



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Ultrastructural study of florid plaques in variant Creutzfeldt-Jakob disease: a comparison with amyloid plaques in kuru, sporadic Creutzfeldt-Jakob disease and Gerstmann-Straussler-Scheinker disease

Citation for published version:

Sikorska, B, Liberski, PP, Sobow, T, Budka, H & Ironside, JW 2009, 'Ultrastructural study of florid plaques in variant Creutzfeldt-Jakob disease: a comparison with amyloid plaques in kuru, sporadic Creutzfeldt-Jakob disease and Gerstmann-Straussler-Scheinker disease' *Neuropathology and Applied Neurobiology*, vol 35, no. 1, pp. 46-59. DOI: 10.1111/j.1365-2990.2008.00959.x

Digital Object Identifier (DOI):

[10.1111/j.1365-2990.2008.00959.x](https://doi.org/10.1111/j.1365-2990.2008.00959.x)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Neuropathology and Applied Neurobiology

Publisher Rights Statement:

Wiley Online Library. OnlineOpen article

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Ultrastructural study of florid plaques in variant Creutzfeldt–Jakob disease: a comparison with amyloid plaques in kuru, sporadic Creutzfeldt–Jakob disease and Gerstmann–Sträussler–Scheinker disease

B. Sikorska*, P. P. Liberski*, T. Sobówt, H. Budka‡ and J. W. Ironside§

*Department of Molecular Pathology and Neuropathology, Chair of Oncology, Medical University of Lodz, †Department of Old Age Psychiatry & Psychotic Disorders, Medical University of Lodz, Lodz, Poland, ‡Institute of Neurology, Medical University of Vienna, and Austrian Reference Centre for Human Prion diseases, Vienna, Austria, and §National Creutzfeldt–Jakob Disease Surveillance Unit, University of Edinburgh, Edinburgh, UK

B. Sikorska, P. P. Liberski, T. Sobówt, H. Budka and J. W. Ironside (2009) *Neuropathology and Applied Neurobiology* 35, 46–59

Ultrastructural study of florid plaques in variant Creutzfeldt–Jakob disease: a comparison with amyloid plaques in kuru, sporadic Creutzfeldt–Jakob disease and Gerstmann–Sträussler–Scheinker disease

Background: Although the histological features of the amyloid plaques in variant Creutzfeldt–Jakob disease (vCJD) are distinct from those in other forms of prion disease [kuru, sporadic Creutzfeldt–Jakob disease (sCJD) and Gerstmann–Sträussler–Scheinker disease (GSS)], their ultrastructural features have only been described in a single case report. **Aims:** To study vCJD plaques systematically and compare them with plaques in kuru, sCJD, GSS and Alzheimer disease (AD). **Methods:** Amyloid plaques were studied by transmission electron microscopy and image analysis in five cases of vCJD, three cases of GSS, two cases of sCJD, one case of kuru and five cases of AD. Immunohistochemistry was performed on paraffin sections from one case of vCJD, two cases of GSS, one case of kuru and two cases of sCJD. **Results:** The florid plaques in

vCJD were either compact or more diffuse; in both forms, the radiating fibrils were organized into thick ‘tongues’, in contrast to kuru plaques. Dystrophic neurites (DNs) containing lysosomal electron-dense bodies or vesicles surrounded florid plaques. Microglial cells were found within florid plaques; occasional amyloid fibrils were identified in membrane-bound pockets of microglial cells. In vCJD, there was significant tau immunoreactivity in DNs around florid plaques while, in sCJD, GSS and kuru, minimal tau immunoreactivity was observed around plaques. **Conclusions:** The ultrastructure of the florid plaques and DNs in vCJD is more reminiscent of neuritic plaques in AD than kuru or multicentric plaques. These findings may reflect differences both in the strains of the transmissible agents responsible for these disorders and in host factors.

Keywords: amyloid, Gerstmann–Sträussler–Scheinker disease, kuru, plaque, prion protein, sporadic Creutzfeldt–Jakob disease, variant Creutzfeldt–Jakob disease, ultrastructure

Published online *Article Accepted* on 20th April 2008

Introduction

Amyloid plaques are a hallmark of transmissible and non-transmissible brain amyloidoses. The former comprise transmissible spongiform encephalopathies (TSEs) or prion diseases, which include kuru [1], Creutzfeldt–Jakob

Correspondence: James W. Ironside, National Creutzfeldt–Jakob Disease Surveillance Unit, University of Edinburgh, Western General Hospital, Edinburgh, EH4 2XU, UK. Tel: +44 131 537 1980; Fax: +44 131 343 1404; E-mail: james.ironside@ed.ac.uk

disease (CJD) [2], Gerstmann–Sträussler–Scheinker disease (GSS) [3,4] and fatal familial insomnia (FFI) [5]. There are also animal diseases – scrapie in sheep and goats [6], transmissible mink encephalopathy [7], chronic wasting disease (CWD) in deer and elk [8–10], and bovine spongiform encephalopathy (BSE) [11]. Of the non-transmissible amyloidoses, Alzheimer’s disease (AD) [12] and familial British dementia [13] are best recognized.

