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Neuropathology of variant Creutzfeldt-Jakob disease

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Abstract. The clinical, neuropathological genetic and biochemical features of variant Creutzfeldt-Jakob disease (vCJD) are compared to the 926 other cases of suspected CJD referred to the National CJD Surveillance Unit laboratory from 1990-2001. Histological studies of the central nervous system, lymphoid tissues and other organs were accompanied by immunocytochemistry for prion protein (PrP); Western blot analysis of PrP^{RES} was performed on frozen brain tissue. The pathology of vCJD showed relatively uniform morphological and immunocytochemical characteristics, with PrP accumulation in lymphoid tissues, but not in other non-neural tissues. PrP^{RES} accumulation in vCJD showed a uniform glycotype pattern distinct from sporadic CJD. All cases of vCJD were methionine homozygotes at codon 129 of the PrP gene. In view of the spread of bovine spongiform encephalopathy in Europe and Japan, continuing surveillance is required for all forms of CJD, with histological and biochemical analysis of suspected cases to allow an accurate laboratory diagnosis.

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INTRODUCTION

Surveillance of Creutzfeldt-Jakob disease (CJD) was reinstated in the UK in 1990 in light of the epidemic of bovine spongiform encephalopathy in cattle and its possible consequences for humans. Identification of a new variant form of Creutzfeldt-Jakob disease (vCJD) in 1996 was based on neuropathological and clinical features in a series of 10 patients (Will et al. 1996) in the UK. At present, 116 cases of vCJD in the UK, six cases in France, one in Italy and one in Ireland have been identified. All patients who have undergone analysis of the prion protein gene (*PRNP*) were homozygous for methionine at the polymorphic codon 129. Since the identification of vCJD in 1996, the evidence for a causal relationship between vCJD and BSE has been considerably strengthened by the results of experimental strain typing and biochemical studies of the disease –associated form of the prion protein (PrP^{RES}) in wild-type and transgenic mice (Collinge et al. 1996, Bruce et al. 1997, Scott et al. 2000). Since it is likely that human exposure to BSE has occurred across Europe in all prion protein genotypes in the population, the development of criteria to detect a BSE-related disease is clearly important for surveillance purposes.

Neuropathological examination is mandatory for the confirmation of a diagnosis of vCJD (WHO 1996). The original neuropathological criteria for vCJD are reviewed in relation to the widening age range of vCJD patients, with particular attention to the differentiation of vCJD from other forms of CJD (Will et al. 2000). These criteria have been applied to all suspected cases of CJD referred to the National CJD Surveillance Unit up to the end of 2001, in whom detailed clinical and genetic data are available.

METHODS

Since 1990, neuropathological review of all cases of CJD in the United Kingdom has been undertaken at the National CJD Surveillance Unit. Since the project began, 926 cases of suspected CJD have been examined. Brain biopsy is seldom performed as a primary investigation in cases of CJD, and the diagnosis is usually confirmed after autopsy. Brain tissue from autopsy cases were fixed in formalin for a minimum of three weeks prior to brain dissection. The brains were sampled extensively, including material from the frontal, parietal, temporal and occipital cortex, the hippocampus, hypothalamus, thalamus, basal ganglia, midbrain, pons, medulla and spinal cord (when available). Other organs were examined histologically if material was available.

All tissue blocks were immersed in 96% formic acid for one hour prior to routine processing. Sections were cut at 5 μm and stained by conventional histological techniques and by immunocytochemistry for PrP using monoclonal antibodies which recognise different PrP epitopes in a standardised validated technique (Bell et al. 1997) (see Table I).

