

# Sporadic and familial CJD: classification and characterisation

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Prion diseases are unique transmissible neurodegenerative diseases that have diverse phenotypes and can be familial, sporadic, or acquired by infection. Recent findings indicate that the PrP genotype and the PrP<sup>Sc</sup> type have a major influence on the disease phenotype in both sporadic and familial human prion diseases. This review attempts to classify and characterise sporadic and familial Creutzfeldt-Jakob disease (CJD) as a function of these two disease determinants. Based on the genotype at codon 129 on both *PRNP* alleles, the size of protease resistant PrP<sup>Sc</sup> fragments and disease phenotype, we divide sporadic CJD into six subtypes: sCJDM1/sCJDMV1, sCJDVV2, sCJDMV2, sCJDM2, sCJDVV1, and sporadic fatal insomnia (sFI). Familial CJD is classified into many haplotypes based on the *PRNP* mutation and codon 129 (and other polymorphic codons) on the mutant allele. The clinical and pathological features are summarised for each sporadic CJD subtype and familial CJD haplotype.

One of the many challenging features of the prion diseases or transmissible spongiform encephalopathies (TSEs) is the prominent heterogeneity. While the pathology of other conformational diseases of the central nervous system, such as Alzheimer's disease, Huntington's chorea and Parkinson's disease, are rather homogenous, prion diseases include a wide spectrum of histopathological phenotypes. The heterogeneity of prion diseases is compounded by several factors. First, prion diseases are unique among conformational diseases as being infectious. At variance with other neurodegenerative diseases, prion diseases include three forms: sporadic, familial and acquired by infection (Table 1). Second, according to the infection portal of entry or the origin of the exogenous infectious prion, the form of prion diseases acquired by infection may display phenotypes that are quite different. This is the case with the iatrogenic Creutzfeldt-Jakob disease (CJD) and the new variant CJD that display quite different pathological features. Third, there are a large number of mutations that are genetically distinct and often are associated with quite different disease phenotypes. Fourth, the sporadic

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*N.B.* Colour figures are referred to as 'Plates' and appear in a 'Colour plate section' at the front of this volume

**Table 1** Classification of human prion diseases

Form	Phenotype
Familial (inherited)	Creutzfeldt-Jakob disease (fCJD) Fatal familial insomnia (FFI) Gerstmann-Sträussler-Scheinker disease (GSS) Mixed or undefined
Sporadic	CJD (sCJD) Typical (MM1/MV1) Early onset (VV1) Long duration (MM2) Kuru plaques (MV2) Ataxic (VV2) Fatal insomnia (sFI)
Acquired	Kuru Iatrogenic CJD (iCJD) New variant CJD (vCJD)

form of prion diseases itself has an unusual degree of phenotypic heterogeneity. The large spectrum of phenotypic variability has made the recognition of prion diseases difficult.

Recent understanding of the molecular mechanisms that play a central role in the pathogenesis of prion diseases has provided insight into the modalities of the phenotypic heterogeneity of these diseases. This, in turn, has led to a more rational and practical classification that is expected to facilitate the identification and diagnosis of human prion diseases.

Codon 129 of the prion protein gene (*PRNP*) is the site of a common methionine (M)/valine (V) polymorphism. In the Caucasian population, 52% of individuals are M homozygous (MM), 36% are heterozygous (MV) and 12% are V homozygous (VV)<sup>1</sup>. We observed that the phenotype of the prion diseases, whether sporadic, familial or acquired by infection, often is different depending on the genotype at codon 129 of the affected subject. Therefore, codon 129 appears to act as a modifier of the disease phenotype in human prion diseases. We also observed that human prion diseases are associated with two types of scrapie prion protein (PrP<sup>Sc</sup>) that are easily distinguishable on Western blot based on the size of the PrP<sup>Sc</sup> domain resistant to digestion by the proteolytic enzyme proteinase K (PK). In PrP<sup>Sc</sup> type 1 the PK-resistant domain has a gel mobility of about 21 kDa with an N-terminus, corresponding to the main PK cleavage site, which commonly starts at residue 82. In PrP<sup>Sc</sup> type 2, the corresponding PK-resistant domain migrates on gel to approximately 19 kDa and its N-terminus starts most often at residue 97<sup>2</sup>. We observed that the disease phenotypes of the patients with prion diseases associated with PrP<sup>Sc</sup> type 1 often are different from the phenotypes associated with PrP<sup>Sc</sup> type 2. This argues that, the PrP<sup>Sc</sup> type is another modifier in human prion diseases. Since the presence of two distinct PK cleavage sites in PrP<sup>Sc</sup> types 1 and 2 is most likely due to the

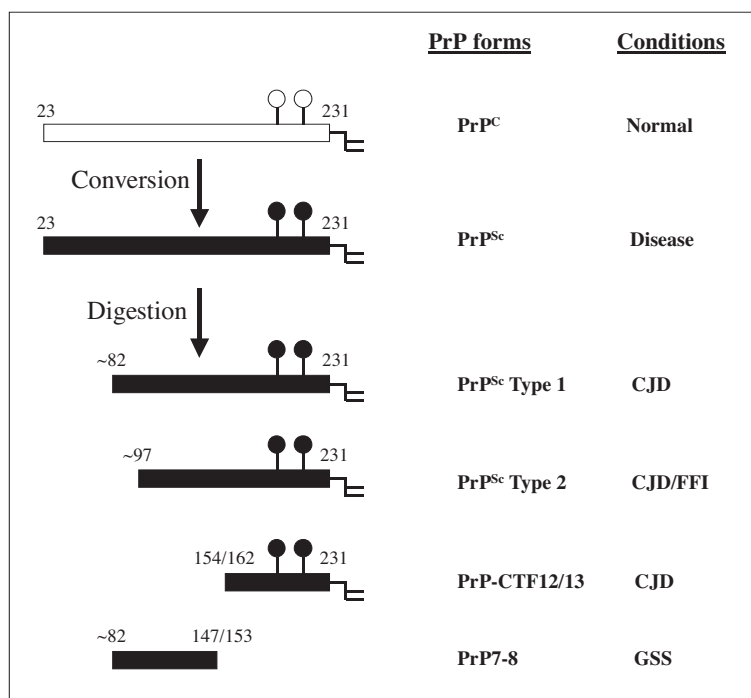
different conformation, PrP<sup>Sc</sup> types 1 and 2 fulfil the characteristics of the 'so-called' prion strains<sup>3-5</sup>. However, the disease-modifying role of the genotype at codon 129 and of the PrP<sup>Sc</sup> type is not totally independent. Approximately 95% of the sporadic CJD patients who are MM homozygous have PrP<sup>Sc</sup> type 1 whereas 86% of the patients who are either VV homozygous or MV heterozygous have PrP<sup>Sc</sup> type 2<sup>6,7</sup>. Therefore, the MM homozygosity favours the formation of PrP<sup>Sc</sup> type 1 and the presence of one or two V at codon 129 favours the formation of PrP<sup>Sc</sup> type 2. Furthermore, amino acid sequencing has revealed that the N-terminus of the PK-resistant core is ragged in both PrP<sup>Sc</sup> types 1 and 2. This results in distinct populations of secondary PK-resistant fragments that start at different residues, in addition to the primary fragments starting at residues 82 and 97 described above<sup>2</sup>. These populations of minor PK-resistant fragments are not generated at random by PK treatment but, like the major fragments starting at residues 82 and 97, are regulated by the genotype at codon 129<sup>2</sup>. Two additional major groups of smaller PK-resistant PrP fragments are also associated with prion diseases. The first group includes two novel C-terminal fragments of PrP (PrP-CTF12/13) that are present along with PrP<sup>Sc</sup> types 1 and 2<sup>8</sup>. Like PrP<sup>Sc</sup> types 1 and 2, PrP-CTF12/13 are primarily generated by *in vitro* limited proteolysis and represent a PK-resistant C-terminal core that includes glycosylated and unglycosylated forms derived from cleavages at residues 154/156 and 162/167, respectively. However, no apparent association between the presence and amount of the PrP-CTF12/13 and disease phenotype has been observed to date<sup>8</sup>. The second group of PK-resistant PrP fragments includes much smaller species of internal fragments with relative molecular mass of 7–8 kDa (PrP7-8) that span from residues 74–90 to residues 146–153<sup>9-11</sup>. The PrP7-8 internal fragments have only been observed in patients affected by mutations in the PrP gene linked to GSS. Figure 1 summarises the characteristics of different PrP<sup>Sc</sup> forms in brains of patients affected by human prion diseases.

