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CLINICAL ARTICLE

Comparative Neuropathology of Kuru with the New Variant of Creutzfeldt-Jakob Disease: Evidence for Strain of Agent Predominating Over Genotype of Host

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The three major influences on the phenotype of the transmissible spongiform encephalopathies are believed to be strain of agent, route of infection and host genotype. We have compared the pathologic profiles and genotypes of the new variant of Creutzfeldt-Jakob disease (vCJD) and kuru. The comparison reveals that there are distinct lesional differences particularly in the prion protein (PrP) load and distribution as seen by immunohistochemistry. The clinico-pathologic phenotypes and the genotypes of these two diseases are sufficiently different to suggest that the strain of agent may play a greater role than any presumptive common route of peripherally acquired infection.

Introduction

The emergence of a new variant form of Creutzfeldt-Jakob Disease (vCJD) raises important questions regarding the clinical and pathological characteristics of any future epidemic. vCJD has been causally linked to bovine spongiform encephalopathy (BSE) (7, 9, 19, 37). Kuru, the prototypic human transmissible spongiform encephalopathy (TSE), is clearly associated with the practice of ritualistic endocannibalism in the Fore group of New Guinea (1). Since vCJD and kuru may share a common mechanism of transmission (ie, a peripheral route of inoculation through the skin, conjunctiva or gastrointestinal tract), it is reasonable to ask to what degree this may influence the clinico-pathologic features of the illness. To investigate this further, we have performed detailed comparative pathological studies, including prion protein (PrP) immunohistochemistry, on eleven archival kuru cases and eleven cases of vCJD.

These diseases are characterised neuropathologically by spongiform change, gliosis and neuronal loss (28). The conversion of the normal host PrP to an abnormal conformer (PrP^{CJD}) is now recognized to play a central role in the process of infectivity and neurodegeneration (13). Amyloid deposits of PrP^{CJD} are an additional neuropathological feature which, although specific, are visualised in a variable proportion of all cases (8, 22, 27). These neuropathological features have been the mainstay for diagnosis for many years, and PrP^{CJD} immunocytochemistry is now a valuable adjunct (3). PrP^{CJD} accumulation occurs in a variety of forms, including a diffuse synaptic pattern, focally as plaques or in a perineuronal distribution (3, 4, 18, 22, 36).

Heterogeneity of the clinical and pathological phenotype occurs, regardless of whether the etiology is sporadic, acquired or inherited (17, 21). The variables determining the extent and distribution of the microscopic pathologic lesions are largely unknown; the strain of agent, route of agent entry, host factors, incubation period and length of illness are all possible contributing factors (21). The host genotype, particularly the naturally occurring ("public") polymorphism on codon 129 in the human PRNP gene is thought to influence disease phenotype, particularly the presence of PrP^{CJD} plaques (12, 14, 26, 31, 33). Experimentally, the strain of agent is known to have a dominant effect when the host genotype is held constant (6). However, in the iatrogenically acquired forms of the human disease, cases centrally inoculated tend to resemble classical CJD with prominent cortical changes whilst peripherally inoculated

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Age at onset (Years)	Sex	Duration of illness (months)	Codon 129
17	f	16	
20	m	17	VV
21	m	13	
22	m	15	
32	f	12	MM
33	f	13	
41	f	13	MM
44	f	19	
44	f	?	
57	f	5	VV
59	f	7	VV

Table 1a. *Kuru.* Age, sex, duration of illness and genotype of cases.

Age at onset (Years)	Sex	Duration of illness (months)	Codon 129
28	m	11	MM
18	m	12	MM
19	m	13	MM
21	f	14	MM
23	m	21	MM
18	f	21	MM
28	m	17	MM
29	m	10	MM
34	f	14	MM
39	f	18	MM
48	m	29	MM

Table 1b. *vCJD.* Age, sex, duration of illness and genotype of cases.

cases usually exhibit more severe cerebellar and basal ganglia lesions, with plaque formation in the cerebellum and cerebrum (5, 35). This study takes advantage of two distinct human variants in which some of the strain/host genotype factors can be teased apart.

