

## Prion disease and Alzheimer's disease: pathogenic overlap

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Review

**Abstract.** Prion diseases are widely recognized for their transmissibility, and it is this feature that has been studied most extensively. In recent years, public health concerns over the transmission of animal forms of prion disease, such as bovine spongiform encephalopathy and chronic wasting disease, to humans has only augmented the notion that prion diseases are primarily infectious. Yet within the spectrum of human prion diseases, often overlooked is the fact that the overwhelming majority of cases are age-dependent sporadic, or inherited processes. Closer examination of the pathophysiological processes involved in prion disease further indicates a neurodegenerative, rather than infectious disease. Indeed, the age requirement, the numerous kindreds carrying point mutations in an amyloidogenic protein, the copper binding properties of the amyloidogenic protein, the evidence of free radical damage, the presence of polymorphisms that influence disease susceptibility, the formation of amyloid plaques, and in some cases the presence of neurofibrillary pathology, are features common to both prion disease and Alzheimer's disease. Therefore, while transmissibility will continue to be a major subject of prion disease research, we suspect that further characterization of its pathophysiological mechanisms will only substantiate the notion that prion disease is fundamentally a neurodegenerative process.

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## INTRODUCTION

Prion diseases are a group of uniformly fatal central nervous conditions defined by the accumulation of abnormal isoforms of the host-encoded prion protein (PrP). The five human phenotypes, based on clinical, pathological, biochemical, and genetic criteria, include: Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker syndrome (GSS), fatal familial and sporadic insomnia (FFI and sFI), kuru, and new variant Creutzfeldt-Jakob disease (nvCJD). While the vast majority of human prion diseases are either sporadic or inherited, transmission of disease by exposure to contaminated material may rarely occur. Transmitted disease has deservedly received a great deal of attention in recent years, in the wake of the BSE epidemic and the appearance of nvCJD (Brown P. et al. 2001, Valleron et al. 2001). However, it is important to keep in mind that transmitted cases remain exceedingly rare in humans, even in Great Britain where the brunt of the BSE epidemic took place. Rather, as studies on prion diseases accumulate, it becomes increasingly apparent that the pathogenesis of this group of diseases shares a number of features in common with the pathogenesis of Alzheimer's disease (AD). Indeed, the similarities are striking. The focus of this review is therefore the neurodegenerative quality of prion diseases and its parallel with AD.

## PRION PROTEIN IN HEALTH AND DISEASE

A large body of evidence implicates PrP, a host-encoded, membrane-bound, glycoprotein of unknown function, as a central pathogenic factor in prion disease (Prusiner 1982, Prusiner et al. 1998). In humans, the normal cellular prion protein (PrP<sup>C</sup>) comprises 209 amino acids, a disulfide bridge between residues 179-214, a glycosylphosphatidyl inositol (GPI) anchor, and two sites of non-obligatory N-linked glycosylation at amino acids 181 and 197 (Caughey et al. 1989, Petersen et al. 1996, Stahl et al. 1987). More than twenty mutations of the PrP gene are now known to cause the inherited disease, and significant genetic linkage has been demonstrated for five of these (Prusiner et al. 1998). Current data suggests that normal cellular PrP (PrP<sup>C</sup>) is converted into PrP<sup>Sc</sup> through a process whereby alpha helical regions are refolded into beta sheets. Fourier transform infrared (FTIR) and circular dichroism

(CD) spectroscopy indicate that PrP<sup>C</sup> contains about 40% alpha helix and minimal beta sheet, while PrP<sup>Sc</sup> contains 30% alpha helix and about 40% beta sheet (Pan et al. 1993). As such, PrP<sup>C</sup> is soluble in denaturing agents and sensitive to protease digestion, whereas PrP<sup>Sc</sup> is relatively insoluble and resistant to proteolytic digestion. It is also noteworthy that the protease resistance of PrP<sup>Sc</sup> is the most sensitive biochemical marker for prion disease (Castellani et al. 1996, 1997, Parchi et al. 1996), and the only molecule consistently associated with infectivity.