Since the PrP genotype and the PrP<sup>Sc</sup> protein type seem to have a major influence on the disease phenotype in both sporadic and familial human prion diseases (see also below), the review that follows is based on the classification and characterisation of sporadic and familial CJD as a function of these two disease determinants.

## Sporadic prion diseases: Creutzfeldt-Jakob disease and fatal insomnia

The term Creutzfeldt-Jakob disease as now currently used was introduced in 1922 following the reports by the two German physicians, Hans Gerhard Creutzfeldt in 1920 and Alfons Maria Jakob in 1921, of six cases with a novel neurodegenerative disease<sup>12-14</sup>. From the beginning,

CJD became controversial. Questions were soon raised concerning the homogeneity of the case reported by the two authors<sup>15</sup>. While the onset in the five cases reported by Jakob was in adulthood and the duration 1 year or less, the Creutzfeldt's case had a likely disease onset at the age of 16 years and a duration of 6 years with remissions<sup>15</sup>. The heterogeneity of CJD was further underlined by the number of variants or subtypes identified by different authors. The most common subtypes include Heidenhain, myoclonic or diffuse cerebral, dyskinetic with or without muscular atrophy, thalamic, cerebellar or ataxic, and panencephalopathic subtypes<sup>15-19</sup>. It also became apparent that the duration could vary greatly ranging from weeks to several years as did the electroencephalographic (EEG) patterns that had the characteristic periodic sharp wave (PSW) complexes only in about 50% of the cases<sup>14,20,21</sup>. Variations in type and distribution of the histopathological lesions not attributable to different duration were also observed. They included the occasional presence of amyloid plaques, and great variation in severity and



**Fig. 1** PrP forms in normal and diseased brains. Normal PrP (PrP<sup>C</sup>) is a glycoprotein with two Asn-linked glycan chains and a GPI anchor. During pathogenesis, PrP<sup>C</sup> is converted to the abnormal PrP<sup>Sc</sup> that can be isolated as protease-resistant core fragments following proteolytic digestion. Several PrP<sup>Sc</sup> subtypes have been identified from brains of patients affected by human prion diseases. They include PrP<sup>Sc</sup> types 1 and 2 that are characteristic of different disease phenotypes, as well as a recently identified smaller C-terminal fragment of 12 or 13 kDa (PrP-CTF12/13), and an internal PrP amyloid peptide of 7–8 kDa (PrP7-8) unique to GSS.

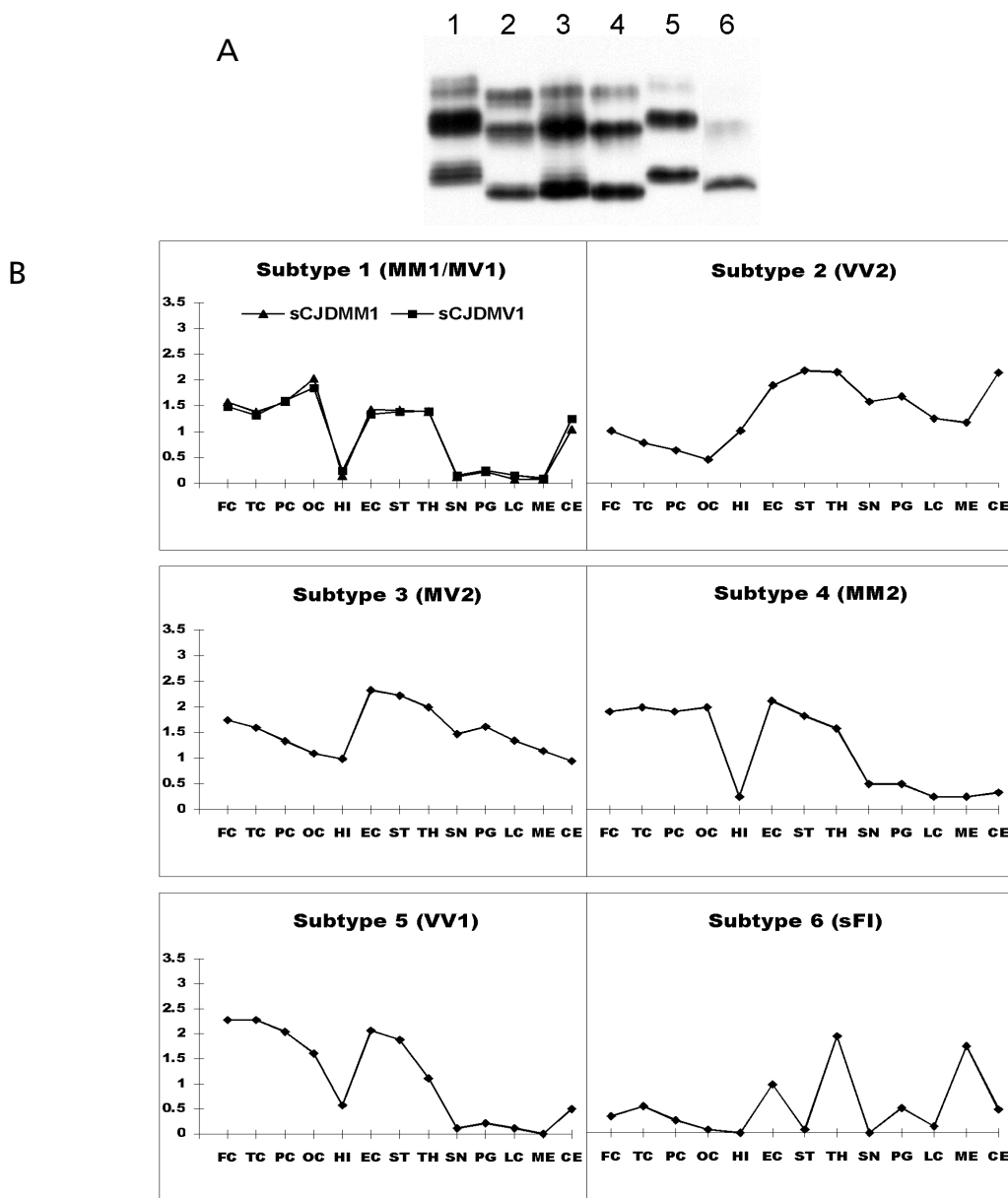
topography of the spongiform degeneration, neuronal loss and astrogliosis, the three cardinal histological lesions of CJD. The three prominent lesions were observed to affect variably either the entire cerebral cortex or only certain layers selectively, and variably extend to basal ganglia and cerebellum<sup>14</sup>. The proliferation of synonyms used to describe CJD, which include ‘vascular encephalopathy’, shows the confusion related to the aetiology and pathogenesis of CJD<sup>22</sup>.

