

# 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<sup>C</sup>) is converted to an alternative form designated PrP<sup>Sc</sup> [22, 27, 30, 100, 129]. It is proposed that PrP<sup>Sc</sup> is the infectious agent acting to replicate itself with high fidelity by recruiting endogenous PrP<sup>C</sup>, 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<sup>Sc</sup> conformations may be possible, only a subset will be compatible with each

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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 PrP<sup>Sc</sup> 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.

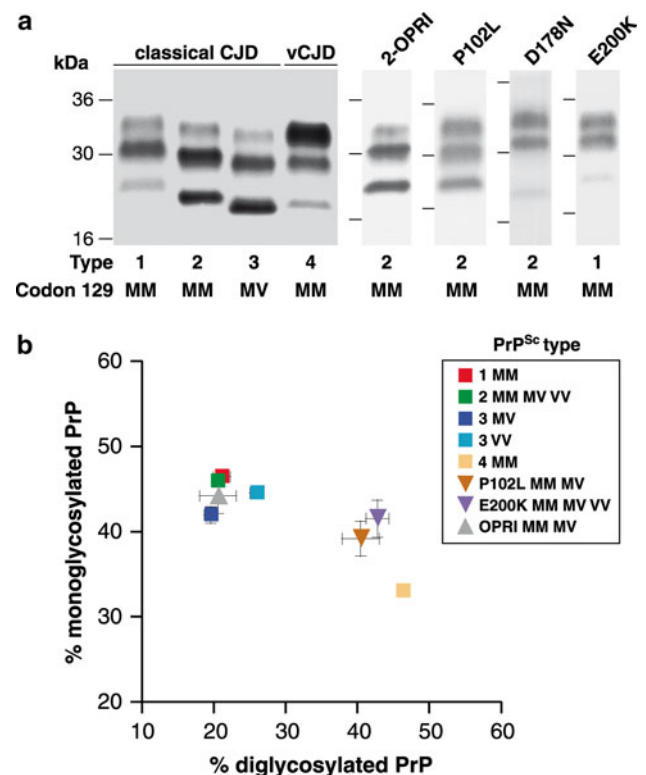
### Aetiologies of human prion disease

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].

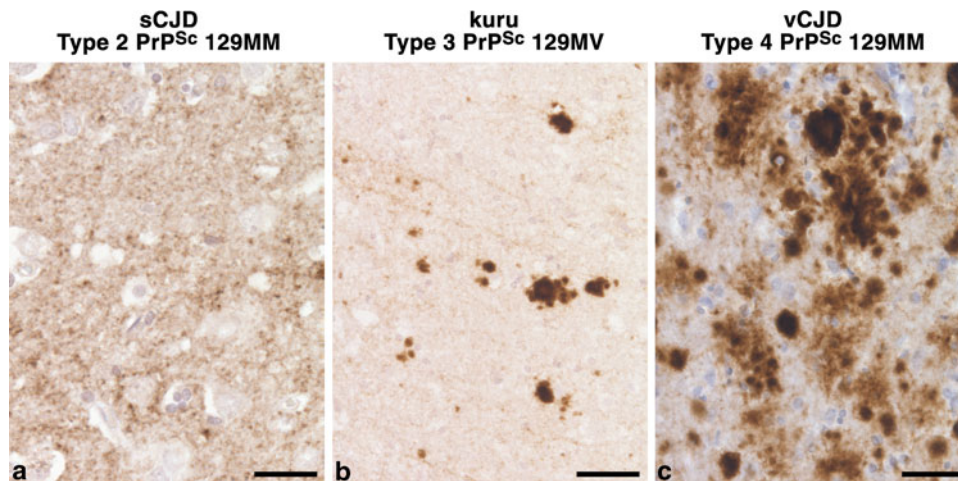
### Prion disease pathology and pathogenesis

