Molecular pathology of human prion disease

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Abstract Human prion diseases are associated with a range of clinical presentations and are classified by both clinicopathological syndrome and aetiology with sub-classification according to molecular criteria. Considerable experimental evidence suggests that phenotypic diversity in human prion disease relates in significant part to the existence of distinct human prion strains encoded by abnormal PrP isoforms with differing physicochemical properties. To date, however, the conformational repertoire of pathological isoforms of wild-type human PrP and the various forms of mutant human PrP has not been fully defined. Efforts to produce a unified international classification of human prion disease are still ongoing. The ability of genetic background to influence prion strain selection together with knowledge of numerous other factors that may influence clinical and neuropathological presentation strongly emphasises the requirement to identify distinct human prion strains in appropriate transgenic models, where host genetic variability and other modifiers of phenotype are removed. Defining how many human prion strains exist allied with transgenic modelling of potentially zoonotic prion strains will inform on how many human infections may have an animal origin. Understanding these relationships will have direct translation to protecting public health.

Introduction

Human prion diseases are invariably fatal conditions that include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker disease (GSS), fatal familial insomnia (FFI), kuru and variant CJD (vCJD) in humans [28]. They are associated with a range of clinical presentations and are classified by both clinico-pathological syndrome and aetiology with sub-classification according to molecular criteria [28, 45, 54, 90, 121, 139]. These diseases have attracted immense research interest for many years not only because of their unique composition and properties but also because of their impact on public health [27, 30, 100, 129]. According to the widely accepted ‘protein-only’ hypothesis [47], host-encoded cellular prion protein (PrP^C) is converted to an alternative form designated PrP^Sc [22, 27, 30, 100, 129]. It is proposed that PrP^Sc is the infectious agent acting to replicate itself with high fidelity by recruiting endogenous PrP^C, and that the difference between these isoforms lies purely in the monomer conformation and its state of aggregation [22, 27, 30, 99, 100, 102, 107, 129].

Central to understanding the molecular basis of prion propagation remains the conundrum of prion strains—how a protein-only infectious agent can encode information required to specify distinct disease phenotypes—and also the so-called species barrier effect which limits cross species infection. While originally considered different aspects of the prion problem, it is now clear that species barriers and prion strains are intimately related by “conformational selection” [26, 30]. Within the protein-only hypothesis of prion propagation prion strains are thought to be encoded by distinct pathogenic PrP conformations or assembly states. Conformational selection proposes that although a wide range of mammalian PrP^Sc conformations may be possible, only a subset will be compatible with each
individual PrP primary structure. Ease of transmission of prions between species (or also within species as a result of PrP polymorphisms), therefore, relates to overlap of permissible PrPSc conformations between the structures of PrP from the source and recipient as well as heterogeneity in cellular mechanisms affecting prion propagation and clearance kinetics [26, 30]. Conformational selection has now been strongly supported by elegant studies of prions in yeast and other fungi [14, 41, 62, 66, 106, 111, 112, 132], and the wider relevance of prion-like mechanisms in other protein misfolding diseases is now becoming clear [30, 82, 85]. Understanding the molecular biology of prion disease will illuminate processes involving protein misfolding and aggregation and protein-based inheritance, which clearly have far-reaching implications in pathobiology, ageing and the evolution of cellular processes.

**Aetiologies of human prion disease**

Human prion diseases are biologically unique and can be divided aetiologically into inherited, sporadic and acquired forms [28]. Approximately 85% of human prion disease occurs sporadically as sporadic CJD at a rate of 1–2 cases per million population per year across the world, with an equal incidence in men and women [16, 27, 28, 38, 121]. Around 15% of human prion disease is associated with autosomal dominant pathogenic mutations in PRNP, and to date, over 30 mutations have been described [27, 28, 53, 65, 80, 121]. Acquired human prion diseases forms have, until recently, been confined to rare and unusual situations. Iatrogenic CJD has arisen as a result of transmission of CJD prions through treatment with pituitary hormones derived from human cadavers, implantation of dura mater grafts, corneal transplantation and the use of contaminated electroencephalographic electrodes [17, 18]. The most well-known incidences of acquired prion disease in humans resulting from a dietary origin have been kuru that was caused by cannibalism among the Fore linguistic group of the Eastern Highlands in Papua New Guinea [2, 3, 35, 36, 79] and more recently the occurrence of vCJD in the United Kingdom and other countries due to human exposure to BSE prions [26, 28, 120, 134]. Remarkably, kuru demonstrates that incubation periods of infection with human prions can exceed 50 years [35, 36].