A major breakthrough came about in the late 1960s when Carleton Gajdusek and his co-workers demonstrated that CJD and other forms of prion disease were transmissible to primates<sup>23,24</sup>. At that time, the transmissibility of CJD and other prion diseases was interpreted within the framework of a viral illness. Subsequent work, largely carried out by Prusiner and his colleagues, has led to the identification of PrP<sup>Sc</sup> and the cloning of the human PrP gene (*PRNP*)<sup>25–27</sup>. The discoveries of the MV polymorphism at codon 129 of *PRNP* and of the PrP<sup>Sc</sup> types associated with human prion diseases provided the base for a novel classification of the CJD subtypes<sup>7,28–30</sup>.

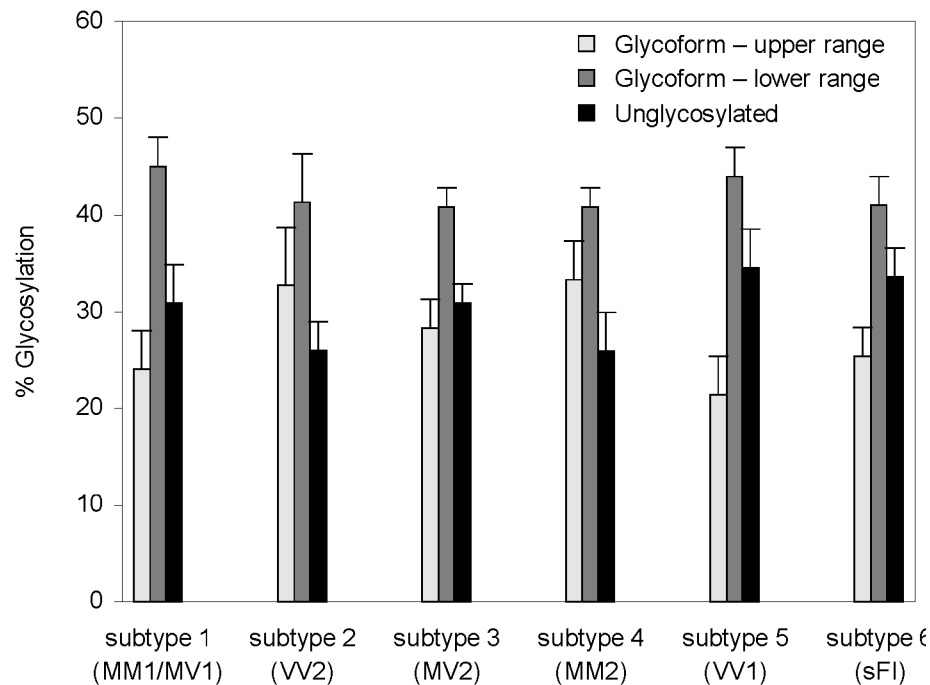
Based on the finding that both genotype at codon 129 and PrP<sup>Sc</sup> types 1 and 2 act as determinants of the disease phenotype, we have divided a large population of patients with the sporadic form of prion disease into six groups, according to whether they carried one of the three (MM, MV, VV) genotypes at codon 129 and either type 1 or type 2 PrP<sup>Sc</sup> (Fig. 2A), whose segregation correlates with the various disease phenotypes previously reported in sporadic CJD (Fig. 2B). Indeed, we observed that patients that were either MM homozygous or MV heterozygous at codon 129 consistently had the typical sporadic CJD (sCJD) phenotype; patients that were either VV homozygous or MV heterozygous and carried PrP<sup>Sc</sup> type 2 had the cerebellar or ataxic form of sCJD even though the histopathology was definitely distinct between the two patient populations. Patients who were VV homozygous and carried PrP<sup>Sc</sup> type 1 and patients who were MM homozygous and carried PrP<sup>Sc</sup> type 2 also had distinct disease phenotypes that had not been previously described. The issue is further compounded by the simultaneous presence of both type 1 and type 2 PrP<sup>Sc</sup> in about 20% of cases of sCJD<sup>31</sup>. Once again, the co-existence of the two types is not random but appears related to the genotype at codon 129. While other PrP<sup>Sc</sup> typing methods and classifications of sCJD have been proposed<sup>32,33</sup>, we believe that our classification of sporadic prion disease into six phenotypically different subtypes represents a realistic and practical system that facilitates the diagnosis of this group of prion diseases based on distinct and easily identifiable molecular features.

### *Subtype 1: sCJDMM1 and sCJDMV1*

This subtype is observed in patients who are MM homozygous or MV heterozygous at codon 129 of the PrP gene and carry PrP<sup>Sc</sup> type 1<sup>6,7</sup>. The



**Fig. 2.** Pr<sup>PSc</sup> types and brain lesion profiles of the six subtypes of sCJD. (A) Immunoblot of PK-resistant PrP<sup>Sc</sup> in the six subtypes of sCJD. Lane 1: subtype 1 (sCJDMM1); lane 2: subtype 2 (sCJDVV2); lane 3: subtype 3 (sCJDMV2); lane 4: subtype 4 (sCJDMM2); lane 5: subtype 5 (sCJDVV1); lane 6: subtype 6 (sFI with 129MM and type 2 PrP<sup>Sc</sup>). (B) Brain lesion profiles of the six subtypes of sCJD (Modified from reference 7). Spongiosis, astrogliosis, and neuronal loss were examined separately for each brain region. Spongiosis was scored on a 0-4 scale: not detectable, mild, moderate, severe, and status spongiosis. Astrogliosis and neuronal loss were scored on a 0-3 scale: not detectable, mild, moderate, and severe. The lesion profiles were based on the final score for each brain region as the average of the three scores. FC: frontal cortex; TC: temporal cortex; PC: parietal cortex; OC: occipital cortex; HI: hippocampus; EC: entorhinal cortex; ST: neostriatum; TH: thalamus; SN: substantia nigra; PG: periventricular grey of midbrain; LC: locus ceruleus; ME: periventricular grey and inferior olive of medulla; CE: cerebellum.



**Fig. 3** Glycoform ratios of PK-resistant PrP<sup>Sc</sup> fragments in the six subtypes of sCJD. The ratios of the three major glycoforms (upper range, lower range, and unglycosylated) of PrP<sup>Sc</sup> in the six sCJD subtypes are compared based on the data in Table 2 of Parchi *et al*<sup>7</sup>. sCJDMM1 and sCJDMV1 are grouped into subtype 1 since they are almost identical in all aspects.

MM and MV CJD-affected subjects have been combined because the comparative study of the pathological features (Fig.2B) as well as the PrP<sup>Sc</sup> gel profile (Fig.3) has revealed no difference between the two patient populations<sup>7</sup>. However, some differences were seen in the clinical features, although the small number of MV patients impedes any conclusion. It is the most common subtype of CJD as it accounts for 60–70% of all cases of sporadic prion disease; over 95% of the patients of this group are 129MM, while the 129MV patients are rare (P. Gambetti *et al.*, unpublished observations)<sup>6,7</sup>. The sCJDMM1/sCJDMV1 subtype corresponds to the variants previously described under the label of myoclonic or ‘classic’ CJD and Heidenhain’s variant. The Heidenhain’s variant has been included in this group because no genotypic and PrP<sup>Sc</sup> type differences were detected between patients with cortical blindness, the main signs of the Heidenhain variant, and the other subjects. The histopathological changes also were indistinguishable.<sup>7</sup>

### *Clinical features*

The mean age at onset of the symptoms is 65 years with a range of 42–91 years; the average clinical duration is 4 months with a range of 1–18 months<sup>7</sup>. The most common presentation in sCJDMM1 patients is cognitive impairment (memory loss, confusion/disorientation, and other types of intellectual decline) which is observed in 70% of the cases; gait or limb ataxia, mental signs (depression, anxiety, psychosis) and visual signs (field defects, distortion, cortical blindness) are the next most common presentations. For sCJDMV1 patients, ataxia rather than cognitive decline as well as sensory defect may be more common at onset. Cognitive impairment, ataxia, myoclonus and pyramidal signs become very common in more advanced stages of the disease. Neurological signs are unilateral at onset in about 25% of cases.

