

# Neuropathology of Human Prion Diseases

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### Contents

1.	Introduction					
2.	Classification of Human Prion Diseases					
	2.1 Sporadic CJD	321				
	2.2 Variably Protease-Sensitive Prionopathy	322				
	2.3 Genetic Prion Diseases	323				
	2.4 latrogenic CJD	324				
	2.5 Variant CJD	324				
	2.6 Kuru	325				
	2.7 Diagnostic Criteria for Human Prion Diseases	325				
3.	The Autopsy in Human Prion Diseases	325				
	3.1 When to Suspect a Prion Disease Prior to Autopsy	325				
	3.2 Autopsy Considerations	326				
4.	Neuropathology					
	4.1 Macroscopic Pathology and Tissue Sampling	326				
	4.2 Brain Biopsies	327				
	4.3 Microscopy	327				
5.	Methods for the Detection of PrP <sup>SC</sup> and PrP <sup>res</sup> in Tissues	336				
	5.1 Immunohistochemistry for PrP	336				
	5.2 Paraffin-Embedded Tissue Blot for PrP <sup>res</sup>	337				
	5.3 Western Blot for PrP <sup>res</sup>	337				
	5.4 Recent Laboratory Techniques for PrP <sup>Sc</sup> Detection in Human Tissues	338				
6.	Conclusions	338				
Ac	Acknowledgments					
Ret	References					

#### Abstract

The human prion diseases comprise sporadic, genetic, and acquired disorders. These are rare conditions with a heterogeneous clinicopathologic phenotype, which can make diagnosis challenging. A combined clinical, genetic, neuropathologic and biochemical approach to diagnosis is therefore essential. Since prion infectivity is the highest in tissues from the central nervous system, special laboratory precautions are required for the

safe handling of these tissues. Neuropathologic assessment is generally performed following autopsy, when the fixed brain should be adequately sampled and studied by conventional stains and immunohistochemistry for the abnormal form of the prion protein. Frozen brain tissue is also required for DNA extraction for prion protein gene sequencing and for Western blot analysis of protease-resistant prion protein. The microscopic assessment of the nature and degree of spongiform change, neuronal loss, gliosis, and abnormal prion protein deposition in the brain can be used to determine the major categories of human prion disease. This information can be combined with clinical, genetic data, and biochemical data to allow an accurate diagnosis of a human prion disease and facilitates subclassification into recognized disease subtypes, for example in sporadic Creutzfeldt–Jakob disease. The spectrum of human prion diseases continues to expand and neuropathology will play a key role in the recognition and understanding of any further novel entities or disease variants that may emerge in the future.

# **1. INTRODUCTION**

The common human neurodegenerative diseases are associated with the accumulation of abnormal disease-associated proteins in the brain, for example  $\beta$ -amyloid and hyperphosphorylated tau in Alzheimer's disease and  $\alpha$ -synuclein in Parkinson's disease. Prion diseases are rare neurodegenerative disorders that are associated with the accumulation of an abnormal isoform of the prion protein (PrP<sup>Sc</sup>), which is derived by misfolding of the normal cellular isoform of the prion protein (PrP<sup>C</sup>).<sup>1</sup> Prion diseases differ from other human neurodegenerative disorders since they are transmissible between individuals and occur in sporadic, genetic, and acquired forms (Table 1).<sup>2</sup> The transmissible agent is unconventional and differs from bacteria and viruses in its physical, chemical, and biological properties. The major, if not the sole, component of the agent is PrP<sup>Sc</sup>, which acts as an infectious misfolded amyloidogenic protein (or prion) as proposed in the prion hypothesis.<sup>1</sup> The prion hypothesis offers a framework within which to classify and investigate human prion diseases (Table 1). Human prion diseases are characterized neuropathologically by spongiform change in affected gray matter regions, accompanied by variable neuronal loss, reactive gliosis, and the accumulation of PrPSc in the brain, as occurs in Creutzfeldt–Jakob disease (CJD).<sup>2</sup>

# 2. CLASSIFICATION OF HUMAN PRION DISEASES

Human prion diseases have previously been classified according to their clinicopathologic phenotype (for example, CJD, Gerstmann–Sträussler–Scheinker syndrome (GSS), or fatal familial insomnia (FFI)) and etiology

Etiology	Disease		
1. Idiopathic (sporadic)	Sporadic Creutzfeldt–Jakob disease (CJD)		
	Sporadic fatal insomnia		
	Variably protease-sensitive prionopathy		
2. Genetic (inherited)	Genetic CJD		
	Gerstmann–Sträussler–Scheinker disease		
	Prion protein cerebral amyloid angiopathy		
	Fatal familial insomnia		
3. Acquired (infectious)	Kuru		
	Iatrogenic CJD		
	Variant CJD		

 Table 1
 Human Prion Diseases Classified According to Etiology

 Etiology
 Disease

(sporadic, familial, and acquired), but current classification is increasingly dependent on both genetic and molecular criteria, specifically the presence of pathogenic mutations, insertions, deletions, and polymorphisms in the prion protein gene (*PRNP*) and the biochemical analysis of the isoforms of disease-associated prion protein in the brain, combined with the results of analysis of the neuropathologic lesions in the brain.<sup>2,3</sup>

