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Pathological spectrum of sporadic Creutzfeldt–Jakob disease

Diane L. Ritchie [1,*], Colin Smith [1,2]

[1] National CJD Research & Surveillance Unit, Centre for Clinical Brain Sciences, Chancellor's Building, University of Edinburgh, Edinburgh BioQuarter, Edinburgh, United Kingdom
[2] Academic Department of Neuropathology, Centre for Clinical Brain Sciences, Chancellor's Building, University of Edinburgh, Edinburgh BioQuarter, Edinburgh, United Kingdom

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ABSTRACT

Human prion diseases are a rare group of transmissible neurodegenerative conditions which are classified according to their aetiology as sporadic, genetic or acquired forms. Creutzfeldt–Jakob disease (CJD) is the most common form of human prion disease, with the sporadic form accounting for ~85% of all reported cases. While advances have been made in the development of clinical tools and biomarkers in the diagnosis of prion disease, allowing greater diagnostic certainty for surveillance purposes, definitive diagnosis requires neuropathological examination of the brain at postmortem. Since the 1990s, efforts have been made to develop a classification system for sporadic CJD (sCJD) based on observed differences in the clinical features and the pathological phenotype (the nature and degree of spongiform vacuolation, neuronal loss, astrogliosis and misfolded prion protein accumulation in the brain), also referred to as the 'histotype'. Six major clinicopathological subtypes of sCJD are internationally recognised, largely correlating with the combination of the two distinct types of the protease-resistant prion protein (PrP^{res} type 1 or 2) and the methionine (M)/valine (V) polymorphism at codon 129 of the prion protein gene (*PRNP*): MM1/MV1, MM2-cortical, MM2-thalamic, MV2, VV1 and VV2. This classification system has been extended to recognise sCJD cases demonstrating both mixed PrP^{res} types or mixed histotypes in the brain of the same individual, as well as including atypical or novel pathological phenotypes. In this review, we will provide an up-to-date overview of the current classification of sCJD based on the prominent neuropathological features. In addition, with levels of infectivity at their highest in the brain, we will also discuss the additional precautions that are recommended when handling and examining postmortem tissues from patients with suspected prion disease.

1. Introduction

Human prion diseases are a group of rare, rapidly progressive and inevitably fatal neurodegenerative conditions. As with other more common neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease, prion diseases are characterised by the accumulation in the brain of abnormal and disease-specific protein aggregates. In prion disease, this abnormal protein comprises a misfolded and partially protease-resistant isoform of a normal endogenous protein, the prion protein (PrP). Nomenclature for this misfolded protein has been the cause of some confusion; the normal cellular protein was termed PrP^{C} and the abnormal, misfolded form PrP^{Sc}.[1] There is a move to use terminology of misfolded prion protein (mfPrP), although this is not yet fully established, and we will refer to PrP^{C} and PrP^{Sc} in this manuscript. Prion diseases are generally classified according to their aetiology, with disease occurring spontaneously [sporadic Creutzfeldt–Jakob disease (sCJD), sporadic fatal insomnia (sFI) and variably protease-sensitive prionopathy (VPSPr)] or associated with a mutation in the *PRNP* gene [Gerstmann–Sträussler–Scheinker syndrome (GSS), fatal familial insomnia (FFI) and familial CJD].[2] A unique feature of prion diseases, and one that has raised the public and scientific profile of this rare group of conditions, is the occurrence of acquired forms of the disease. The acquired forms have resulted from the accidental human-to-human exposure to the infectious agent during medical or surgical procedures (iatrogenic CJD)[3] or by ingestion (kuru).[4] However, the infectious agent has also spread as a zoonotic infection to humans as witnessed with the variant CJD (vCJD) epidemic, which is causally linked to bovine spongiform encephalopathy (BSE), a prion disease in cattle.[5–8]

* Address for correspondence: National CJD Research & Surveillance Unit, Centre for Clinical Brain Sciences, Chancellor's Building, University of Edinburgh, Edinburgh BioQuarter, Edinburgh, EH16 4SB, United Kingdom.
E-mail address: diane.ritchie@ed.ac.uk (D.L. Ritchie).

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One hundred years have passed since the term 'Creutzfeldt–Jakob disease', the archetypal form of human prion disease, first appeared in the literature.[9] The name, derived from combining earlier independent case reports of neurologists Hans Creutzfeldt and Alfons Jakob, described six patients with an unusual and fatal neurological disorder of unknown aetiology.[10–13] Since these original case reports, significant advances have been made in the development of clinical, molecular and pathological tools in the diagnosis of these conditions, so much so, that retrospective review of these six cases has revealed that not all would meet the present-day diagnostic criteria for a prion disease.[14,15] Such developments have resulted in the identification and classification of an ever-expanding spectrum of sporadic, genetic and acquired forms of prion disease, each characterised by common biological features that include a prolonged asymptomatic phase which may extend up to several decades, followed by a clinical phase that may vary in length from a few months to several years.[3,16–18] Different forms of prion disease also share a common histopathology that is predominantly confined to the central nervous system (CNS) and includes spongiform vacuolation, reactive proliferation of astrocytes and microglial, neuronal loss and, in certain forms of prion disease, the formation and deposition of amyloid plaques within the brain.[2] The accumulation and detection of PrP^{Sc} in the CNS is an important diagnostic feature, common to the vast majority of prion diseases.[2] In this review, we will provide an up-to-date overview of the current phenotypical spectrum of sCJD, the most common form of CJD. In particular, we will focus on the pathological phenotype of sCJD and comment briefly on the current status of diagnostic tests.

2. Sporadic Creutzfeldt–Jakob disease

The sporadic form of CJD comprises around 85% of all reported cases of CJD, with a reported worldwide incidence of approximately 1–2 per million of the population.[19,20] Recent data from a large-scale international surveillance study reveal a steady increase in the incidence of sCJD cases, most likely due to better case ascertainment, improved diagnostic tools and an ageing population.[19,21] sCJD occurs most frequently in adults in the seventh decade of life (mean age at death of 67), but cases in adults over 80 years of age and in younger adults have been reported.[22–26] A potential cause of sCJD has yet to be identified. Other than age and a number of recognised polymorphisms on the *PRNP* gene, case–control studies have failed to identify any significant risk factors or sources of transmission.[27–29] Evidence for a history of surgery has produced inconsistent reports,[30–32] and whilst transfusion transmission of vCJD has been reported,[33–36] there remains no definitive evidence for transfusion-based transmission of sCJD.[31,35,37–40] However, experimental evidence from transgenic animal models has detected infectivity in plasma from a small series of sCJD patients.[41] No occupational risk factors have been identified, including in medical staff and healthcare workers.[42] Therefore, it remains that sCJD most likely occurs from the spontaneous conversion of PrP^{C} to PrP^{Sc} or an as yet unidentified somatic mutation in *PRNP*.