The EEG shows periodic sharp wave (PSW) complexes within the first 3 months of disease in about 80% of the cases<sup>7</sup>. The test based on the cerebrospinal fluid (CSF) level of the 14-3-3 protein, a surrogate marker of CJD, has a sensitivity of about 95%<sup>34,35</sup>, making the concurrent presence of PSW complexes on EEG and positive 14-3-3 tests virtually diagnostic of sCJD<sup>36</sup>.

### *Histopathological features*

Subtype 1 sCJD is characterised by the presence of fine spongiform degeneration, astrogliosis and neuronal loss. The spongiform degeneration is made of fine vacuoles and is fairly homogeneously distributed within the affected regions. In the cerebral neocortex, it affects all layers except for the first; in the cerebellum, it affects the molecular layer. The topography of the lesions shows that the rostral are more severely affected than the caudal brain regions. The cerebral neocortex, especially in the occipital lobe, is more severely affected than basal ganglia, thalamus and cerebellum while the brain stem is virtually spared. The lesions are very severe in the entorhinal cortex, whereas the hippocampal cortex is spared.

Immunohistochemistry for PrP shows a punctate pattern of staining called synaptic, with a degree of intensity that overall is directly related to the severity of the histological regions. However, the immunostaining is often not uniform as relatively large regions may remain unstained or show variable staining intensities (Plate XVII). This may explain the negative PrP immunostaining in 15–20% of the brain biopsies from cases of sCJD (P. Gambetti, unpublished observations).

### *Subtype 2: sCJDV2*

This is the next most common subtype. It accounts for 16% of all cases (P. Gambetti, unpublished observations)<sup>7</sup>, and matches the previously described cerebellar or ataxic variant.



### *Clinical features*

The mean age at onset is about 60 years with a range of 41–81 years; the clinical duration is 6 months with a 3–18 month range. Ataxia was among the presenting signs in all 45 VV patients examined; cognitive impairment and oculomotor signs were present in about one-third of the patients while myoclonus was rare. With disease progression, dementia was observed in all patients, and myoclonus and pyramidal signs affected the majority of the patients. In contrast, cortical signs such as aphasia, apraxia and visual defects were never observed.

The EEG shows non-specific slowing in the great majority of patients while the presence of PSW complexes is limited to less than 10% of the cases. The sensitivity of the 14-3-3 test is about 80%, significantly lower than that of sCJDMM1<sup>34</sup>.

### *Histopathological features*

The lesion triad of fine spongiform degeneration, astrogliosis and neuronal loss also is present in this subtype. The spongiform degeneration is made of fine vacuoles as in sCJDMM1 but, in the cortex, often has a laminar distribution; it preferentially affects deep layers and is generally more severe in the frontal than in the occipital cortex. At variance with the sCJDMM1, the cortex of the hippocampus gyrus shows spongiform degeneration, although the lesions in the entorhinal cortex are far more severe. The topography of the lesions shows that the caudal brain regions are more severely affected than the rostral regions. The cerebral neocortex is generally more severely affected in the frontal than in the occipital lobe and the severity of the lesions is a function of the disease duration so that the cerebral cortex is often spared in cases with rapid course. Overall, the cerebral neocortex is less affected than basal ganglia and thalamus while the brain stem also shows lesions in the dorsal regions and in the substantia nigra; the cerebellar cortex is atrophic.

The PrP immunohistochemistry is characterised by the presence of focal PrP aggregates that look like plaques but do not contain PrP amyloid, since they are Congo red and thioflavine S negative. Other distinctive features are strong immunoreaction around some neuronal perikaria and apical dendrites as well as a laminar distribution of the immunostaining in the deep cortical layers corresponding to the spongiform degeneration in cases with a less than 1-year duration (Plate XVII). Furthermore there is intense immunostaining along cell processes, probably neuronal, especially prominent in the basal ganglia and thalamus. A diagnostic feature of this subtype is the immunostaining pattern of the cerebellum that shows intense immunostaining in the Purkinje and upper granule cell layers due to the presence of numerous plaque-like formations as well as of the dentate and olivary nuclei that stand out from the adjacent parenchyma. The distribution of the PrP<sup>Sc</sup>, like the distribution of the spongiform degeneration, is affected by the duration of the disease<sup>6</sup>. In

cases of less than 5 months' duration, PrP<sup>Sc</sup> is present in relatively small amounts in the cerebral neocortex and in higher, but uniform, amount in other grey-brain regions, a distribution quite different from that of the sCJDM1 and sCJDMV1 subjects in which the highest amount of PrP<sup>Sc</sup> is located in the cerebral cortex. With longer disease duration, the PrP<sup>Sc</sup> distribution also markedly increases in the neocortex and becomes homogeneous throughout the brain grey matter regions.

### *Subtype 3: sCJDMV2*

This subtype accounts for 9%<sup>7</sup> (P. Gambetti, unpublished observations) of all cases of sporadic prion disease. It is phenotypically similar to sCJDVV2, but is easily distinguishable based on significantly longer duration and presence of Kuru plaques in the cerebellum that suggest that the PrP<sup>Sc</sup> associated with these two CJD subtypes are different although both are of type 2. Along with the sCJDVV2, it matches the variant previously described as cerebellar or ataxic.

#### *Clinical features*

The mean age at onset, about 60 years with a range of 40–81 years, is similar to that of sCJDVV2. In contrast, the clinical duration differs significantly as in sCJDMV2 patients it is 17 months with a 5–72 month range, almost 3-fold longer than in the sCJDVV2 patients. Ataxia also is the most common presenting sign (80% of the cases), but cognitive and mental signs are more common than in the sCJDVV2 patients (74% *versus* 27% and 34% *versus* 19%, respectively). As in sCJDVV2 patients, ataxia and cognitive deterioration are present in all patients as the disease progresses. Myoclonus, pyramidal signs are also common as are aphasia and apraxia that are absent in sCJDVV2 patients, while no visual signs are observed as in sCJDVV2 patients.

The EEG and 14-3-3 data are similar to those of the sCJDVV2 patients<sup>34</sup>.

#### *Histopathological features*

Histopathologically, sCJDMV2 is fairly similar to sCJDVV2. The two main changes that distinguish the sCJDMV2 subtype from the sCJDVV2 subtype are: (i) the presence of Kuru plaques in the Purkinje cell layer and superficial granule cell region of the cerebellum; and (ii) the lack of significant cerebellar cortical atrophy. Furthermore, coarse spongiosis, which may be focal or fairly widespread, is occasionally observed, and brain stem lesions are less severe.

The PrP immunohistochemistry also is similar to that of sCJDVV2. However, the immunostaining of the cerebellar cortex is more intense and the PrP aggregates are more compact due to the presence of the Kuru plaques. The laminar immunostaining of the cortex is more

variable and the characteristic immunostaining pattern associated with the coarse spongiosis is seen where this type of spongiosis is present.