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 PrP<sup>Sc</sup> 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. **a** Proteinase K digested brain homogenates analysed with monoclonal antibody 3F4 and enhanced chemiluminescence showing the PrP<sup>Sc</sup> types seen in sporadic, acquired and inherited prion disease. Types 1–3 PrP<sup>Sc</sup> are seen in the brain of classical forms of CJD (either sporadic or iatrogenic CJD), while type 4 PrP<sup>Sc</sup> is uniquely seen in vCJD brain. Distinct PrP<sup>Sc</sup> types are seen in cases with *PRNP* point mutations. Classification according to Hill et al. [53, 54]. **b** Proteinase K digestion of brain homogenate and analysis with anti-PrP monoclonal antibody 3F4 by enhanced chemifluorescence enables calculation of the proportions of di-, mono-, and non-glycosylated PrP. The key shows PrP<sup>Sc</sup> type or mutation and *PRNP* codon 129 genotype (*M* methionine, *V* valine). Data points represent the mean relative proportions of di- and mono-glycosylated PrP as percentage  $\pm$  SEM. In some cases, the error bars were smaller than the symbols used



**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 PrP<sup>Sc</sup> detected in each sample (using the London classification of human PrP<sup>Sc</sup> types [54]) is designated above each brain sample. The most common subtype of sporadic CJD (**a**) typically shows a diffuse, synaptic

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<sup>Sc</sup> 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<sup>Sc</sup> 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

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  $\mu$ m; **b** 50  $\mu$ m

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 asymptotically 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,

86, 135] with residue 129 acting to directly restrict the propagation of particular prion strains through conformational selection [26, 27, 30, 119] as well as heterozygosity conferring resistance by inhibiting homologous protein–protein interactions [25, 30, 86]. About 38% of northern Europeans are homozygous for the more frequent methionine allele, 51% are heterozygous and 11% homozygous for valine. Homozygosity at *PRNP* codon 129 predisposes to the development of sporadic and acquired CJD [25, 38, 68, 79, 86, 135] and is most strikingly observed in vCJD, where all neuropathologically confirmed cases studied so far have been homozygous for codon 129 methionine of *PRNP* [28, 81, 119, 136].

The hypothesis that alternative conformations or assembly states of PrP provide the molecular substrate for a significant part of the clinicopathological heterogeneity seen in human prion diseases and that this relates to the existence of distinct human prion strains is supported by considerable experimental evidence [30, 34, 90, 113] and also by the demonstration of protein conformation-based inheritance mechanisms of yeast prions [106, 112, 132]. Different human PrP<sup>Sc</sup> isoforms, referred to as molecular strain types, have been identified in the brain of patients with phenotypically distinct forms of CJD [34, 45, 54, 88, 90, 92, 113, 122, 139] and are classified by both the fragment size and ratio of the three principal PrP bands seen after protease digestion. To date, we have characterised four types of human PrP<sup>Sc</sup> that can be commonly identified in sporadic and acquired human prion diseases [27, 34, 54, 121] (Fig. 1) although much greater heterogeneity seems likely [121]. Sporadic and iatrogenic CJD and kuru are associated with PrP<sup>Sc</sup> types 1–3, while type 4 PrP<sup>Sc</sup> is uniquely associated with vCJD and is characterised by a fragment size and glycoform ratio that is similar to PrP<sup>Sc</sup> seen in BSE and BSE when transmitted to several other species [34, 54, 122, 127]. An earlier classification of PrP<sup>Sc</sup> types seen in classical CJD described only two banding patterns [88] with PrP<sup>Sc</sup> types 1 and 2 that we describe corresponding with the type 1 pattern of Gambetti and colleagues and our type 3 fragment size corresponding to their type 2 pattern [87, 90]. Consensus on the nomenclature of human PrP<sup>Sc</sup> types has been hindered by the fact that the N-terminal conformation of some PrP<sup>Sc</sup> subtypes seen in sporadic CJD can be interconverted in vitro via changes in metal-ion occupancy [54, 122] or solvent pH [21, 84, 138]. While it has proposed that pH alone determines the N-terminal structure of PrP<sup>Sc</sup> in sporadic CJD [21, 84], this interpretation has not been supported by other studies [69, 98, 122], and the conformations of PrP<sup>Sc</sup> types 1 and 2 that we describe in *PRNP* methionine homozygous patients show critical dependence upon the presence of copper or zinc ions under conditions, where pH 7.4 is tightly controlled [122]. While type 4 PrP<sup>Sc</sup>

is readily distinguished from the PrP<sup>Sc</sup> types seen in classical CJD and kuru by a predominance of the di-glycosylated PrP glycoform, type 4 PrP<sup>Sc</sup> also has a distinct proteolytic fragment size [54] although this is not recognised by the alternative classification which designates type 4 PrP<sup>Sc</sup> as type 2b [87].