Human TSEs occur as sporadic, hereditary and infectious (iatrogenic) disorders [14,15]. CJD is a sporadic disorder (sCJD) in around 85% of cases; some 10–15% of CJD cases are hereditary, linked to over 20 mutations in the prion protein gene (*PRNP*); and the remaining cases are iatrogenic following neurosurgical procedures, administration of human growth hormone and gonadotrophins, and corneal or dura mater transplants [16]. Other familial forms of human TSEs include FFI and GSS. In 1996, a new variant form of CJD (now known as ‘variant’ CJD, or vCJD) was discovered, which results from exposure to BSE, probably by the oral route [17].

Amyloid plaques are restricted to certain subsets of human prion diseases: in sCJD, amyloid plaques occur in approximately 10–15% of cases that have been characterized as the MV2 subtype, according to the codon 129 polymorphism in the *PRNP* gene and the isoform of the disease-associated prion protein found on Western blot analysis of the brain [18]. These plaques closely resemble those seen in all cases of kuru (hence, kuru plaques) [19,20], but are structurally distinct at the light microscopic level from the florid plaques in vCJD [21] and the multicentric plaques in GSS [3,4,22]. There are no plaques in FFI; indeed, there are only relatively mild pathological changes in FFI anyway. In prion diseases in animals, amyloid plaques are common in different strains of rodent scrapie [23] and occur in all cases of CWD in mule deer [10]. In scrapie in sheep, they are only rarely present, and are absent in BSE in cattle; however, one recent novel form of cattle TSE reported in Italy (bovine amyloidotic spongiform encephalopathy or BASE) is char-

acterized by amyloid plaques in the brain [24]. Most cases of ruminant TSE infections do not give rise to amyloid plaques.

The ultrastructure of kuru and multicentric plaques has been studied for decades, but the florid plaques that are characteristic of vCJD have been described at the ultrastructural level in only one case report [25]. To rectify this situation, we studied vCJD plaques systematically by light and electron microscopy and compared them with plaques in sCJD, GSS, kuru and AD. The hypothesis for this study is that vCJD amyloid plaques possess ultrastructural features different from amyloid plaques occurring in other human prion diseases; these ultrastructural differences are reflected in their unique light microscopic appearances.

Material and methods

Five cases of vCJD, three cases of GSS (one autopsy and two biopsy specimens), two cases of sCJD with plaques and one case of kuru [20] were used in this study. The clinical data of the vCJD cases are summarized in Table 1. One GSS case belonged to the original Austrian ‘H’ family [4,22]; the remaining two cases belonged to the German ‘Sch’ family, which has been previously described [26,27]. Both Austrian and German GSS families were carriers of 102^{Leu} mutation in the *PRNP* gene; the codon 129 was homozygous for methionine in both the Austrian case and German cases [4,16,28]. All GSS cases fulfilled the neuropathological diagnostic criteria of Budka *et al.* [29]. Both of the sCJD cases were heterozygous at codon 129 in the *PRNP* gene. We also used archival brain specimens of a 16-year-old man who died from kuru in 1968 [20]. Furthermore, we studied five well-preserved biopsy and autopsy specimens of Alzheimer disease (AD) from the files of Department of Molecular Pathology and Neuropathology, Medical University, Lodz, Poland.

For electron microscopy, approximately 2-mm³ samples of grey matter from different brain regions were prepared.

