Molecular and clinical classification of human prion disease

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While rare in humans, the prion diseases have become an area of intense clinical and scientific interest. The recognition that variant Creutzfeldt-Jakob disease is caused by the same prion strain as bovine spongiform encephalopathy in cattle has dramatically highlighted the need for a precise understanding of the molecular biology of human prion diseases. Detailed clinical, pathological and molecular data from a large number of human prion disease cases have shown that distinct abnormal isoforms of prion protein are associated with prion protein gene polymorphism and neuropathological phenotypes. A molecular classification of human prion diseases seems achievable through characterisation of structural differences of the infectious agent itself.

Prion diseases are fatal neurodegenerative disorders that include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), fatal familial insomnia (FFI), kuru and most recently variant CJD (vCJD) in humans. The central feature of prion diseases is the post-translational conversion of a normal host-encoded, glycosylphosphatidylinositol (GPI)-anchored glycoprotein, the cellular prion protein (PrPC), to an abnormal isoform, designated PrPSc. This transition appears to involve only conformational change rather than covalent modification and confers PrPSc with partial resistance to proteolytic degradation and detergent insolubility. Prion diseases are biologically unique in that the disease process can be triggered through inherited germline mutations in the human prion protein gene (PRNP), infection (by inoculation, or in some cases by dietary exposure) with tissue containing PrPSc or by rare sporadic events that generate PrPSc. A wealth of experimental evidence indicates that an abnormal PrP isoform is the principal, if not the sole, component of the transmissible infectious agent, or prion. The mechanism of neurodegeneration that accompanies the accumulation of PrPSc in the brain remains unknown. The existence of multiple strains or isolates of prions has been difficult to accommodate within a protein-only model of prion
propagation and understanding how a protein-only infectious agent can encode distinct disease phenotypes in humans has been of considerable biological interest. A great deal of experimental evidence now suggests that prion strain diversity is encoded within PrP itself and phenotypic diversity in human prion diseases relates to differing physicochemical properties of abnormal PrP isoforms. Furthermore, the propagation of distinct abnormal PrP isoforms may be determined by the host genome.

**Aetiology of human prion diseases**

Human prion diseases can be divided aetiologically into inherited, sporadic and acquired forms. About 85% of cases of human prion disease occur sporadically as Creutzfeldt-Jakob disease (sporadic CJD) at a rate of roughly 1 case per million population per year across the world, with an equal incidence in men and women. The aetiology of sporadic CJD is unknown, although hypotheses include somatic PRNP mutation, or the spontaneous conversion of PrP<sub>C</sub> into PrP<sub>S</sub> as a rare

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**Pathogenic mutations**

**Polymorphic variants**

Fig. 1 Pathogenic mutations and polymorphisms in the human prion protein. The pathogenic mutations associated with human prion disease are shown above the human PrP coding sequence. These consist of 1, 2 or 4–9 octapeptide repeat insertions (ORPI) within the octapeptide repeat region between codons 51 and 91, a 2 octapeptide repeat deletion (OPRD) and various point mutations causing missense or stop amino-acid substitutions. Point mutations are designated by the wild-type amino acid preceding the codon number, followed by the mutant residue, using single letter amino-acid nomenclature. Polymorphic variants are shown below the PrP coding sequence. Deletion of one octapeptide repeat is not associated with prion disease in humans.
stochastic event. Homozygosity at a common coding polymorphism at codon 129 of PRNP encoding either methionine or valine (Fig. 1) predisposes to the development of sporadic and acquired CJD\textsuperscript{11–14}. Additionally, a PRNP susceptibility haplotype has been identified indicating additional genetic susceptibility to sporadic CJD at or near to the PRNP locus\textsuperscript{15}.