Materials and Methods

Eleven archival cases of kuru were studied utilizing material collected in New Guinea in the 1960's and stored in formalin in the Department of Pathology, the University of Melbourne. Details of the cases were sparse (see table 1). Sections were taken from coronally cut cerebrum including the cingulate gyrus, insular cortex, basal ganglia and thalamus at the level of the mamillary bodies and hippocampus and entorhinal cortex at the level of the lateral geniculate bodies. Sections were also taken from the lateral cerebellum, midbrain, medulla, pons and spinal cord where available. Eleven cases of new variant CJD were studied in collaboration with the CJD Surveillance Unit, Edinburgh. These were similarly sectioned although more inclusive block taking was performed including sections of superior parietal and occipital cortex. All cases were paraffin embedded following immersion in formic acid for one hour. They were stained using haematoxylin and eosin and then for PrP^{CJD} with the monoclonal antibody, KG9 (courtesy of Dr C Birkett, Institute of Animal Health, Compton UK) utilizing the hydrolytic autoclaving method (18, 4) and glial fibrillary acidic protein (GFAP) (DAKO) subsequent to microwave enhancement. Selected cases were stained using the Gallyas silver method. Haematoxylin and eosin, PAS, and luxol fast blue preparations were available for review on original kuru slides from the same cases, where further sampling included the occipital cortex. The original paraffin blocks on these cases were not available.

PrP genotyping. Genomic DNA was extracted from destained haematoxylin and eosin-stained microscopic slides made from paraffin-embedded brain tissue according to a standard protocol (34). The portion of the PRNP gene open reading frame spanning codons 95 to 150 was amplified in two steps by PCR using Taq DNA poymerase. The first PCR amplification was performed using the oligonucleotide primers: 5'- aggatggaacactggggg-3' and 5'-gattgtgatattgacgcagtc-3' under the following conditions: initial denaturation at 95°C for 3 min, then 35 cycles of denaturation at 94°C for 30 sec,annealing at 55°C for 30 sec, extension at 72°C for 30 sec, and a final extension at 72°C for 5 min (GeneAmp PCR System 9600, Perkin-Elmer). A second PCR amplification of amplicons from the first PCR was performed using oligonucleotide primers: 5'- caccacagtcagtggaac-3' and 5'-atagtaacggtcctcatagtca-3' under the same conditions described above, except that the annealing temperature was at 60°C. The restriction endonuclease Mae II (Boehringer- Mannheim, USA) was used for the identification of the methionine(M)/valine(V) coding alternative at polymorphic codon 129

Results

Kuru. *Histology.* Sections revealed a relatively constant pattern of spongiform change in all cases. Spongiform change was present in laminae 3-5 of sections of cingulate, occipital, entorhinal, and insular gyri with all laminae involved in the subiculum. The hippocampus was unaffected with no evidence of spongiform change or neuronal loss in CA1, 2 or 3 or the dentate fasciculus. The putamen and caudate nuclei showed prominent and severe spongiform change, with occasional putamenal neurons showing intraneuronal vacuoles. A lesser