Table 1. Basic clinical data on variant Creutzfeldt–Jakob disease (vCJD) cases

vCJD cases	96/02	96/03	96/07	96/45	96/110
Age (years)	41	29	30	31	35
Duration (months)	18	23	18	9	14
Sex	F	F	F	M	F
<i>PRNP</i> codon 129 genotype	MM	MM	MM	MM	MM

Table 2. Antibodies used for immunohistochemistry on paraffin sections

<i>Antigen</i>	<i>Company</i>	<i>Clone</i>	<i>Host</i>	<i>Dilution</i>
HLA DR	DAKO	CR3/43	Mouse IgG1	1 : 100
GFAP	DAKO	Polyclonal	Rabbit	1 : 3000
APP	Chemicon	22C11	Mouse IgG1	1 : 500
NFP	DAKO	2F11	Mouse IgG1	1 : 50
Tau	Pierce endogen	AT8	Mouse IgG1	1 : 200
PrP	Cayman	12F10	Mouse IgG2a	1 : 500
Ubiquitin	DAKO	Polyclonal	Rabbit	1 : 200

HLA DR, human leucocyte antigen DR; GFAP, glial fibrillary acidic protein; APP, amyloid precursor protein; NFP, neurofilament protein.

From vCJD, and the GSS case from the H family, we always studied at least four regions – frontal, temporal, occipital cortices and the cerebellum. Wherever possible, four blocks were studied from every region and at least two grids were prepared from every block. From the vCJD cases, we studied collectively 50 blocks. From the brain biopsy of the GSS case from the Sch family, only the frontal cortex was available. From the kuru case, one block from the frontal cortex and one block from cerebellum were studied. From the remaining cases, only single blocks were available. The tissue blocks were immersion-fixed in 2.5% glutaraldehyde for less than 24 h, embedded in Epon and routinely processed for electron microscopy; from a kuru case and one of the sCJD cases, material was reprocessed for electron microscopy from formalin-fixed paraffin-embedded blocks as described previously [30]. Grids were examined and photographed in JEOL JEM 100 CX and JEOL JEM 1011 transmission electron microscopes at 80 kV. Analysis of fibril diameter was performed using iTEM Soft Imaging System for transmission electron microscopy, Olympus, Japan and the diameters of 100 fibrils were measured in each case. For statistical analysis, only cases fixed in glutaraldehyde were included. A non-parametric Mann–Whitney *U*-test was used to compare group mean values for fibril diameters.

Additionally, we performed immunohistochemistry on paraffin sections of the frontal cortex and cerebellum from one vCJD case, two sCJD cases, two GSS cases and one kuru case. All the paraffin blocks used for immunohistochemistry were obtained from autopsy brain tissue, routinely fixed in 4% formaldehyde and embedded in paraffin. Antibodies used in the study are described in Table 2. For visualization of all immunolabelling, the LSAB+ Kit with horseradish peroxidase (DAKO, Ely, Cambridgeshire, UK) was used.

Results

Plaques in specimens of GSS and sCJD were of two major types: the first was stellate unicentric kuru plaques composed of a dense centre of interwoven fibrils and radiating fibrils at the periphery, and they were seen in both GSS and sCJD (Figure 1). The second type was found only in GSS specimens – multicentric plaques composed of several merging unicentric plaques (Figure 2). A particular plaque type – unicentric stellate amyloid plaques (florid plaques) – were readily identified in all vCJD specimens (Figure 3); they were most abundant in the occipital cortex and the cerebellum. These were either compact (resembling kuru plaques) or more diffuse; even in the more compact plaques, the radiating fibrils were organized into thick ‘tongues’ in contrast to kuru plaques. Irrespective of the region studied, no obvious differences were seen in the general ultrastructural features of the plaques in vCJD. The radiating tongues of amyloid that give the florid plaque a stellate appearance were also thicker than those in GSS and vCJD. It was noteworthy that, apart from florid plaques, smaller amyloid (fibrillar) structures – which have been called cluster plaques – were also seen in vCJD; however, it is impossible to be sure that those are not the poles of larger plaques cut tangentially. Numerous empty vacuoles were seen within and around florid plaques, which appeared as rather dilated ‘empty’ processes (as judged by remnant of subcellular organelles like mitochondria within them) and not typical ‘spongiform’ vacuoles. The latter term is used to describe ‘empty’ spaces containing curled membrane fragments and ‘fluffy’ material [31]. Thus, the nature of vacuoles surrounding florid plaques remains uncertain.