About 15% of human prion diseases are associated with autosomal dominant pathogenic mutations in PRNP\textsuperscript{8,9}. How pathogenic mutations in PRNP cause prion disease has yet to be resolved; however, in most cases, the mutation is thought to lead to an increased tendency of PrP\textsuperscript{C} to form PrP\textsuperscript{Sc} though there is evidence\textsuperscript{16,17} to suggest that this may not be solely attributable to decreased thermodynamic stability of mutated PrP\textsuperscript{C}. Experimentally manipulated mutations of the prion gene can lead to spontaneous neurodegeneration without the formation of detectable protease-resistant PrP\textsuperscript{18,19}. These findings raise the question of whether all inherited forms of human prion disease invoke disease through the same mechanism; in this regard, it is currently unknown whether all are transmissible by inoculation\textsuperscript{9}.

Although the human prion diseases are transmissible diseases, acquired forms have, until recently, been confined to rare and unusual situations. The two most frequent causes of iatrogenic CJD occurring through medical procedures have arisen as a result of implantation of dura mater grafts and treatment with human growth hormone derived from the pituitary glands of human cadavers\textsuperscript{20,21}. Less frequent incidences of human prion disease have resulted from iatrogenic transmission of CJD during corneal transplantation, contaminated electroencephalographic (EEG) electrode implantation and surgical operations using contaminated instruments or apparatus\textsuperscript{20,21}. 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\textsuperscript{9,14,22}, and more recently the occurrence of vCJD in the UK and other European countries which appears causally related to human exposure to BSE in cattle\textsuperscript{7,23–26}. Incubation periods of acquired prion diseases in humans can be extremely prolonged, and it remains to be seen if a substantial epidemic of vCJD will occur within the UK and elsewhere.

**Clinical features of human prion diseases**

Human prion diseases of distinct aetiologies are associated with a range of clinical presentations which are now seen as clinicopathological syndromes rather than individual disease entities (Table 1).
Prions for physicians

Table 1 Diagnosis of prion disease

**Sporadic (classical) CJD**
- Rapidly progressive dementia with two or more of the following: myoclonus, cortical blindness, pyramidal signs, cerebellar signs, extrapyramidal signs, akinetic mutism
- Most cases aged 45–75 years at onset
- Serial electroencephalogram shows pseudoperiodic complexes in most cases
- CSF 14-3-3 protein usually positive
- CT and MRI brain scan normal, or atrophy, or abnormal signal basal ganglia
- PRNP analysis: no pathogenic mutations, most are codon 129 homozygotes
- Brain biopsy in highly selected cases (to exclude treatable alternative diagnoses): PrP immunocytochemistry or Western blot for PrPSc types 1–3

**Iatrogenic CJD**
- Progressive cerebellar syndrome and behavioural disturbance or classical CJD-like syndrome with history of iatrogenic exposure to human prions (pituitary-derived hormones, tissue grafting or neurosurgery)
- May be young
- EEG, CSF and MRI generally less helpful than in sporadic cases
- PRNP analysis: no pathogenic mutations, most are 129 homozygotes
- Brain biopsy in highly selected cases (to exclude treatable alternative diagnoses): PrP immunocytochemistry or Western blot for PrPSc types 1–3

**Variant CJD (human BSE)**
- Early features: depression, anxiety, social withdrawal, peripheral sensory symptoms
- Cerebellar ataxia, chorea or athetosis often precedes dementia, advanced disease as sporadic CJD
- Onset most often in young adults
- EEG shows non-specific slow waves, CSF 14-3-3 may be elevated
- MRI brain scan: may be high T2-weighted signal in posterior thalamus bilaterally
- PRNP analysis: no mutations, all codon 129 methionine to date
- Tonsil biopsy: characteristic PrP immunostaining and PrPSc on Western blot (type 4t)

**Inherited prion disease**
- Highly varied clinical syndromes between and within kindreds: should consider in all presenile dementias and ataxias irrespective of family history
- PRNP analysis: diagnostic, codon 129 genotype may predict age at onset in presymptomatic testing