#### *Subtype 4: sCJDMM2*

The fourth subtype is found in 2–8% of cases (P. Gambetti, unpublished observations)<sup>7</sup>. This subtype was not recognised prior to 1999, although the type of spongiform degeneration that characterises this subtype was reported in earlier studies<sup>7,14,37</sup>.

##### *Clinical features*

The mean age at onset is 65 years with a 49–77 year range, and the average disease duration is 16 months with a 9–36 months range. Presentation is dominated by cognitive impairment, observed in all cases examined<sup>7</sup>, followed by aphasia in one-third of the cases. Through the course of the illness, myoclonus and pyramidal signs also become common, and Parkinsonism, apraxia and seizures are present in about 30% of the cases, but ataxia is uncommon.

The EEG characteristically shows non-specific slowing; only occasionally there are non-periodic paroxysmal discharges while PSW complexes have not been reported. The 14-3-3 tests were positive in 6 of 8 cases examined<sup>34</sup>.

##### *Histopathological features*

The hallmark of the sCJDMM2 subtype is the spongiform degeneration with large vacuoles, identified as status spongiosis by previous authors<sup>38</sup> and, more recently, coarse spongiosis<sup>6</sup>. The vacuoles are several times larger than the characteristic vacuoles of the typical spongiform degeneration of sCJDMM1, and are widespread in the cerebral cortex basal ganglia and thalamus. They are often confluent and result in the formation of rounded islands of tissue surrounded by vacuoles reminiscent of the ‘florid’ plaques of vCJD but with the substantial difference that PrP amyloid plaques are not present. The coarse spongiform degeneration is often accompanied by intense astrogliosis. There is minimal pathology in the brain stem while the cerebellum is virtually unaffected. Overall, the lesion profile in sCJDMM2 is similar to that of the subtype 1 (see above).

PrP immunohistochemistry reveals two basic patterns an intense staining of the rim of the large vacuoles<sup>39</sup> and a spotted pattern with loose plaque-like formations.

#### *Subtype 5: sCJDVV1*

This is the most uncommon sCJD subtype as it affects about 1% of all cases of sporadic prion disease but it is of great interest because it may

include patients with early onset of the clinical disease<sup>7</sup>. This subtype was not identified prior to 1999.

#### *Clinical features*

Reported age at onset and duration are 39 years (range, 24–49 years) and 15 months (range, 14–16 months), which would characterise this subtype as early onset<sup>7</sup>. However, these data, based on three cases, need to be confirmed in a large cohort. Presentation is characterised by dementia mainly of the fronto-temporal type, which may evolve for some months without significant motor signs. Myoclonus and pyramidal signs eventually appear.

EEG shows slowing but not PSW complexes. The 14-3-3 tests were positive in the three cases examined<sup>34</sup>.

#### *Histopathological features*

The apparent hallmark of this subtype is the dissociation between the histological lesions that include severe fine spongiform degeneration, gliosis, and occasionally neuronal loss, and the PrP immunostaining that is faint and has a synaptic pattern. Neurofilament-containing ballooned neurons are also observed in the neocortex. The distribution of the lesions reveals the following differences with that of the sCJDMM1 subtype: (i) the severity of the lesions decrease (rather than increase) from the frontal to the occipital cortex; (ii) the hippocampal cortex is more affected; and (iii) the thalamus and cerebellum are less affected.

### *Subtype 6: sporadic fatal insomnia (sFI)*

This subtype is also rare as it accounts for about 2% of all cases of sporadic prion disease. A total of nine proven cases of this subtype have been reported<sup>40–42</sup>. Its phenotype is indistinguishable from that of fatal familial insomnia (FFI) and is referred to as sporadic fatal insomnia (sFI)<sup>41</sup>. Phenotypically, sFI corresponds to the thalamic variant of CJD of old classifications<sup>7,14,43</sup>. sFI is linked to the 129MM genotype and PrP<sup>Sc</sup> type 2. Although the sFI genotype and protein type are the same as those of subtype 4, the two PrP<sup>Sc</sup> species associated with these two subtypes are different (see below) and likely to be distinct prion strains.

#### *Clinical features*

The mean age at onset is 50 years (range, 36–70 years) and the mean duration 24 months (range, 15–53 months). The clinical presentation commonly includes ataxia, visual signs and cognitive impairment. Most patients eventually shows insomnia, dementia, and motor signs including ataxia, dysarthria, tremor, myoclonus and spasticity<sup>41,42</sup>.

The EEG shows non-specific slowing. Polysomnographic recordings carried out in one case were indistinguishable from those of FFI<sup>42</sup>.

### *Histopathological features*

The major pathology is centred in the thalamus especially the medial dorsal and anterior ventral nuclei, that show severe astrogliosis and neuronal loss but generally without spongiform degeneration. The inferior olives also show similar lesions. Other brain regions are less affected. Spongiform degeneration and gliosis are moderate or minimal and focal in the cerebral cortex, where they preferentially affect anterior neocortical regions and the entorhinal cortex<sup>41</sup>. Astrogliosis and neuronal loss are also minimal in basal ganglia and cerebellum.

As in FFI, PrP<sup>Sc</sup> associated with sFI is of type 2 and is present in amounts that, except for entorhinal cortex, are 10–50 times lower than in sCJDMM1. However, PrP<sup>Sc</sup> associated with sFI shows a different glycoform ratio<sup>41</sup>. While in FFI PrP<sup>Sc</sup> is characterised by a marked under-representation of the unglycosylated form, in sFI the ratio of the three glycoforms (diglycosylated, monoglycosylated and unglycosylated) expressed as a percentage distribution of the forms is 26:40:34, similar to that of other sCJD subtypes<sup>41</sup>. The different PrP<sup>Sc</sup> glycoform ratio is a consistent finding helpful in distinguishing sFI from FFI on immunoblot.

One sFI case has been transmitted to transgenic mice expressing a mouse-human chimeric PrP with methionine at codon 129. The affected mice developed a disease characterised by the presence of PrP<sup>Sc</sup> type 2 as in the donor, and a distribution of the histology lesions and of PrP<sup>Sc</sup> that is similar to that of FFI, arguing that the PrP<sup>Sc</sup> species associated with sFI and FFI belong to the same strain<sup>40</sup>.

The finding that sFI and sCJDMM2 share the same 129 genotype and PrP<sup>Sc</sup> type but phenotypically are quite distinct raises questions concerning the molecular basis of phenotypic diversity in these two subtypes. However, using one- and two-dimensional gel electrophoresis we have shown that the PrP<sup>Sc</sup> species present in these two conditions differ in the glycans they carry, providing a possible role of glycans in phenotypic determination<sup>7,44</sup>.