Biochemical Analysis

Frozen brain tissue was stored at –80°C and investigated for the presence of protease-resistant PrP (PrP^{RES}) by Western blotting as previously described (Ironside et al. 2000). Briefly, 10% brain homogenates were subjected to limited proteolysis by digestion with Proteinase K (BDH). Electrophoresis was performed on a 12%T acrylamide SDS-PAGE mini-gel format (Bio-Rad Laboratories) and proteins transferred to Hybond ECL membranes (Amersham Parmacia Biotech). The anti-PrP monoclonal antibody 3F4 (Senetek) was used at a 1:10,000 dilution. Detection employed ECL+ reagents and Hyperfilm ECL (Amersham Pharmacia Biotech). PrP^{RES} was visualised by the inclusion of streptavidin peroxidase at a dilution of 1:1,500 in the secondary incubation. The molecular weight was estimated by reference to biotinylated ECL

Table I

Details of antibodies used for immunocytochemistry

Antibody	Source	Dilution	Incubation
PrP (KG9)	TSE Resource Centre Compton, UK	1:50	overnight at room temperature
PrP (3F4)	Senetek, USA	1:2000	overnight at room temperature
PrP (6H4)	Prionics, Switzerland	1:3000	overnight at room temperature

molecular weight markers (Amersham Pharmacia Biotech.).

RESULTS

Pathology of vCJD cases

The clinical and neuropathological features of vCJD cases in the UK are summarised in Tables II and III. The brain weight in most cases was within the normal ranges for age. However, in cases with a lengthy clinical history (>18 months) there was evidence of cerebellar atrophy (particularly involving the vermis), with a corresponding reduction in myelinated axons in the white matter. Cerebral cortical atrophy was identified in patients who had survived for 28 months and over. Incidental findings in vCJD include a small right temporal cavernous angioma and a small focus of chronic inflammation within the brainstem in the absence of a widespread encephalomyelitis.

Spongiform change was focally distributed within the cerebral cortex in vCJD, particularly in the occipital and inferior frontal regions. Extensive confluent spongiform change was not a prominent feature in the cerebral cortex (in comparison to cases of sporadic CJD). The hippocampus was relatively spared. In cerebellar cortex, spongiform change was a prominent feature in the hemispheres and vermis with no particular anatomical predilection. Extensive spongiform change was always present in the caudate nucleus and putamen. In the thalamus, focal spongiform change involved many of the anterior and medial nuclei, often in the absence of plaques. In the hypothalamus, spongiform change was most evident in the paraventricular and supraoptic nuclei. Spongiform change was also detected in the periaqueductal grey matter of the midbrain and the pontine nuclei, but was not present in either the medulla or spinal cord.

Table II

Clinical diagnostic criteria for vCJD

I	A	Progressive neuropsychiatric disorder
	B	Duration of illness > 6 months
	C	Routine investigations do not suggest an alternative diagnosis
	D	No history of potential iatrogenic exposure
	E	No evidence of a familial form of TSE
II	A	Early psychiatric symptoms[a]
	B	Persistent painful sensory symptoms[b]
	C	Ataxia
	D	Myoclonus or chorea or dystonia
	E	Dementia
III	A	EEG does not show the typical appearance of sporadic CJD[c] (or no EEG performed)
	B	Bilateral pulvinar high signal on MRI scan
IV	A	Positive tonsil biopsy[d]

DEFINITE:	I A and neuropathological confirmation of vCJD[e]
PROBABLE:	I and 4/5 of II and III A and III B OR I and IV A[d]
POSSIBLE	I and 4/5 of II and III A

a, depression, anxiety, apathy, withdrawal, delusions; b, this includes both frank pain and/or dysaesthesia; c, generalised triphasic periodic complexes at approximately one per second; d, tonsil biopsy is not recommended routinely, nor in cases with EEG appearances typical of sporadic CJD, but may be useful in suspect cases in which the clinical features are compatible with vCJD and MRI does not show bilateral pulvinar high signal.

Table III

Diagnostic neuropathological features of vCJD

Cerebral and cerebellar cortex:	Multiple florid plaques in H&E sections
	Numerous small cluster plaques in PrP stained sections
	Amorphous pericellular and perivascular PrP accumulation
Caudate nucleus and putamen:	Severe spongiform change
	Perineuronal and axonal PrP accumulation
Posterior thalamic nuclei and midbrain:	Marked astrocytosis and neuronal loss
Brainstem and spinal cord:	Reticular and perineuronal PrP accumulation in grey matter
PrP^{RES} accumulation in lymphoid tissues throughout the body	
Predominance of di-glycosylated PrP^{RES} in central nervous system and lymphoid tissues	