Sporadic CJD is a heterogenous form of prion disease that can be further classified into different subtypes based on variations in clinical features (age at onset, clinical presentation, disease duration) and varying patterns of neuropathology. These different clinicopathological subtypes largely correlate with the combination of two molecular features: (i) the methionine (M)/valine (V) polymorphism at codon 129 of *PRNP* which results in three possible genotype combinations (129MM, 129MV and 129VV); (ii) the biochemical profile of the abnormal PrP, specifically the electrophoretic mobility associated with the protease-resistant core of the protein (PrP^{res}). The vast majority of the prion field follow the classification system described by Piero Parchi and Pierluigi Gambetti that recognises two PrP^{res} types associated with sCJD: PrP^{res} type 1 with a relative molecular mass of ~21 kDa, and PrP^{res} type 2 with a molecular mass of ~19 kDa (Fig. 1A).[43–45] Combining the *PRNP* codon 129 genotype and PrP^{res} type results in a molecular classification of six sCJD subtypes: sCJD MM1, MM2, MV1, MV2, VV1 and VV2 (Fig. 1B). The most frequently occurring sCJD subtypes are those in patients who are homozygous for methionine at *PRNP* codon 129, supporting methionine homozygosity as a susceptibility factor for sCJD, as well as other forms of prion disease.[46,47] The relationship of these molecular subtypes to the different clinicopathological phenotypes of sCJD will be discussed in more detail later in this review.

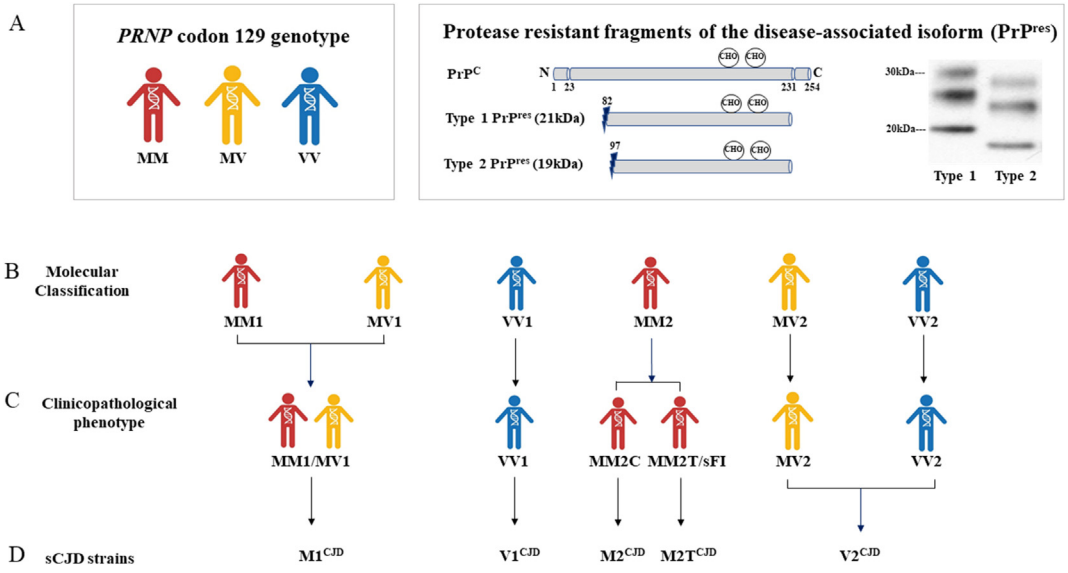

Fig. 1. Diagrammatic representation of the molecular and clinicopathological classification of sporadic CJD and the associated prion strains. (A) The three possible methionine/valine polymorphism at codon 129 of *PRNP* are shown along with a diagrammatic representation of the normal cellular prion protein (PrP^{C}) and the two protease-resistant core fragments (PrP^{res} type 1 and type 2) of the disease-associated isoform (PrP^{res}) after digestion with proteinase K. Also shown is a representative western blot of proteinase K-treated samples of PrP^{res} types 1 and 2 detected in brain homogenate from sCJD patients using the monoclonal antibody 3F4. (B) The six molecular subtypes of sCJD as classified by pairing the *PRNP* codon 129 genotype with the two distinct PrP^{res} types. (C) Subsequent classification of sCJD by variations in the clinical and pathological phenotypes. The six subtypes are closely associated with those described in the molecular classification. (C) Distinct strains of sCJD as defined by distinct transmission properties following inoculation into animal models. CJD, Creutzfeldt–Jakob disease.

3. Clinical diagnosis of sCJD

Early diagnosis of sCJD and other forms of prion diseases is often challenging due the rapid progression of the disease and a broad clinical heterogeneity that includes progressive multifocal neurological signs and symptoms, including rapidly progressive cognitive decline with dementia, visual abnormalities, movement disorders, myoclonus and ataxia, all symptoms that are common to other neurological conditions. Furthermore, reports of atypical clinical presentation in sCJD patients with extended disease durations, or in some rare genetic forms, can add to the complexities of an early clinical diagnosis.[48] A number of *in vivo* tests are available to support a diagnosis of sCJD and crucially to help differentiate from other rapidly progressive dementias and those potentially treatable conditions.[49,50] Traditional methods include electroencephalography (EEG), which shows the appearance of characteristic periodic sharp wave complexes in sCJD[51–53] and elevated levels of biomarkers of rapid neuronal injury [including 14-3-3, phosphorylated tau (p-tau), S-100, neuron-specific enolase] in cerebrospinal fluid (CSF).[52,54–56] More recently, hyperintensity signals in the caudate, putamen and the cerebral cortex on magnetic resonance imaging (MRI) have been incorporated.[57–60] Individually, the relative sensitivities and/or specificity of each of these three clinical tests is low; therefore, in the current diagnostic criteria used by the International CJD Surveillance Network, a combination of clinical presentation and results from these investigations are necessary to classify a patient with 'probable' or 'possible' sCJD (Table 1).[61]

The clinical diagnostic criteria were revised in 2017 to recognise the development of the highly sensitive protein misfolding amplification technologies (Table 1).[61] These techniques, which mimic prion replication *in vitro,* have proved transformative in the clinical diagnosis, and in the search for a disease-specific biomarker in prion disease. One amplification assay, the CSF real-time quaking-induced conversion (RT-QuIC) assay, is a multiwell, plate-based assay able to amplify minute quantities of PrP^{Sc} (the 'seed') present in CSF to detectable levels. Using bacterially expressed recombinant PrP protein as a 'substrate', and employing using intermittent periods of incubation and automated shaking for disaggregation, the misfolded aggregates generated can be measured in real-time by the presence of thioflavin-T (Th-T) levels in the assay which are incorporated into the growing amyloids.[62–64] Systematic retrospective and prospective studies have demonstrated that the CSF RT-QuIC assay has a high specificity (~100%) and sensitivity (~73–89%) in the diagnosis of sCJD and is now an integral part of the current diagnostic criteria.[65–67] Whilst CSF is the primary substrate of choice for RT-QuIC, several studies have since shown that olfactory mucosa,[68–73] skin specimens,[74,75] nerves[76] and tear fluids[77] also test positive by RT-QuIC in sCJD patients.

