

Clinical and Neuropathological Investigations in Creutzfeldt-Jakob Disease

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a group of rare and invariably fatal degenerative diseases of the central nervous system affecting humans as well as a number of animal species.¹ Enormous public and scientific attention has focused on prion diseases, not only because of their unique biological properties, but also because of their impact on animal and public health, particularly with the emergence of bovine spongiform encephalopathy (BSE)² and variant Creutzfeldt-Jakob disease (variant CJD) in the United Kingdom.³ Unlike other forms of CJD, infectivity is readily detectable within lymphoid tissues in variant CJD,⁴ raising concerns over the potential spread of variant CJD by iatrogenic means, particularly through surgical procedures and surgical instruments, as the infectious agent shows an alarming resistance to conventional decontamination methods. More recently it has been shown that variant CJD also appears to be transmissible by blood transfusion, heightening concerns over secondary human-to-human spread of the disease via contaminated blood products.^{5,6}

In humans, prion diseases occur in three main groups; they may occur sporadically, by autosomal dominant inheritance through mutations or insertions in the prion protein gene (PRNP), or by secondary transmission through either dietary or medical exposure to the infectious agent.⁷ Traditionally, human prion diseases are classified according to their major clinical features into Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), fatal familial insomnia (FFI) and kuru (Table 1). All forms of prion disease share four neuropathological features (spongiform vacuolation, neuronal loss, astrocytic and microglial proliferation and in certain cases the presence of amyloid plaques), which although characteristic of these disorders are not entirely specific.⁸

All prion diseases are associated with the conversion of the normal cellular host encoded prion protein, PrP^C, to an abnormal disease-associated isoform, PrP^{Sc}. PrP^{Sc} is not only a diagnostic marker of disease, but has been proposed as the sole or principal component of the transmissible agent in prion disease. According to the 'prion hypothesis', PrP^{Sc} is derived from the normal cellular protein (PrP^C) by a post-translational mechanism, which appears to involve a conformational change.⁹ This involves refolding of the protein to a structure containing a high beta sheet content, which readily forms aggregates and is more resistant to denaturation by proteases than PrP^C.

Genetic and molecular aspects of sporadic CJD

The most common form of human prion disease is sporadic CJD, which accounts for around 85% of all human prion diseases, with a world wide incidence of around 1-

1.5 cases per million of the population per annum. Like all human prion diseases, much phenotypic heterogeneity exists within sporadic CJD in terms of clinical and pathological features.¹⁰ This heterogeneity has been linked with the polymorphism found at codon 129 on PRNP which encodes either methionine (M) or valine (V).¹¹ This polymorphism has also been identified as an important risk factor in sporadic CJD; most cases occur in individuals who are homozygous for methionine at codon 129, who present with the most 'typical' clinical and pathological features. Cases of sporadic CJD in heterozygotes and valine homozygotes are rarer and display more 'atypical' phenotypes (Table 2).¹²

The physicochemical properties of PrP^{Sc} also play an important role in influencing the disease phenotype in sporadic CJD. Western blot analysis of the protease resistant core of PrP^{Sc}, referred to as PrPres, has identified two kinds of heterogeneity within the brains of patients with CJD. Firstly, differences occur in the mobility of the protease resistant core, presumably relating to different PrPres fragment sizes after proteinase K-mediated N-terminal truncation, and secondly, variation occurs in the relative abundance of the three PrP glycoforms (diglycosylated, monoglycosylated and nonglycosylated). Following the classification of Parchi et al.¹³ two distinct PrP^{Sc} types or PrPres isotypes, have been identified after proteinase K digestion: one with a mobility on western blot of around 21kDa named PrPres type 1, and the second, which is slightly smaller with a molecular weight of around 19kDa named PrPres type 2 (Figure 1).¹³ This classification system has been further subdivided to incorporate PrPres isotype combined with codon 129 PRNP genotype (MM, MV, VV) resulting in six different sporadic CJD subtypes.¹⁴ Examination of the clinical and pathological data from each of these subtypes shows that although not all have a distinct phenotype, there does appear to be a good correlation between clinical and neuropathological features and disease subtype (Table 2). More recently, the observation of CJD patients with more than one PrPres isotype within the brain has combined to increase the heterogeneity and complexity observed in sporadic CJD.