**Sporadic CJD**

Classical sporadic CJD presents as a rapidly progressive multifocal dementia usually with myoclonus. The onset is usually in the 45–75 year age group with peak onset between 60–65 years. The clinical progression is typically over weeks progressing to akinetic mutism and death often in 2–3 months. About 70% of cases die in under 6 months. Prodromal features, present in around a third of cases, include fatigue, insomnia, depression, weight loss, headaches, general malaise and ill-defined pain sensations. In addition to mental deterioration and myoclonus, frequent additional neurological features include extrapyramidal signs, cerebellar ataxia, pyramidal signs and cortical blindness. Raised cerebrospinal fluid 14-3-3 protein, neuronal-specific
enolase (NSE), and S-100, although not specific for CJD, may be helpful diagnostically in the appropriate clinical context. The EEG may show characteristic pseudoperiodic sharp wave activity that is helpful in diagnosis but present only in about 70% of cases. Magnetic resonance imaging (MRI) may show signal changes in the basal ganglia that, though not specific, can be diagnostically helpful. Neuropathological confirmation of CJD is by demonstration of spongiform change, neuronal loss and astrocytosis. PrP amyloid plaques are usually not present in CJD though PrP immunohistochemistry will nearly always be positive

Atypical forms of sporadic CJD are well recognised. Of cases of CJD, 10% have a much more prolonged clinical course with a disease duration of over 2 years. About 10% of CJD cases present with cerebellar ataxia rather than cognitive impairment, so-called ataxic CJD. Heidenhain's variant of CJD refers to cases in which cortical blindness predominates with severe involvement of the occipital lobes. The panencephalopathic type of CJD refers to cases with extensive degeneration of the cerebral white matter in addition to spongiform vacuolation of the grey matter and has been predominately reported from Japan. Amyotrophic variants of CJD have been described with prominent early muscle wasting. However, most cases of dementia with amyotrophy are not experimentally transmissible and their relationship with CJD is unclear

Inherited prion disease

Over 50 autosomal dominant pathogenic PRNP mutations have been described (Fig. 1). In the appropriate clinical setting, identification of a pathogenic PRNP mutation provides diagnosis of inherited prion disease and sub-classification according to mutation; PRNP analysis is also used for presymptomatic genetic testing in affected families. Traditionally, inherited prion diseases have been classified by the presenting clinical syndrome, falling into three main sub-divisions of either GSS, CJD or fatal familial insomnia (FFI). GSS can be clinically differentiated from sporadic CJD as it commonly presents as a chronic cerebellar ataxia with pyramidal features with dementia occurring much later in a clinical course that is typically longer than that seen in classical CJD. FFI is characterised by progressive untreatable insomnia, dysautonomia and dementia, selective thalamic degeneration and is most commonly associated with a missense mutation at codon 178 of PRNP, though sporadic FFI with no causative mutation in PRNP has been reported. Remarkably, some families show extensive phenotypic variability which can encompass both CJD- and GSS-like cases as well
as other cases which do not conform to either CJD or GSS phenotypes\textsuperscript{40}. Progressive dementia, cerebellar ataxia, pyramidal signs, chorea, myoclonus, extrapyramidal features, pseudobulbar signs, seizures and amyotrophic features can be seen in variable combinations. Such atypical prion diseases may lack the classical histological features of a spongiform encephalopathy entirely although PrP immunohistochemistry is usually positive\textsuperscript{41}. The existence of phenotypic overlap between individuals with different mutations and even in family members with the same PRNP mutation indicates that accurate classification of inherited human prion diseases should be based upon mutation alone\textsuperscript{40}. Because of the extensive phenotypic variability seen in inherited prion disease and its ability to mimic other neurodegenerative conditions, notably Alzheimer’s disease, prion protein gene analysis should be considered in the investigation of all presenile ataxias and dementias, even in the absence of an apparent family history of neurodegenerative illness\textsuperscript{40–43}.

\textit{Iatrogenic CJD}

Clinical presentation in iatrogenic forms of human prion disease appear to be related to their aetiology and in particular the route of exposure to human prions\textsuperscript{9,21,44}. Peripheral routes of infection are typically associated with longer incubation periods and usually present with a Kuru-like syndrome in which ataxia rather than dementia is the prominent early clinical feature. In contrast, patients associated with dura mater graft related exposure to human prions in which infectivity is placed in close proximity to the brain typically have a clinical presentation of sporadic CJD, although exceptions with unusual clinical features have been reported\textsuperscript{45}.