Although valuable in the diagnosis of sCJD, RT-QuIC is not a technique available in all diagnostic laboratories. Whilst this assay does show an almost 100% sensitivity for the most common subtypes of sporadic and genetic forms of CJD, there are still uncertainties over the sensitivity for some forms of prion disease including the rarer sporadic and genetic subtypes, FFI and vCJD.[67] In one study, RT-QuIC-negative cases of sCJD tended to have a younger age of onset with over-representation of VV1 and MM2 subtypes.[78] Although attempts have been made, CSF RT-QuIC is currently unable to accurately discriminate between the different sCJD subtypes.[79,80] As with other clinical tests for sCJD, the levels of sensitivity achieved mean that an RT-QuIC-positive result may only classify a patient with a 'probable' diagnosis of sCJD (Table 1). Whilst neuropathological examination of the brain is desirable for a definite diagnosis of sCJD and is required for accurate subtyping, the 2017 revised diagnostic criteria for surveillance of CJD recognise the impact of RT-QuIC in diagnosis;[61] essentially, rapidly progressive cognitive decline with a suggestive clinical phenotype and a positive RT-QuIC is highly likely to be sCJD, allowing greater accuracy during life, an important requirement for potential clinical trials, and greater certainty for surveillance programs where post-mortem examination may not be available.[19]

Table 1
Current diagnostic criteria for sCJD

'Definite sCJD'	'Probable sCJD'	'Possible sCJD'
• Progressive neurological syndrome and • Neuropathologically confirmed or • Immunohistochemically confirmed or • Biochemically confirmed	• I + two of II and typical EEG[a] or • I + two of II and typical MRI scan[b] or • I + two of II and positive CSF 14-3-3 or • Progressive neurological syndrome and a positive RT-QuIC	• I + two of II + duration ≤ 2 years

I Rapidly progressive cognitive impairment
II A Myoclonus
B Visual or cerebellar problems
C Pyramidal or extrapyramidal features
D Akinetic mutism

CSF, cerebrospinal fluid; DWI, diffusion-weighted imaging; EEG, electroencephalography; FLAIR, fluid-attenuated inversion recovery; MRI, magnetic resonance imaging; RT-QuIC, real-time quaking-induced conversion assay; sCJD, sporadic Creutzfeldt–Jakob disease.
Adapted from European Centre for Disease Prevention and Control.[61]
[a] Generalised periodic complexes.
[b] High signal in caudate/putamen on MRI brain scan or at least two cortical regions (temporal, parietal, occipital) either on DWI or FLAIR.

4. Examination of the brain in suspected prion diseases: postmortem considerations

Brain tissue from patients suspected of having a prion disease is typically examined at postmortem, but brain biopsies are still examined on rare occasions, primarily to exclude a prion disease where the differential diagnosis may include a potentially treatable condition. Prion diseases are not easily transmitted from person to person. They are not transmissible from person to person through normal contact or through environmental contamination, and there remains no convincing evidence to suggest that the prion agent may be transmitted via aerosols, yet conducting postmortems on individuals with suspected prion disease can prove challenging. The high titres of infectivity associated with an unfixed brain, combined with the use of knife blades/saws and other sharp instruments at postmortem, has resulted in reluctance by many mortuaries and mortuary technicians to conduct such autopsies. While 'high-risk' postmortem suites are desirable for suspected prion disease cases, any general postmortem suite can be used with the addition of some important health and safety measures and the provision of appropriate guidance for mortuary staff.[81] Central amongst these measures is the use of disposable personal protective equipment which includes cut-resistant gloves, gowns/aprons and full-face visors or a ventilated helmet. The remarkable resistance of the infectious agent to conventional methods of decontamination supports the use of a dedicated set of instruments, or where possible, disposable instruments. Inactivation protocols such as cleaning with 1 M sodium hydroxide and porous-load autoclaving at 134–138°C for 18–20 min can be used on instruments that cannot be disposed, but these should still be designated for use on suspected cases of prion disease only. To minimise any contamination to the surrounding environment, it is also recommended that the postmortem is not carried out whilst other postmortems are in progress and that the deceased remains in an open body bag with absorbent material during the procedure. Regardless of these challenges, the postmortem rate remains relatively high in comparison to the general autopsy rate.[82] It should be noted that the transmissibility of other misfolded proteins has been demonstrated, and the neuropathology and research community may wish to consider the potential health and safety risks associated with any brain in which the misfolded protein may be found.[83]

Macroscopic examination of the brain in sCJD and other prion diseases usually shows no specific findings, other than subtle age-related changes, most likely due to the often-rapid progression from clinical presentation to death. In some patients with extended disease duration, there may be evidence of cerebral and cerebellar atrophy which in rare cases, such as the 'panencephalopathic CJD', may also lead to collapse of the cortical architecture and in some cases, degeneration in the white matter.[84] For microscopic analysis, widespread sampling of the brain, to include all cortical lobes, the cerebellar hemisphere, brain stem, thalamus and basal ganglia, is recommended owing to the heterogeneity in the pattern and severity of the histopathology (spongiform vacuolation, astrocytosis and neuronal loss) and PrP^{Sc} accumulation. This widespread sampling is also valuable for further investigations, should the clinical diagnosis of a prion disease not be supported by neuropathological examination. Immersion of fixed postmortem tissue blocks for 1 h in 96% formic acid prior to processing and embedding is a protocol recommended for tissue samples collected from suspected cases of CJD as a method introduced to reduce the levels of potential infectivity. Following treatment with formic acid, tissues can be investigated in any general category-II pathology laboratory. Additionally, a small sample of grey matter enriched tissue, commonly the frontal cortex, frozen at postmortem, is essential for the detection and biochemical analysis of the protease-resistant PrP. The availability of frozen tissue also provides a source of DNA for *PRNP* sequencing. A complement of fixed and frozen samples allows a comprehensive diagnosis and subclassification of disease. Such widespread examination of the brain is also important for the continued surveillance of novel and atypical forms of prion disease.

5. Routine tools in the diagnosis of prion disease

It remains that a definite diagnosis of prion disease depends on neuropathological examination of the brain. Histological examination for the characteristic spongiform change, neuronal loss and gliosis play an important role in the diagnosis, but none of these are specific for prion disease on their own. The vast majority of tools target the detection of PrP^{Sc}.

5.1. Immunohistochemistry

The detection of PrP^{Sc} in the brain by immunohistochemistry (IHC) remains a fundamental tool in the diagnosis of prion disease.[2] PrP^{Sc} accumulates in a range of distinct patterns in sCJD, ranging from widespread synaptic and perivacuolar deposits to perineuronal and plaque-like accumulations (Fig. 2).[2] The observation of different patterns and types of PrP^{Sc} accumulation contribute to the subclassification of disease as well as in the detection of novel or atypical forms of prion disease. A large number of commercially available anti-PrP antibodies are available, each recognising different epitopes on the PrP.[85] It is recommended that more than one PrP antibody be used in the investigating cases of suspected prion disease to allow for differential immunoreactivity, most clearly demonstrated in cases of VPSPr.[86] At present, most commercially available antibodies are not able to distinguish between the normal and the abnormal PrP. Therefore, a number of antigen-retrieval protocols have been developed in order to minimise the detection of PrP^{C} whilst optimising the sensitivity for PrP^{Sc}. Heat-retrieval methods such as hydrated autoclaving or microwaving in citric acid are the most commonly used not only to expose the antigen epitopes but also to help in the denaturation of PrP^{C}. Routinely, these are combined with a 5-min incubation in 96% formic acid to enhance the immunostaining. Immersion of tissue sections in proteinase K is an additional step recommended in order to digest the normal PrP^{C}, but the concentration and time of exposure are kept to a minimum (approximately 5 μg/mL for 5 min) to preserve tissue morphology and prevent adverse digestion of the tissue. Whilst the principles of IHC in the diagnosis of prion disease have remained unchanged, the methodology has developed over time with the utilisation of highly sensitive polymer detection kits.[87] Furthermore, formic acid treatment of tissue samples prior to processing to paraffin wax blocks permits the use of automatic staining technology which has improved the reproducibility and efficiency of the assay.