### *Sporadic CJD associated with both PrP<sup>Sc</sup> types 1 and 2*

Types 1 and 2 PrP<sup>Sc</sup> co-exist in up to 25% of cases with sCJD<sup>7,45</sup>. The two PrP<sup>Sc</sup> types may be present in the same anatomical region (*i.e.* the same cortical gyrus) or may occupy different regions such as cortical and subcortical regions<sup>45</sup>. Type 1 appears to be better represented than type 2 in MMsCJD subjects, while VV subjects seem to have more type 2<sup>31</sup>. The clinical and pathological phenotype in each subject depends on the

**Table 2** Genotype and phenotype of familial CJD and FFI (reprinted with permission from Kong *et al*<sup>52</sup>.)

Genotype	Onset (years)	Duration	Clinical and pathological features
Familial CJD			
P105T	30–42	NA	* NA ** NA
R148H–129M	63	18 months	* Like sCJDMV2 ** Like sCJDMV2
D178N–129V	26–56	9–51 months	* Dementia, ataxia, myoclonus, extrapyramidal and pyramidal signs ** Spongiosis, neuronal loss and astroglia in the cerebral cortex (most severe), striatum, and thalamus (least severe), while the cerebellum is spared
V180I–129M	66–85	1–2 years	* Similar to typical sCJD but with a slower progression ** Like typical sCJD
T183A–129M	45	4 years	* Personality changes followed by dementia and Parkinsonism ** Atrophy with spongiform degeneration in the cerebral cortex and, to a lesser extent, in the basal ganglia
T188A–129M	82	4 months	* Like sCJDMM1 ** Immunohistochemistry for PrP negative, no immunoblot
T188K	59		* Dysphasia, rapidly progressive dementia, and negative family history ** NA
T188R			* NA ** NA
E196K–129M	63–77	~1 year	* Rapidly progressive dementia, ataxia, no PSW on EEG ** NA
E200K–129M	35–66	2–41 months	* Similar to typical sCJD; atypical signs such as supranuclear palsy and peripheral neuropathy in some cases ** Like typical sCJD
V203I–129M	69	~1 month	* Sudden confusion hallucinations, abnormal motor functions, myoclonus, PSW on EEG, negative family history ** NA
H208R–129M	60	7 months	* Like typical sCJD ** Like typical sCJD
V210I–129M	49–70	3–5 months	* Like typical sCJD ** Like typical sCJD
E211Q–129M	42–81	3–32 months	* Like typical sCJD, PSW on EEG ** NA
M232R–129M	55–70	4–24 months	* Like typical sCJD ** Like typical sCJD
FFI			
D178N–129M	20–71	6–33 months	* Reduction of total sleep time, enacted dreams, sympathetic hyperactivity, myoclonus, ataxia; late dementia, pyramidal and extrapyramidal signs in the cases with a relatively long duration (> 1 year) ** Preferential thalamic and olivary atrophy; spongiform changes in the cerebral cortex in the subjects with a duration of symptoms longer than 1 year

\*Clinical and \*\*pathological features. NA, not available.

PrP<sup>Sc</sup> type that predominates<sup>31</sup>. The phenotype of most MM1+2 sCJD patients largely mimics the phenotype of MM1 subjects while that of the VV1+2 cases is similar to that of the sCJDVV2 subtype<sup>31</sup>. Similarly, PrP

immunostains with a combination of the synaptic and perivacuolar patterns in the MM1+2 cases helps to distinguish these cases<sup>31</sup>. Since PrP immunostaining in the sCJDV1 is less distinctive, the immunohistochemical recognition of the VV1+2 subjects is more difficult<sup>31</sup>. The finding that the genotype at codon 129 seems to influence the PrP<sup>Sc</sup> type that, in turn, determines the phenotype is consistent with the molecular mechanisms of phenotypic determination outlined above.

## Familial prion diseases: Creutzfeldt-Jakob disease and fatal insomnia

The first familial case of CJD was recorded in 1924 by Kirschbaum<sup>15</sup>. However, it was Meggendorfer who showed, in 1930, that the subject described by Kirschbaum was a member of a large kindred that became known as the 'Backer' family<sup>46</sup>. This family was subsequently proven to carry an inherited form of CJD<sup>47</sup>. About 50 years later, the transmissibility of familial and sporadic CJD was demonstrated<sup>48-51</sup>. These experiments established for the first time that familial prion diseases have the unique properties of being both inherited and infectious. Cloning of the PrP gene<sup>27,28</sup> led to the demonstration that inherited forms of human prion diseases, such as familial CJD and FFI, are linked to pathogenic mutations in the human PrP gene (*PRNP*)<sup>52</sup>. The transmissibility of familial prion disease by an infectious mechanism was then explained in the context of the prion hypothesis: PrPs bearing the pathogenic mutations possess a high propensity to convert spontaneously to the infectious isoform<sup>53</sup>. The majority of studies carried out over the past decade support this mechanism.

A large number of mutations and polymorphisms have been reported in the *PRNP* gene<sup>52</sup>. The mutations include 24 missense point mutations, 27 octapeptide repeat mutations with insertions of 1, 2 and 4-9 additional repeats, 2 octapeptide repeat mutations with deletion of two repeats and two nonsense mutations. Three missense polymorphisms located at codons 129 (M/V), 171 (N/S), and 219 (E/K), and the deletion of one 24-bp octapeptide repeat is known along with 12 silent polymorphisms<sup>52</sup>. Table 2 lists the mutations with CJD or FFI phenotypes.

In familial prion diseases, polymorphism at codon 129 affects the phenotype when located on the mutant allele, while on the normal allele it may influence age at onset and duration of the disease. Based on the effects on the disease phenotype of the polymorphism at codon 129 (as well as other polymorphisms), we have used both the pathogenic mutation and the codon 129 (and other mutant/polymorphic codons, when necessary) on the mutant allele (*i.e.* the haplotype in this discussion) to identify more accurately each individual familial prion disease.

### *CJD with the E200K–129M haplotype (CJD<sup>E200K–129M</sup>)*

CJD<sup>E200K–129M</sup> is the most common form of familial CJD. It is particularly prevalent in Jews of Libyan and Tunisian origin that have an incidence of CJD about 100 times higher than the world-wide average, and in Slovaks<sup>54,55</sup>. The penetrance of this mutation is 90–100%, if the possibility of late age at onset of this disease is taken into account<sup>56,57</sup>.

#### *Clinical features*

CJD<sup>E200K–129M</sup> is similar to sCJD<sup>MM1</sup>, the most common subtype of sCJD<sup>6,7,58</sup>. The mean age at onset is 58 years (range, 33–84 years) and the mean duration 6 months (range, 2–41 months)<sup>59,60</sup>. The presentation includes cognitive and mental abnormalities (80–83% of patients), cerebellar signs (43–55%), visual signs (19%), and myoclonus (12%)<sup>59,61</sup>. During the course of the disease, all patients develop dementia, 73% have myoclonus, 79% cerebellar signs, 40% seizures, while sensory and cranial nerve involvement is present in 24%<sup>61</sup>. CJD<sup>E200K–129M</sup> patients also show signs of motor and sensory peripheral neuropathy rarely found in sCJD patients.

The EEG shows slowing in all patients while about 75% display PSW complexes<sup>61–63</sup>. Brain atrophy is detected in about one-third of patients with computed tomography (CT)<sup>64</sup>. The CSF 14-3-3 protein is elevated in almost all the CJD<sup>E200K</sup> cases<sup>65,66</sup>. Insomnia has been reported as a prominent sign in one patient with severe pathology of the thalamus.

#### *Histopathological features*

Spongiform degeneration, astrogliosis and neuronal loss in various combinations of severity are invariably present<sup>6,7</sup>. They are generally severe and widespread in the cerebral cortex and milder in the striatum, diencephalon, and cerebellum. The extent of astrogliosis and neuronal loss appears to be related to disease duration.

PrP immunohistochemistry is consistently positive throughout the brain with a punctate or ‘synaptic’ pattern apparently correlated to the histological lesions. Different PrP patterns in the cerebellum have been reported for CJD<sup>E200K–129M</sup> subjects with Met or Val at codon 129 on the normal allele, with a synaptic pattern for 129MM subjects and granules and plaque-like structures for 129MV subjects<sup>69</sup>. PrP immunostaining is also seen in the substantia gelatinosa of the spinal cord. The peripheral neuropathy is both axonal and with segmental demyelinating<sup>64,68,69</sup>.

