Brain Pathology 7: 547-553 (1997)

RESEARCH ARTICLE

Pathology and Immunocytochemistry of a Kuru Brain

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We report here results of modern staining techniques including anti-prion protein (PrP) immunocytochemistry to a set of archival brain specimens of a 16 year-old male who died from kuru in 1967. Brain suspensions transmitted disease to chimpanzees and New World monkeys. The PrP gene is homozygous for valine at the polymorphic codon 129. Histology shows neuronal loss, spongiform change, and astrogliosis. Lesions are maximal in parasagittal and interhemispheric areas of frontal, central and parietal cortex, cingulate cortex, striatum, and thalamus, and are accentuated in middle and deep cerebral cortical layers. PrP accumulates as diffuse synaptic type deposits and mostly unicentric plaques. PrP deposition is maximal in parasagittal and interhemispheric areas of frontal, central and parietal cortex, cingulate cortex, basal ganglia, and cerebellar cortex. Plaques are prominent in the striatum, thalamus, and granular layer of cerebellar cortex. Meticulous examination reveals only rare "florid" plaques with surrounding vacuolation.

We conclude that 1) pathology including immunomorphology of PrP deposition in this kuru brain is within the lesion spectrum of Creutzfeldt-Jakob disease although plaques are unusually prominent and widespread; 2) kuru does not share the neuropathological hallmarks of the new Creutzfeldt-Jakob disease variant recently reported in the UK and France; 3) topographic prominence of PrP deposition parallels that of spongiform change and/or astrogliosis.

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Introduction

Human transmissible spongiform encephalopathies (TSEs) or prion diseases comprise Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler Scheinker disease (GSS), fatal familial insomnia, and kuru. Some clinicopathological features of a new CJD variant, most recently described in the UK (30) and France (11), resemble those reported in kuru. Kuru was transmitted by ritualistic endocannibalism in the Fore people in Papua-New Guinea (16). In all types of TSEs, the hallmark of disease is accumulation of a pathological protease-resistant isoform of cellular prion protein (PrP) in the CNS (12). Anti-PrP immunocytochemistry has emerged as sensitive tool for demonstration of PrP accumulation in routinely processed and archived brain tissue (6,24). Kuru plaques which were observed by conventional stains in 68% of kuru brains (4) bind anti-prion-protein antisera (20,29). However, a detailed description of the immunomorphology of PrP deposition in kuru is still lacking. With the cessation of cannibalism, kuru has gradually disappeared. Unfortunately, most paraffin embedded blocks of kuru brains have been widely disseminated during passage of time and are now unretrievable. We had the opportunity to apply modern staining techniques including anti-PrP immunocytochemistry to what appears to be one of the last sets of archival kuru brain specimens.

Material and methods

This brain had been studied in the Laboratory of Central Nervous System Studies, NIH, Bethesda, USA. Available paraffin embedded tissue blocks comprise three coronal slices of the cerebrum at the levels of the genus corporis callosi, central thalamus, and pulvinar thalami. Other blocks contain the midbrain, pons, medulla oblongata, cerebellum, and upper cervical spinal cord.

Histology. Conventional stains performed on 5 µm thick sections include H&E, luxol fast blue/nuclear fast red, PAS, alcian blue, Congo red, Kanzler, and Bodian and Bielschowsky silver impregnations. Immunocytochemistry used monoclonal antibodies against common leukocyte antigen (CLA) (PD7/26 and 2B11, Dako), CD68 (macrophages; KP1, Dako), HLA class II ß chains (HLA-DR) (CR3/43, Dako), vimentin (V9, Dako), neurofilament proteins (NFP) (2F11, mainly phosphorylated epitopes, Dako), ß/A4