\textit{Kuru}

The central clinical feature of kuru is progressive cerebellar ataxia and, in sharp contrast to sporadic CJD, dementia is late and may be absent. A prodrome and three clinical stages consisting of an ambulatory stage, a sedentary stage and a tertiary stage have been described\textsuperscript{9,22}.

\textit{Variant CJD}

The early clinical presentation of vCJD resembles kuru more than classical CJD and consists of behavioural and psychiatric disturbances, peripheral sensory disturbance and cerebellar ataxia. Common early psychiatric features include dysphoria, withdrawal, anxiety, insomnia,
and apathy. Neurological symptoms have preceded psychiatric symptoms in 15% of cases studied, and are present in combination with psychiatric symptoms in 22% of cases from the onset of disease. No common early neurological features have been reported, but paraesthesiae and/or pain in the limbs is seen in around half of the cases. However, a significant proportion of patients exhibited neurological symptoms within 4 months of clinical onset; these included poor memory, pain, sensory symptoms, unsteadiness of gait and dysarthria. Disorientation, hallucinations, paranoid ideation, confabulation, impaired self-care, and the commonest neurological features (cerebellar signs, chorea, dystonia, myoclonus, upper motor neuron signs and visual symptoms), developed late in the course of the illness. The duration of disease is longer in vCJD with mean patient survival times of about 13 months, compared with about 4 months for classical CJD. Moreover, whereas classical CJD is predominantly a late-onset disease with a peak onset at 60–65 years, the median age of onset of vCJD is 26 years. The EEG is not helpful in the diagnosis of vCJD; whilst generalised slowing is usually present, the characteristic periodic changes associated with classical CJD are not. The CSF 14-3-3 protein is not helpful, and may often be negative. MRI, however, is useful in the diagnosis of vCJD; in the majority of cases, high signal is noted in the posterior thalamus (pulvinar) bilaterally on dual echo (T2 or proton density-weighted) MRI. Other common MRI features of vCJD are medial thalamic and peri-aqueductal grey matter high signal, and the notable absence of cerebral atrophy. All vCJD cases to date are homozygous for methionine at PRNP codon 129. A reduced frequency of human leukocyte antigen (HLA) class-II type DQ7 has been described in patients with vCJD, but not in those with classical CJD; this may have important implications for understanding host susceptibility to infection by BSE prions. A firm tissue based diagnosis of vCJD can be made during life by tonsil biopsy, with demonstration of a characteristic sub-type of PrPSc (see below).

Molecular classification of human prion diseases

The marked clinical heterogeneity observed in human prion diseases has yet to be explained. However, it has been clear for many years that distinct isolates, or strains, of prions can be propagated in the same host and these are biologically recognised by distinctive clinical and pathological features. Therefore, it is likely that a proportion of clinicopathological heterogeneity seen in sporadic CJD and other human prion diseases relates to the propagation of distinct human prion strains. Within the framework of the protein-only hypothesis of prion
propagation, the biological properties of distinct prion strains are thought\textsuperscript{1,2,4} to be encoded by differing physicochemical properties of PrP\textsuperscript{Sc}. The identification of strain-specific PrP\textsuperscript{Sc} structural properties would thus allow an aetiology-based classification of CJD by typing of the infectious agent itself.

Following the demonstration that different sub-types of PrP\textsuperscript{Sc} are associated with two strains of transmissible mink encephalopathy in hamsters\textsuperscript{50,51}, several human PrP\textsuperscript{Sc} types have been identified in the brain that are associated with different phenotypes of CJD\textsuperscript{23,52–56}. The different