#### 2.1 Sporadic CJD

Sporadic CJD (sCJD) is the commonest form of human prion disease, occurring most frequently in the seventh decade of life, with an incidence of 1–2 per million population per annum.<sup>2</sup> Cases of sCJD appear to arise spontaneously, perhaps due to a somatic mutation in the *PRNP* or a consequence of spontaneous misfolding of PrP<sup>C</sup>, resulting in the generation of PrP<sup>Sc</sup>. The commonest presenting clinical feature is rapidly progressive dementia, accompanied by other neurologic signs and symptoms, including myoclonus, ataxia, and visual problems. Death usually occurs within 6 months of the disease onset. Electroencephalography (EEG) and cerebrospinal fluid (CSF) 14-3-3 protein analysis are helpful investigations in the clinical diagnosis of sCJD (see below); the detection of PrP<sup>Sc</sup> in the CSF by the real-time quaking-induced conversion assay (RT-QuIC) has recently proven to be of high specificity and sensitivity as a diagnostic tool.<sup>4</sup> Magnetic resonance imaging (MRI) of the brain in sCJD characteristically shows signal

	ММ	MM	MV	
Normal population <sup>a</sup>	39	11	50	
Sporadic CJD <sup>b</sup>	60	20	20	
Variant CJD <sup>c</sup>	99		1	

 Table 2 PRNP Codon 129 Polymorphisms in Normal Populations and in Prion Diseases

 PRNP Codon 129 Polymorphisms (%)

<sup>a</sup>Data pooled from five studies (http://www.cjd.ed.ac.uk/sites/default/files/report24.pdf).

<sup>b</sup>UK deaths from sporadic CJD 1996–2014 for whom genetic data are available (*n*=856) (http://www.cjd.ed.ac.uk/sites/default/files/report24.pdf).

<sup>c</sup>National CJD Research & Surveillance Unit 2017 (http://www.cjd.ed.ac.uk/sites/default/files/world figs\_0.pdf).

CJD: Creutzfeldt-Jakob disease; M: methionine; V: valine.

hyperintensities in the cortical ribbon and in the basal ganglia on diffusionweighted imaging.<sup>2</sup>

However, sCJD is heterogeneous in terms of clinicopathologic phenotype; individual cases broadly conform to a range of subtypes which are classified according to the *PRNP* codon 129 polymorphism (Table 2) and the isoform (type) of the protease-resistant prion protein ( $PrP^{res}$ ) in the brain, and are associated with characteristic neuropathologic features (see below).<sup>3</sup>

#### 2.2 Variably Protease-Sensitive Prionopathy

Variably protease-sensitive prionopathy (VPSPr) is the most recently identified human prion disease.<sup>5</sup> VPSPr appears to be a sporadic disease of unknown etiology. Patients do not have any risk factors for acquired human prion diseases, nor have there been any *PRNP* mutations associated with VPSPr. Clinical diagnostic criteria for VPSPr have not yet been defined; patients are generally elderly and present with a range of neurological abnormalities including movement disorders, ataxia, and cognitive decline. The duration of the clinical illness is longer than for sCJD. A diagnosis of VPSPr is difficult to make in life and many of the reported cases have been diagnosed after autopsy based on the neuropathological findings and brain PrP biochemistry (see below). Retrospective studies have suggested that VPSPr is a rare disorder, but its rather poorly defined clinical features suggest that it may be underascertained and hence no accurate figures for its incidence and prevalence are available.<sup>6</sup>

# 2.3 Genetic Prion Diseases

Genetic prion diseases are inherited diseases that occur as autosomal dominant disorders with high penetrance. Genetic prion diseases are estimated to represent approximately 10%–15% of all forms of human prion disease.<sup>2</sup> Not all cases have a family history of prion disease, but some patients give a family history of other neurological disorders, possibly as a result of misdiagnosis of prion diseases in the past. Cases without a family history may occur as a consequence of spontaneous mutation. Over 40 different pathogenic *PRNP* mutations (missense, insertion, deletion, and amber mutations) have been identified to date.<sup>2</sup> The age at onset of clinical symptoms is remarkably variable, even within affected families.

A wide range of clinicopathologic phenotypes have been reported, including genetic CJD, GSS, and FFI. The *PRNP* polymorphisms at codons 129 and 219 on both the mutant and nonmutated alleles have a substantial influence on the clinicopathologic phenotype.