To demonstrate differences in amyloid fibril thickness, we performed analysis of fibril diameter using a morpho-

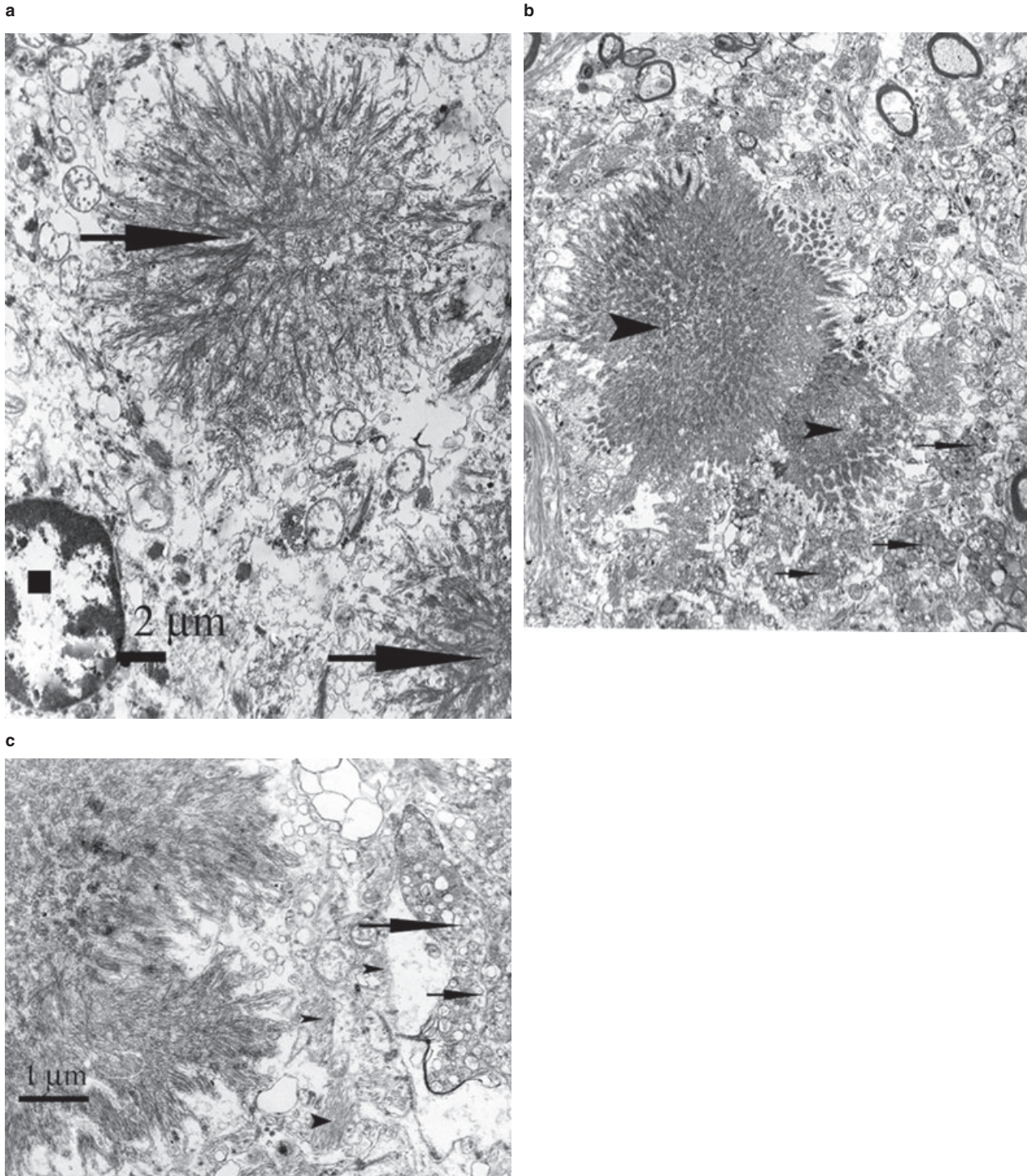


Figure 1. Gerstmann–Sträussler–Scheinker disease. (a, b, c) Different views of amyloid plaques. Note that some plaques are surrounded by a pale ‘halo’ composed of astrocytic processes (c, arrowheads). (c) Dystrophic neurites around a plaque (arrows).