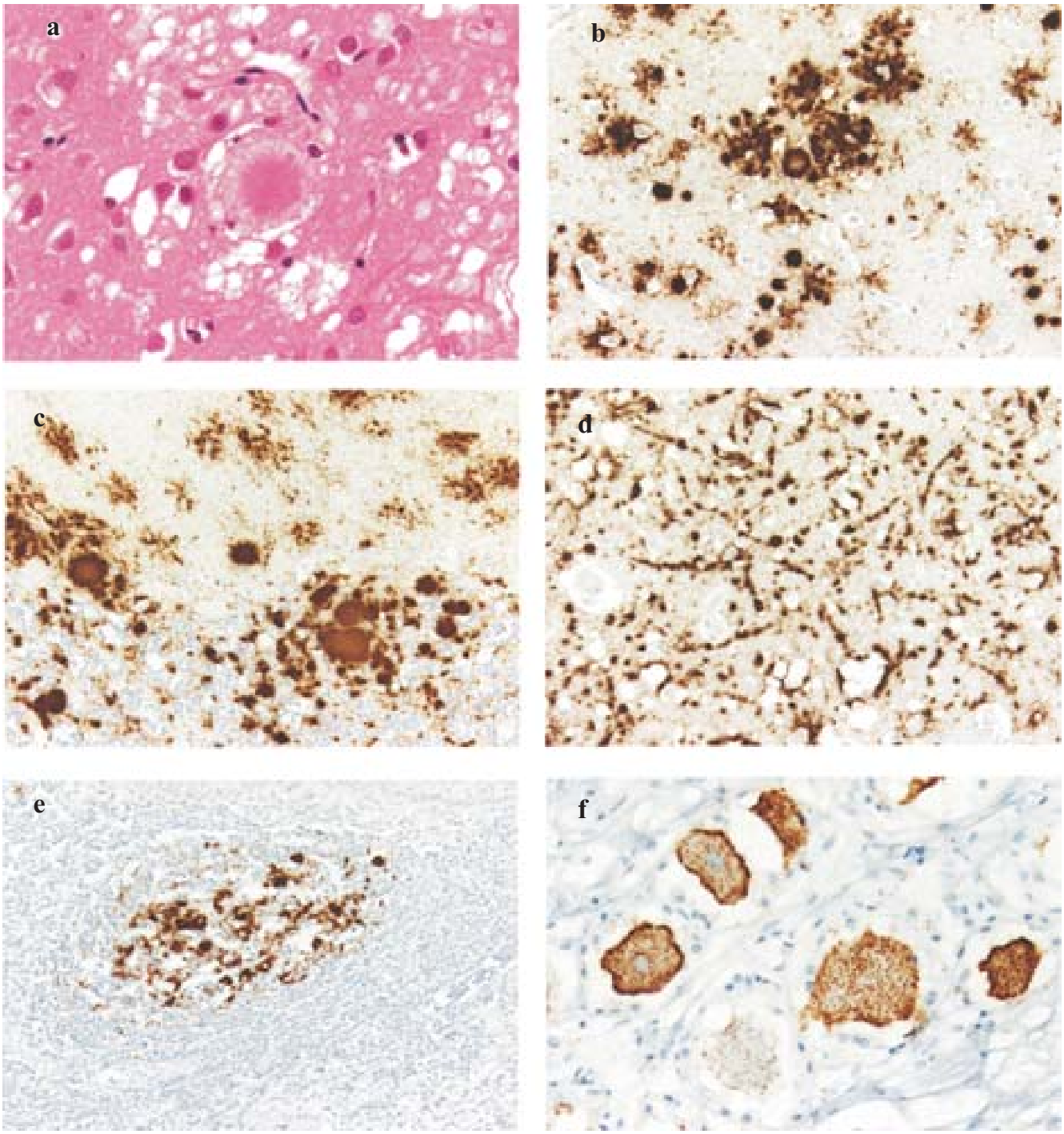

Fig. 1. a, a typical florid plaque in the cerebral cortex (centre) comprises an eosinophilic core with a pale radial periphery, surrounded by spongiform change. Haematoxylin and eosin; b, low magnification view of PrP immunocytochemistry in the cerebral cortex in vCJD. In addition to the staining of plaques, multiple amorphous deposits are located around neurones and blood vessels. KG9 antibody; c, immunocytochemistry for PrP in the cerebellar cortex shows large numbers of plaques, particularly in the granular layer (bottom) with numerous amorphous perineuronal deposits in the granular layer (top). 3F4 antibody; d, PrP deposition in the basal ganglia shows no plaque structures, but there is perineuronal staining and a linear decoration of axons. KG9 antibody; e, folicular dendritic cells and tingible body macrophages within the germinal centre of a tonsil from a patient with vCJD stain strongly for PrP. KG9 antibody; f, positive punctate staining for PrP in the spinal dorsal root ganglia in vCJD. As well as the punctate ganglion cell staining, some satellite cells also appear to be positive. KG9 antibody.

In the dorsomedial and posterior regions of the thalamus there was severe and extensive neuronal loss with marked astrocytosis (Zeidler et al. 2000). The distribution of astrocytosis did not relate to the presence of amyloid plaques or spongiform change. In the midbrain, severe neuronal loss and astrocytosis occurred in the colliculi and the periaqueductal grey matter. Neuronal loss in the cerebral cortex was most evident in the primary visual cortex, with accompanying astrocytosis. The neuronal populations in the hippocampus were relatively well-preserved. All layers of the cerebellar cortex suffered neuronal loss, particularly the granular cell layer. In cases with a lengthy clinical course, there was severe neuronal loss in the cerebral and cerebellar cortex with marked astrocytosis, consistent with the macroscopic finding of atrophy in these structures. Neuronal loss and astrocytosis were not prominent in the pontine nuclei, but were identified in the inferior olivary nuclei in the medulla.

Florid plaques were identified on haematoxylin and eosin stains in all autopsy cases of variant CJD. As previously reported (Ironside et al. 1996, Ironside 1998), these structures comprise an eosinophilic central core with radiating fibrils which are surrounded by a rim or corona of spongiform change (Fig. 1a). Florid plaques were most numerous in the occipital cortex, particularly at the bases of sulci, but were present in all cerebral lobes in all cortical layers. In the cerebellum, florid plaques were most easily identified in the molecular layer, but were also present as aggregates in the granular layer. No evidence of an amyloid angiopathy was identified.