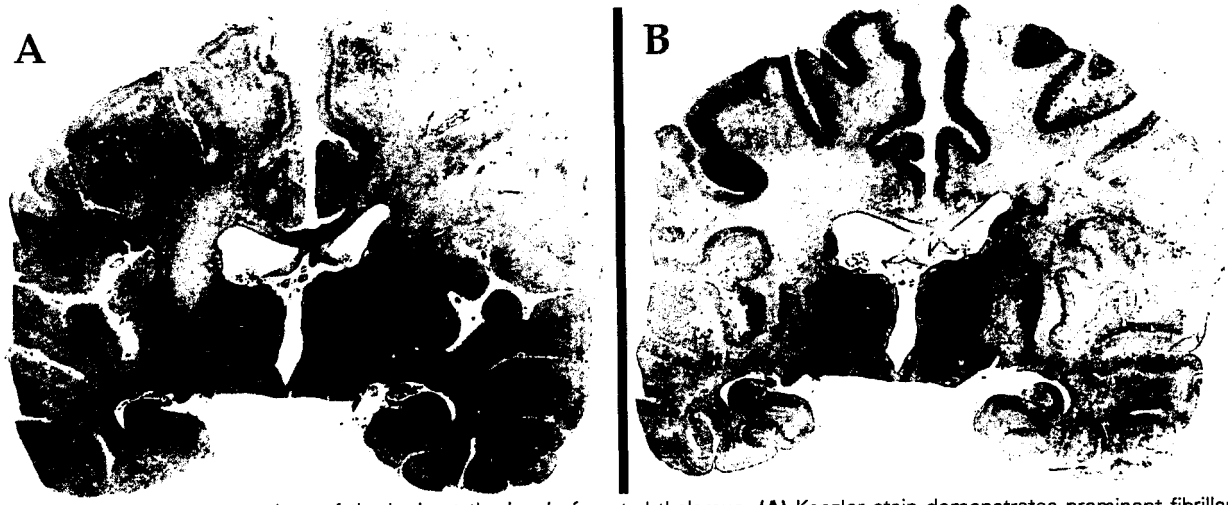

Figure 1. Whole mount sections of the brain at the level of central thalamus. **(A)** Kanzler stain demonstrates prominent fibrillary gliosis which is laminar in the middle and deep layers of parasagittal and interhemispheric neocortex and cingulate cortex, and more diffuse in the thalamus and underneath the insular and temporal cortices. **(B)** Anti-PrP immunostain reveals prominent PrP deposits in the parasagittal and interhemispheric neocortex and cingulate cortex, and insular region, with laminar accentuation in deep layers, in the striatum and thalamus, lateral geniculate body, and pre-/parasubiculum.

protein (6F/3D, Dako), and PrP (3F4, Dr. R. Kascsak, Staten Island, NY). Polyclonals comprised anti-GFAP (Dako), -S100 protein (Dako), -ferritin (Sigma), -tau protein (Dako), and -ubiquitin (Dako). Monoclonal antibodies were followed by the avidin biotin peroxidase complex technique, polyclonals by the peroxidase-anti-peroxidase technique. Selected anti-ferritin and -GFAP immunostained sections were counterstained with alcian blue dye. For anti-PrP immunocytochemistry, sections were pretreated with a three-tiered protocol of hydrated autoclaving, concentrated formic acid, and guanidine isothiocyanate (18). PrP immunoreactive control tissues included brain from the original GSS family (21), negative control tissue included brains from AIDS and Alzheimer's disease patients, and from patients having died from bronchopneumonia, acute cardiac arrest and pulmonary embolism. The type of PrP immunoreactivity was categorized as previously described (8).

Molecular analysis. Genomic DNA was extracted from paraffin-embedded brain tissue. The 3'-portion of the PRNP gene open reading frame was amplified by PCR using Taq polymerase and oligonucleotide primers 5'-TACTGAGCGGCCGCCAACATGAAGCACATGG CTGGT and 5'-TACTGAGTCGACCCTTCCTCATCCCACTATCAGG-3' at following conditions: an initial denaturation at 95°C x 5 min., then 94°C x 1.5 min/60°C x 1.5/72°C x 3 min for 40 cycles; and a final extension at 72°C x 7 min (Perkin Elmer 480 thermal cycler). The restriction enconuclease Mae II (Boehringer-Mannheim, USA) was used for detection of a methionine or valine coding triplet at polymorphic codon 129.