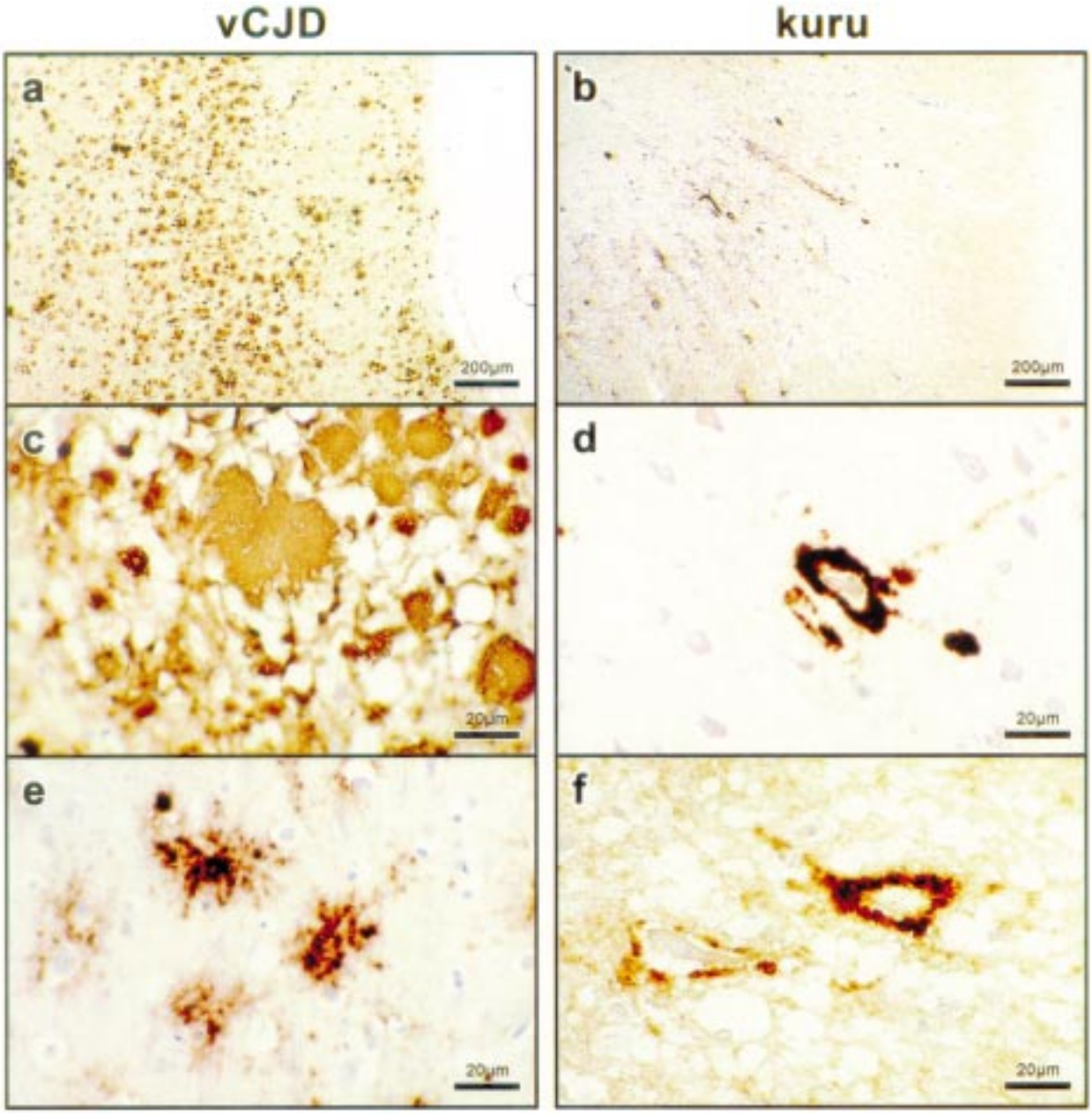


Figure 1. ***Cortex.*** *vCJD.* **a**) Diffuse cortical PrP deposit. KG9 immunoperoxidase. **c**) 'Florid' plaque PrP deposition with intervening spongiform change in the neuropil. KG9 immunoperoxidase. **e**) Linear-granular diffuse plaques. KG9 immunoperoxdase. *Kuru.* **b**) Laminar 3-5 accentuation of KG9 immunoreactivity. KG9 immunoperoxidase. **d**) Perineuronal and dentritic PrP deposition of the pyramidal neurons in laminae 3-5. KG9 immunoperoxidase. **f**) Perineuronal and synaptic pattern with intervening spongiform change in laminae 3-5. KG9 immunoperoxidase.

degree of spongiform change was noted in the thalamus in all cases with the medial nuclei appearing more affected than the lateral nuclei. The cerebellar molecular layer showed a prominent spongiform change with intense molecular and granular layer gliosis associated with granular cell and variable Purkinje cell loss (Figure 3b). Within the midbrain, minor depigmentation of the neurons of the substantia nigra was seen. Spongiform change was moderate to severe in the periaqueductal grey matter and colliculi associated with moderate gliosis. Minor spongiosis and gliosis were seen in the basis pontis, central tegmental area and inferior olivary nucle-