**Prion disease pathology and pathogenesis**

The brains of patients with prion disease frequently show no recognisable abnormalities on gross examination at necropsy; however, microscopic examination typically reveals characteristic histopathologic changes, consisting of neuronal vacuolation and degeneration, which gives the cerebral grey matter a microvacuolated or ‘spongiform’ appearance, and a reactive proliferation of astroglial cells (for review see [19, 20]). Although spongiform degeneration is frequently detected, it is not an obligatory neuropathologic feature of prion disease; the presence of astro-gliosis and micro-gliosis, although not specific to the prion diseases, is more constantly seen. The lack of a lymphocytic inflammatory response is also an important characteristic. Demonstration of abnormal PrP immunoreactivity, or more specifically biochemical detection of PrPSc in brain material by immunoblotting techniques (Fig. 1) is diagnostic of prion disease, and some forms of prion disease are characterised by deposition of amyloid plaques composed of insoluble aggregates of PrP [19, 20]. Amyloid plaques are a notable

![Fig. 1 Immunoblot analysis of human prion disease brain.](image-url)
feature of kuru and GSS [20, 48], but they are less frequently found in the brains of patients with sporadic CJD which typically show a diffuse pattern of abnormal PrP deposition [20, 54] (Fig. 2). The histopathological features of vCJD are remarkably consistent and distinguish it from other human prion diseases with large numbers of PrP-positive amyloid plaques that differ in morphology from the plaques seen in kuru and GSS in that the surrounding tissue takes on a microvacuolated appearance, giving the plaques a florid appearance [59, 134] (Fig. 2). Abundant florid plaques are established as the neuropathological hallmark of vCJD [59] and, to date, have only been found in association with BSE infection in hosts homozygous for PrP with methionine at residue 129 (in humans, primates or transgenic mice [6, 67, 119, 134]).

Distinct forms of prion disease show differences in lymphoreticular involvement that appear to be related to the divergent properties of distinct prion strains [1]. For example, the tissue distribution of PrP Sc in vCJD differs strikingly from that in classical CJD and inherited prion disease [51–53, 55, 57, 61, 124] with uniform and prominent involvement of lymphoreticular tissues, with the highest amounts (up to 10% of brain concentrations) in tonsil [52, 124]. In contrast, in sporadic CJD, PrP Sc has only been irregularly detected by immunoblotting in non-central nervous system tissues at very much lower levels [46, 94]. Tonsil biopsy is used for antemortem diagnosis of vCJD and, to date, has shown 100% sensitivity and specificity [52, 121, 124], and tonsil is the tissue of choice for prospective studies investigating the prevalence of vCJD prion infection within the UK and other populations [24, 42, 56]. The fact that lymphoreticular prion infection is not a feature of iatrogenic CJD [51, 52] or kuru [15, 36] argues that the distinct pathogenesis of vCJD relates to the effect of prion strain rather than to a peripheral route of infection [15, 36, 127]. The demonstration of extensive peripheral tissue involvement in the pathogenesis of vCJD raises concern that asymptomatically infected carriers may be transmitting the disease to others via blood transfusion, as now appears to have occurred [71, 93, 136], or other iatrogenic routes including contaminated surgical and medical instruments [4, 26, 120, 123].

Determinants of phenotypic variability in human prion disease

The clinical presentation of human prion disease varies enormously, and there is considerable overlap observed between individuals with different disease aetiologies [28, 80, 120, 121] and even in family members with the same pathogenic PRNP mutation [29, 31, 32, 65, 77, 80, 125]. Progressive dementia, cerebellar ataxia, pyramidal signs, chorea, myoclonus, extrapyramidal features, pseudobulbar signs, seizures and amyotrophic features can be seen in variable combinations. Criteria used for diagnosis of human prion disease have been defined [28, 131], and definite diagnosis of sporadic and acquired prion disease relies upon neuropathological examination and the demonstration of abnormal PrP deposition in the central nervous system by either immunoblotting or immunohistochemistry [20, 28, 60, 128, 131]. Polymorphism at residue 129 of human PrP [encoding either methionine (M) or valine (V)] powerfully affects susceptibility to human prion diseases [25, 38, 68, 79, 123].