In addition to the distinct human PrP<sup>Sc</sup> types associated with sporadic and acquired prion disease, molecular strain typing has also provided insights into the phenotypic heterogeneity seen in inherited human prion diseases [53, 65]. Patients with inherited prion disease caused by point mutations have glycoform ratios of PrP<sup>Sc</sup> fragments distinct from those seen in both classical CJD and vCJD [53]. Individuals with the same *PRNP* mutation can also propagate PrP<sup>Sc</sup> with distinct fragment sizes [53, 96, 97]. Detection of PrP<sup>Sc</sup> 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 PrP<sup>Sc</sup> types have been complicated. A major confounding issue in this regard has been resolving whether relatively subtle biochemical differences in PrP<sup>Sc</sup> 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 PrP<sup>Sc</sup> 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 PrP<sup>Sc</sup> 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 PrP<sup>Sc</sup>, there is strong agreement between laboratories



**Table 1** Factors influencing phenotypic variability in human prion disease

Aetiology: sporadic, acquired or inherited
Route of infection in acquired disease
Location of first propagation in inherited and sporadic disease
Transmission barrier effect (kinetics of prion propagation)
Infecting prion strain type
Prion strain mutation or adaptation in host
Multiple strain infection and strain competition within host
PrP genotype of source of infecting prion
Host <i>PRNP</i> genotype: >30 pathogenic mutations; coding polymorphisms; interaction mutations/polymorphisms; codon 129 zygosity; B haplotype; other non-coding changes
Host genome type—other loci: known major effects on incubation period and strain selection
Differential recruitment of wild-type PrP in inherited prion disease
Subclinical (carrier) state
Co-existent non-prion pathology (chronic inflammation)

that phenotypic diversity in human prion disease relates in significant part to the existence of distinct prion strains [34, 40, 45, 50, 54, 69, 88, 90, 98, 104, 113, 139]. Despite these advances, however, the ability of genetic background to influence prion strain selection [6, 72–74, 81, 109], coupled with the knowledge that route of transmission in acquired human prion disease may dramatically influence clinical and neuropathological presentation [17, 126, 127, 133], strongly emphasises the requirement to remove host genetic variability and other modifiers of phenotype (Table 1) by identifying distinct human prion strains in appropriate transgenic models [5–7, 11, 13, 33, 58, 63, 64, 76, 114, 119].

Whether the diversity of phenotypes seen in human disease can be faithfully recapitulated by transgenic modelling remains an open question. In this regard, the issue of prion strain selection or mutation will be a major factor. As recently hypothesised [30], prion strains may not exist as previously thought as molecular clones with a single PrP<sup>Sc</sup> type (where strain mutation in a different host would involve generation of a distinct PrP<sup>Sc</sup> type), but may consist of an ensemble of molecular species (containing a dominant PrP<sup>Sc</sup> type that is preferentially propagated by its usual host) from which a less populous subspecies may be selected by an alternative host, resulting in a strain shift or mutation. In this regard, different cellular populations and tissues within a single host would provide different environments for strain selection as recently demonstrated in vitro [70]. Given the degree of molecular diversity observed in human prion disease, it may be extremely difficult to isolate the full complement of human prion strains in transgenic mice having a single genetic background.

## Future perspectives

Despite major advances, the molecular basis of mammalian 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 PrP<sup>C</sup> to PrP<sup>Sc</sup> 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 [16, 27, 78, 125], other causes for at least some cases, include environmental exposure to human prions [37, 75, 95] or exposure to animal prions. In this regard, the number of prion strains causing sheep scrapie has yet to be established [8, 10, 12], 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 [130]. 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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