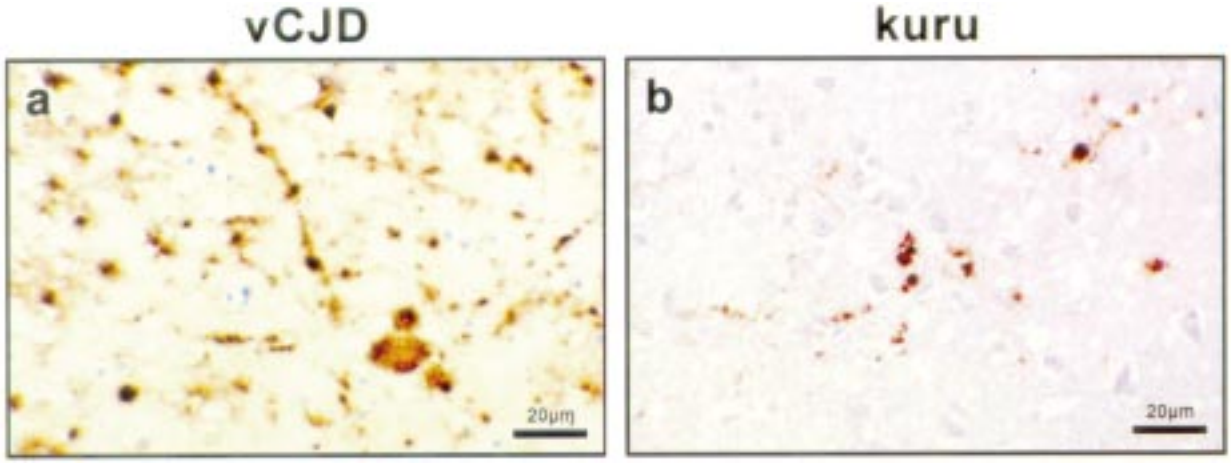


Figure 2. *Putamen.* **a**) vCJD showing granular linear PrP deposits. KG9 immunoperoxidase. **b**) Kuru showing small granular PrP plaques. KG9 immunoperoxidase.

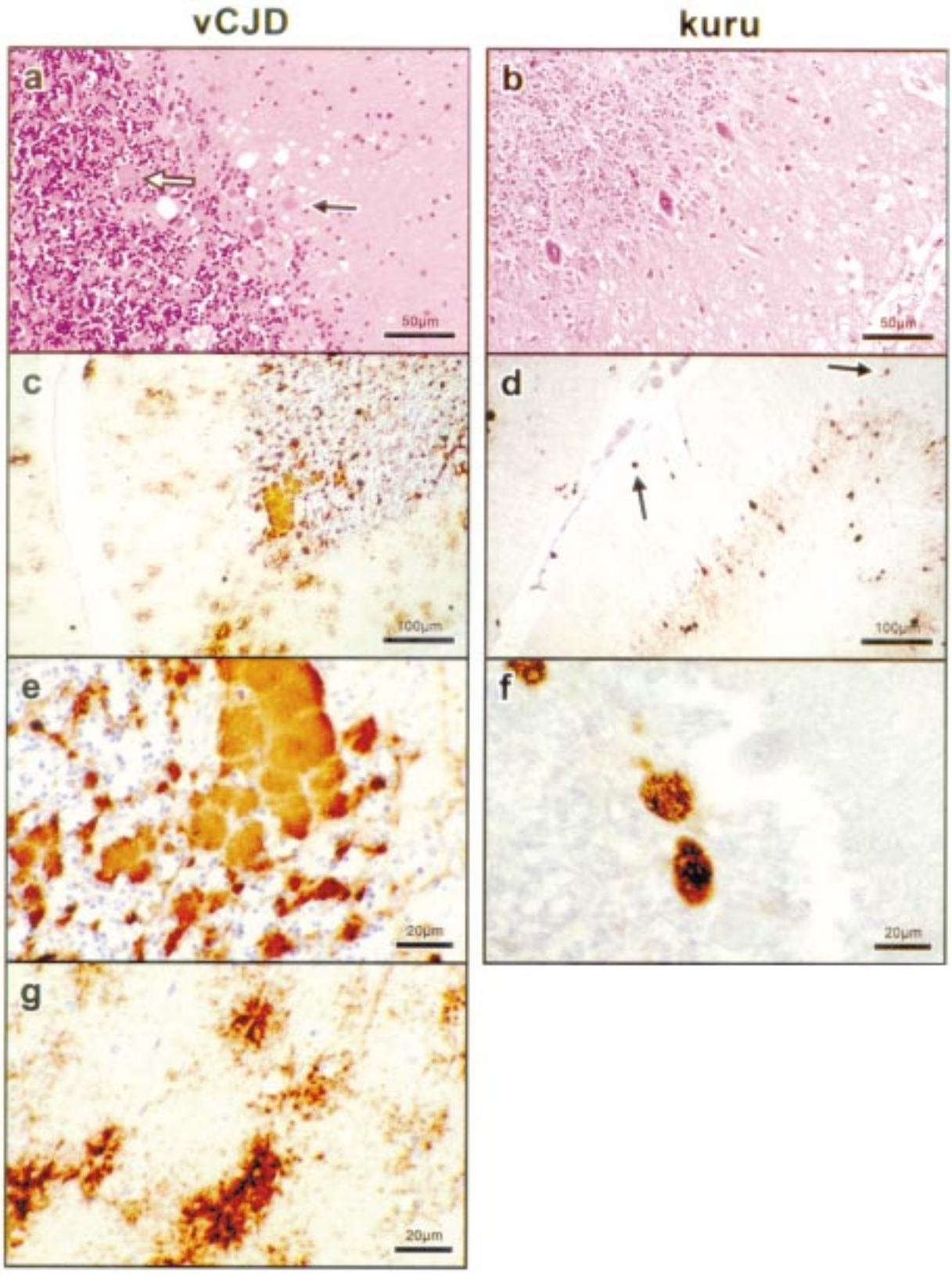
us in the pons and medulla. Within limited sections of spinal cord available neuronal preservation was apparent with minimal evidence of gliosis and minor spongiform change in the substantia gelatinosa. Typical kuru plaques were seen using haematoxylin and eosin stains within the granular layer of the cerebellum with occasional plaques in the molecular layer. Plaques were PAS positive, argyrophilic and present in the cerebellum of all cases. Kuru plaques were rarely seen in the cortex and putamen. Demyelination was not apparent in any of the regions examined.