The presence of distinct strains of the infectious agent in prion diseases has been established for some time, particularly in relation to scrapie in sheep. However, the presence of individual strains remains difficult to explain within the bounds of the prion hypothesis, which proposes that all the information required for individual strain phenotypes is contained within the prion protein itself.⁹ In sporadic CJD, the different conformations of PrPres as determined by western blot analysis have been proposed to represent different biological profiles of the transmissible agent, which may in turn relate to different biological strains. Confirmation that these different molecular conformations or isoforms of the prion protein do indeed correspond to distinct strains will require analysis of the biological properties (such as incubation period and pat-



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Diane Ritchie started her career in prion diseases at the Institute for Animal Health, Neuropathogenesis Unit, under the guidance of Professor Moira Bruce looking at experimental models of scrapie. Currently she is studying at the National CJD Surveillance Unit looking at human prion diseases with Professor James Ironside, where she has refined the PET blot technique for use on human tissue.

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Aetiology	Disease
Sporadic	Sporadic Creutzfeldt-Jakob Disease Sporadic Fatal Insomnia
Familial	Familial CJD Gerstmann-Sträussler-Scheinker Fatal Familial Insomnia
Acquired	Kuru (human source) Iatrogenic CJD (human source) Variant CJD (bovine source)

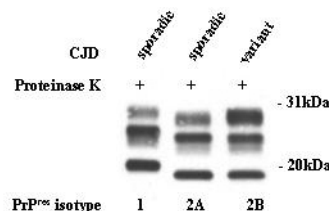
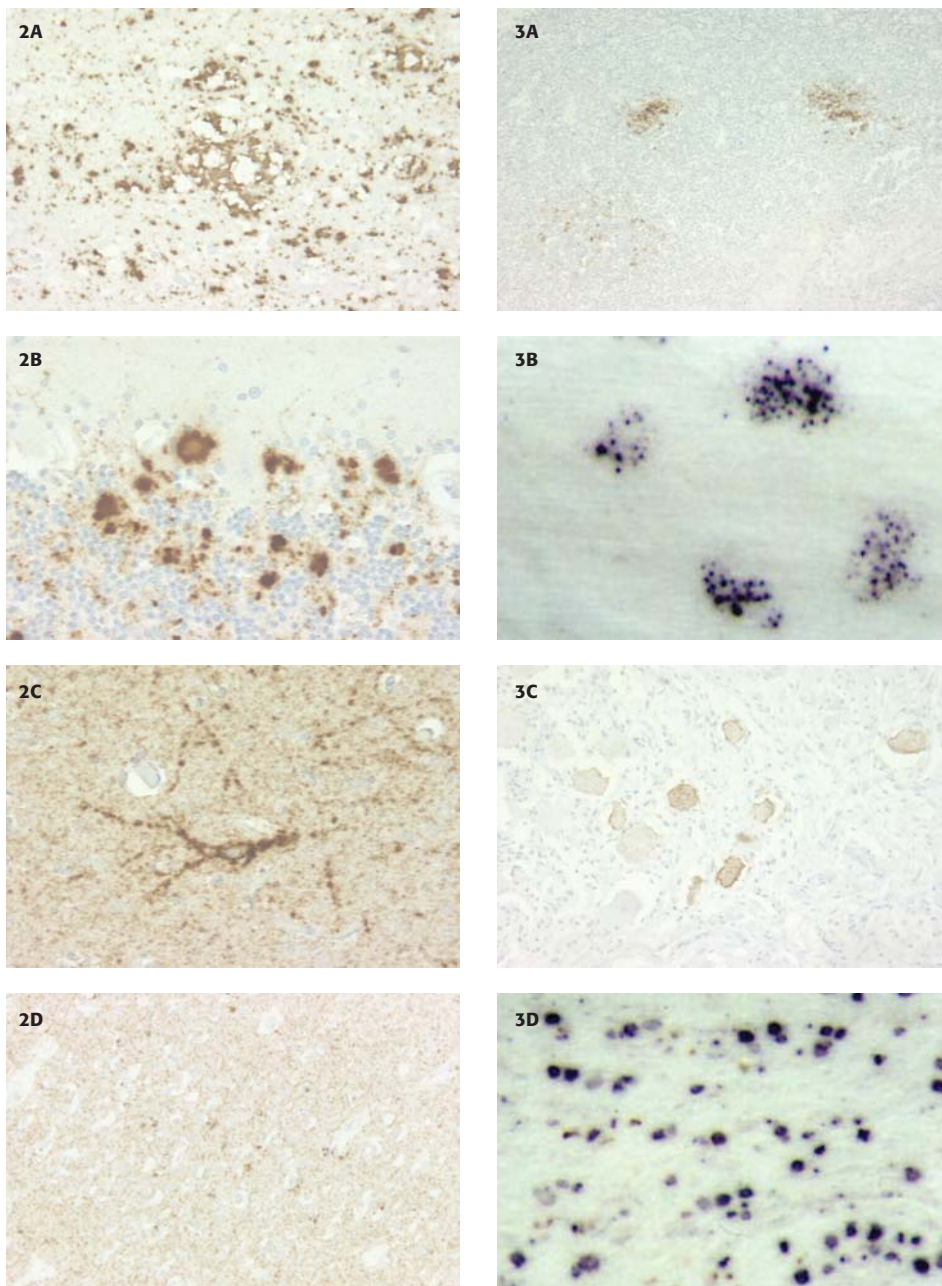