The PrP<sup>Sc</sup> in CJD<sup>E200K–129M</sup> is of type 1 and is characterised by the under-representation of the unglycosylated form<sup>30,70</sup>, similar to that observed in FFI (see below) and in vCJD<sup>30,32</sup>. Brain homogenates from CJD<sup>E200K–129M</sup> patients have been used successfully to infect apes<sup>71</sup> and humanised transgenic mice<sup>72</sup>.



### *CJD with the E200K–129V haplotype (CJD<sup>E200K–129V</sup>)*

The E200K–129V haplotype has been reported<sup>52</sup> in five subjects from apparently unrelated families, and all have type 2 PrP<sup>Sc</sup>. In contrast to those of E200K–129M, the phenotypes of the E200K–129V haplotype are similar to those of sCJDV2<sup>7</sup>, presenting as ataxia at onset followed by myoclonus and PSW complexes on EEG examination. PrP<sup>Sc</sup> accumulates predominantly in the cerebellum where it forms plaque-like structures.

### *CJD with the D178N–129V haplotype (CJD<sup>D178N–129V</sup>)*

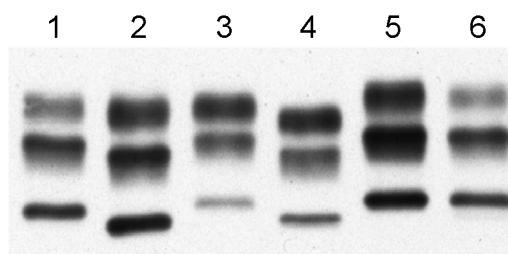
The D178N–129V haplotype has been observed in a total of 12 apparently unrelated kindreds from Germany, America, France, Israel, and Finland<sup>7</sup>, including the Backer family<sup>46,47,73</sup>. CJD<sup>D178N–129V</sup> shares the D178N PRNP mutation with FFI that has codon 129M on the mutated allele (see below). Genetic linkage analysis is consistent with the D178N–129V haplotype being the cause of the disease<sup>74</sup>.

#### *Clinical features*

Initial signs include cognitive impairment, especially memory loss, often associated with depression, irritability, and abnormal behaviour, followed by ataxia, speech impairments with dysarthria and aphasia, tremor, and myoclonus. EEG examination reveals generalised slow-wave activity without PSW complexes in most cases. Insomnia has not been reported in CJD<sup>D178N–129V</sup>. The identity of codon 129 on the normal allele appears to modify the age at onset and disease duration: the mean age at onset is  $39 \pm 8$  years (range, 26–47 years) for the 129VV patients and  $49 \pm 4$  years ( $P < 0.01$ ; range, 45–56 years) for the 129VM patients; the mean duration is  $14 \pm 4$  months (range, 9–18 months) for 129VV patients and  $27 \pm 14$  months ( $P < 0.05$ ; range, 7–51 months) for the 129VM patients<sup>14,29,47</sup>. However, clinical signs and histopathology appear to be independent of the zygosity at codon 129.

#### *Histopathological features*

CJD<sup>D178N–129V</sup> usually displays spongiosis associated with prominent gliosis, often in the form of gemistocytic astrocytes, and variable degrees of neuronal loss<sup>75</sup>. Enlarged or ballooned neurons with argyrophilic and Lewy body-like inclusions composed of neurofilaments are sometimes present (P. Parchi and P. Gambetti, unpublished observations). The topography of these lesions is consistent and fairly distinctive. It is characterised by widespread involvement of the cerebral cortex including subiculum and entorhinal cortex of the hippocampus as well



**Fig. 4** Immunoblot of PrP<sup>Sc</sup> in sCJD and fCJD with PrP<sup>Sc</sup> types 1 or 2. Lane 1, sCJD 129MM, PrP<sup>Sc</sup> type 1; lane 2, sCJD 129VV, PrP<sup>Sc</sup> type 2; lane 3, fCJD E200K-129M, PrP<sup>Sc</sup> type 1; lane 4, fCJD E200K-129V, PrP<sup>Sc</sup> type 2; lane 5: fCJD 144bp-Insert-129M, PrP<sup>Sc</sup> type 1; lane 6, fCJD V210I-129M, PrP<sup>Sc</sup> type 1. All samples were PK-treated (reprinted with permission from Kong *et al*<sup>52</sup>).

as spongiosis in the fascia dentata and severe spongiosis with variable degrees of gliosis of the putamen and the caudate nucleus; the thalamus is moderately affected while cerebellum and brain stem show little or no pathology. The PrP immunostaining pattern is punctate, and its intensity matches the severity of the histopathology. However, the cerebellum shows minimal, but definite, immunostaining despite the lack of structural changes. There are no PrP plaques.

PrP<sup>Sc</sup> in CJD<sup>D178N-129V</sup> patients examined to date is of type 1 with under-representation of the unglycosylated form (Fig. 4)<sup>30,70,76</sup>. It is derived exclusively from the mutant PrP<sup>77</sup>, suggesting no direct participation of PrP<sup>C</sup> expressed by the normal allele.

CJD<sup>D178N-129V</sup> has been transmitted to squirrel monkeys<sup>21,78</sup> that have 129MM, but transmission to transgenic mice expressing the human/mouse chimeric PrP-129M has failed<sup>79</sup>.

### *CJD with the V210I-129M haplotype (CJD<sup>V210I-129M</sup>)*

The V210I-129M haplotype has been reported in 23 subjects<sup>52</sup>. The V210I mutation exhibits low penetrance.

#### *Clinical features*

The mean age at onset is 59 years with a range of 46–80 years; the average duration is 6 months with a range of 2 to more than 24 months. The initial clinical signs include memory, behavioural and gait disturbances, sudden sensory and motor deficits, clumsiness, dystonic movements, and dysarthria, followed by myoclonus, dysarthria, mutism, cerebellar signs, and diffuse white matter degeneration in the later stages<sup>80</sup>. PSW complexes are present on EEG<sup>81–84</sup>. Global or

temporal cortical hypoperfusion was detected by single-photon emission computed tomography (SPECT)<sup>84</sup>.

### *Histopathological features*

CJD<sup>V210I-129M</sup> is characterised by spongiosis and gliosis of the grey matter, often more prominent in the cerebral cortex and molecular layer of the cerebellum<sup>81,82,84</sup>.

The V210I PrP<sup>Sc</sup> is of type 1<sup>6,84,85</sup> and derived from both the mutant and normal PrP<sup>77,86</sup>. CJD<sup>V210I-129M</sup> has been transmitted to humanised transgenic mice<sup>90</sup> that showed a PrP<sup>Sc</sup> distribution similar to that produced by sCJD and different from that of other familial prion diseases<sup>84</sup>.

### *Familial CJD with rare mutations*

#### *The V180I–129M haplotype (CJD<sup>V180I-129M</sup>)*

A total of seven cases with this haplotype have been reported. Six are Japanese and one American; all have no family history. Four subjects carried 129M on the normal allele<sup>87–90</sup>, and one subject had a second mutation (M232R) on the other allele (see below). The distinguishing features of this haplotype are the relatively long duration (1–2 years) and the possible presence of Kuru plaques and plaque-like formations in 129MV subjects<sup>88–90</sup>.