5.2. Paraffin-embedded tissue blot

A further technique that may be applied to formalin-fixed tissue in the diagnosis of prion diseases is the paraffin-embedded tissue (PET) blot.[88] In the PET blot, tissue sections are blotted onto nitrocellulose membrane rather than glass slides. The superior adherence of tissue sections to the nitrocellulose allows a much more intense pretreatment with proteinase K (approximately 25 μg/mL for 18 h at 60°C); this assures the complete digestion of the normal PrP, leaving only the disease-associated form (Fig. 3A). Such an increase in specificity for PrP^{Sc} makes the PET blot a particularly valuable tool for use on biopsy material, where levels of PrP^{C}

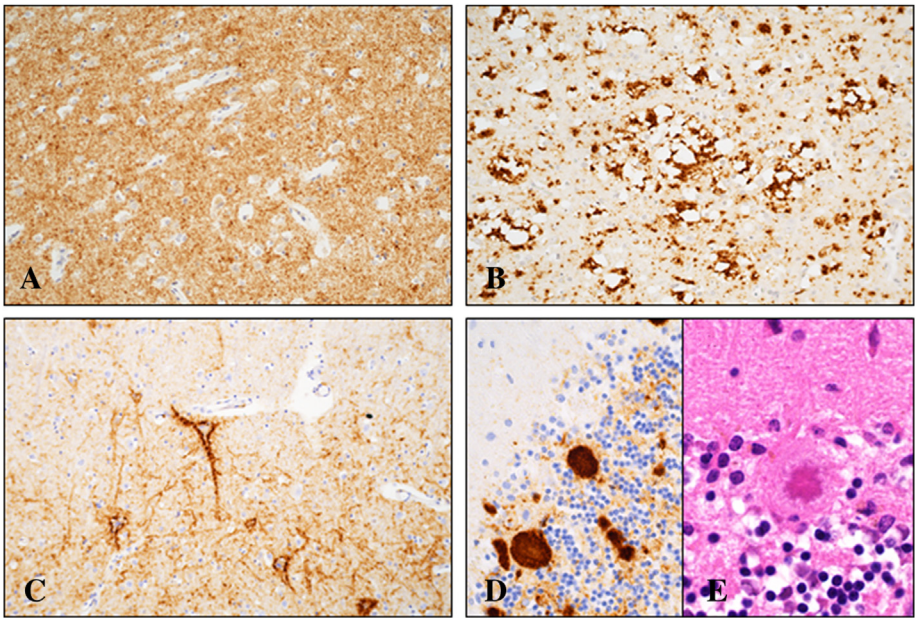

Fig. 2. Variable patterns of PrP^{Sc} accumulation in the brain in sporadic CJD. (A) Widespread granular or synaptic-type accumulation throughout the neuropil of the cerebral cortex. (B) Intense perivacuolar deposits of PrP^{Sc} around areas of confluent vacuolation. (C) Perineuronal labelling of pyramidal neurons accompanied by synaptic accumulations of PrP^{Sc} in the temporal cortex. (D) Intense PrP^{Sc} labelling of kuru-like plaques and smaller plaque-like deposits in the molecular layer of the cerebellum. (E) In contrast to the plaque-like deposits, kuru plaques are visible on H&E stain (H&E, ×400 ROI). PrP was detected using 12F10 anti-PrP monoclonal antibody. Magnification: ×200 (A,B); ×400 (C,D).
CJD, Creutzfeldt–Jakob disease; H&E, haematoxylin and eosin; PrP, prion protein.

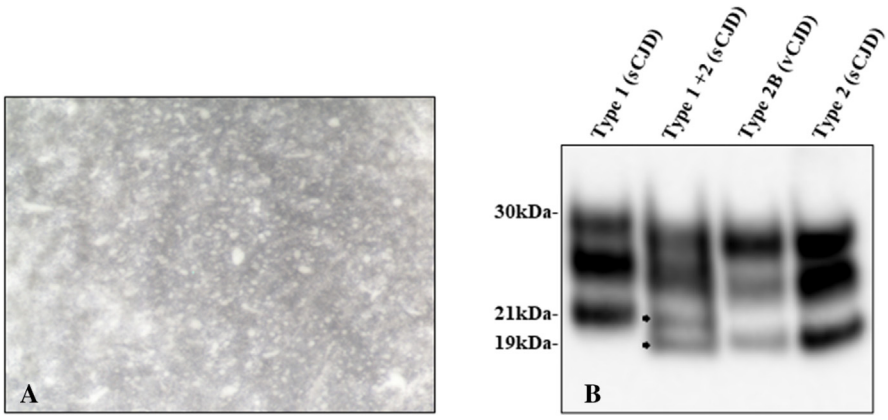

Fig. 3. Tools in the diagnosis of sporadic CJD (sCJD) and other human prion diseases. (A) Synaptic accumulation of PrP^{Sc} in the frontal cortex in sCJD. PrP^{Sc} was detected by the paraffin-embedded tissue (PET) blot. Whilst this technique is specific for PrP^{Sc}, the cellular morphology is inferior to that of immunohistochemistry (magnification, ×200). (B) Western blot analysis of protease-resistant prion protein (PrP^{res}) types in sporadic CJD (sCJD) following proteinase K digestion. Three cases of sporadic CJD are represented showing PrP^{res} type 1 and PrP^{res} type 2, along with a case of sporadic CJD in which both type 1 and 2 were present in an extract of brain from the same individual. A brain sample from a case of variant CJD (vCJD) is also added as a control for the glycosylation ratio. Variant CJD cases are characterised by the presence of a dominant diglycosylated band and are referred to as 'type 2B', to distinguish them from type 2 (often referred to as type 2A) in sporadic CJD cases. Western blots performed using 3F4 anti-PrP monoclonal antibody and provided by kind permission of Dr Marcelo Barria and Ms Angela Chong.
CJD, Creutzfeldt–Jakob disease; PrP, prion protein.

are commonly upregulated, or in cases where non-specific labelling is problematic on IHC.[85,89] The sensitivity of the PET blot is also superior to that of IHC and is often applied in cases where levels of PrP^{Sc} are low.[88] However, the PET blot is not used routinely in the diagnosis of disease due to the large volumes of antibodies required, the extended length of the assay when compared to IHC and the lack of cellular detail for the subclassification.

5.3. *Western blot analysis*

The preservation of frozen tissue samples at postmortem is also an important component for the detection and characterisation of PrP^{Sc} types by western blot for the diagnosis and subclassification of prion disease. Furthermore, in the absence of a blood sample, frozen tissue is also a useful source of DNA for *PRNP* codon 129 analysis or full sequencing. A molecular 'type' in prion disease refers to the characterisation of the protease-resistant core of the PrP that is generated from brain homogenates (normally 10% weight to volume) following treatment with proteinase K (~50 μg/mL for 1 h at 37°C) and western blotting. Two variables are assessed: firstly, the size of the PrP^{res} fragment, which results from differences observed in the mobility of protease-resistant fragment after gel electrophoresis; and secondly, the relative abundance of the different glycosylated PrP isoforms (non-glycosylated, monoglycosylated and diglycosylated) referred to as the glycoform ratio (Fig. 1A, Fig. 3B). A number of protocols have been described, the vast majority of which follow the molecular typing scheme described by Parchi and Gambetti that recognises the two PrP^{res} types associated with sCJD (type 1 and type 2).[43,90,91] The mobility of the non-glycosylated fragment of sCJD type 2 cases is indistinguishable from that observed in vCJD cases. However, vCJD can be distinguished from sCJD type 2 cases by a predominance of the diglycosylated fragment. To indicate this difference in the glycoform ratio, the type 2 PrP^{res} associated with vCJD cases is termed type 2B (Fig. 3B).[90] In rare cases where only frozen tissue samples are available, the detection of PrP^{res} by western blot is sufficient to confirm a diagnosis of prion disease. However, confirmation of the form of prion disease or the subclassification of disease relies upon the correlation of the biochemical, genetic and pathological phenotype.