- Genetic CJD closely resembles sCJD, in its clinical and pathologic features; *PRNP* sequencing in cases of apparent sCJD with no family history may reveal occasional cases with a *PRNP* mutation association with genetic CJD, the commonest of which is the E200K mutation.
- GSS presents clinically as a progressive spinocerebellar syndrome with variable neurologic signs and symptoms including pyramidal signs and progressive cognitive decline, but dementia are not always present. Some affected families have previously been given a diagnosis of other forms of hereditary ataxia, such as spinocerebellar ataxia. The P102L mutation was the first mutation to be associated with GSS; an increasing number of point mutations also associated with GSS have subsequently been identified.<sup>2</sup>
- FFI is characterized clinically by sleep disturbance and dysautonomia, with other variable neurologic signs and symptoms including myoclonus, cognitive abnormalities, and pyramidal signs. FFI was recognized clinically for many years before the underlying *PRNP* abnormality was identified: the *PRNP* D178N–129M haplotype.<sup>2</sup>
- The insertional, deletion, and amber *PRNP* mutations are associated with variable clinical phenotypes, some of which resemble sCJD while others resemble GSS; in the past, patients with these mutations have been misclassified clinically as Alzheimer's disease or Huntingdon's disease.
- A novel clinicopathologic phenotype of genetic human prion disease has recently been reported, characterized by diarrhea, autonomic neuropathy,

and a systemic PrP amyloidosis. A previously unreported *PRNP* Y163X truncation mutation was found in affected family members.<sup>7</sup>

#### 2.4 latrogenic CJD

Iatrogenic CJD (iCJD) was first reported in 1974 in a patient who had received a corneal transplant from a donor with sCJD. Over 450 cases of iCJD have subsequently been reported, with occasional cases representing transmission by contaminated neurosurgical instruments and intracerebral electrodes. However, the majority of cases have occurred in recipients of human dura mater grafts human or pituitary growth hormone. Incubation periods vary from around 2–4 years in cases associated with neurosurgery or intracerebral electrodes to over 3 decades in some human pituitary hormone recipients. Iatrogenic CJD has a variable clinicopathologic phenotype; some patients have clinical features similar to those in sCJD, whereas progressive cerebellar signs and symptoms with other focal neurologic abnormalities and late dementia are common in human pituitary hormone recipients who develop iCJD.<sup>2</sup>

#### 2.5 Variant CJD

Variant CJD (vCJD) was first reported in 1996 as a novel prion disease that was linked to dietary exposure to the bovine spongiform encephalopathy (BSE) agent the United Kingdom. Over 230 cases of vCJD have since been identified worldwide, with 178 cases occurring in the United Kingdom.<sup>8</sup> Three cases of vCJD have been transmitted by blood transfusion in the United Kingdom. Although the number of new vCJD cases worldwide is declining, the number of future vCJD cases is uncertain; evidence of asymptomatic vCJD infection has been found in around 1 in 2000 of UK appendix specimens tested.<sup>9</sup>

Variant CJD has a younger age of onset than sCJD, with most cases occurring in the third decade of life. In contrast, the clinical illness is longer (mean 14 months) and usually begins with psychiatric and/or sensory manifestations followed by ataxia, pyramidal and extrapyramidal signs and cognitive impairment. Cranial MRI shows hyperintensity in the posterior thalamus (the "pulvinar sign") on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images.<sup>2</sup> All confirmed cases of vCJD worldwide were *PRNP* codon 129 methionine homozygotes, but in 2016 a case of definite vCJD with a *PRNP* codon 129 heterozygote (methionine/valine) genotype was identified,<sup>10</sup> raising the possibility of further similar cases in the future (Table 2).

# 2.6 Kuru

Kuru occurred as an epidemic among the Fore tribe of Papua New Guinea in the 1950s as a neurologic disorder associated with ritualistic cannibalism.<sup>2</sup> Kuru was responsible for many deaths in the women and children of the tribe. The incidence of kuru declined after this ritual was discouraged. The final patients with kuru died in the early 21st century, with incubation periods extending over 4 decades. Kuru is now extinct and will therefore not be considered at any greater length here.

# 2.7 Diagnostic Criteria for Human Prion Diseases

Human prion diseases benefit from internationally agreed diagnostic criteria that refer to clinical features, duration of illness, results of clinical investigations, *PRNP* analysis, and neuropathology to allow suspected cases to be classified as "possible," "probable," or "definite."<sup>11</sup> A "definite" diagnosis requires neuropathological confirmation.

Useful clinical investigations include:

- 1. EEG: periodic triphasic complexes in 2/3 of sCJD cases.
- 2. MRI of the brain: increased signal in T2 sequences in the basal ganglia and cortex in sCJD; increased signal in T2/FLAIR sequences in the posterior thalamus and midbrain in vCJD (the "pulvinar sign").
- **3.** CSF protein analysis: elevated levels of 14.3.3 and S100 proteins in sCJD; normal 14.3.3 levels with elevated phospho-tau levels in vCJD.<sup>2</sup>
- **4.** RT-QuIC positivity for PrP<sup>Sc</sup> in CSF or other tissues.<sup>4,12</sup>

For more detailed information, please see: "Diagnostic criteria for human prion disease."<sup>11</sup>

# 3. THE AUTOPSY IN HUMAN PRION DISEASES

# 3.1 When to Suspect a Prion Disease Prior to Autopsy

The differential clinical diagnosis of a progressive neurodegenerative disorder not infrequently includes sCJD and other human prion diseases. The likelihood of a prion disease under these circumstances can be assessed by reference to the diagnostic criteria for human prion diseases.<sup>11</sup> However, sCJD is not a uniform disorder and not all cases show the typical changes described in the investigations above; furthermore, some patients may have a prolonged duration of illness, while others have atypical presentations, such as sudden stroke-like features. Genetic prion diseases usually, but not always, have a family history of a neurodegenerative disorder. Although this may have been identified as genetic CJD, GSS of FFI, the disease occurring in earlier affected family members may have been misdiagnosed, particularly if *PRNP* sequencing had not been performed. Phenotypic variability also occurs in genetic prion diseases, even within a single affected family.