#### *The T183A–129M haplotype (CJD<sup>T183A-129M</sup>)*

This has been observed in 17 subjects, of whom 12 were from a Brazilian kindred of Spanish and Italian origin and the others from the US, Germany, and Venezuela<sup>52</sup>. Distinguishing features are the long duration (up to 9 years) and the clinical picture (including imaging) resembling frontotemporal dementia. The PrP immunostaining pattern is punctate<sup>91</sup>, and PrP<sup>Sc</sup> is of type 2 in one case examined<sup>92</sup>.

#### *The M232R–129M haplotype (CJD<sup>M232R-129M</sup>)*

This has been reported in eight Japanese subjects with no family history of neurodegenerative diseases<sup>93</sup>. Five patients were 129MM, one 129MV, one 219EK with the 219K on the normal allele<sup>88</sup>, and one with the V180I mutation on the other allele<sup>87</sup>. An additional subject carrying the M232R–129M mutation had diffuse Lewy body and progressive dementia but displayed no other characteristics of CJD<sup>94</sup>. At variance with the previous two haplotypes, the presence of PSW complexes at EEG is common. CJD<sup>M232R-129M</sup> has been transmitted to wild-type mice at an efficiency apparently comparable to that of sCJD<sup>93</sup>.

#### *V180I–129M and M232R–129M*

This is a unique, twice-mutated genotype involving both alleles that has been reported in an 84-year-old subject with clinical and pathological

features of the typical sCJD<sup>87</sup>. The high molecular-weight glycoform of PrP<sup>Sc</sup> is reported absent<sup>87</sup>.

*Deletions of two octapeptide repeats (CJD<sup>Del2-129M</sup>)*

Such mutations involving the R2 and R3 or both R2 repeats have recently been reported in two families<sup>95,96</sup>. The disease phenotype, including PrP<sup>Sc</sup> type, mimics that of the typical type 1 sCJD<sup>96</sup>.

*Other rare or novel PRNP mutations with CJD-like phenotypes*

The following PRNP mutations have been reported in only one or a few patients each. R148H-129M is similar to sCJDMV2 in all aspects<sup>7,97</sup>. R208H-129M<sup>98,99</sup>, T188A-129M<sup>100</sup> and E211Q-129M<sup>101,102</sup> are very similar to sCJDMM1 in all aspects examined. Q160Stop-129M<sup>103</sup>, E196K-129M<sup>101</sup>, V203I-129M<sup>101</sup>, T188K<sup>103</sup>, and probably P238S<sup>104</sup>, T188R<sup>103,104</sup>, and P105T ([http://www.mad-cow.org/prion\\_point\\_mutations.html](http://www.mad-cow.org/prion_point_mutations.html)) display some signs of neurodegeneration. In addition, I138M and G142S were reported in patients with illness unrelated to prion diseases ([http://www.mad-cow.org/prion\\_point\\_mutations.html](http://www.mad-cow.org/prion_point_mutations.html)).

*Fatal familial insomnia*

The D178N-129M haplotype associated with FFI is among the most prevalent familial CJD after CJD<sup>E200K</sup>. It has been reported in at least 25 pedigrees and 5 additional subjects with no family history from different countries and races<sup>52</sup>.

*Clinical features*

The disease presents between 20 and 72 years (average, 49 years) with no significant difference between 129MM and 129MV subjects. In contrast, the duration was  $11 \pm 4$  months in the 40 subjects with 129MM, significantly shorter than the  $23 \pm 19$  months in the 17 subjects with 129MV ( $P < 0.001$  based on Student's *t*-test). However, the ranges significantly overlap in the two groups. The signs include insomnia and oneiric or stuporous episodes with hallucinations and confusion, autonomic dysfunction (systemic hypertension, irregular breathing, diaphoresis, pyrexia and impotence), and often myoclonus, spasticity and seizures. Some of the signs differ between 129MM and 129MV subjects. Insomnia, myoclonus and autonomic malfunction are often more severe in 129MM subjects, while in 129MV subjects ataxia, dysarthria and seizures often predominate.

Routine EEG activity is generally slowed and may demonstrate PSW complexes associated with myoclonus in patients with long disease duration. Polysomnography is an invaluable diagnostic tool in FFI as it shows markedly shortened sleep time and disorderly transition between sleep stages<sup>105</sup>. These changes are less severe in the 129MV patients. Similarly helpful is PET scanning that demonstrates hypometabolism in the thalamus and cingulate cortex, more widespread in 129MV patients than in 129MM patients<sup>106</sup>. SPECT has detected a dramatic decrease in dopamine and serotonin transporters in the thalamic–hypothalamic region of two FFI patients<sup>107</sup>.

### *Histopathological features*

FFI is characterised by loss of neurons and astrogliosis in the thalamus in all cases and in the inferior olives in most cases<sup>16,106,108–111</sup>. The severity of the histopathology in the cerebral cortex increases with the disease duration, often with spongiosis and astrogliosis in the entorhinal cortex and the piriform and para-olfactory cortices. Mild neuronal loss, which is likely due to apoptosis<sup>112</sup>, is also seen in the cerebellum<sup>16,106,108–111</sup>, peri-aqueductal grey of the midbrain, the reticular formation and raphe of the brain stem<sup>16</sup>. PrP deposits are usually absent, but may be detected in the molecular layer of the cerebellum with a strip-like pattern and in the subiculum-entorhinal region<sup>16</sup>.

The PrP<sup>Sc</sup> is of type 2 with marked under-representation of the unglycosylated form<sup>2,30,109</sup> (Fig. 4).

FFI has been transmitted to transgenic mice expressing a chimeric human/mouse PrP<sup>79</sup>, with PrP<sup>Sc</sup> type and deposition as well as pathology mimicking those of FFI.

### *Familial CJD associated with insertional mutations*

At least 32 families are known to have a prion disease associated with 1–9 (but not 3) extra 24-bp repeats in the *PRNP* gene<sup>52</sup>. In 26 families, the repeat expansion is coupled with methionine at codon 129, while in 6 other families the inserts were found in the valine allele. As in other inherited prion diseases, the amino acid at codon 129 may influence the phenotypic effects of the repeat-expansion mutations.

If all insertion mutations are considered together, the disease phenotype is highly variable because clinical and pathological features include either a typical CJD phenotype or a phenotype more consistent with GSS while rare cases lack specific histopathology<sup>32,113,114</sup>. The phenotypic heterogeneity becomes less pronounced when cases are grouped according to the number of repeats.

### *Clinical features*

In cases with insertions of 4 or fewer repeats, the mean age at onset is 62 years (range, 82–52 years) and the duration 6 months (range, 2–14 months) excluding 2 cases with an exceptional 7-year duration<sup>52</sup>; patients commonly present with rapidly progressive dementia, ataxia, visual disturbances and myoclonus with PSW on the EEG. Disease penetrance is low. In contrast, the mean age at onset in patients with 5 or more extra repeats is 32 years (range, 21–61 years) and a mean disease duration of 6 years (range, 3 months to over 19 years)<sup>52</sup>. The vast majority of these patients present with a slowly progressive syndrome characterised by mental deterioration, cerebellar and extra-pyramidal signs often lacking the PSW complexes on EEG examination.

### *Histopathological features*

Histopathologically, the cases with 4 or fewer extra repeats are indistinguishable from classical CJD, whereas the cases with 5–7 extra repeats are heterogeneous and may show either CJD-like change, GSS-like features, or cannot be easily classified as GSS or CJD changes<sup>52</sup>.

PrP<sup>Sc</sup> of types 1 or 2 (or close to them) has been demonstrated in cases with 1 and 4–7 extra repeat insertions<sup>52</sup>.

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