5.4. *Potential new diagnostic tools: misfolded protein amplification*

As described earlier, the RT-QuIC amplification assay has been adopted as a clinical test for sCJD using CSF samples; it can also be applied to samples of frozen brain homogenate. A second *in vitro* method, known as protein misfolding amplification assay (PMCA), shows high sensitivity in the amplification of vCJD prions, but is less efficient in the amplification of sCJD prions, and as yet, a standardised diagnostic PMCA protocol for the detection of prions present in biological fluids or tissues has not been implemented.[92] An alternative assay, the conformational detection assay (CDI) is a 96-well plate-based assay that detects PrP^{Sc} based on conformational differences with the endogenous PrP.[93] However, similar to the *in vitro* amplification methods, CDI is not implemented as a standard diagnostic technique. As such, it remains that the current confirmation of a prion disease relies upon immunohistochemical detection on fixed tissue samples and western blot analysis on frozen brain homogenates.

6. Subclassification of sCJD

sCJD can be divided into six molecular subgroups by pairing the methionine/valine *PRNP* codon 129 polymorphism and the PrP^{res} type detected by western blot analysis of brain homogenates (PrP^{res} type 1 or type 2). These six molecular subtypes (MM1, MV1, VV1, MM2, MV2 and VV2) largely correlate with distinct clinicopathological phenotypes of sCJD, but with some minor modifications (Fig. 1A–C).[45] These include (i) combining the sCJD MM1 and MV1 cases into a single subgroup as they share common clinical and pathological features, and (ii) the separation of sCJD MM2 cases into a 'cortical' (MM2C) and a 'thalamic' (MM2T) subtype, with each having markedly different pathological phenotypes.[45] Traditionally, the MM2 thalamic subtype was included as a distinct subgroup of sCJD. However, the clinical and neuropathological similarity to the genetic prion disease, FFI, has seen thalamic cases often reclassified as sporadic fatal insomnia (Fig. 1).[94–96] Consequently, five major sCJD clinicopathological subtypes are reported (MM1/MV1, MM2C, MV2, VV1 and VV2) based on suggestive clinical features and a distinctive and readily identifiable pathology in the brain, often referred to as the 'histotype' (Fig. 1C).[2,45] Confirmation that these clinicopathological subtypes represent different strains of sCJD has come from experimental transmission studies in which four different prion strains were identified: these comprise the M1 strain (sCJD MM1/MV1 subtypes), V2 strain (sCJD VV2 and MV2 subtypes), M2 strain (sCJD MM2C subtype) and the V1 strain (sCJD VV1 subtype).[97–99] Experimental transmission of brain homogenate from MM2/sFI cases also showed transmission properties distinct from the other sCJD, but which were similar to that of FFI in the same animal models, confirming a distinct strain of prion agent.[94,100–102]

7. Pure sCJD subtypes

The identification and classification of sCJD subtypes based on neuropathological phenotype alone, has proven to be extremely reliable

and accurate.[95] Moreover, when compared to PrP^{res} typing or *PRNP* codon 129 genotyping, examination of the histotypes allows the identification of atypical and novel phenotypes of sCJD and other prion diseases. The archetypal histotype of the five 'pure' sCJD subtypes, defined by the detection of a single PrP^{res} type following widespread sampling and examination of the brain are described in the following.

7.1. sCJD MM1/MV1

The most common and classical subtype of sCJD, representing ~60–70% of all sCJD cases, are found in *PRNP* codon 129MM (~57%) and 129MV (~8%) individuals in combination with PrP^{res} type.[12,103] The similarity in the clinical and pathological features of sCJD MM1 and MV1 patients resulted in the consolidation in a single subgroup. The duration of illness in this subtype is often rapid, on average, around 4 months. The pathological phenotype of sCJD MM1/MV1 is characterised by a widespread distribution of classical spongiform change, comprising widespread microvacuolation (vacuoles of ~2–5 μm in diameter), throughout the neuropil of the cortical layers, often most severe in the frontal and occipital lobes (Fig. 4A). The hippocampus is generally spared, but spongiform change is present to a variable extent in the basal ganglia, thalamus and in the molecular layer of the cerebellum, often in a focal distribution (Fig. 4B). Immunohistochemically, there is a general widespread synaptic/granular pattern of PrP^{Sc} accumulation throughout the grey matter regions of the brain, often more easily visualised in the cortical regions and in the cerebellum (Fig. 4C,D). The cerebellum often shows a more intense pattern of punctate deposits in the molecular layer.

7.2. sCJD VV2

This represents the second most common subtype of sCJD, accounting for around 10–15% of cases.[2,103] The clinical duration of illness in this subtype is on average 6 months. A mild to moderate spongiform degeneration, predominantly microvacuolar in type, is distributed in a distinctive linear distribution in cortical layers 4–6, most prominent in the frontal and temporal cortices (Fig. 4E). There is often the most severe vacuolation in the cerebellum which is accompanied by marked neuronal loss in the granular layer. Spongiform pathology is also marked in the