The clinical history should also be reviewed for other potential risk factors for a human prion disease, such as a previous history of treatment with human growth hormone, or previous neurosurgery that includes a dura mater graft. Not all the relevant clinical details for these and other risk factors may be available before an autopsy is to be performed, in which case a precautious approach (see below) is required in view of the uncertainty concerning potential exposure to human prions.

#### 3.2 Autopsy Considerations

Special equipment is required when performing an autopsy on a case of suspected human prion disease, preferably including a dedicated "high risk" autopsy room with trained mortuary staff, personal protective equipment, and single use-disposable instruments. If a dedicated autopsy room is not available, the autopsy may proceed in the mortuary as long as no other autopsies are being performed at the same time and care is taken to minimize any potential contamination of the mortuary environment.

The autopsy should be performed according to WHO guidelines for personal protection and the cleaning and decontamination of reusable instruments and the mortuary environment<sup>13</sup>; if potential CJD autopsies are performed frequently, consideration should be given for establishing and using a dedicated set of instruments that are not used in general autopsies. In addition to retaining fixed and frozen brain tissue in accordance with the consent given for the autopsy (see below), sampling of other tissues can also be of diagnostic value, particularly the lymphoreticular system (spleen, tonsil, and lymph nodes) in suspected cases of vCJD.

# 4. NEUROPATHOLOGY

#### 4.1 Macroscopic Pathology and Tissue Sampling

Macroscopic examination of the brain in cases of prion disease shows no specific abnormalities, with age-related changes present. Cerebral and cerebellar atrophy may be present in cases with a long duration of illness, when the term "panencephalopathic CJD" may be appropriate. Interestingly, the hippocampi in such cases usually do not appear to be proportionally atrophied. Cerebellar atrophy may present in some cases of sCJD (particularly the VV2 subtype) and in other groups of human prion disease with significant cerebellar involvement, including GSS, vCJD, and iCJD in human growth hormone recipients.

Extensive sampling of the brain for microscopy is recommended in cases of suspected prion disease, as the nature and distribution of the lesions can be variable in each disease entity, and between cases of the same disease. Blocks should be taken from all regions of the cerebral cortex, the hippocampus, basal ganglia, thalamus, cerebellum, and brain stem. Formic acid pre-treatment of fixed tissue blocks prior to processing and embedding in paraffin wax is advised to significantly reduce potential infectivity, including blocks of any non-CNS tissues sampled.<sup>13</sup>

#### 4.2 Brain Biopsies

Brain biopsies in patients with suspected prion disease are not performed as a routine investigation, since the neurosurgical instruments that make contact with brain tissue have to be destroyed after use if the diagnosis is confirmed, to eliminate the possibility of subsequent iatrogenic transmission following reuse.<sup>13</sup> Routine hospital processing and decontamination of instruments do not remove prion infectivity, which binds avidly to steel surfaces. However, occasionally a brain biopsy may be required to exclude the possibility of an alternative treatable condition (such as cerebral vasculitis) in the differential diagnosis. In this situation, it is important to obtain both fixed and frozen tissue from the biopsy specimen to allow both neuropathological and biochemical investigations to aid diagnosis; the fixed tissue can be pretreated with formic acid prior to embedding to reduce prion infectivity.<sup>13</sup>

#### 4.3 Microscopy

#### 4.3.1 Microscopic Pathology

The characteristic neuropathological features of human prion diseases comprise spongiform change in affected gray matter regions, with reactive astrocytosis and microglial proliferation, accumulation of PrP<sup>Sc</sup> in a variety of patterns, and amyloid plaque formation in some cases.<sup>2</sup> These features vary in their nature, distribution, and severity between different disease entities and are also highly variable within a single brain, reinforcing the need for adequate sampling of the brain for histological diagnosis.

#### 4.3.2 Spongiform Change

Spongiform change is characterized by numerous rounded vacuoles in the gray matter that can vary from 2 to  $20\,\mu\text{m}$  in diameter. These occur in variable numbers and can range from small discrete vacuoles (microvacuolar spongiform change) to large coalescent vacuoles (confluent spongiform change) that give a sponge-like appearance (Fig. 1). It is important not to confuse tissue fixation and processing artifacts such as retraction artifact with



**Fig. 1** (A) Microvacuolar spongiform change in the cerebral cortex in the MM/MV1 subtype of sCJD (hematoxylin and eosin). (B) Granular/synaptic PrP accumulation in the MM/MV1 subtype of sCJD (12F10 antibody). (C) Confluent spongiform change in the MM2 (cortical) subtype of sCJD (hematoxylin and eosin). (D) Perivacuolar accumulation of abnormal PrP around a region of confluent spongiform change (12F10 antibody). (E) Perineuronal accumulation of abnormal PrP in the VV2 subtype of sCJD (12F10 antibody). (F) Intense labeling of a kuru-type plaque in the cerebellum in the MV2 subtype of sCJD (12F10 antibody).