Fig. 2 Human PrP\textsuperscript{Sc} types. (a) Western blot developed with monoclonal antibody 3F4 showing human PrP\textsuperscript{Sc} types 1-4. PrP\textsuperscript{Sc} types 1-3 are seen in the brain of classical forms of CJD (either sporadic or iatrogenic CJD), PrP\textsuperscript{Sc} type 4 is uniquely seen in vCJD brain. (b) Western blot developed with monoclonal antibody 3F4 showing type 4t PrP\textsuperscript{Sc} seen uniquely in tonsil of vCJD cases. (c) Relative proportions of di- and monoglycosylated PrP\textsuperscript{Sc} glycoforms following partial digestion with proteinase K. Error bars show SEM and where not visible were smaller than the symbols used to designate the mean.
fragment sizes seen on Western blots, following treatment with proteinase K, suggests that there are several different human PrP\textsuperscript{Sc} conformations, referred to as molecular strain types. These types can be further classified by the ratio of the three PrP bands seen after protease digestion, corresponding to amino-terminally truncated cleavage products generated from di-, mono-, or non-glycosylated PrP\textsuperscript{Sc}. Four types of human PrP\textsuperscript{Sc} have now been reliably identified using molecular strain typing (Fig. 2a)\textsuperscript{23,25,54,56}. Sporadic and iatrogenic CJD are associated with PrP\textsuperscript{Sc} types 1–3, while type 4 human PrP\textsuperscript{Sc} is uniquely associated with vCJD and is characterised by a fragment size and glycoform ratio that is distinct from PrP\textsuperscript{Sc} types 1–3 observed in classical CJD (Fig. 2a)\textsuperscript{23,25,54,56}. The methionine/valine polymorphism at codon 129 of \textit{PRNP} is associated with different PrP\textsuperscript{Sc} types. PrP\textsuperscript{Sc} types 1 and 4 have so far only been detected in methionine homozygotes, type 3 cases are predominantly associated with at least one valine allele, while type 2 is seen in any \textit{PRNP} codon 129 genotype\textsuperscript{23,48,49,54,56}. PrP\textsuperscript{Sc} types 1 and 2 are associated with two clinically distinct sub-types of sporadic CJD and have N-terminal structures determined by the co-ordination of metal ions\textsuperscript{54,56}. This represents a novel mechanism for post-translational modification of PrP, and for the generation of multiple prion strains in humans. Importantly, the identification of strain-specific PrP\textsuperscript{Sc} structural properties has enabled investigation of the influence of human PrP primary structure, in particular polymorphic residue 129, in determining PrP\textsuperscript{Sc} structure. Transgenic mice expressing human PrP with either valine or methionine at residue 129 have revealed that this polymorphism constrains both the propagation of distinct human PrP\textsuperscript{Sc} conformers and the occurrence of associated patterns of neuropathology (unpublished data)\textsuperscript{7,23,25}. These data strongly support the biological relevance of molecular strain typing which can now be applied to rapid molecular diagnosis of classical CJD or vCJD and to produce a new classification of human prion diseases.

In addition to the identification of human PrP\textsuperscript{Sc} types 1–4, molecular strain typing has provided insights into the phenotypic heterogeneity seen in inherited human prion diseases\textsuperscript{57}. In agreement with existing evidence that human prion strain diversity is generated through variance in PrP\textsuperscript{Sc} conformation and glycosylation, cases of inherited prion disease caused by point mutations in the \textit{PRNP} gene show glycoform ratios distinct from those observed in sporadic CJD and vCJD. Additionally, individuals with the same \textit{PRNP} mutation can propagate PrP\textsuperscript{Sc} with distinct fragment sizes. These intriguing observations provide the first clues into how diverse clinical phenotypes may arise in patients with the same \textit{PRNP} mutation and have identified key isolates whose transmission properties can be rigorously investigated in appropriate lines of transgenic mice expressing wild-type or mutated human PrP.

Molecular strain typing has major implications for epidemiological surveillance 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<sup>8</sup>, other causes for at least some cases, including environmental exposure to human or animal prions, has not been ruled out by existing epidemiological studies<sup>58</sup>. Sub-classification of sporadic CJD based upon PrP<sup>Sc</sup> type immediately allows a more precise molecular classification of human prion disease and re-analysis of epidemiological data using these molecular sub-types may reveal important risk factors obscured when sporadic CJD is analysed as a single entity. For example, it will be important to review the incidence of sporadic CJD associated with PrP<sup>Sc</sup> type 2 and other molecular sub-types in both BSE-affected and unaffected countries in the light of recent findings<sup>7</sup> suggesting that human BSE prion infection may result in propagation of either type 4 PrP<sup>Sc</sup> or type 2 PrP<sup>Sc</sup>. Individuals that propagate type 2 PrP<sup>Sc</sup> as a result of BSE exposure may present with prion disease that would be indistinguishable on clinical, pathological and molecular criteria from that found in classical CJD.