Results

Patient. Kupenota was born in the Kume village in the South Fore. His mother died of kuru when he was a small boy. He had been a normal child through adolescence, when he left the kuru region at about the age of 15 years to work on a coffee plantation in Goroka, from which he returned to his home village shortly before the onset of kuru.

His kuru began insidiously in mid-1966. By mid-September 1966 he showed marked ataxia and kuru tremors and was accepted as a classical case of severe kuru, still ambulatory then of three months duration. From that stage his disease progressed rather slowly, with unremittingly and increasingly severe tremors and locomotor ataxia. By one year from the time of onset he was unable to walk with bilateral support and was confined to his house for most of the day. Dysarthria, which appeared already within 6 months of onset, progressed to total loss of speech; yet he was still able to eat well and only lost the ability to feed himself at about this time. His slight obesity, which he had developed in the early stage of the disease, now disappeared and he became totally sedentary, and he was carried out of the house for urination and defecation.

Terminally, he was still mentally alert and able to respond with grunts to his name and to indicate affirmation or negation by head movement and gruntings. He could not turn over or remain seated during the last month of his disease, but had only developed small decubitus ulcers before he died.

He died after a terminal week of irregular episodes of Cheyne-Stokes respiration and increasing dysphagia, which caused some aspiration even when drinking fluids. He was still well oriented in time, place and person just before his death.

Brain suspension of Kupenota transmitted disease to chimpanzees, squirrel monkeys and spider monkeys (2,5,17). A scheme of primary transmission of kuru from Kupenota to New World monkeys (17)