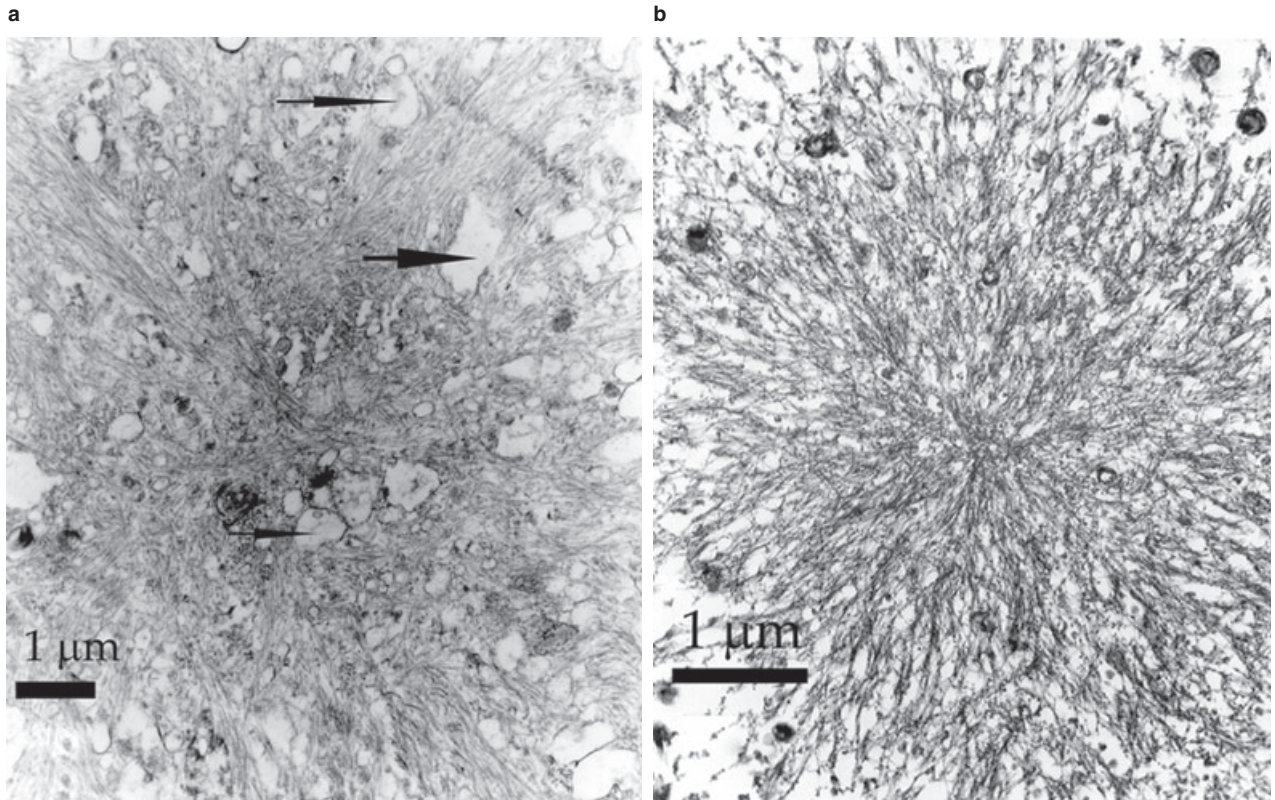


Figure 2. Amyloid plaques in (a) variant Creutzfeldt-Jakob disease (vCJD) in comparison to (b) Gerstmann-Sträussler-Scheinker disease. Note the vacuoles (arrowheads) around the vCJD plaque (a).

metric system for transmission electron microscopy by measuring the diameters of 100 fibrils in every case. The mean diameter of a single fibril in GSS was 17.64 nm (range 12–22.99 nm) while, in vCJD, it was 21.37 nm (range 17.23–24.37 nm) and in AD – 14.24 nm (range 11.41–17.35 nm). As normality assumption for data distribution was not met for the AD and vCJD groups (Shapiro–Wilk W test significant) and variances could not be assumed as equal (Levene’s test for equality of variances significant for all three between groups comparisons), a non-parametric Mann-Whitney U -test was used to compare groups’ means as a *post hoc* procedure after a Kruskal–Wallis one-way analysis of variance ($\chi^2 = 215.2$; $df = 2$, $P < 0.001$).

In GSS, the mean value for fibril diameter (17.6 ± 2.5 nm) was significantly larger than in AD (14.2 ± 1.4 nm), Mann-Whitney U -test, $P < 0.001$. In vCJD, the group mean value (21.4 ± 1.8 nm) was significantly bigger than in both GSS ($P < 0.01$) and AD ($P < 0.001$). Thus, the statistical analysis showed significant differences between these groups. The mean values

for fibril diameters in kuru plaques in cases of kuru and sCJD were 23.40 and 27.06 nm, respectively, although these values were not statistically analysed due to the changes evoked by different fixation conditions from vCJD, GSS and AD cases. Fibrils from amyloid material retrieved from paraffin blocks are usually much thicker than in corresponding material directly fixed in glutaraldehyde; e.g. in GSS plaques, the fibril diameter from paraffin-embedded tissues is approximately 50 nm, approximately three times more than from the non-paraffin-embedded material. From these observations, we may therefore speculate that the real diameter of amyloid fibrils in kuru plaques from a case of kuru may be around 7–8 nm, consistent with data already published from material directly fixed in glutaraldehyde, which give a fibril diameter of 7 nm [32].