spongiform change. The rounded vacuoles of spongiform change should also be distinguished from gray matter vacuolation in a range of unrelated conditions, including cerebral edema and metabolic or toxic encephalopathies. In these conditions, vacuolation is usually also present in the white matter, unlike in human prion diseases. The vacuolation in metabolic encephalopathies and toxic disorders is usually accompanied by other histological abnormalities that allow a distinction from human prion diseases. However, other neurodegenerative disorders can exhibit focal gray matter vacuolation that is indistinguishable from spongiform change, particularly in the temporal cortex in cases of Lewy body dementia. Immunohistochemistry for disease-associated prion protein in such cases (and in the other nonprion disorders mentioned above) is negative, and a full histological assessment will allow an appropriate diagnosis to be made.

Status spongiosis is the neuropathological term used when severe cerebral cortical gray matter vacuolation is accompanied by extensive neuronal loss, gliosis, and collapse of the cortical architecture. Status spongiosis is characteristically present in panencephalopathic sCJD, but it is a nonspecific finding that can occur in other disorders associated with severe cortical neuronal loss, including frontotemporal lobar degeneration and severe hypoxic brain damage.

#### 4.3.3 Amyloid Plaques

A variety of amyloid plaques with different morphologies has been identified in some sporadic, genetic, and acquired human prion diseases (Figs. 1 and 2). These plaques are composed of  $PrP^{Sc}$  arranged in dense  $\beta$ -sheet aggregates, which differ in composition and morphology from the amyloid plaques in other neurodegenerative disorders such as Alzheimer's disease and are most frequently present in the cerebellar cortex. Tinctorial stains for amyloid plaques such as the Congo red stain can be used to demonstrate these plaques, but they are most easily visualized using immunohistochemistry for disease-associated prion protein. A range of distinct plaque morphologies have been described:

• Kuru-type plaques, composed of a solid core and radiating fibrils, were first described in kuru, but are also present as a diagnostic feature in the MV2 subtype of sCJD,<sup>3</sup> and in some iCJD cases, including dura mater graft and human pituitary growth hormone recipients.<sup>2</sup> They occur most frequently in the cerebellar cortex (granular layer and molecular layer), but can occasionally be found in other gray matter regions, including the cerebral cortex and basal ganglia.



**Fig. 2** (A) Numerous microplaques in the cerebellar cortex in VPSPr (*PRNP* codon 129 VV) (12F10 antibody). (B). Multicentric amyloid plaques in the cerebellar cortex in GSS (*PRNP* codon 129 P102L) (12F10 antibody). (C) Unusual linear deposits of abnormal PrP in the cerebellar flat cortex in genetic CJD with four octapeptide repeat inserts in the *PRNP* (12F10 antibody). (D). Florid plaques in the cerebral cortex in vCJD surrounded by a "halo" of spongiform change (hematoxylin and eosin). (E) PrP immunohistochemistry shows widespread accumulation of PrP<sup>Sc</sup> in florid plaques and in multiple smaller deposits in vCJD (12F10 antibody). (F) A paraffin-embedded tissue blot demonstrates protease-resistant prion protein accumulation in the germinal centers of the spleen in vCJD (3F4 antibody).

• Microplaques, which are a diagnostic feature of VPSPr and occur most frequently in the molecular layer of the cerebellar cortex.<sup>5</sup> These small lesions may also be present in other gray matter regions, including the cerebral cortex, basal ganglia, and thalamus.

- Multicentric plaques, which are large irregular lesions with multiple cores that give a multicentric appearance on both tinctorial stains and immunohistochemistry for PrP. They are diagnostic of GSS and can be found most frequently in the cerebellar cortex, also occasionally in the cerebral cortex and basal ganglia. Multicentric plaques are associated with several different *PRNP* mutations, the most common of which is the P102L mutation.<sup>2</sup>
- Florid plaques, which are composed of a central eosinophilic amyloid core with radiating peripheral fibrils surrounded by a corona of vacuolation. They are a diagnostic feature of vCJD in both *PRNP* codon 129 MM and MV genotypes and are present in large numbers in the cerebral cortex and cerebellar cortex.<sup>2</sup>

Immunohistochemistry for prion protein is essential for the neuropathological diagnosis of human prion diseases and is described in further detail below.