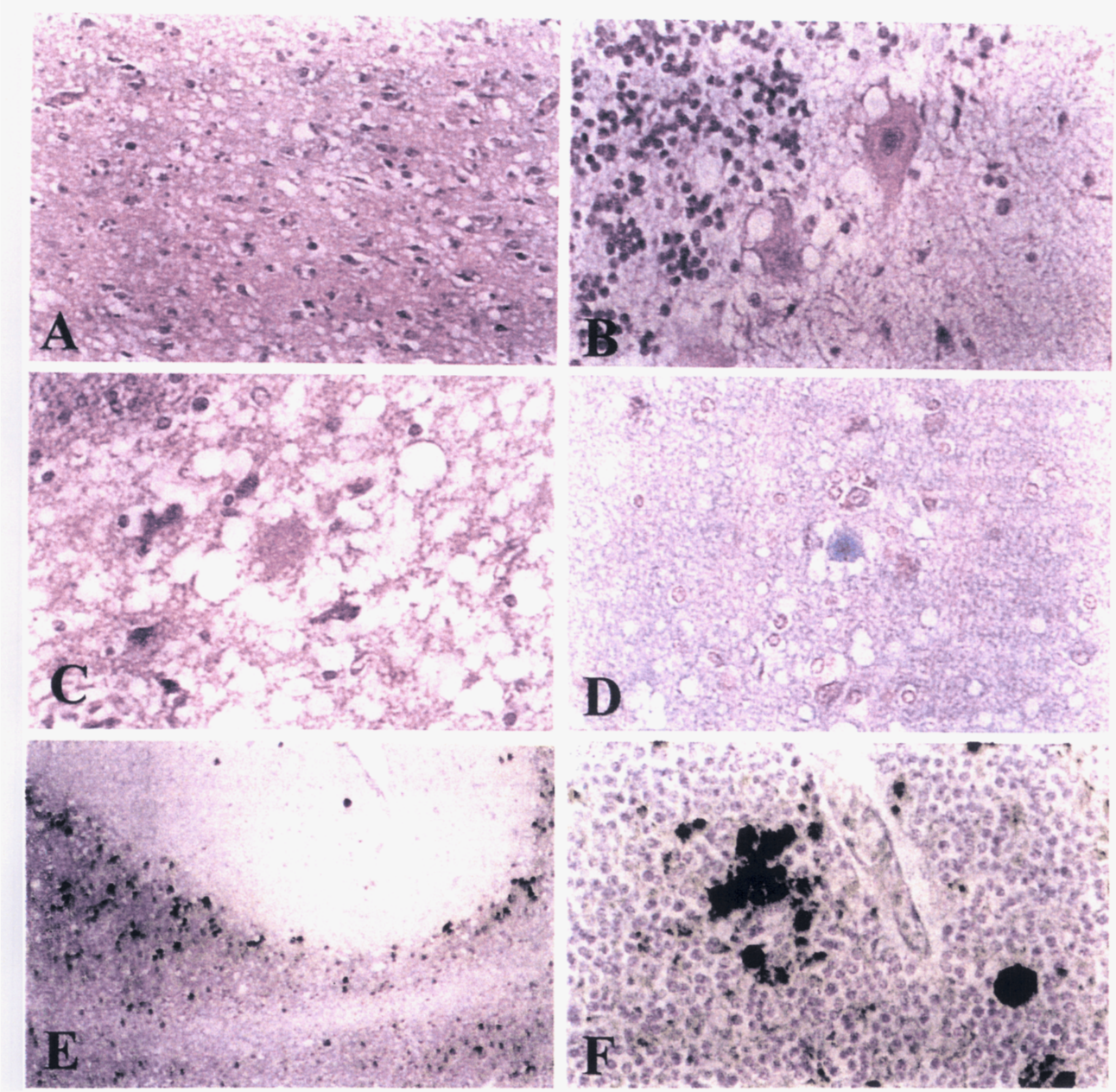

Figure 2. (A) Characteristic spongiform change (SC) of cerebral cortex (HE, x100). **(B)** Prominent cytoplasmic vacuolation of cerebellar Purkinje cells (HE, x130). **(C)** Prominent diffuse SC is accentuated around a large plaque in the striatum (HE, x130). **(D)** "Florid" plaque: a central unicentric plaque is surrounded by a rim of SC (alcian blue and nuclear fast red, x130). **(E)** PrP deposition in the cerebellar cortex: in addition to fine granular diffuse (synaptic-type) PrP deposition in the granular layer, PrP plaques are numerous in the granular layer and less frequent in the molecular layer (immunocytochemistry with 3F4, x25). **(F)** A spherical unicentric PrP plaque in addition to multicentric deposits in the cerebellar granular layer (3F4, x130).

and a Woelke-Heidenhain stained whole mount section of Kupenota's brain (4) were illustrated.

Molecular analysis. Polymorphic codon 129 of the PrP gene was homozygous for the valine coding triplet GTG.

Neuropathology. Grossly, the brain shows slight atrophy with enlargement of internal and external CSF spaces. Hippocampi are macroscopically inconspicuous. On whole mount sections, prominent fibrillary gliosis is laminar in parasagittal and interhemispheric areas of frontal, central and parietal cortex, cingulate cortex, and more diffuse in thalami and underneath insular and temporal cortices (Fig. 1A). Anti-PrP immunostaining of whole mount sections shows prominent diffuse PrP deposition in the thalamus, striatum, corpus geniculatum laterale, pre-/parasubiculum, and mainly in parasagittal and interhemispheric areas of frontal, central and parietal cortex, cingulate cortex with laminar distribution (Fig. 1B).

Histological tissue lesioning is characterized by

Table. Regional severity of tissue lesioning and PrP deposition.