Interestingly, florid plaques were neuritic – i.e. they contained dystrophic neurites; the latter contained lysosomal electron-dense bodies or vesicles (Figure 3). Dystrophic neurites (DNs) were found either at the margin of plaques or immersed within the centre of the plaque. In the non-

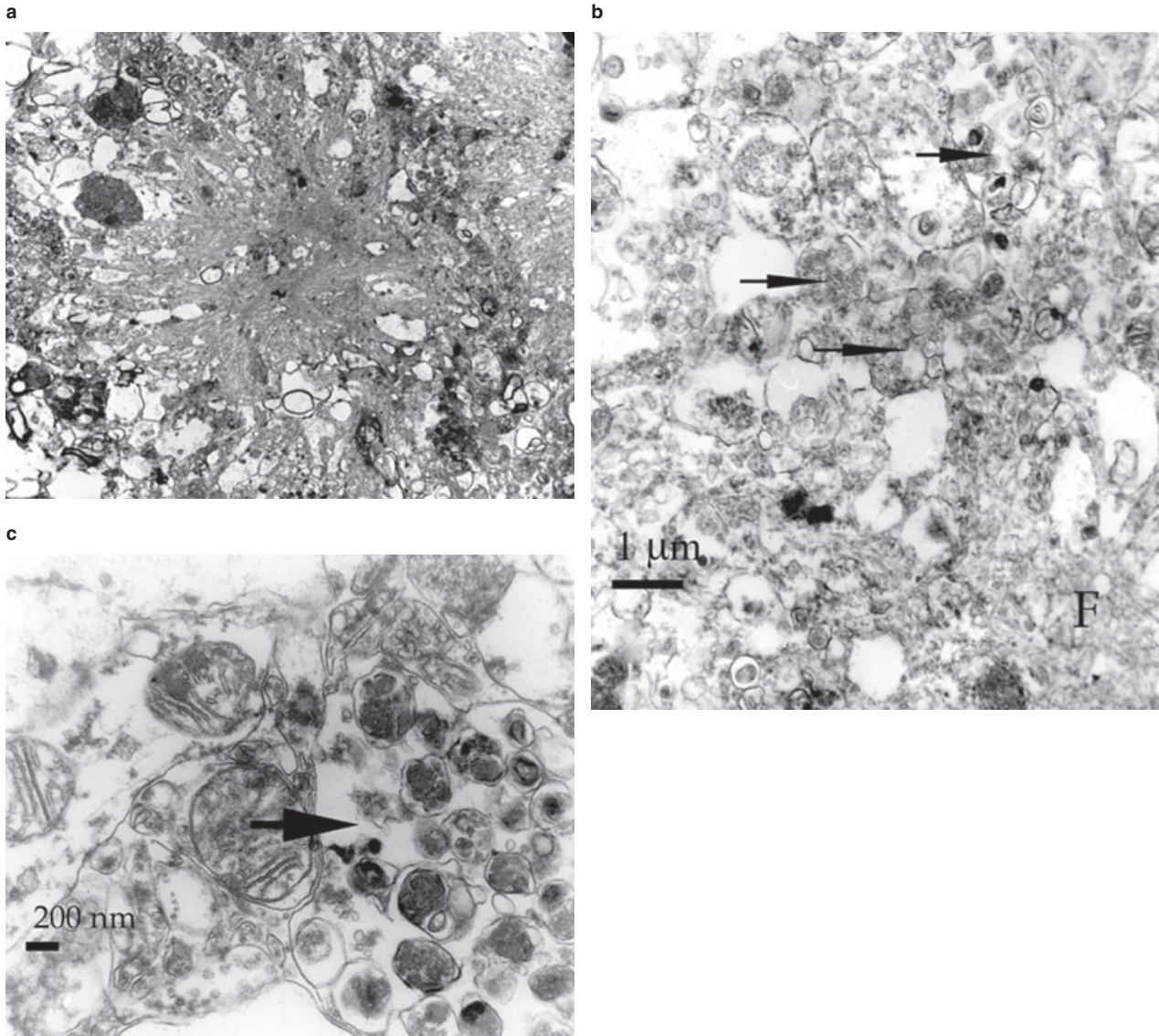


Figure 3. Variant Creutzfeldt–Jakob disease (vCJD). Lower (a) and higher (b) magnifications of amyloid plaques. Note that the florid plaque in vCJD is more 'diffuse' than specimens in Figures 1–2. (c) A dystrophic neurite (arrow) with dense lysosomal bodies (arrowhead). F, amyloid fibrils.

