions;⁵⁶ and together with the up to 100-fold variability within each phenotypic group, these lower levels of PrP^{Sc} may explain why some sCJD cases are difficult to transmit, and why lower endpoint titers are obtained with human prions in transgenic mice expressing human or chimeric PrP^C.^{37,71,81,103}

Up to 90% of the pathogenic prion protein in sCJD is protease-sensitive⁸¹ and we found the highest concentrations in Type 2 PrP^{Sc} (129M) (Fig. 1).⁹² The broad range of absolute and relative levels of rPrP^{Sc} and sPrP^{Sc} offers evidence of a broad spectrum of PrP^{Sc} molecules differing in protease sensitivity in each group with an identical polymorphism at codon 129 of the PRNP gene and an identical WB pattern. Moreover, these findings signal the existence of a variety of sCJD PrP^{Sc} conformers; and since protease sensitivity is one of the characteristics of prion strains, they also suggest that distinct sCJD prion strains exist.^{56,57,81,82,104,105}

The heterogeneity of PrP^{Sc} conformations we found with CDI within sCJD patients homozygous for codon 129 polymorphism of the PRNP gene is remarkable, having a range corresponding to that of stabilities found in approximately 30 distinct strains of de-novo and natural laboratory rodent prions studied up to now.^{56,72,92,106} The intriguing differential effect of PK treatment

with increasing stability of Type 1 and decreasing stability of Type 2 PrP^{Sc} (129M) suggests that in contrast to Type 1, the protease-resistant core of Type 2 was profoundly destabilized. Together with the increased frequency of exposed epitopes in codon 129 MM samples with Type 2 rPrP^{Sc} after PK treatment, these observations may indicate one of three possibilities: that the ligand protecting the 3F4 epitope was removed by PK treatment; that epitope 108–112 was protected by the N-terminus of PrP^{Sc}; or that conformational transition resulted in more exposed 108–112 epitope. Whether the epitope hindrance in undigested PrP^{Sc} is the result of lipid, glycosaminoglycan, nucleic acid or protein binding to the conformers unique to the MM2 sCJD PrP^{Sc} remains to be established. Since sCJD cases with Type 2 PrP^{Sc} (129M) have remarkably extended disease durations, the molecular mechanism underlying these effects calls for detailed investigation. Cumulatively, our findings indicate that sCJD PrP^{Sc} exhibits extensive conformational heterogeneity and suggest that a wide spectrum of sCJD prions cause the disease. Whether this heterogeneity originates in a stochastic misfolding process that generates many distinct self-replicating conformations^{37,62} or in a complex process of evolutionary selection during development of the disease⁷³ remains to be established.

We discovered protease-sensitive conformers of PrP^{Sc} while developing a conformation-dependent immunoassay (CDI), which does not require proteolytic degradation of ubiquitous PrP^C.⁵⁶ Although the original definition of sPrP^{Sc} was purely operational, considerable additional data demonstrate that (1) sPrP^{Sc} replicates *in vivo* and *in vitro* as an invariant and major fraction of PrP^{Sc}; (2) sPrP^{Sc} separates from rPrP^{Sc} in high speed centrifugation and (3) the proteolytic sensitivity of PrP^{Sc} can reliably differentiate various prion strains.^{56,57,81,82,104,105} Accumulation of sPrP^{Sc} precedes protease-resistant product (rPrP^{Sc}) in prion infection,^{64,107} and up to 90% of PrP^{Sc} accumulating in CJD brains consists of sPrP^{Sc}.⁸¹ Thus, the detection by CDI of sPrP^{Sc} as a disease-specific marker is widely regarded as a more reliable basis for diagnosing prion diseases. This improved detection led to the discovery of a new human prion disorder, variably protease-sensitive prionopathy (VPSPr).^{17,56,81,90,108} It is noteworthy that synthetic prions generated *in vitro* during polymerization of recombinant mouse PrP into amyloid fibers produced prions composed exclusively of sPrP^{Sc} upon inoculation into wild mice.¹⁰⁶

Our recent data indicate that the levels as well as stability of sPrP^{Sc} are a good predictor of the progression rate in sCJD (Fig. 1).⁹² Despite the inevitable influence of variable genetic background and the potential difficulties in evaluating initial symptoms, the disease progression rate and incubation time

jointly represent an important parameter, which is influenced by replication rate, propagation and clearance of prions from the brain.^{64,109} The correlations among the levels of sPrP^{Sc}, the stability of sPrP^{Sc}, and the duration of the disease found in this study all indicate that sPrP^{Sc} conformers play an important role in the pathogenesis. When sPrP^{Sc} is less stable than rPrP^{Sc}, the difference in stability correlates with less accumulated sPrP^{Sc} and shorter duration of the disease. Conversely, when sPrP conformers are more stable than rPrP^{Sc}, we observed the opposite effect—more accumulated sPrP^{Sc} and extended disease duration (Fig. 1).

In laboratory rodent prion models, we found that levels of sPrP^{Sc} varied with the incubation time of the disease⁵⁶ and we hypothesized that the molecular mechanism of this link may be related to the replication or clearance rate of prions.^{56,64,81} Our recent data on sCJD prions extend this observation and indicate that higher levels of less stable sPrP^{Sc} lead to faster progression of the disease.⁹² These observations are in accord with the experiments on yeast prions and suggest that the stability of misfolded protein is inversely related to the replication rate.¹¹⁰ Although the modulating effect of prion clearance in the mammalian brains is likely,⁶⁴ the data from both yeast and human prions lead to the hypothesis that the less stable prions replicate faster by exposing more available sites for growth of the aggregates. In mammalian prions, this effect leads to shorter incubation time and faster progression of the disease.

Future Directions

Additional steps are necessary to improve our understanding of the phenotypic diversity of sCJD. One step is to determine whether the mixed WB patterns of Type 1–2 rPrP^{Sc} in the same or different anatomical areas represent a unique conformation or a mixture of conformers, and to map the distribution in the individual brain.⁸⁹ Additionally, novel conformational approaches using tandem protein misfolding cyclic amplification (PMCA) and CDI should allow analysis of the impact of different PrP^{Sc} polymorphisms and conformations on the replication rate of human prions. Such studies have clearly broader implications, as recent data suggest that the process of intercellular induction of protein misfolding is relevant in the pathogenesis of growing number of other neurodegenerative diseases.^{39–43}

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