Area	Spong. change	Astrogliosis	Neuronal loss	PrP deposition
• frontal cortex				
-dorsomedial	+++	+++	+++	sss/ppp
-lateral	+ to ++	++	++	p
-basal	+	+	+	s to ss/p to pp
• central cortex				
-dorsomedial	+++	+++	+++	sss/ppp
-lateral	+ to ++	++	++	ss/pp
• insular cortex	++	+	++	ss, pp
• temporal cortex	- to +	- to +	- to +	- to s/- to p
• parietal cortex	+ to ++	+ to ++	+	s/p
• entorhinal cortex	++	+	+	s
• hippocampus				
-pre/parasubiculum	+++	+++	+++	sss/ppp
-stratum pyramidale	- to +	- to +	- to +	s/p
-stratum rad.&lacun.	+++	++	+	s/ppp
-dentate gyrus	++	+	-	s/p
• striatum	+++	++	+++	sss/ppp
• thalamus	+	+++	+++	sss/ppp
• corpus genic. laterale	-	++	+ to ++	sss
• cerebellar cortex				
-archicerebellum	+	+ to ++	++	sss/ppp
-paleocerebellum	+	+ to ++	++	sss/ppp
-neocerebellum	+	+ to ++	++	ss/ppp
• dentate nucleus	+	++	++	ss/pp
• mesencephalon				
-superior colliculi	+	+	+	p
-inferior colliculi	++	++	+	p
-periaqueductal grey	++	++	+	pp
-reticular formation	++	++	+	pp
-substantia nigra	++	+	+	s
• pons				
-locus coeruleus	++	++	+	s/pp
-reticular formation	++	++	+	s/p
-raphe	++	++	+	s
-pontine nuclei	- to +	+++	+++	ss
• medulla oblongata				
-tegmental nuclei	- to +	+ to ++	- to +	s/p
-inferior olivary nuclei	- to +	+++	+++	ss
• cervical spinal grey	- to +	+ to ++	+	s/p

Table. Spongiform change, astrogliosis, neuronal loss: - absent, + slight, ++ moderate, +++ prominent. PrP deposition: s, ss, sss slight, moderate, prominent synaptic type of PrP deposition, respectively. p, pp, ppp slight, moderate, prominent plaque type PrP deposition, respectively.

spongiform change (SC) (Fig. 2A), astrogliosis (AG) and neuronal loss (NL) of variable severity in grey matter structures of cerebrum, cerebellum, and brainstem. The intensity of SC correlates with that of AG in most but not all areas (Table).

In the cerebral cortex, most prominent lesions symmetrically involve the parasagittal and interhemispheric areas of the frontal, central and parietal cortex, and the cingulate cortex. SC, AG and NL show laminar accentuation in the middle and deep cortical layers. A few neurons contain small cytoplasmic vacuoles; other neurons are swollen and strongly immunoreactive with anti-NFP. In the thalamus, a few axonal spheroids are detectable and few neurons have small cytoplasmic vacuoles. In archi-, paleo- and neocerebellar cortex, a few Purkinje cells contain prominent vacuoles (Fig. 2B). The granular cell layer includes a few swollen axons of Purkinje cells ("torpedoes"). The molecular layer exhibits slight to moderate SC. A few neurons of substantia nigra and locus ceruleus contain small cytoplasmic vacuoles. In the pontine base, NL and AG are most prominent in the

central zone. In brainstem and cervical spinal cord, ascending and descending nerve fibre tracts are well preserved; gliosis is prominent in inferior olivary nuclei.

Conventional stains detect plaques in the parasagittal and interhemispheric neocortex, cingulate cortex, pre-/parasubiculum, basal ganglia (Fig. 2C), thalamus and cerebellar cortex. The plaques occur as distinct, dense compact structures or as less dense, granulofibrillary deposits (Fig. 2C). The granulofibrillary plaques are spheric or irregularly outlined. The compact plaques are either spheric and unicentric with a homogenous dense center surrounded by a halo of radiating spikes (classical kuru-type plaques), or are irregularly outlined and infrequently show small satellite deposits. A few plaques are multicentric. The plaques are non-argyrophilic and PAS positive. Some of the compact plaques (including a portion of the kuru plaques) are slightly congophilic and exhibit faint apple-green birefringence under polarized light, whereas the granulofibrillary plaques are all non-congophilic and non birefringent. In H&E stain, only some of the compact plaques and a few large sized granulofibrillary plaques are evident. In the striatum, relatively large sized granulofibrillary plaques are predominant and prominent diffuse SC is slightly accentuated around the plaques (Fig. 2C). Alcian blue is the most sensitive conventional stain for detection of both compact and granulofibrillary plaques (Fig. 2D). In the cerebral cortex, thalamus, and molecular layer of cerebellar cortex, rare plaques surrounded by a zone of SC ("florid plaques") are detectable after meticulous search (Fig. 2D). In H&E stained sections, however, no definite "florid plaques" are detectable. All types of plaques are strongly positive with anti-PrP (Fig. 2E,F) and anti-ApoE, and negative with anti-ß/A4. Anti-PrP detects numerous granulofibrillary plaques which are not visible in the H&E stain. Silver stains and anti-tau, -ubiquitin, and -NFP immunocytochemistry do not reveal a neuritic component of kuru plaques. Most of the plaques are surrounded by a meshwork of astrocytic cell processes. A small portion of plaques show intimate contact with microglial cell bodies and/or microglial cell processes.