Figure 1: Western blot analysis of PrPres from the frontal cortex of two different sporadic CJD (s) patients, showing the type 1 and type 2 mobility variants. The distinctive type 2B pattern found in variant CJD (v) patients, with a predominance in the diglycosylated PrPres is also shown.

Table 2: Clinical and pathological features of sporadic CJD subtypes (adapted from Parchi et al, Ann Neurol 1999)¹⁴

Sporadic CJD Subtype	Mean disease duration (months)	Clinical symptoms	Patterns of PrP deposition
MM1/MV1	3.9	Cortical visual impairment (41% of cases), rapidly progressive dementia, involvement of the pyramidal and extrapyramidal systems, Myoclonus.	Widespread and intense perivacuolar deposits around areas of confluent spongiform change with synaptic labelling throughout the cerebral cortical layers.
MM2 (cortical variant)	15.7	Progressive dementia.	Intense perivacuolar labelling around areas of confluent spongiform change.
MM2 (thalamic variant)	15.6	Ataxia and cognitive impairment with the addition of insomnia.	PrP depositions less intense in this subtype; widespread synaptic positivity particularly targeting the occipital cortex; cerebellum relatively spared.
MV2	17.1	Dementia at clinical onset (50% of cases) often with ataxia or extrapyramidal signs.	Intense labelling of kuru plaques, most obvious in the cerebellum. Synaptic positivity present in the granular layer of the cerebellum.
VV1	15.3	Progressive dementia.	Weak and widespread synaptic labelling; cerebellum is relatively spared.
VV2	6.5	Progressive ataxia with dementia developing during later stages.	Perineuronal positivity within the cerebral cortex and intense plaque like deposits in the basal ganglia.



terns of neuropathology) after transmission to laboratory mice. In variant CJD, which is recognised as a distinct human prion strain closely related to the BSE strain in cattle,¹⁵ the biological profile on western blot analysis is also distinct from other human prion diseases, with a mobility much like that found in type 2 sporadic CJD cases, but with a unique glycoform ratio in which there is a predominance of the di-glycosylated band.¹⁶ The PrPres isotype of variant CJD patients is referred to as type 2B to distinguish it from the type 2 found in sporadic CJD (Figure 1). The PrPres isotype of variant CJD cases resembles that of BSE in cattle and in a range of other species, which has helped confirm that BSE was undoubtedly the source of variant CJD.¹⁶

Diagnosing human prion disease

Although clinical criteria for the diagnosis of human prion disease with a high degree of certainty have been agreed,¹⁷ a definitive diagnosis requires the examination of biopsy or post-mortem brain material for the presence of PrP^{Sc}. Immunohistochemical detection using antibodies raised against the prion protein is invaluable in the pathological diagnosis of

Figure 2: Immunohistochemistry for the prion protein (PrP) in sporadic CJD subtypes. All sections are immunolabelled with the KG9 anti-PrP antibody and counterstained with Haematoxylin. (A) Frontal cortex in the sporadic CJD MM1 subtype showing intense perivacuolar positivity around areas of confluent spongiform change. Original magnification x200. (B) Cerebellum in the sporadic CJD MV2 subtype showing intense positivity of kuru plaques in the granular layer. Original magnification x400. (C) Perineuronal labelling in the occipital cortex in the sporadic CJD VV2 subtype. Also shown is the widespread deposition of synaptic positivity. Original magnification x400. (D) Faint synaptic labelling within the cerebral cortex in the sporadic CJD VV1. Original magnification x200.