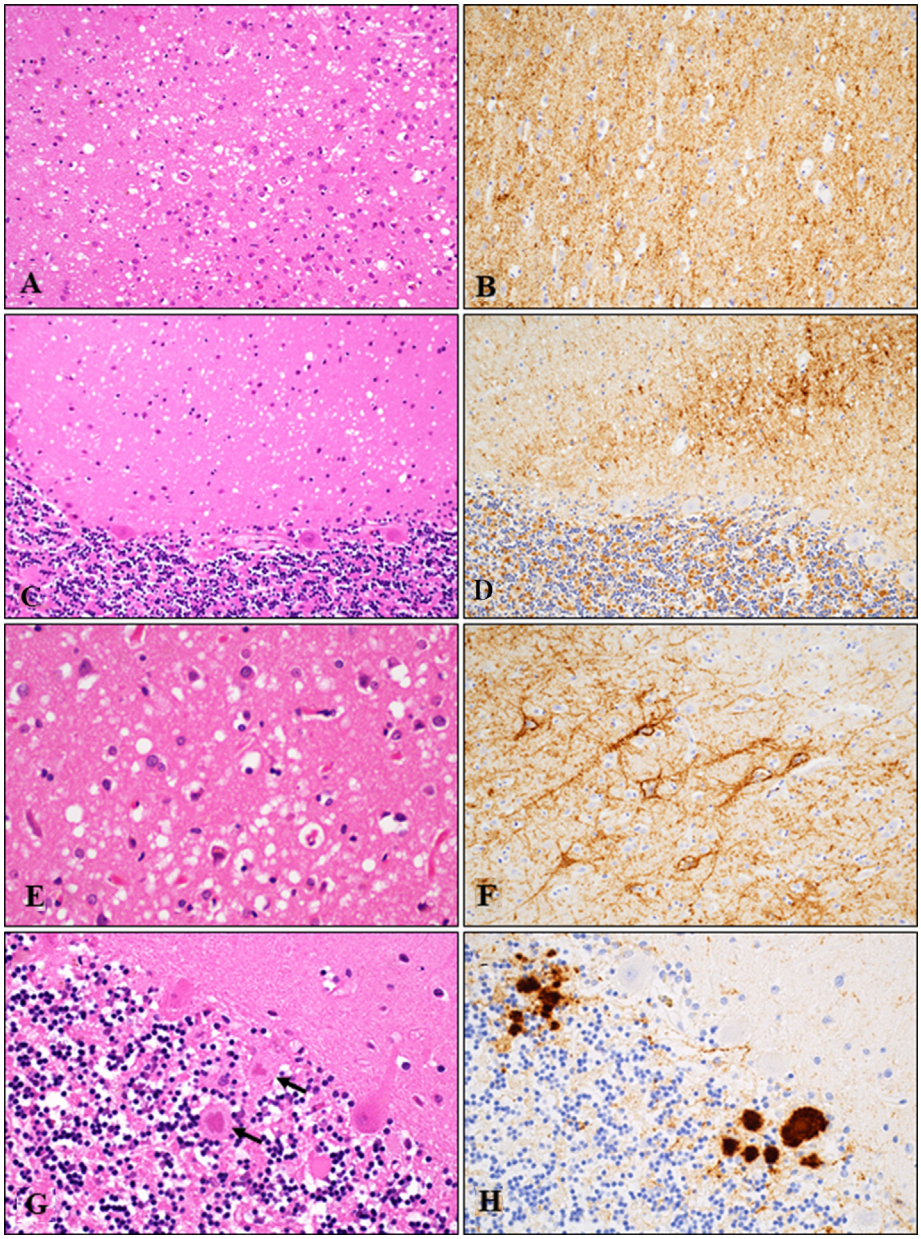

Fig. 4. Prominent neuropathological features in the most common 'pure' sporadic CJD (sCJD) subtypes. (A,B) Widespread micro-vacuolation and accompanying gliosis in the frontal cortex of sCJD MM1 with a general synaptic pattern of PrP accumulation. (C,D) sCJD MM1 demonstrating microvacuolation in the molecular layer of the cerebellar cortex with a granular/synaptic accumulation of PrP in both the granular and molecular layers. (D,E) Microvacuolation in the neuropil of the temporal cortex in sCJD VV2 with a perineuronal and synaptic pattern of PrP immunoreactivity. (F,G) Kuru plaques (arrow) in the cerebellum in the sCJD MV2 subtype that stain intensely on PrP immunohistochemistry alongside smaller plaque-like deposits. PrP was detected using 12F10 anti-PrP monoclonal antibody. Magnification: ×200 (A,B,C,D,F); ×400 (E,G,H).
CJD, Creutzfeldt–Jakob disease; PrP, prion protein.

subcortical grey matter regions of the thalamus, basal ganglia and hippocampus, with accompanying neuronal loss and astrogliosis. IHC highlights a linear pattern of perineuronal labelling with decoration of the apical ascending dendrites in the deep neuronal layers of the grey matter accompanied by synaptic deposits of PrP^{Sc} throughout the neuropil (Fig. 4F). Small, intensely stained PrP plaque-like deposits are also present throughout the grey matter regions, particularly marked in the basal ganglia, thalamus and cerebellum. These plaque-like deposits are not visible on haematoxylin and eosin (H&E) stain.

7.3. sCJD MV2

This subtype represents ~10% of all sCJD cases, with an average clinical duration of around 17–18 months.[2,103–105] The pathological hallmark of this subgroup is the presence of 'kuru-type' amyloid plaques, solid rounded structures composed of a PrP^{Sc} core and radiating fibrils. Kuru-type plaques are most frequently observed in the granular and purkinje cell layer of the cerebellar cortex, but may also occur in the basal ganglia, thalamus and more rarely in the cerebral cortex (Fig. 4G). Kuru-type plaques are visible on H&E stain and using traditional tinctorial stains such as Congo red, periodic acid-Schiff and Thioflavin S; however, they are most easily visualised by PrP IHC (Fig. 4H). Other pathological features are in keeping with the VV2 subtypes with the presence of multiple plaque-like accumulations of PrP^{Sc}. A rarer neuropathological subset of sCJD MV2 cases is also recognised in which no kuru-type plaques are present, but has a histotype that is dominated by a cortical pathology.[106] In such cases, large vacuoles (15–20 μm in diameter), that generally coalesce, are observed in the cerebral cortex, basal ganglia and thalamus, in a pattern reminiscing that of sCJD MM2 cases. These regions stain intensely in a perivacuolar pattern of accumulation following PrP IHC. This rare subset of MV2 cases is designated MV2 'cortical' (MV2C) to distinguish them from the predominant and archetypal kuru-type plaque (MV2K) variant.

7.4. sCJD MM2

The MM2 subgroup comprises one of the rarer forms of sCJD, responsible for around 5% of all cases.[2,103] This subgroup is further divided into the MM2-cortical (MM2C) and MM2-thalamic (MM2T) forms based on two distinct clinical and pathological phenotypes. The rarer MM2T variant is more commonly classified as sFI as it shares a strikingly similar phenotype and transmission properties to the genetic prion disease, FFI.[45,94,101,107] Neuropathologically, there is neuronal loss, often severe in the mediodorsal and anterior nuclei of the thalamus and in the olivary nucleus, which is generally accompanied by severe astrogliosis that may be demonstrated by IHC for glial fibrillary acidic protein (Fig. 5A,B). Spongiform change and other pathological features in other brain regions are often mild or absent. The accumulation of PrP^{Sc} in MM2T/sFI cases is often patchy, in a synaptic deposition of accumulation restricted to the entorhinal cortex, but may also be difficult to detect. In the more prevalent MM2C sCJD variant, the pathological hallmark is the presence of large and coalescent vacuolations throughout the cortical regions and to a lesser extent in the basal ganglia and thalamus (Fig. 5C). In some MM2C cases with extended disease durations, the severity of the vacuolation and neuronal loss can progress to a condition known as status spongiosis. The cerebellum shows spongiform change in a patchy distribution. PrP immunostaining in sCJD MM2C cases is associated with intense perivacuolar deposits, mirroring that of the rare sCJD MV2C (Fig. 5D).