Morphological spectrum and distribution of PrP deposits. PrP accumulates in grey matter structures of cerebrum, cerebellum, brainstem, and spinal cord. PrP deposition patterns are diffusely fine granular (synaptic type) (Fig.1 B, 2E) and plaques (Fig. 2E,F). A few patchy/perivacuolar deposits are restricted to the periaqueductal grey and tegmentum of midbrain and pons. Prominent PrP deposition is confined to areas with prominent SC and/or AG (Fig. 1A,B; Table).

In the cerebral cortex, PrP accumulation shows laminar accentuation in deep cortical layers (Fig. 1B). Synaptic PrP is frequently accentuated perineuronally and periaxonally. A few PrP plaques extend to the subcortical white matter. In cerebellar cortex, plaques are prominent in granular and Purkinje cell layers and less prominent in the molecular layer (Fig. 2E). A few plaques extend to the subcortical white matter. In the dentate nucleus, synaptic PrP accumulates peri-neuronally and periaxonally. The adjacent white matter contains some plaques. In the brainstem, PrP frequently shows perineuronal and periaxonal accentuation. In the cervical spinal cord, synaptic PrP is slightly accentuated in the substantia gelatinosa. In addition, small plaque-like patches of PrP are diffusely distributed in the spinal grey and extend, in the posterior horn, to close vicinity of the surface of the spinal cord. PrP deposits are not detectable in nerve roots.

Morphology of plaques with immunocytochemistry for PrP. The size of the plaques ranges from small granules to a diameter of 40 µm. The largest plaques are detectable in the thalamus and cerebellar cortex. The plaques occur as granulofibrillary deposits and dense compact forms which are most commonly unicentric with homogenous center surrounded by radiating spikes (kuru-type plaques) or small granules. Also, some compact plaques have an irregularly curved outline due to close vicinity and/or coalescence of two or more unicentric plaques. A few granulofibrillary and compact plaques are multicentric (Fig. 2F) and form chains in the cerebellar cortex.

Discussion

Basic patterns of immunohistochemically detectable cerebral PrP deposition in CJD include synaptic, plaque and patchy/perivacuolar deposits (8). In the individual case, the three PrP deposition types occur separately or in various combinations. In this kuru brain, PrP accumulates as synaptic type deposits and plaques. The frequency of plaques in sporadic CJD is only 5-10% (12). Plaques in sporadic CJD comprise indistinct-patchy and distinct dense forms; the latter are most frequently spheric and unicentric with a homogenous core surrounded by a halo of radiating fibrils (kuru-type plaques) (12). Numerous and widespread PrP plaques including kuru-type plaques and multicentric plaques are a constant finding in iatrogenic CJD of growth hormone recipients (7). According to our experience (9), rare sporadic CJD cases also may have a widespread distribution of plaques; in the cerebellum, most plaques are found in the granular layer. Thus, pattern and distribution of PrP deposition, and morphology of PrP plaques in this kuru brain, are within the spectrum seen in sporadic and iatrogenic CJD. However, as compared with sporadic CJD, plaques are here unusually prominent and widespread.