Immunocytochemistry

Immunocytochemistry for PrP showed strong staining of the florid plaques in the grey matter in all areas of the cerebral and cerebellar cortex, and also revealed numerous smaller plaques arranged in irregular clusters (Figs. 1b, c). In addition, there was widespread deposition of PrP in a pericellular distribution, apparently around small neurones and astrocytes (Fig. 1d). These deposits were not visualised on routine stained preparations but some could be identified on Gallyas silver impregnation. In the basal ganglia and thalamus, there was a linear and perineuronal pattern of PrP accumulation (Fig. 1d), in marked contrast with the lower levels of PrP accumulation in cases of sporadic CJD which were methionine homozygotes at codon 129 in the PrP gene. In the hippocampus, the cornu ammonis showed little PrP deposition, but there was a dense accumulation in the dentate fascia and the subiculum. Synaptic and neuronal positivity was also present in the midbrain, pons and medulla, particularly in the pontine nuclei. In the spinal cord, PrP positivity was present in the grey matter regions at all levels, particularly in the substantia gelatinosa. No PrP immunoreactivity was detected in the dura mater. Spinal dorsal root ganglia stained positively for PrP (Fig. 1f).

Non-CNS tissues

The commonest immediate cause of death in the vCJD patients was bronchopneumonia. Occasional cases exhibited mild fatty change in the liver. Routine morphological analysis of the peripheral nerves revealed no evidence of a neuropathy. Positive staining for PrP was identified (as previously reported) in follicular dendritic cells within germinal centres of the tonsil (Fig. 1e), appendix, spleen and lymph nodes from the cervical, mediastinal, para-aortic and mesenteric regions (Hilton et al. 1999, Ironside et al. 2000). Positive staining for PrP was also identified in follicular dendritic cells in Peyer's patches in the ileum, although marked autolysis prevented detailed analysis. PrP immunocytochemistry in other organs (the heart, lung, skeletal muscle, salivary gland, oesophagus, stomach, liver, gall bladder, pancreas, kidney, adrenal gland, thyroid gland, parathyroid gland, bladder, testes, and pelvic organs (vagina, cervix, uterus, Fallopian tubes and ovaries) and skin) was all negative in vCJD. In sporadic CJD, all lymphoid tissues, and other organs showed no immunoreactivity for PrP.