### Susceptibility and phenotype in prion disease vary with prion protein "strains"

The *PRNP* codon 129 polymorphism influences a number of factors, including susceptibility and phenotype. For example, the homozygous state is a risk factor for the disease; less than 10% of CJD subjects are heterozygous (methionine or valine) at codon 129, while 51% of the Western European population is heterozygous (Fink et al. 1991, Parchi et al. 1996). Additionally, all nvCJD subjects to date are homozygous methionine at codon 129. Patients with the D178N mutation have autosomal dominant familial prion disease, however the disease phenotype varies from fatal familial insomnia (FFI) to Creutzfeldt-Jakob disease (CJD178), depending on whether the mutant allele contains methionine or valine at codon 129 (Goldfarb et al. 1992, Monari et al. 1994). Heterozygous (129M/V) FFI subjects have a significantly longer disease duration and greater cortical pathology compared with homozygous subjects (M/M) (Parchi et al. 1995). In addition to codon 129, the electrophoretic mobility of PrP<sup>Sc</sup> has been shown to play a significant role in sporadic CJD subtype, and likelihood of CSF expression of 14-3-3 protein (Castellani et al. submitted, Parchi et al. 1996, 1999). Taken together, these data indicate the existence of "strains" of prion disease, based solely on features of the protein itself.

### Host factors

While the association of PrP<sup>Sc</sup> with disease is irrefutable, several lines of evidence indicate non-PrP factors. Studies in transgenic mice chimeric for mouse and human PrP suggest that an important "host component" facilitates transmission (Telling et al. 1995). The nature of the host component, designated "protein X" by Prusiner and colleagues, is an open question, but it is believed to

be a molecular chaperone (Kaneko et al. 1997, Prusiner et al. 1998). In this regard, it is also noteworthy that the putative "yeast prion" Sup35 interacts directly with hsp104 (Schirmer and Lindquist 1997), a heat shock protein in yeast, previously studied for its role in acquired thermotolerance with no known homologue in mammals, resulting in protein folding and protease resistance. Other studies using an *in vitro* conversion reaction showed that hsp 104 and GroEl both facilitate the conversion of normal yeast prion protein to the protease resistant form (DeBurman et al. 1997). Whether a classical molecular chaperone facilitates PrP conversion in mammalian disease remains to be determined, although chaperone proteins HSP-70 and the ER protein BiP have been suggested as possible mediators of PrP conversion (Jin et al. 2000, Ma and Lindquist 2001).

Degradation within the proteasome may represent another important mediating factor, as enhanced aggregation and retrograde transport of PrP within the cytosol occurs in cells with proteasome inhibition (Ma and Lindquist 2001). Moreover, accumulation of cytosolic PrP species in cell culture enhances cytotoxicity, while transgenic mice overexpressing the cytoplasmic form of PrP (PrP<sub>23-230</sub>) develop neurodegeneration (Ma et al. 2002). More recent studies, however, dispute the primary toxicity of cytosolic PrP (Roucou et al. 2003).

## PRION DISEASE AND ALZHEIMER'S DISEASE: PATHOGENIC OVERLAP

While Alzheimer's disease and prion disease differ in terms of incidence and to a lesser extent duration of symptoms, both processes demonstrate: (i) an age requirement; (ii) sporadic occurrence in the majority of cases; and (iii) inherited disease based on mutations in an amyloidogenic protein in a minority of cases. Aside from kuru and rare iatrogenic cases, prion disease tends to present in middle age and older patients (Parchi et al. 1999). Therefore, it is not surprising that age-related pathological processes such as oxidative stress, protein cross-links, and adduct formation that are widespread early findings in Alzheimer's disease (Sayre et al. 1997, Smith et al. 1996, 1997), also occur in prion disease (Choi S.I. et al. 1998, Choi Y.G. et al. 2000, Guentchev et al. 2002, Kim et al. 2000). In addition, sometimes overlooked is the fact that about 85 percent of human prion disease cases are sporadic, i.e., there is no exposure to contaminated material and no detectable muta-

tion leading to disease. The lack of clusters of CJD throughout the world aside from kindreds with familial CJD (e.g., E200K mutation) is also consistent with the sporadic disease concept and suggests that prion disease is primarily a neurodegenerative process.