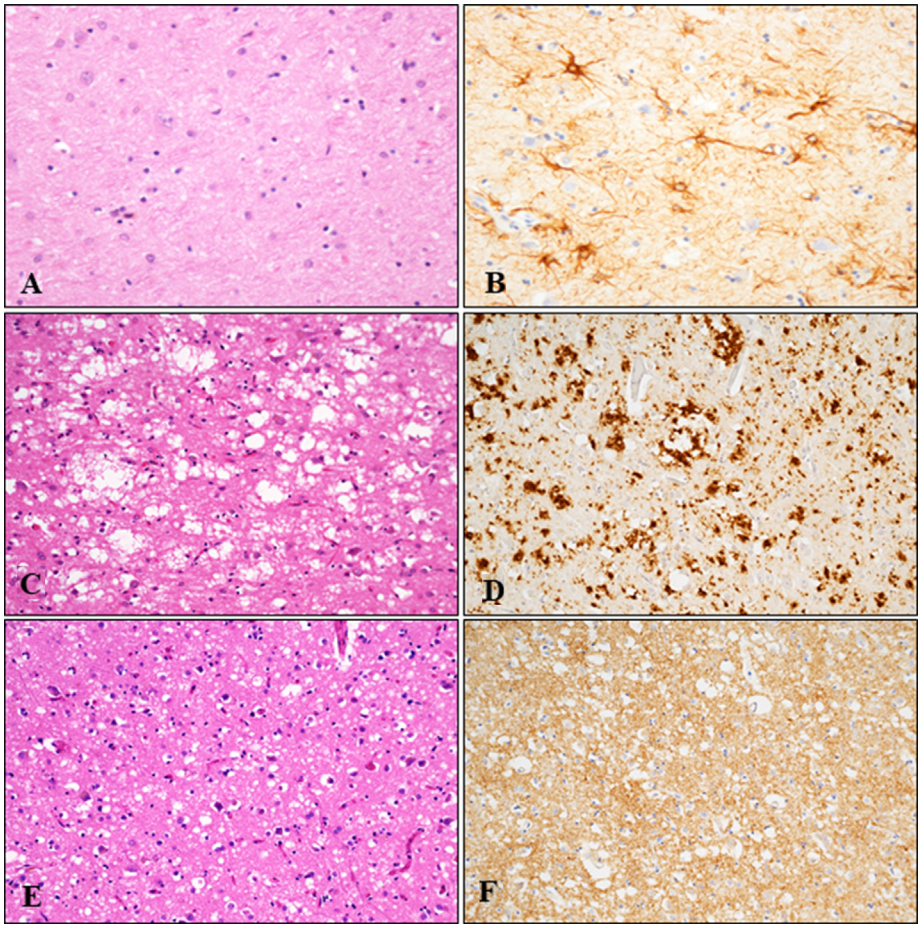

Fig. 5. Prominent neuropathological features in the rarer 'pure' sporadic CJD (sCJD) subtypes. (A,B) The MM2-thalamic subtype of sCJD, more commonly referred to as sporadic fatal insomnia, demonstrating marked neuronal loss and gliosis in the mediodorsal and anterior nuclei of the thalamus. (C,D) Large and often confluent vacuolation in the frontal cortex in sCJD MM2-cortical subtype with an intense perivacuolar pattern of PrP immunoreactivity. (E,F) The rare sCJD VV1 subtype of sCJD is characterised by microvacuolation throughout the neuropil in the cerebral cortex with a widespread synaptic pattern of PrP accumulation. PrP was detected using 12F10 anti-PrP monoclonal antibody. The gliosis was demonstrated by immunohistochemistry for glial fibrillary acidic protein. Magnification: ×200 (C,D,E,F); ×400 (A,B).
CJD, Creutzfeldt–Jakob disease.

7.5. sCJD VV1

Patients who are homozygous for valine at *PRNP* codon 129, with a PrP^{res} type 1, represent the rarest subtype of sCJD (~1% of all reported cases). Commonly, these patients have a younger disease onset and a long disease duration, averaging around 21 months. The pathological phenotype is characterised by moderate to severe microvacuolation throughout the cerebral cortex (most obvious in the frontal and occipital cortex) and in the basal ganglia (Fig. 5E). The cerebellum, basal ganglia and thalamus are relatively spared. PrP^{Sc} accumulates in a widespread synaptic or granular pattern of accumulation (Fig. 5F).

8. Co-occurrence of sCJD subtypes

The classification of sCJD has been expanded to recognise the existence of 'mixed' subtypes, cases in which both PrP^{res} type 1 and type 2 co-occur in the brain of the same patient. Original studies estimated that the co-occurrence of PrP^{res} types was a feature of approximately 5% of sCJD cases.[45,108] Subsequent studies, involving more extensive sampling of the brain, reported that the proportion of sCJD cases with co-occurring PrP^{res} type was much higher (between 15% and 45%).[108–113] Furthermore, the application of PrP^{res} type-specific antibodies suggested that the co-occurrence of PrP^{res} types may indeed be a feature of all sCJD cases;[86,114,115] however, questions were raised regarding the ability of these type-specific antibodies to accurately discriminate between true PrP^{res} types and partially digested fragments.[111,116] The proportion of PrP^{res} type 1 and PrP^{res} type 2 detected in the same brain can vary, with this variation thought to influence the disease phenotype. Neuropathologically, cases with a mixture of PrP^{res} types show the co-existence of histotypes that are characteristic for the pure sCJD subtypes, but in a proportion that often reflects the most dominant PrP^{res} type.[111,117,118] Additionally, the observation of focal areas of mixed pathology in the brain may also be indicative of mixed PrP^{res} types that may go undetected by Western blot analysis due to restricted sampling.

The co-occurrence of PrP^{res} types has been identified in individuals from each of the three *PRNP* codon 129 genotypes which expands the classification system to include MM1+2C, MV1+2C and VV1+2 cases.[117] The MM1+2C cases are the most common of the mixed sCJD types