Biochemistry

In agreement with Parchi et al. (Parchi et al. 1997, Parchi et al. 1999a) we have only observed the presence of either one of two different mobility variants in case of sporadic CJD: the first having a non-glycosylated PrP^{RES} of ~21 kDa and termed type 1, and the other of ~19 kDa termed type 2 (Fig. 2, lanes B and C). In our experience, the PrP^{RES} isoform pattern from patients with vCJD is closely similar in terms of mobility to the type 2 isoform seen in sporadic CJD. However, as described previously (Collinge et al. 1996, Parchi et al. 1997, Ironside et al. 2000) vCJD samples do show a glycoform profile characterised by the predominance of

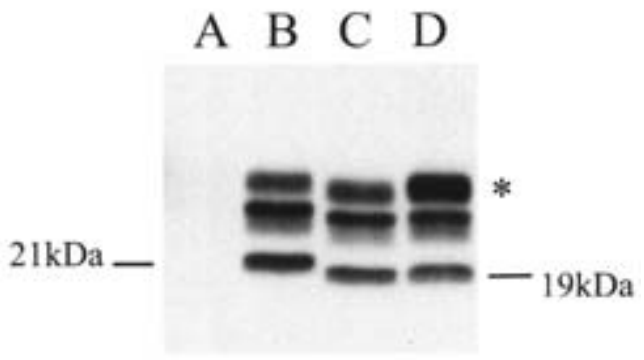

Fig. 2. A, western blot analysis of protease resistant prion protein (PrP^{RES}) in post-mortem cerebral cortex from patients without Creutzfeldt-Jakob disease; B, C, with sporadic Creutzfeldt-Jakob disease, or D, with variant Creutzfeldt-Jakob disease. PrP^{RES} is absent from the control case (A) but is present in the cases of Creutzfeldt-Jakob disease (B, C, D). The PrP^{RES} isotype found in sporadic Creutzfeldt-Jakob disease has either a 21 kDa non-glycosylated PrP^{RES} (B) or a 19 kDa non-glycosylated isoform with the predominance of the diglycosylated isoform (marked with an asterisk in D).

di-glycosylated PrP^{RES}, termed type 2B (Parchi et al 1997) (Fig. 2, lane D). The diagnostic use of this type 2B glycoform pattern is clear since we have found this characteristic hyperglycosylation in every case of vCJD and have not yet encountered it in any other case of CJD as defined by clinical and pathological criteria. Some atypical cases of sporadic CJD have exhibited clinical features which initially resembled those of vCJD. However, on Western blot analysis, none of these atypical cases show the characteristic type 2B glycosylation pattern of vCJD.

Comparison of vCJD cases with other cases of CJD diagnosed in the UK since 1990 showed no major overlap in terms of neuropathological or biochemical features. Furthermore, only cases of vCJD have PrP^{RES} detectable in lymphoid tissues, with a predominance of the di-glycosylated form of PrP^{RES} in Western blots after protease K digestion. The laboratory diagnostic features of vCJD are summarised in Table III.

DISCUSSION

Laboratory studies in vCJD have helped define neuropathological and biochemical features which are characteristic and allow a clear distinction from other forms of CJD. The uniformity of these features is in marked contrast to sporadic CJD (the main differential diagnosis), and is consistent with a single strain of causal agent, namely BSE. The significance of the florid plaque as a neuropathological hallmark of vCJD is also confirmed. Florid plaques are not unique to vCJD, and were first described in transmissions of Icelandic scrapie to mice (Fraser 1979). They also occur in chronic wasting disease (Williams et al. 1993), a disorder which appears unrelated to BSE. Since the initial description of florid plaques in vCJD, similar lesions were identified in the brains of macaques following experimental intracerebral transmission of BSE (Lasmezas et al. 1996). Florid plaques have been recently reported in small numbers in occasional cases of iatrogenic CJD in dura mater graft recipients in Japan; however, these iatrogenic cases do not exhibit any of the other distinguishing neuropathological features of vCJD (Schimizu et al. 1999). The small cluster plaques in the cerebral and cerebellar cortex visible on PrP immunocytochemistry may be a more specific pathological marker for vCJD, and have not been reported to occur in any other form of human prion disease. The amyloid plaques occurring in kuru can be distinguished easily from florid plaques by their restricted distribution in the cerebral cortex and cerebellum, their generally smaller size, and the absence of surrounding spongiform change (McLean et al. 1997).