## Amyloid fibril formation

Significant genetic linkage has been demonstrated between amyloid  $\beta$  protein precursor (A $\beta$ PP) mutations (and overexpression in the case of Down's syndrome) and AD, and between *PRNP* mutations and prion disease as noted above. Both processes result in accumulation of the respective proteins with increased beta sheet content, although participation by the protein encoded on the normal allele varies. Mutations in AD tend to be clustered in and around the amyloidogenic region of A $\beta$ PP, whereas the same can be said of prion disease particularly in the case of GSS (Jobling et al. 2001). Interestingly, allelic polymorphisms in both conditions (e.g., Prnp codon 129 in prion disease, presenilin 1 (Lambert et al. 2001) and ApoE in AD) confer susceptibility to disease and influence phenotypic characteristics, although evidence for allelic variation in A $\beta$ PP itself is lacking (Liddell et al. 1995). It is also noteworthy that small differences in protein size effect major differences in cerebral targeting of amyloid/PrP deposition. For instance, there is a tendency for amyloid- $\beta_{1-42}$  in AD to deposit in plaques and amyloid- $\beta_{1-40}$  to deposit in cerebral vessels. Similarly in sporadic CJD, major differences in targeting of gray matter occur as a function of PrP<sup>sc</sup> type 1 (21 kD) or PrP<sup>sc</sup> type 2 (19kD) (Parchi et al. 1996). Perhaps the most compelling evidence linking AD to a prion disease is the F198S GSS kindred, wherein PrP<sup>sc</sup> plaques in gray matter are accompanied by tau-positive neurofibrillary pathology, amyloid- $\beta$  deposits, and gradual memory loss with motor signs of about 2-3 years duration as opposed to the duration of less than 1 year associated with typical prion diseases (Ghetti et al. 1989).

## Copper metabolism and oxidation

Mutations in amyloid- $\beta$  and prion protein suggest that perturbation in the normal cellular function of these proteins underlies disease, although the precise function of each is unresolved. Recent data suggest that A $\beta$ PP functions as a kinesin-I membrane receptor, mediating axonal transport of beta-secretase and presenilin-1

(Kamal et al. 2001), while neurophysiological abnormalities in PrP null mice indicate a potential role for PrP in neural transmission (Collinge et al. 1994). Importantly, however, accumulating evidence indicates that both amyloid- $\beta$  and PrP<sup>C</sup> are critical to oxidative homeostasis, likely mediated by copper (see Fig. 1). This is consistent with the established early involvement of oxidative stress in AD at multiple levels and recent data suggesting that oxidative impairment in prion disease may be a pivotal event (Pogocki 2003, Wong et al. 2001).

Consistent with the oxidative stress hypothesis in both AD and prion disease is perturbation in copper metabolism, now recognized as an important feature of both processes. Copper is a redox-active trace metal ion with roles in assimilation of iron into both microbial and mammalian cells, modulation of transcriptional activation and protein trafficking, and is required for a number of cellular enzymes critical to brain function. Teleologically speaking, the inherent toxicity of highly redox-reactive copper requires that cells evolve special ways of transporting copper required for essential biological functions. Indeed, elaborate cellular machinery are involved in recruiting, trafficking, compartmentalizing, and, ultimately, inserting copper into appropriate proteins. These include binding of copper to shuttling proteins that facilitate safe transport by sequestration.

Within the central nervous system, it is important to recognize that ceruloplasmin-bound copper comprises less than 1% of bound copper in the brain (Loeffler et al. 1996); therefore, it is reasonable to speculate that copper binding by other proteins assumes greater importance in preventing oxidative damage. Not surprisingly, the role of both PrP<sup>C</sup> and soluble amyloid- $\beta$  in copper binding has thus begun to emerge. Amyloid- $\beta$  has been shown to bind copper with a stoichiometry of 1:3, at His<sub>6</sub>, His<sub>13</sub>, and His<sub>14</sub>, whereas PrP contains one nona- and four octapeptide repeats between codons 50 and 91 that specifically bind multiple copper ions *via* histidine. In both instances, copper binding induces conformational changes that enable SOD-like or Cu/Zn SOD activity. Therefore, metal binding (copper and zinc) to amyloid- $\beta$  generates an allosterically ordered membrane-penetrating oligomer linked by SOD-like bridging histidine residues (Curtain et al. 2001). Similarly, the N-terminal octapeptide repeat domain of PrP<sup>C</sup> binds multiple copper ions *via* histidine residues effecting a conformational change and SOD-like activity (Brown D.R. et al. 2001), while reduction of SOD-like activity and Cu/Zn SOD activity has been demonstrated in scrapie-infected brains (Wong et al. 2001). Taken together, these data indicate that amyloid- $\beta$  and PrP<sup>C</sup> function to maintain oxidative balance, and that perturbations in both proteins result in copper-driven oxidative damage.