Immunohistochemistry. Sections revealed a variation in both the form and distribution of PrP^{CJD} within different brain areas in kuru, although the changes seen in all cases were relatively constant. The main forms of PrP^{CJD} deposition were synaptic, plaque and perineuronal. Plaques were defined as localized depositions of PrP^{CJD}, which varied up to 30μm in size and were mostly unicentric, with occasional groups of smaller plaques. Within the cingulate, insular and entorhinal gyri, PrP^{CJD} immunostaining showed a weak laminar 3-5 synaptic pattern with perineuronal PrP^{CJD} outlining large pyramidal cells and their dendrites (Figure 1 b,d,f). Scattered, infrequent plaques were seen in all laminae. The subiculum showed an intense synaptic pattern in all laminae with sparse perineuronal staining, but there was no PrP^{CJD} deposition in the pyramidal layer and dentate fasciculus of the hippocampus. Within the basal ganglia and thalamus a faint synaptic pattern was seen in most cases with scattered to moderate numbers of small PrP^{CJD} plaques (Figure 2b). The cerebellum showed a pronounced plaque and synaptic deposition localized to the granular layer with only infrequent molecular layer plaques (Figure 3d,f). Within the brainstem and spinal cord, synaptic PrP^{CJD} of varying degree was seen in the substantia nigra, periaqueductal grey matter, colliculi, basis pontis and central tegmental area, the inferior olivary nucleus and the spinal grey matter with emphasis on the substantia gelatinosa. Specific cranial nerve nuclei did not appear affected although very small PrP^{CJD} deposits were present in the adjacent parenchyma. Linear PrP^{CJD} was seen in some transverse white matter tracts with a very occasional plaque (figure 4b).

PRNP genotyping. The fragment of the PRNP gene including the polymorphic codon 129 was successfully amplified in 5 of 7 tested kuru cases. Restriction endonuclease digestion analysis revealed that three kuru patients were homozygous for valine and two were homozygous for methionine (See Table 1a).