Ultrastructural studies of florid plaques in vCJD have revealed a close accumulation of amyloid fibrils in the central core. One recent study has also revealed neuritic dystrophy around florid plaques in the cerebellum (Liberski et al. 2000). This interesting observation requires further study, since neuritic dystrophy is not evident on light microscopy (and is not associated with positive staining for phosphorylated tau). The association between amyloid plaques and dystrophic neurites in vCJD is similar to that in Alzheimer's disease, highlighting the potential similarities in mechanisms of degeneration in these disorders.

The pattern of thalamic neuronal loss and gliosis in vCJD (Zeidler et al. 2000) is distinct from the thalamic lesions in both fatal familial insomnia (Gambetti et al. 1995) and sporadic fatal insomnia (Mastrianni et al. 1999), which focus on the anterior and medial thalamic nuclei. The clinical significance of this observation is of potential interest in relation to the sensory abnormalities experienced by patients with vCJD, which do not occur in neuroanatomical dermatomes and may therefore represent thalamic pain. Furthermore, this pathological

finding correlates with the anatomical distribution of the "pulvinar sign" on MRI in vCJD (Zeidler et al. 2000).

The epidemiological features of vCJD indicate that a dietary route of exposure is the most likely route of exposure to the BSE agent in the cases identified so far (Will et al. 1996), particularly in a cluster of 5 cases identified in a 4 year period in a rural region of England. However, the identification of other routes of exposure to BSE in future cases cannot be excluded and is part of an ongoing case-control study. In the limited data available on kuru (another acquired human prion disease which was probably transmitted by the oral route), codon 129 genotype did not appear to exert a major influence on the clinical or neuropathological features of the disease in one series (McLean 1997), although in another larger series it was suggested that kuru plaques were more evident (but not exclusively present) in individuals who were valine homozygotes or heterozygotes at codon 129 (Cervenakova et al. 1999)

Our experience suggests that a characteristic PrP^{RES} glycoform ratio can usefully distinguish between vCJD and sporadic CJD as reported previously (Collinge et al. 1996, Parchi et al. 1997, Ironside et al. 2000); however, a glycoform pattern similar to that of vCJD is known to characterise fatal familial insomnia (Parchi et al. 1995, Telling et al. 1996) and GSS (Parchi et al. 1999b) and we have recently made a similar finding in an unusual Dutch case of sporadic CJD (Head et al. 2001). It is questionable whether this pattern will be conserved in patients with "human BSE" who are valine homozygotes or heterozygotes at codon 129, although transmission studies using transgenic "humanised" mice suggest that it might (Hill et al. 1997). The presence of PrP^{RES} in the lymphoreticular system (Hilton et al. 1999, Hill et al. 1999) might similarly be a useful diagnostic criterion, although studies of scrapie in sheep have suggested that the distribution of PrP^{RES} in lymphoid tissues is dependent on the host *PRNP* genotype. It should also be noted that the overall levels of both of PrP^{RES} and infectivity in the lymphoreticular system are significantly lower than in the CNS in vCJD (Bruce et al. 2001, Wadsworth et al. 2001), requiring increased sensitivity in the Western blotting assay and careful assessment by PrP immunocytochemistry. An integrated approach to the post-mortem diagnosis of vCJD, relying on a combination of histology, immunohistochemistry, PrP gene sequencing and PrP^{RES} analysis. As more cases of BSE and vCJD are identified in an increasing number of countries, detailed laboratory investigation of all suspected CJD cases is required in order to maintain vigilance concerning the effects of the BSE agent in man.

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