**Molecular diagnosis of vCJD through *ante-mortem* tonsil biopsy**

Concomitant with studies on human PrP<sup>Sc</sup> from brain has been investigation of PrP<sup>Sc</sup> in peripheral tissues in human prion diseases. These studies have established that the pathogenesis of vCJD differs significantly from that of other forms of CJD (Fig. 3). PrP<sup>Sc</sup> is readily detectable in lymphoreticular tissues in vCJD and not in classical CJD or inherited cases of human prion disease<sup>49,59,60</sup>. A distinctive PrP<sup>Sc</sup> type, designated type 4t, is consistently seen in both *ante-mortem* and *post-mortem* tonsil from patients with vCJD (Fig. 2b)<sup>49,60</sup>. Type 4t PrP<sup>Sc</sup> in tonsil differs in the proportions of the PrP glycoforms from type 4 PrP<sup>Sc</sup> seen in vCJD brain (Fig. 2c) implying the superimposition of tissue- and strain-specific effects on PrP glycosylation<sup>49</sup>. Tonsil biopsy is used for *ante-mortem* diagnosis of vCJD and, to date, all patients with a positive tonsil biopsy have been confirmed as vCJD at autopsy, if performed, or have had a subsequent clinical course consistent with vCJD. Importantly, all patients with a negative biopsy have been confirmed to have other diagnoses at autopsy, or the subsequent clinical course (significant improvement or recovery) excludes diagnosis of vCJD (unpublished)<sup>26,49,60</sup>. To date, tonsil biopsy has shown 100% sensitivity and specificity for diagnosis of vCJD and may allow diagnosis at an early clinical stage. Early diagnosis obviates the need for further investigation, which may include brain biopsy, necessary to exclude
alternative potentially treatable conditions and will be increasingly important with the advent of putative treatments and clinical trials where the aim must be to intervene early before extensive CNS damage has occurred. In natural sheep scrapie and in experimental murine models of scrapie, where there is also a prominent involvement of lymphoreticular tissues, PrPSc is detectable early in the incubation period long before clinical onset suggesting that PrPSc may be detectable in human tonsil and other lymphoreticular tissues a considerable period before patient presentation. Large-scale, anonymous screening of routine surgical tonsillectomy tissues for PrPSc may provide early warning of a high level of preclinical vCJD prion infection, and several such studies are in progress.

**Potential iatrogenic transmission of vCJD prions**

The demonstration of extensive lymphoreticular involvement in the peripheral pathogenesis of vCJD, raises concerns that iatrogenic transmission of vCJD prions through medical procedures may be a
major public health issue. Precautionary risk reduction measures have already been taken with respect to blood and blood products; however, major concerns remain relating to possible iatrogenic transmission of vCJD prions via contaminated surgical instruments. Transmission of vCJD to both transgenic and conventional mice involves a transmission barrier that severely limits the ability to detect low titre infectivity. Pending development of a transmission barrier-free model for bioassay of vCJD prions, high sensitivity immunodetection of PrPSc has been used to provide an upper limit on PrPSc levels in peripheral tissues, including blood, to inform risk assessment models. PrPSc is largely confined to the central nervous and lymphoreticular systems in vCJD. While a range of surgically important tissues have levels of PrPSc 10⁴–10⁵-fold lower than found in brain, the demonstration of PrPSc in rectal tissue points to a risk of iatrogenic transmission via surgical instruments used for gastrointestinal biopsy. Similarly, ophthalmic surgical instruments used in procedures involving optic nerve and the posterior segment of the eye, in particular the retina, also may represent a potential risk for iatrogenic transmission of vCJD.

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