#### 4.3.4 Sporadic CJD

Sporadic CJD has a variable clinical and pathological phenotype, which has been found to broadly correspond to a subclassification based on the results of PRNP sequencing and Western blot analysis for PrPres.<sup>3</sup> The combinations of the PRNP codon 129 polymorphism (Table 2) and the PrPres isoform (Fig. 3) allows for six combinations (MM1, MV1, MM2, MV2, VV1, and VV2) that appear to be associated with certain neuropathologic features, often accompanied by suggestive clinical findings<sup>3</sup> (Fig. 1). There is some overlap of the features between these groups; for example, the MM1 and MV1 subgroups share common clinical and neuropathologic features and are often combined into a single subgroup, MM/MV1. In contrast, the MM2 subgroup is heterogeneous in terms of both clinical and pathological features and is usually subdivided into the MM2 cortical (MM2C) and MM2 thalamic (MM2T) subgroups. The MM2T subgroup corresponds to a phenotype that is closely resembles the genetic prion disease FFI and is now often referred to as sporadic fatal insomnia.<sup>2,3</sup>

The diagnostic neuropathologic features in the sCJD subgroups defined in this classification are summarized in Table 3. However, not all cases of sCJD fit easily into this classification, since some cases have atypical clinical and pathologic features. Also, Western blot analysis of the brain can show combinations of types 1A and 2A PrP<sup>res</sup> either in different areas of the same



Fig. 3 (A) A diagrammatic representation of the main protease-resistant prion protein (PrP<sup>res</sup>) types found in the human prion disease brain as determined by proteinase K digestion and Western blot analysis. The classification of the banding pattern has two components, one numerical depending on the migration of the bands and the other alphabetical depending on their relative abundance. The pattern is termed type 1 if the nonglycosylated (*bottom*) band is  $\sim$ 21 kDa, type 2 if the nonglycosylated band is  $\sim$ 19 kDa or type 1+2 if both bands are found. Type 2 can occur as a doublet and is termed type 2(d). The pattern is given the suffix A if the *middle* or *bottom* (mono- or nonglycosylated) bands predominate, B if the top (diglycosylated) band predominates or A/B if the glycosylated bands (*middle* and *top*) predominate at the expense of the nonglycosylated (bottom) band. A pattern dominated by a low molecular mass unglycosylated band is termed 8 kDa. The faint ladder of bands that sometimes accompanies the 8 kDa band is shown in gray. Types 1A, 2A, 2A(d), and 1+2(A) are characteristic of sporadic and iatrogenic CJD. Type 2B is associated with variant CJD. Types 1B, 1A/B, and 2A/B are often found in genetic CJD, GSS, and FFI, respectively. The 8 kDa pattern is characteristic of some cases of GSS and of VPSPr. (B) shows a representative Western blot image of sCJD MM1 subtype (1A), sCJD VV2 subtype (2A), and vCJD (2B).

brain, or even in the same brain region in sCJD (Fig. 3). Although an updated classification system taking some of these findings into account has been proposed by Parchi *et al.*<sup>14</sup> this has not yet been universally adopted.

Subtype	Median Age at Onset (Years)	Median Duration of Illness (Months)	Frequency (%)	Neuropathologic Diagnostic Features	PrP Deposition Patterns
MM1 MV1	66 73	3 5	57 6	Widespread microvacuolar spongiform change in cerebral cortex and cerebellum	Granular, synaptic
MM2 cortical	52	17	7	Confluent spongiform change in cerebral cortex	Perivacuolar
MM2 thalamic (sporadic fatal insomnia)	53 <sup>b</sup>	16 <sup>b</sup>	<1	Thalamic neuronal loss and gliosis in anterior nuclei. Limited spongiform change	Granular, synaptic
VV1	53	10	2	Microvacuolar spongiform change: Cerebral cortex (temporal lobe)	Synaptic
VV2	66	6	14	Linear microvacuolar/confluent spongiform change in cerebral cortex layer 3; severe cerebellar neuronal loss and gliosis	Perineuronal, synaptic, plaque-like
MV2	65	11	14	Kuru-type plaques in cerebellar cortex Widespread microvacuolar $\pm$ confluent spongiform change	Kuru plaques, synaptic, plaque-like

 Table 3 Sporadic CJD Subtypes Classified by PRNP Codon 129 Genotype and PrP<sup>res</sup> Type<sup>a</sup>

<sup>a</sup>Cases with mixed PrP<sup>Sc</sup> subtypes are not included. <sup>b</sup>Data represent a single case. Data from the UK National Creutzfeldt–Jakob Disease Research & Surveillance Unit (www.cjd.ed.ac.uk/).

#### 4.3.5 VPSPr

VPSPr is the most recently identified human prion disease, which (as its name suggests) is defined not by its clinical or pathologic features, but by the findings on Western blot examination for  $PrP^{res}$  in the brain<sup>5</sup> (Fig. 3). In contrast to sCJD, VPSPr occurs most commonly in the *PRNP* codon 129VV genotype, with the codon 129 MM genotype being the least frequent. VPSPr is characterized neuropathologically by the presence of spongiform change with intermediately sized vacuoles and amyloid microplaques, which occur most commonly in the cerebellum<sup>6</sup> (Fig. 2). The neuropathology of VPSPr is influenced by the *PRNP* codon 129 genotype, with the MM genotype usually containing more plaques in the cerebrum.<sup>6</sup> The PrP<sup>Sc</sup> in the brain in VPSPr is relatively poorly resistant to proteinase K digestion, resulting in the appearance of characteristic, N-and C-terminally truncated ~8 kDa PrP<sup>res</sup> bands in Western blots, sometimes accompanied by a faint ladder of bands extending into the 18–30 kDa range<sup>5</sup> (Fig. 3).