New Variant CJD (vCJD). *Histology.* The pathology seen in all cases was uniform, as previously described

Figure 3. (Opposing page) ***Cerebellum.****vCJD.* **a**) Focal spongiform change in the molecular layer with an adjacent plaque (black arrow) with Purkinje cell drop out and Bergmann layer proliferation with a torpedo body (white arrow). Haematoxylin and eosin. **c**) Diffuse deposition of PrP in all layers of cerebellum. KG9 immunoperoxidase.**e**)'Florid' plaque formation in the granular layer. KG9 immunoperoxidase. **g**) Linear-granular pattern with central dendritic sparing within the molecular layer. KG9 immunoperoxidase. *Kuru* **b**) Diffuse spongiform change in an atrophic, gliotic molecular layer with focal Purkinje cell loss and Bergmann proliferation. Haematoxylin and eosin immunoperoxidase. **d**) PrP deposition accentuated in the granular layer with occasional PrP plaques in the molecular layer (arrow). KG9 immunoperoxidase. **f**) Scattered single PrP plaques with a faint synaptic staining in the granular layer. KG9 immunoperoxidase.


vCJD
kuru
a
b
50µm
50µm
c
d
100µm
100µm
e
f
20µm
20µm
g
20µm

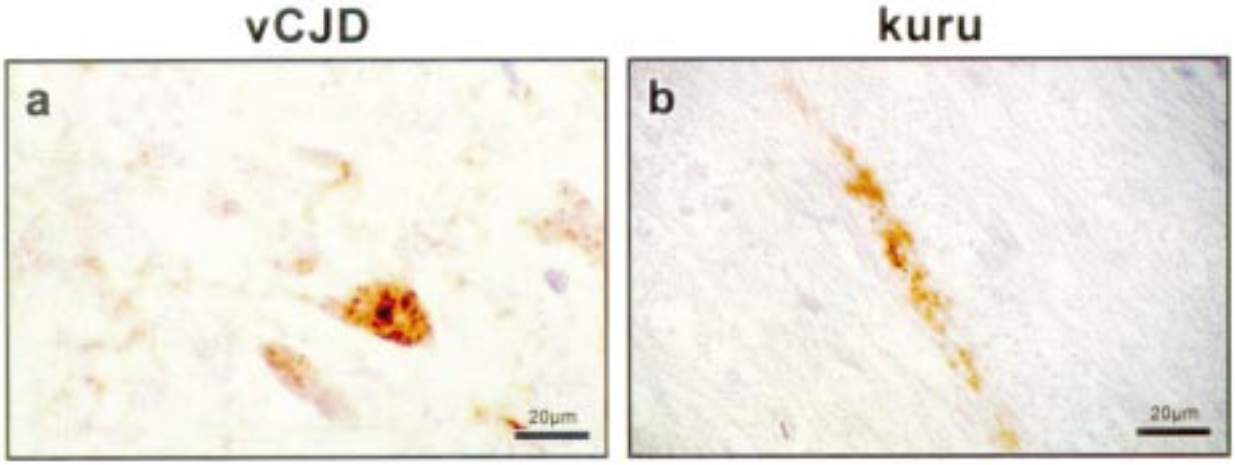


Figure 4. *Pons.* **a**) vCJD showing intraneuronal granular PrP immunostaining of pontine neurons. KG9 immunoperoxidase. **b**) Kuru showing linear pattern of PrP immunostaining along the line of white matter tracts. KG9 immunoperoxidase.

(21, 37). Focal areas of spongiform change associated with gliosis were present in all laminae within all sections of frontal, parietal, temporal and occipital cortex. Central to these foci, eosinophilic plaques occurred either singularly or in clusters. These plaques were round with peripheral radiating spicules around an eosinophilic core. They were PAS positive, congophilic and argyrophilic. Gallyas silver stains also showed finer diffuse fibrillary plaques of varying size with a fine granular appearance to the plaque fibrils within all the laminae of the cortex. Intense spongiform change was present in the putamen and caudate nucleus associated with severe gliosis. Plaques were not evident at these sites. Within the thalamus, spongiform change was most prominent in the dorsomedial nucleus and posterior pulvinar. The molecular layer of the cerebellum showed foci of spongiform change associated with plaques similar to those in the cerebral cortex. The spongiform change was focal, and much of the molecular layer appeared unremarkable (Figure 3a). Patchy Purkinje cell loss was evident, with occasional torpedoes on surviving cells. Within the granular layer numerous plaques were seen, with reactive gliosis in the granular and molecular layers associated with plaques. Astrocytosis of the white matter was also evident. With silver stains, plaques showed peripheral radial spicules that were argyrophilic as were fine feathery diffuse deposits. Within the brain stem, the midbrain showed spongiform change and gliosis in the superior colliculi and substantia nigra. Plaques were not present at these sites. The pontine nuclei also exhibited gliosis and a minor degree of spongiform change. There was a loss of neurons from the inferior olivary nucleus with minor spongiform degeneration and dense synaptic PrP deposition. Specific cranial nerve nuclei did not appear affected. The spinal cord showed no convincing evidence of either spongiform change or amyloid plaque formation.