This kuru brain shares with CJD and GSS further characteristic features: 1) The hippocampi are well preserved, as has been previously described in kuru (4), CJD, and GSS (8,21). 2) Plaques (including preamyloid plaques) in our kuru patient strongly

bind anti-apoE. ApoE immunoreactivity has been described in kuru type plaque amyloid of CJD (28). According to our experience, apoE binds also to plaques of GSS. Thus, apoE is present in plaques of all types of human TSEs and appears a sensitive marker for amyloid and preamyloid plaques. 3) It has a few swollen neurons which immunocytochemically contain NFP. Swollen neurons have been observed in kuru (3), CJD and GSS (21,27). Accumulation of NFP in swollen neurons has been demonstrated for CJD and GSS (21,27). 4) Some plaques in this kuru brain are associated with interdigitating microglia and astrocytic processes. The relationship of microglial cells and plaques in kuru has been described previously (19). Association of microglia and astrocytes with plaques is also a feature of CJD and GSS (1,20,25).

Recently, a new variant of CJD has been reported in the UK (30) and France (11). It is characterized by distinct neuropathology including extensive distribution of kuru-type plaques throughout the cerebrum and cerebellum and prominent PrP deposits especially in the molecular layer of cerebellar cortex. The most characteristic feature is plaques with a surrounding zone of SC, similar to "florid" plaques in scrapie (15). These plaques are easily recognizable in H&E stained sections (30). This kuru brain shares with the new CJD variant the widespread distribution of plaques. In contrast to the new CJD variant, however, PrP deposits are not prominent in the molecular layer of cerebellar cortex, and "florid" plaques are very rare and not obvious in routine stains.

In the new variant of CJD (30), codon 129 of the PrP gene has been constantly homozygous for methionine (M/M), whereas codon 129 in the present kuru case is homozygous for valine (V/V). In a group of 38 kuru patients, the genotypes at codon 129 were M/V in 50%, M/M in 30% and V/V in 20% of the cases; in a group of 46 controls of the same ethnic background, the genotypes were M/V in 48%, M/M in 30% and V/V in 22% of the cases (P. Brown and L. Cervénaková, unpublished data). Thus there is no difference between kuru patients and controls, and kuru affects persons irrespective of codon 129 genotype. This is in contrast to sporadic and iatrogenic CJD in the Caucasian population in which homozygosity strikingly predominates (7,14), whereas the normal Caucasian population has a similar distribution of genotypes at codon 129 of the PrP gene as the Fore people with and without kuru. In sporadic CJD of Japanese people, valine at codon 129 of the PrP gene is associated with deposition of PrP plaques (26) . It remains to be determined whether valine at codon 129 is also a factor for accumulation of PrP plaques in kuru.

In contrast to CJD, the distribution of tissue lesions in kuru brains has been described as relatively constant with prominent involvement of the parasagittal, interhemispheric, cingulate and insular cortex, parahippocampus, and basal ganglia (4,23). This kuru brain has the same lesion profile and thus seems representative of the disease. In experimental kuru, brain lesioning is remarkably stereotyped with evidence for selective vulnerability of specific neuronal subpopulations (5). Moreover, tissue pathology in this kuru brain corresponds with the distribution of PrP deposition; prominent PrP deposition is restricted to areas with prominent SC and/or AG. There is experimental evidence that local accumulation of PrP induces the characteristic neuropathology of prion diseases (10,13,22). In contrast, the intensities of SC and AG are not parallel: in the thalamus, AG is severe, whereas SC is slight; in the striatum, SC is severe, and AG only moderate.

We found slight PrP accumulation in the substantia gelatinosa of the spinal cord. This pattern of PrP accumulation is similar to that reported in some sporadic CJD cases and in growth hormone-associated CJD (18). Such PrP deposition in the spinal cord was speculated to indicate spinal entry of the agent from a peripheral site of inoculation (18).

Acknowledgement

This work is part of the European Union Biomed-1 Concerted Action "The human prion diseases: from neuropathology to pathobiology and molecular genetics" (Project Leader: H. Budka).

Mrs H. Flicker is kindly acknowledged for excellent technical assistance.

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