#### 4.3.6 Genetic CJD

Microscopic examination of the brain shows features similar to sCJD, usually corresponding to the MM/MV1 subtype, with microvacuolar spongiform change and granular/synaptic accumulation of disease-associated PrP on immunohistochemistry. The microscopic features can vary depending on the associated *PRNP* mutation.<sup>2</sup> The commonest *PRNP* mutation to be associated with genetic CJD is the E200K mutation, which has a worldwide distribution. The mutant allele is usually *PRNP* codon 129-M. Western blot analysis of brain PrP<sup>res</sup> characteristically shows type 1B or 1A/B type (Fig. 3).

#### 4.3.7 GSS

Multicentric PrP amyloid plaques in the cerebellar cortex are the diagnostic neuropathological feature of GSS (Fig. 2). Spongiform change is often absent in the cerebellum and is variable in its distribution in other brain regions. Some of the associated *PRNP* mutations exhibit particular neuropathologic features, such as neurofibrillary tangles in the cerebral cortex in cases with the *PRNP* F198S mutation. Western blot analysis of the brain PrP<sup>res</sup> in GSS demonstrates a range of findings, including types 1B, 1A/B, or the 8 kDa pattern, the latter usually found in brain regions containing amyloid plaques (Fig. 3).

#### 4.3.8 FFI

FFI is characterized by severe thalamic gliosis and neuronal loss in the anterior thalamic nuclei and occasionally in other thalamic and hypothalamic nuclei. Spongiform change and disease-associated PrP accumulation in the thalamus are difficult to detect, but may be present in the entorhinal cortex or cerebellum. FFI is associated with the *PRNP* D178N-129M haplotype. The *PRNP* D178N mutation is also found in some patients with genetic CJD, but in a D178N-129V haplotype.<sup>2</sup> Western blot analysis in FFI shows a brain PrP<sup>res</sup> type 2A/B (Fig. 3).

#### 4.3.9 Insertional, Deletion, and Amber PRNP Mutations

This group of genetic prion disease exhibits a range of neuropathologic features that vary according to the associated genetic abnormality. Some of the smaller insertional mutations have features resembling sCJD, but in cases with four octapeptide repeat inserts or more PrP immunohistochemistry can demonstrate characteristic linear deposits in the cerebellar molecular layer (Fig. 2). In some of the rare amber mutations disease-associated PrP accumulates in the walls of intracerebral blood vessels, resulting in a PrP amyloid angiopathy.<sup>2</sup> Western blot analysis of brain PrP<sup>res</sup> in patients with insertional mutations usually shows PrP<sup>res</sup> types resembling sCJD type 1A or 2A (Fig. 3).

#### 4.3.10 Variant CJD

Large numbers of florid plaques within the cerebral cortex and the cerebellum are a diagnostic neuropathologic feature of vCJD (Fig. 2). Other characteristic features include extensive disease-associated PrP accumulation as small cluster plaques and diffuse deposits in the brain on immunohistochemistry or paraffin-embedded tissue (PET) blot analysis (Fig. 2). Spongiform change is widespread, particularly in the caudate nucleus and putamen. The thalamus shows severe neuronal loss and gliosis in the pulvinar, corresponding to the characteristic MRI abnormalities. Western blot analysis shows a uniform brain PrP<sup>res</sup> type 2B (Fig. 3).

Disease-associated PrP is also present outside the CNS in follicular dendritic cells within germinal centers in lymphoreticular tissues including the tonsil, lymph nodes, and spleen<sup>15</sup> (Fig. 2). Western blot analysis of these tissues also demonstrates PrP<sup>res</sup> type 2B (Fig. 3). Tonsil biopsy has been employed as a diagnostic test for some patients with suspected vCJD, using both immunohistochemistry for disease-associated PrP and Western blot analysis for PrP<sup>res</sup>.

#### 4.3.11 latrogenic CJD

The neuropathology of iCJD in general resembles that of sCJD, but can vary depending on the source of infection and the patient's *PRNP* codon 129 subtype.<sup>2</sup> Kuru-type plaques can occur in the cerebellum in some human growth hormone and dura mater graft recipients, not only in patients with a *PRNP* codon 129 MV genotype, but also in patients with a *PRNP* codon 129 MM genotype. This finding may be influenced by the strain of the transmissible prion agent involved. Western blot analysis shows a range of brain PrP<sup>res</sup> isoforms resembling those found in sCJD, but *PRNP* codon 129 MM iCJD cases with kuru-type plaques in the cerebellum may contain a PrP<sup>res</sup> type that is intermediate in size between type 1 and type 2, either on its own or as a doublet (Fig. 3).

Recent studies of iatrogenic CJD in human growth hormone and human dura mater graft recipients have found evidence of increased A $\beta$  deposition in the brain, both as diffuse and neuritic plaques and as amyloid angiopathy.<sup>16</sup> Since low levels of A $\beta$  can be present in both the pituitary gland and dura mater, these findings raise the possibility that iatrogenic amyloid seeding of A $\beta$  has occurred in the brains of these patients by a prion-like mechanism.