Immunohistochemistry. All cases showed a remarkably similar PrP^{CJD} deposition both in extent, morphology and topography. Within all areas of cerebral cortex there was extensive PrP^{CJD} deposition formed predominantly by dense solid round plaques of varying size, with peripheral radiating spicules. In many areas, plaques were arranged in small clusters surrounded by vacuoles, the 'florid plaque' (Figure 1c). Other intervening fine diffuse plaques composed of finely punctated fibrils were also frequently seen (Figure 1e). Plaques occurred in all laminae although there appeared to be some concentration in lamina 1 and laminae 2 - 4 with plaques predominating over lesser amounts of perineuronal and linear PrP^{CJD} (Figure 1a).

Within the putamen and caudate nucleus PrP^{CJD} immunostaining was intense with a different overall morphology to that of the cerebral cortex (Figure 2a). The predominant pattern was interweaving granular linear deposits sparing the white matter tracts. Granules were occasionally up to 10um in diameter and occasional PrP^{CJD} plaques with more classical morphology were present. The thalamus and hypothalamus showed both an intense granular linear background staining with scattered cluster plaques and single plaques.

Within the cerebellum the $PrPC^{JD}$ deposition patterns within the molecular and granular cell layers were different (Figure 3c). The molecular layer showed numerous diffuse plaques with individual fibrils within the plaques having a finely punctate PrP^{CJD} deposition.

Some PrP^{CJD} deposits appeared to radiate transversely from short linear spaces that did not appear to be endothelial lined and were more likely peridendritic (Figure 3g). Within the granular layer, PrP^{CJD} was seen to be intensely synaptic and formed both unicentric and cluster plaques (Figure 3e). Fine granular deposits of PrP^{CJD} were seen in the cerebellar white matter that appeared to be running in parallel with the axons. An intense deposition of PrP^{CJD} occurred in a synaptic pattern in the dentate nucleus. Within the brain stem and spinal cord, synaptic PrP^{CJD} was seen in the periaqueductal grey matter, tegmentum and basis pontis, inferior olivary nuclei and spinal grey and substantia gelatinosa. Pontine neurons revealed a granular intracytoplasmic PrP^{CJD} with a synaptic background PrP^{CJD} and occasional small granular plaques (Figure 4a). The intracytoplasmic location and specificity of immunostaining requires further evaluation. Linear PrP^{CJD} was seen in some transverse white matter tracts and patchy synaptic PrP^{CJD} was present in the pontine tegmentum, similar to that in kuru cases. Granular neuronal PrP^{CJD} was also seen in the pontine nuclei. A comparative analysis of the immunohistochemistry in kuru and vCJD is presented in Tables 2a and b.

PRNP genotyping. Previous analyses of the vCJD cases had revealed all cases to be methionine homozygotes at codon 129 (37) (See Table 1b).

PrP^{CJD} load	vCJD	Kuru
frontal cortex	+++	+
basal ganglia	+++	+
thalamus	+++	+
cerebellar molecular layer	+++	+
cerebellar granular layer	+++	++ - +++
basis pontis	++	+
spinal grey matter	++	+
substantia gelatinosa	+	+

Table 2a. PrP^{CJD} load assessed on a scale of + to +++. Comparison of the PrP^{CJD} load in vCJD and kuru by immunohistochemistry.

PrP^{CJD} pattern	vCJD	Kuru
florid plaques	+	-
linear/granular diffuse plaques	+	-
dentritic	+	+
perineuronal	+	+
cortical L3-5 laminar accentuation	-	+
linear white matter in brain stem and spinal cord	+	+
granular intracytoplasmic	+	-

Table 2b. PrP^{CJD} patterns either present (+) or not present (-). Comparison of the PrP^{CJD} patterns in vCJD and kuru by immunohistochemistry.