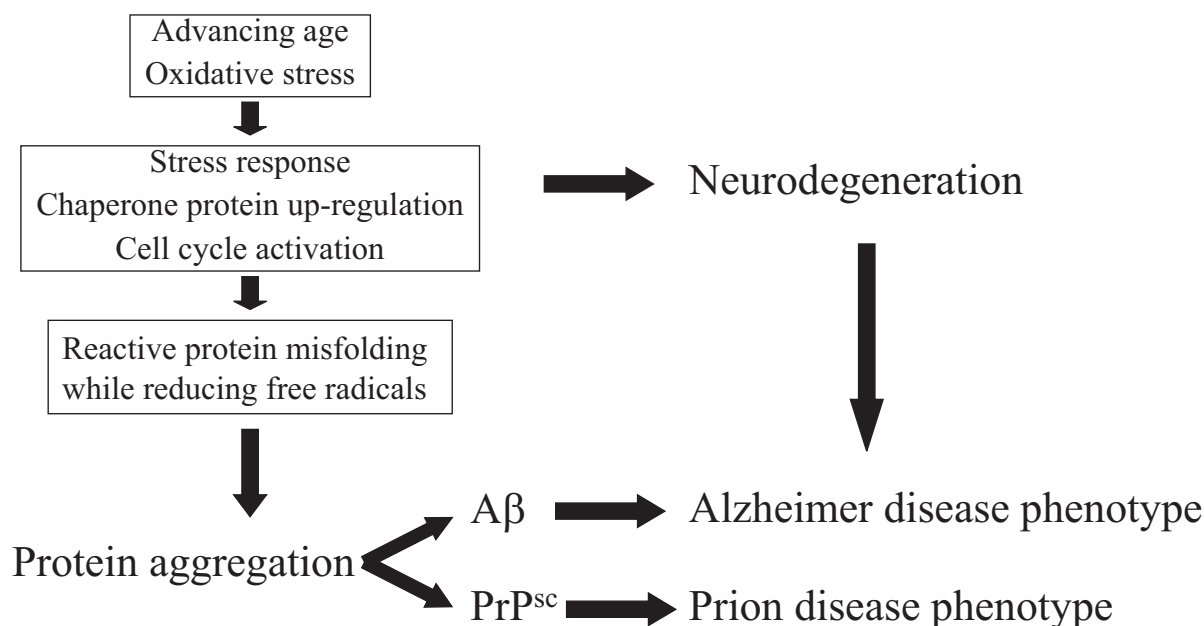


Fig. 1. Neurodegenerative disease pathophysiology. Biochemical and pathological processes culminating in Alzheimer's disease and prion disease are essentially the same aside from the accumulating protein and thus the disease phenotype.



While amyloid- $\beta$  in AD can be cytotoxic by a free radical mechanism (Rottkamp et al. 2002), we have recently shown that amyloid- $\beta$  also acts as an antioxidant by binding and sequestering free radicals (Cuajungco et al. 2000, Joseph et al. 2001, Nunomura et al. 2000, 2001, Perry et al. 2000, Rottkamp et al. 2001, Smith et al. 2000). This is consistent with previous studies demonstrating copper binding by amyloid- $\beta$  plaques (Cherny et al. 1999), and our accumulating evidence that pathological lesions of AD represent an adaptive response to free radical damage rather than an accumulation of toxic substances (Smith et al. 2000). While the mechanisms of cytotoxicity of PrP<sup>sc</sup> remain an open question, it is noteworthy that most cases of Creutzfeldt-Jakob disease, including the classic, rapidly progressive phenotype, are devoid of Congo red-positive amyloid- $\beta$  plaques (Parchi et al. 1999), indicating profound brain dysfunction in the absence of histochemically-apparent amyloid fibril formation. Additionally, those cases that do accumulate Congo red-positive amyloid plaques (10-15% of sporadic CJD cases with kuru plaques, Gerstmann-Straussler-Scheinker syndrome, kuru, variant Creutzfeldt-Jakob disease) generally have a longer disease duration than classical, rapidly progressive CJD. This is consistent with our hypothesis that amyloid plaques in neurodegenerative disease, including prion disease, represent an adaptive response to free radical-mediated toxicity, and that amyloid fibrils are not primarily responsible for brain injury.

## CONCLUSIONS

Prion diseases comprise a heterogeneous group of conditions of humans and animals defined by brain accumulation of PrP<sup>sc</sup> and transmissibility. While the etiology of prion diseases remains to be elucidated, close examination of disease mechanisms demonstrates striking similarities to AD as we have presented above. Transmissibility remains a characteristic of prion disease that has not been replicated in AD. However, when one considers the rarity of transmitted disease in humans, and the difficulty with which transmission is accomplished either iatrogenically or in the laboratory, it is reasonable to suggest that transmissibility is only tangentially related to prion disease pathogenesis. As the biochemistry of this group of disorders continues to become elucidated, transmissibility of prion diseases may become more of an artifact of a neurodegenerative pro-

cess that otherwise follows accepted biochemical principles.

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