# 5. METHODS FOR THE DETECTION OF PrP<sup>sc</sup> AND PrP<sup>res</sup> IN TISSUES

#### 5.1 Immunohistochemistry for PrP

Immunohistochemistry for PrP detection is essential in the neuropathologic diagnosis of human prion diseases.<sup>2</sup> Disease-associated prion protein can accumulate in the brain in a variety of patterns, ranging from widespread synaptic and perineuronal accumulations to perivacuolar, granular, and plaque-like accumulation. The recognition of these different patterns of accumulation is required both for diagnosis and for the identification of the different subtypes of sCJD<sup>3</sup> (Table 3; Fig. 1).

Most of the available antibodies to PrP do not distinguish between the normal cellular form of the protein, PrP<sup>C</sup>, and the disease-associated iso-form, PrP<sup>Sc</sup>, so it is necessary to introduce pretreatment steps to optimize the labeling of the disease-associated isoform and to minimize the labeling of the normal isoform.<sup>17</sup> The pretreatment protocols generally include steps to denature PrP<sup>C</sup> in the tissue sections before the antibodies are applied, for example by autoclaving the sections, or the use of proteinase K to digest PrP<sup>C</sup> in the tissue sections.<sup>17</sup> The available anti-PrP antibodies recognize

a range of different epitopes on the protein. Consequently, the use of these different antibodies may produce differential labeling in tissue sections, as observed recently in VPSPr, where the use of four different antibodies resulted in differential labeling of the microplaques in the brain.<sup>6</sup> Immuno-histochemistry can also be used to detect disease-associated prion protein in non-CNS tissues, particularly in the germinal centers of lymphoid tissues and autonomic ganglia in vCJD<sup>15</sup> (Fig. 2).

#### 5.2 Paraffin-Embedded Tissue Blot for PrP<sup>res</sup>

In order to improve the sensitivity and specificity in the detection of protease-resistant PrP (PrP<sup>res</sup>) in tissue sections, a method known as the paraffin-embedded tissue (PET) blot has been modified for use on human tissues<sup>18</sup> (Fig. 2). This method involves cutting and deparaffinizing the section, mounting it on a nitrocellulose membrane and performing a longer digestion with proteinase K than is possible on glass-mounted slides, which helps denature  $PrP^{C}$  more completely before immunostaining.<sup>18</sup> The PET blot sections are visualized on a dissecting microscope, since the membrane is not fully translucent. This method has proven to be very helpful in cases where the interpretation of PrP immunohistochemistry has been problematic, such as in small brain biopsy samples, or where only very limited amounts of disease-associated prion protein are present, in a tissue section for example in non-CNS tissues in vCJD<sup>18</sup> (Fig. 2).

#### 5.3 Western Blot for PrP<sup>res</sup>

The identification and classification of  $PrP^{Sc}$  in the brain by Western blot analysis is essential for the diagnosis and classification of human prion diseases.<sup>2</sup> This makes it essential to store at least one frozen tissue sample from each biopsy or autopsy cases of suspected prion disease; the frozen tissue can also be used for DNA extraction and *PRNP* sequencing or codon 129 analysis if required.

Western blotting is used to detect PrP<sup>Sc</sup> in CNS and non-CNS tissues, and to characterize or type the protease-resistant fragments that are generated on the basis of their molecular weight and glycosylation status (Fig. 3). Several different Western blot protocols and nomenclatures for typing have been published, but the Parchi et al. classification is the widest use.<sup>3</sup> In addition to being diagnostically informative, PrP<sup>res</sup> typing can also be used to explore the relationships between PrP<sup>Sc</sup> biochemistry, neuropathology, and the clinical disease phenotype in human prion diseases (Fig. 3).

# 5.4 Recent Laboratory Techniques for PrP<sup>Sc</sup> Detection in Human Tissues

A range of other techniques have been developed recently for the detection of PrP<sup>Sc</sup> in human prion disease tissues. While all are used as research tools, some are now being used diagnostically, representing good examples of translational research.

- Real-time quaking-induced conversion (RT-QuIC). This in vitro PrP<sup>Sc</sup> amplification method is now in use in a highly sensitive and specific CSF test for sCJD.<sup>4</sup> It has also been used to detect PrP<sup>Sc</sup> in nasal brushings containing olfactory epithelium from patients with sCJD.<sup>12</sup>
- Protein misfolding cyclical amplification. This in vitro amplification technique<sup>19</sup> can specifically detect PrP<sup>Sc</sup> in CNS and non-CNS tissues in vCJD. More recently, it has been used to detect PrP<sup>Sc</sup> in the urine of patients with vCJD (but not in sCJD patients)<sup>20</sup> and in the blood in vCJD,<sup>21</sup> raising the possibility of its use in a blood-based assay for vCJD.

# 6. CONCLUSIONS

The diagnosis of human prion diseases in a Neuropathology Laboratory now requires histological, immunohistochemical, biochemical, and genetic investigations. These established methods are being supplemented by a range of recent techniques that enhance the sensitivity and specificity of PrP<sup>Sc</sup> detection. However, the established neuropathological techniques are still required for the full characterization of human prion diseases, the recognition of disease subtypes, and the identification of novel prion diseases in the 21st century.

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