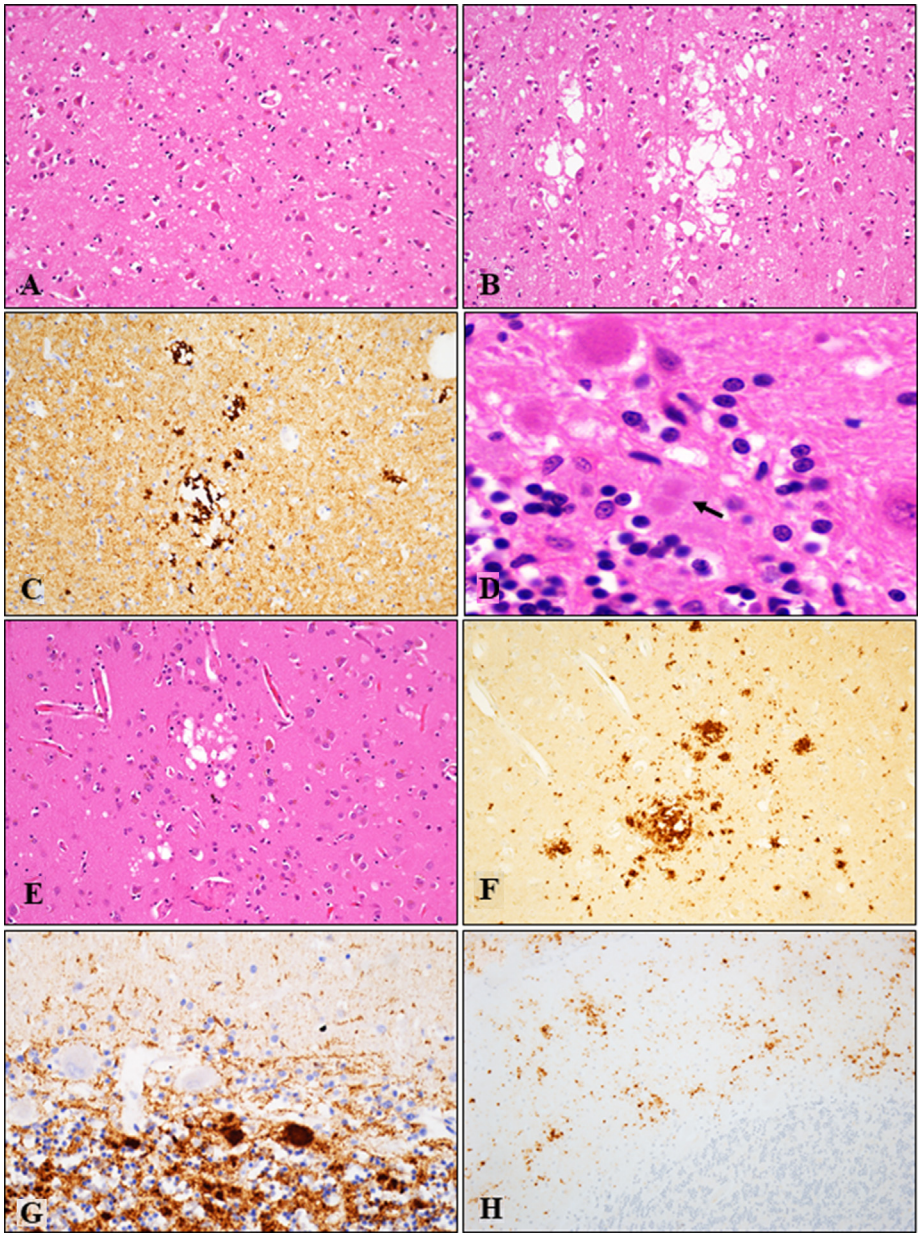

Fig. 6. Neuropathological features in 'mixed' sporadic CJD (sCJD) subtypes. (A–C) The frontal cortex in sCJD MM1+2C showing regions with a predominance of microvacuolation, consistent with the MM1 subtype, alongside areas of confluent vacuolation as observed in sCJD MM2 cases. PrP immunohistochemistry demonstrates widespread synaptic pattern of accumulation with focal areas of intense perivacuolar staining. (D–G) sCJD MV2K+C subtype with the presence of kuru plaques in the cerebellum with confluent vacuolation present in the cerebral cortex, both of which show an intense pattern of PrP immunoreactivity. (H) The cerebellum in VPSPr showing the intense labelling of microplaques in the molecular layer. PrP was detected using the 12F10 anti-PrP monoclonal antibody. Magnification: ×200 (A,B,C,E,F,H); ×400 (G); ×400 ROI (D).
CJD, Creutzfeldt–Jakob disease; PrP, prion protein; VPSPr, variably protease-sensitive prionopathy.

reported, estimated to be a feature of around 43% of all sCJD MM cases. MM1+2C cases show a pathological phenotype that reflects a combination described for pure MM1 and MM2-cortical subtypes. Small microvacuolation as well as some larger confluent vacuoles are a feature of the cerebral cortex in a ratio that reflects the dominant PrP^{res} (Fig. 6A,B). This mixed histotype is also reflected in the PrP IHC which shows a combination of both synaptic PrP^{Sc} accumulations and intensely stained areas of perivacuolar accumulations in a variable pattern throughout the cortical regions (Fig. 6C). Mixed sCJD VV1+2 cases are rarer, comprising around 23% of all sCJDVV cases and are largely identified by the presence of both PrP^{res} types following western blot analysis.[118] However, pathological investigations can demonstrate the presence of subtle pathological features characteristic of both the VV1 and VV2 subtypes.[95,117]

A further two mixed sCJD subtypes have been classified based on histotype alone as they are associated with PrP^{res} type 2 subtypes. The rare MV2K+C subtype has an easily recognisable histotype characterised by the presence of kuru plaques in the cerebellum and widespread confluent vacuolation that stains intensely in a perivacuolar pattern of PrP^{Sc} in the cerebral cortex and basal ganglia (Fig. 6D–G). Small plaque-like deposits in the cerebellum, putamen and thalamus are also characteristic for these cases.[95,104,119–122] Lastly, the rare MM2T+2C subtype has been described in only a small number of cases and is characterised by the combination of severe neuronal loss and gliosis in the thalamic and inferior olivary nucleus, with areas of confluent vacuolation and corresponding perivacuolar accumulations of PrP in the cortical regions.[117,123–125]

9. Atypical sCJD pathological phenotypes

The classification system set out for sCJD originated in 1996 and has remained relatively unchanged but for the inclusion of mixed subtypes. However, even with such a rare disease, not all cases of sCJD cases exhibit the characteristic features described in the classification system. Atypical cases are increasingly being recognised in terms of both their clinical and neuropathological features.[126] Whilst the vast majority encompass single case reports, a small group of sCJD MM1 cases have been reported with a rare histotype characterised by PrP amyloid plaques present in subcortical and deep nuclei white matter (p^{WM}-CJDMM1)[127–131] or in cerebellar grey matter (p^{GM}-CJDMM1).[131] Recent reports suggest there may also be some subtle differences in the size of the unglycosylated PrP^{res} fragment in a proportion of the p^{WM}-sCJDMM1 cases,[131] but this may be linked to technical differences, specifically differences in the conditions of proteolytic digestion. Whilst clinically these cases have an extended disease duration, results from experimental transmission suggest consistent strain properties with sCJDMM1 and p^{WM}-sCJDMM1.[130]

10. Variably protease-sensitive prionopathy

VPSPr was first described in 2008 and is increasingly considered to be either a novel sporadic prion proteinopathy or a phenotype within the spectrum of sCJD.[132] Whilst codon 129 valine homozygosity (VV) is over-represented (~65% of reported cases), all genotypes have been described in VPSPr cases.[133] The clinical presentation is non-specific, often with psychiatric symptoms with an atypical dementia; most VV and MV cases show psychiatric symptoms, cognitive decline and/or aphasia, whereas MM cases may show aphasia, parkinsonism and ataxia.[134] Most cases are associated with a long clinical duration. Early reported cases describing analysis of CSF with RT-QuIC were negative,[79] but second-generation RT-QuIC studies are reported to be positive in cases of VPSPr.[135]

One of the most prominent features of VPSPr cases is the presence of a low 8 kDa PrP^{res} fragment after digestion with proteinase K and western blot, commonly accompanied by a ladder of higher molecular weight bands. However, the neuropathological features of VPSPr cases are also unique. Histological examination will show vacuolation in the cerebral cortex, subcortical grey matter and the cerebellar molecular layer. IHC for PrP^{sc} shows a pattern different to that seen in other forms of sCJD; there is fine granular staining with more prominent aggregates and microplaques, a prominent feature of the cerebellum (Fig. 6H). The intensity and pattern of PrP^{Sc} is variable depending on the anti-PrP antibody used. The microplaques are not easily observed by H&E staining alone. As the name suggests, the PrP^{sc} shows variable sensitivity to PK digestion, with VV isoforms being more sensitive than MV, which in turn is more sensitive than MM.[133]

11. Summary

sCJD, although the most common of all prion diseases, remains a rare disease, but neuropathologists are likely to see cases during their professional career. Whilst the clinical diagnosis of sCJD has improved significantly, subclassification of disease still requires examination of the brain and the correlation of the histological, immunohistochemical and molecular features. With no effective treatment for sCJD or any form of prion disease, current research in prion disease has focused on the development of novel drugs targeting PrP^{Sc}, proposed clinical trials for antisense oligonucleotide therapies (targeting *PRNP* mRNA) and a monoclonal antibody which targets PrP^{C} (PRN100).[136]

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