![Fig. 2 Distinct patterns of PrP deposition in brain in human prion disease. The disease aetiology, PRNP codon 129 genotype of the patient (M methionine, V valine) and the type of PrPSc detected in each sample (using the London classification of human PrPSc types [54]) is designated above each brain sample. The most common subtype of sporadic CJD (a) typically shows a diffuse, synaptic pattern of abnormal PrP deposition, while kuru (b) shows not only variably diffuse PrP deposition but also striking formation of PrP plaques in various areas of the brain. These PrP plaques are consistently distinct from those seen in vCJD (c), where PrP plaques are often surrounded by conspicuous vacuolation, designated ‘florid plaques’. Scale bars a, c 25 μm; b 50 μm.](image-url)
Different human PrPSc isoforms, referred to as molecular inheritance mechanisms of yeast prions [106, 112, 132], also by the demonstration of protein conformation-based considerable experimental evidence [30, 34, 90, 113] and significant part of the clinicopathological heterogeneity assembly states of PrP provide the molecular substrate for a [28, 81, 119, 136].

been homozygous for codon 129 methionine of PRNP mutations have glycoform ratios of PrPSc fragments distinct from those seen in both classical CJD and vCJD [53, 65]. Patients with inherited prion disease caused by point mutations have glycoform ratios of PrPSc fragments distinct from those seen in both classical CJD and vCJD [53].

Individuals with the same PRNP mutation can also propagate PrPSc with distinct fragment sizes [53, 96, 97]. Detection of PrPSc in the molecular mass range of ca. 21–30 kDa is, however, not a consistent feature, and some cases, in particular those in which amyloid plaques are a prominent feature, show smaller protease resistant fragments of ca. 7–15 kDa [53, 65, 89, 96, 97, 110]. The propagation of pathological isoforms of wild-type PrP may also make a significant contribution to phenotypic variability in inherited prion disease [23, 43, 108, 125].

Classification of human prion disease

Efforts to produce a unified international classification and nomenclature of human PrPSc types have been complicated. A major confounding issue in this regard has been resolving whether relatively subtle biochemical differences in PrPSc are of biological importance and accurately reflect the propagation of distinct human prion strains. This is particularly true in sporadic CJD [21, 34, 44, 54, 88, 90, 91, 138], where progress has been severely hampered by a lack of transgenic modelling data to firmly distinguish the identity of distinct prion strains and their defining molecular and neuropathological phenotypes. This fundamental problem coupled with the difficulties and variability of the biochemical methods used to distinguish PrPSc types [21, 54, 88, 91, 118, 122, 138] has so far precluded an internationally accepted classification system for human prion strains. In this regard, the increasingly recognised co-occurrence of different PrPSc types in the same brain [38, 49, 54, 90, 96, 98, 101, 105, 118, 125, 137] and the recognition that protease-sensitive pathological isoforms of PrP may have a significant role in both animal and human prion disease [9, 39, 44, 83, 103, 104, 115–117] have further confounded progress. Although agreement has yet to be reached on methodological differences, nomenclature and the biological importance of relatively subtle biochemical differences in PrPSc, there is strong agreement between laboratories.
that phenotypic diversity in human prion disease relates in significant part to the existence of distinct prion strains. Despite these advances, however, the ability of genetic background to influence prion strain selection, coupled with the knowledge that route of transmission in acquired human prion disease may dramatically influence clinical and neuropathological presentation, strongly emphasises the requirement to remove host genetic variability and other modifiers of phenotype. In this regard, the issue of prion strain diversity remains unknown. To date, the conformational repertoire of pathological isoforms of wild-type human PrP and the various forms of mutant human PrP has not been fully defined. Biochemical investigation of disease-related PrP isoforms in patients allied with detailed clinical and neuropathological analysis will continue to inform on the diversity of phenotypes seen in human prion disease. As it has now become clear that prion strain type, host genetic makeup and numerous other factors may significantly influence prion disease phenotype, it is expected that the actual number of distinct human prion strains may be far less than the number of identified phenotypes. Detailed transgenic modelling will therefore be crucial to establishing how many human prion strains exist and what the defining molecular features of PrP are for each strain. This information allied with comprehensive transgenic modelling of human BSE infection and other relevant, potentially zoonotic, prion strains will inform on how many human prion strains may have an animal origin. Understanding the risks that existing and emerging animal prion diseases pose will have direct translation to protecting public health.

Development of an accurate classification for human prion disease will have major implications for epidemiological research into the causes of sporadic CJD, whose aetiology remains obscure. While spontaneous conversion of PrPSc to PrPSc as a rare stochastic event, or somatic mutation of the PrP gene, resulting in expression of a pathogenic PrP mutant are plausible explanations for sporadic CJD, other causes for at least some cases, include environmental exposure to human prions or exposure to animal prions. In this regard, the number of prion strains causing sheep scrapie has yet to be established, and epidemiological data cannot exclude this as a cause of a minority of cases. Similarities between types of sporadic CJD and types of sheep scrapie have been reported. As future research begins to provide a more precise understanding of the origins of human prion disease, this will facilitate re-analysis of epidemiological data to reveal important risk factors that might have been obscured by analysing sporadic CJD as a single entity.

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