florid plaques of vCJD, DNs were also observed, mainly at the plaque margins. Microglial cells (Figure 4), not unlike those in GSS [33], were found within vCJD plaques and, in certain specimens, amyloid fibrils were clearly present in membrane-bound pockets of microglial cells. In two specimens from the temporal cortex in vCJD, Hirano bodies were found in cell processes in the neuropil close to florid plaques (Figure 5). In kuru, astrocytic processes were frequently seen at the margin of plaques. The ultrastructural differences between the various types of plaques are illustrated in Figure 6 and summarized in Table 3.

Recently, we realized that autophagy is an important contributor to the pathology of TSEs [34,35]. To this end, we observed many multivesicular bodies, which are involved in the process of microautophagy, in the vCJD cases [36]. Those subcellular organelles were often found within electron-lucent vacuoles of different sizes located at the margin of florid plaques (Figure 7). In AD, the ultrastructure of amyloid plaques was similar to that in earlier descriptions [37], with dense amyloid cores surrounded by corona of DNs; in contrast to the DNs of TSEs, those in AD contained paired helical filaments (PHFs).

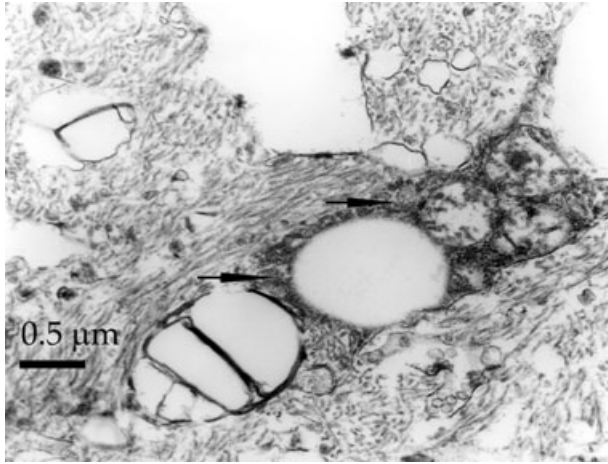


Figure 4. Variant Creutzfeldt–Jakob disease. Dense cytoplasm of a microglial cell within an amyloid plaque. Note the large electron-lucent vacuoles within the cytoplasm of the microglial cell.

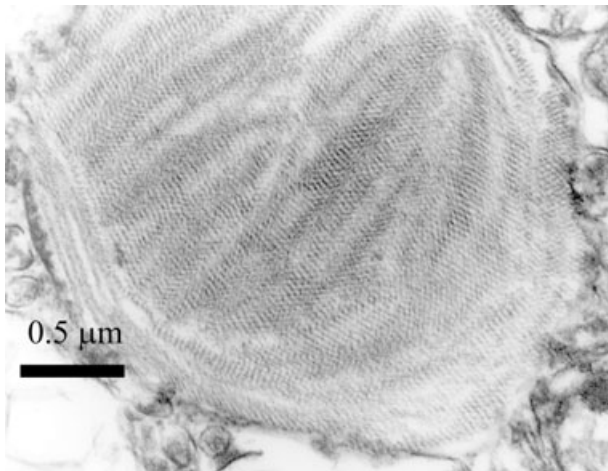


Figure 5. Variant Creutzfeldt–Jakob disease. A typical Hirano body in a cell process adjacent to a florid plaque.

Table 3. Comparison of ultrastructural features in various types of amyloid plaques in human TSEs

	Florid plaques (vCJD)	Kuru plaques (sCJD)	Multicentric plaque cores (GSS)	Kuru plaques (kuru)	Aβ plaques (AD)
Plaque size	Large	Medium*	Small, forming larger clusters	Small*	Various sizes
Average fibril diameter (range)	21.37 nm(17.23–24.37)	9–10 nm†	17.64 nm(12.0–22.99)	7–8 nm†	14.24 nm(11.41–17.35)
Dystrophic neurites	+++	Single	++	Single	+++
Vacuoles	+++	-	-	-	-
Microglia	+++	+	+++	++	+++
Astrocytic processes	-	+	+	+++	+

*In specimens retrieved from paraffin blocks.

†Estimated.