Discussion

Differences in disease phenotype in sporadic and acquired human transmissible spongiform encephalopathies are well recorded (5, 31, 35). The three major influences on the phenotype are believed to be strain of agent, route of infection and host genotype. Neuropathological comparison of vCJD and kuru reveal that these diseases show distinct differences, particularly with PrP^{CJD} immunohistochemistry where there is a much greater PrP load in all brain areas in vCJD compared to kuru, with the exception of the cerebellar granular layer. Further topographical and morphological differences add to the distinction between these two diseases (see Table 2a and b). The underlying differences in neuropathologic profiles are in keeping with the different clinical features in these two disorders. Although both diseases exhibit prominent cerebellar ataxia, vCJD lacks the chronic emotional lability characteristics of kuru, and is characterised instead by psychiatric and sensory symptoms at onset with subsequent myoclonus and other movement disorders followed by dementia and akinetic mutism (37). If vCJD and kuru do share a common route of infection, then the explanation for the different clinicopathologic phenotypes must lie elsewhere.

The 11 cases of kuru show a homogeneous pattern with an accentuation of cerebellar changes. Of the five cases in which the codon 129 was assessed there were two M-M and three V-V (Table 1a). Further analyses of codon 129 in 42 cases of kuru show 17 M-V, 12 M-M and 11 V-V (16, 25, 30 and P Brown and L Cervenakova, unpublished data). The PRNP allelic variations in kuru therefore lends support to the agent strain being a dominant factor in determining phenotype. The presence of M-M homozygotes in kuru also suggests it is not the presence of valine 129 that determines peripheral selection of the PrP^{CJD} as previously postulated to account for the high frequency of a valine 129 in other cases of iatrogenic CJD (10). In vCJD, all cases have been M-M homozygotes (37). However vCJD has occurred within a very limited time frame compared to kuru (11). It therefore remains to be determined if homozygosity at codon 129 dictates a shorter incubation period. Moreover, the prolonged incubation period in kuru (over 40 years in some cases) may be significant

when attempting to estimate future numbers of vCJD cases in the United Kingdom (11).

The results of this study (including PrP^{CJD} immunohistochemistry) are consistent with another recently described kuru case (16) and 34 historical cases (2, 15, 23, 24, 29). The earlier cases were noted to have laminar spongiform change in the cingulate gyrus, with otherwise minimal cortical involvement and prominent lesions in the basal ganglia and the dorsomedial and posterior thalamus, with the greatest changes in the cerebellum. However, sparing of the hippocampus as seen in the present series of cases and others (16, 23, 24) is different to the severe changes noted in CA1 in the two kuru cases described by Lantos (25). Amyloid kuru plaques were not always noted in the historical descriptions. However, PrP plaques are demonstrable by immunohistochemistry in all cases, and are accentuated in the cerebellar granular layer. The similarity of these 45 kuru cases with sporadic CJD cases with a type-2 PrP^{CJD} glycosylation pattern (31) may be further evidence of transmission of a similar strain of agent. This is supported by two recently studied kuru brains which were both PrP^{CJD} type 2A (30). Scrapie and BSE strain transmission studies in mice have also shown maintenance of phenotypic characteristics throughout ten or more serial passages, implying the various isolates maintain strain-specific properties (7).

The perineuronal, apical dentritic and synaptic pattern of PrP deposition in kuru suggest a synaptic pattern of accumulation. Cortical neurons in laminae 3 and 5 are particularly affected: the extension of these apical dentrites to lamina 1 and the size and position of the labelled cortical neurons suggest these are most likely pyramidal cells. Specific targeting of neuronal subtypes has been postulated to be a determining factor at a cellular level for the topographic distribution of PrP^{CJD} (13). This may relate to differing surface expression of glycosylated PrP (10). This specific neuronal involvement within cortical laminae 3-5 also supports spread of the PrP^{CJD} from area to area via neuroanatomical pathways of synapsing neurons (13), rather than by diffusion. Similar evidence for synaptic accumulation of PrP^{CJD} occurs in vCJD, particularly in the basal ganglia, cerebellar dentate nucleus and brainstem.

In conclusion, this study has shown clear-cut and consistent differences in the neuropathologic profiles of kuru and vCJD that are not explicable on the basis of a common peripheral route of agent exposure. We suggest that the differing strains of the PrP^{CJD} agent could play a major role in determining the phenotype in these two peripherally acquired infectious diseases.

Acknowledgments

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