Figure 3: Detection of the prion protein (PrP) in peripheral organs in variant CJD comparing the PET blot analysis (3F4 anti-PrP antibody) and immunohistochemistry (KG9 anti-PrP antibody). (A) Immunohistochemistry and (B) PET blot analysis in the tonsil in variant CJD. (C) Immunohistochemistry and (D) PET blot analysis in a dorsal root ganglion in variant CJD.

prion diseases, but since all readily available anti-PrP antibodies recognise both PrP^C and PrP^{Sc}, a number of pre-treatments (autoclaving, formic acid treatment, and partial digestion with proteinase K) are required to denature any PrP^C, leaving PrP^{Sc} for diagnosis. Immunohistochemistry has demonstrated the numerous patterns of PrP^{Sc} accumulation within sporadic CJD ranging from the light synaptic depositions to the more intense and distinctive kuru plaques (Figure 2). These different patterns of PrP deposition have been studied extensively and attempts have been made to correlate these with the individual disease subtypes (Table 2).¹⁴ The advent of immunohistochemistry has witnessed an ever-increasing number of anti-PrP antibodies targeting different epitopes on the prion protein; these combined with the increasing number of improved immunodetection kits available has improved the sensitivity of immunohistochemistry. In certain cases, the detection of PrP^C in immunohistochemistry can prove problematic in the interpretation of staining results. Since only limited proteinase K digestion can be performed on tissue sections for histology, PrP^C is not always completely degraded, particularly in brain biopsy material. This problem is not encountered with western blot analysis of

frozen tissue, where a more rigorous digestion with proteinase K results in the complete digestion of PrP^C leaving only the protease resistant core of PrP^{Sc}. A combination of technical aspects from immunohistochemistry and western blot techniques has led to the development of the paraffin embedded tissue blot technique (PET blot).^{18,19} This method uses fixed paraffin tissue sections blotted on to nitrocellulose membrane to investigate the presence of PrP^{Sc}. As in western blot methods for PrP^{Sc}, the PET blot has an extensive pre-treatment step with proteinase K ensuring the complete digestion of PrP^C, but has the advantage of retaining some of the tissue architecture and some of the cellular detail of immunohistochemistry. The PET blot method has been utilised in a number of studies and has demonstrated increased sensitivity and specificity in the detection of PrP^{Sc}, for example in peripheral organs in variant CJD (Figure 3).^{19,21}

Future developments

The recent detection of PrP^{Sc} in tissues such as muscle in sporadic CJD²² has reinforced the need for more sensitive and specific detection techniques for PrP^{Sc}. Many exciting developments have been made using experimental models of prion disease in the development of

diagnostic screening tests and screening assays for PrP^{Sc}, which may also prove applicable in human prion diseases. The recent detection of low levels of PrP^{Sc} within blood samples of scrapie-infected hamsters has been described using protein misfolding cyclic amplification (PMCA) technology.^{23,24} This in vitro method has the ability of converting undetectable levels of PrP^{Sc} into larger PrP^{Sc} aggregates, by incubating with an excess of PrP^C. Repeated step-wise sonication of the newly formed PrP^{Sc} aggregates allows the conversion of further PrP^C molecules, resulting in larger PrP^{Sc} aggregates at levels which are easily detected by western blotting. This work is continuing by looking at the detection of PrP^{Sc} in blood from infected animals during the presymptomatic incubation period as well as investigating the detection of PrP^{Sc} in plasma and blood products. Whilst a diagnostic blood test for CJD may not be imminent, the potential for a blood-detection method is of prime concern in prion research and could potentially offer a valuable minimally invasive pre-clinical test for variant CJD, which may also have important implications in verifying the safety of donated blood and blood products, and in estimating the number of individuals in the UK who are infected with BSE.

Enormous public and scientific attention has focused on prion diseases, not only because of their unique biological properties, but also because of their impact on animal and public health

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