TSEs, transmissible spongiform encephalopathy; vCJD, variant Creutzfeldt–Jakob disease; sCJD, sporadic Creutzfeldt–Jakob disease; GSS, Gerstmann–Sträussler–Scheinker disease; AD, Alzheimer disease.

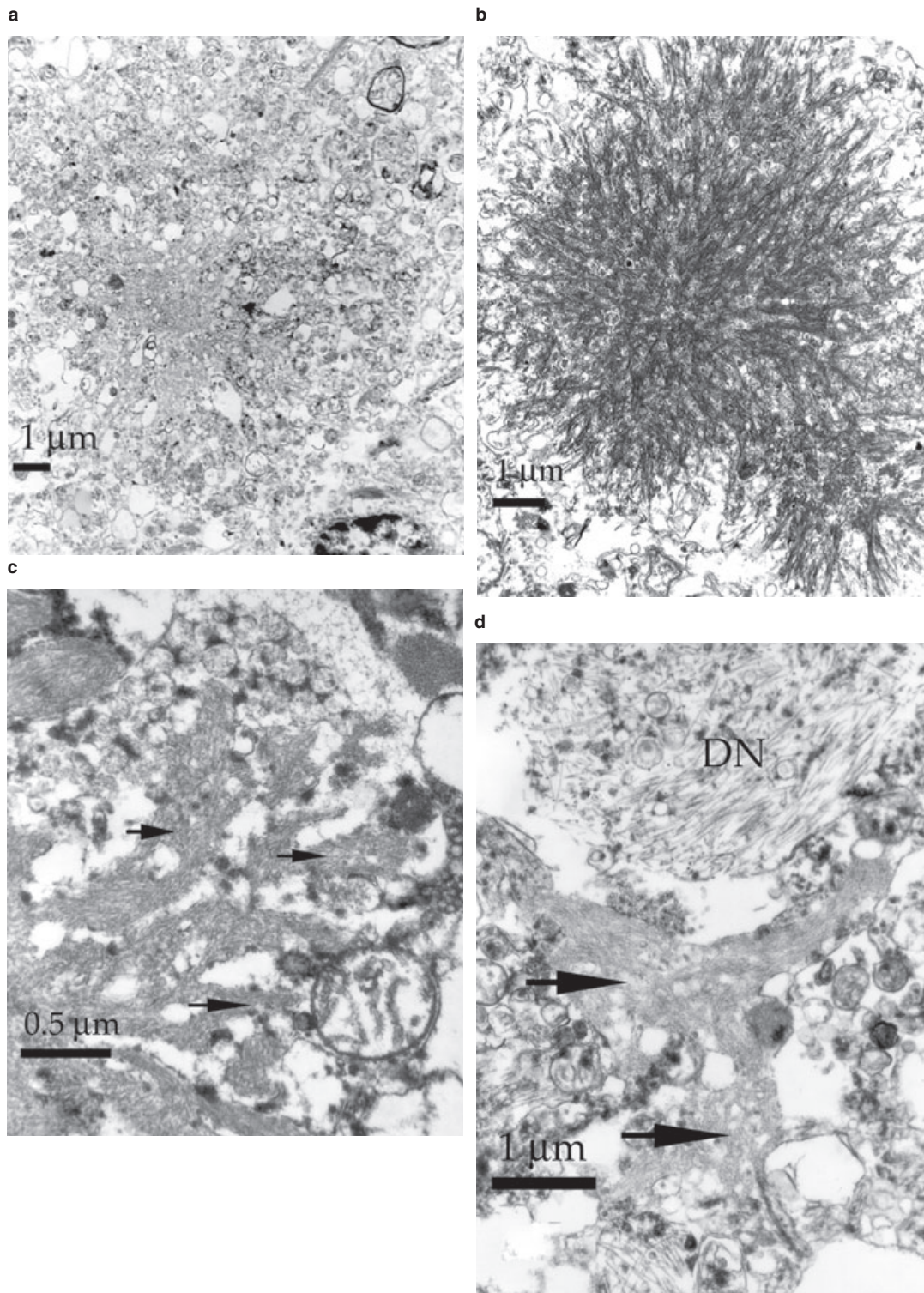


Figure 6. Comparison of plaque ultrastructure in (a) variant Creutzfeldt–Jakob disease (vCJD), (b) Gerstmann–Sträussler–Scheinker disease and (c) Alzheimer’s disease. Amyloid is labelled by arrows in (c) and (d), the latter also showing a dystrophic neurite (DN) in vCJD.

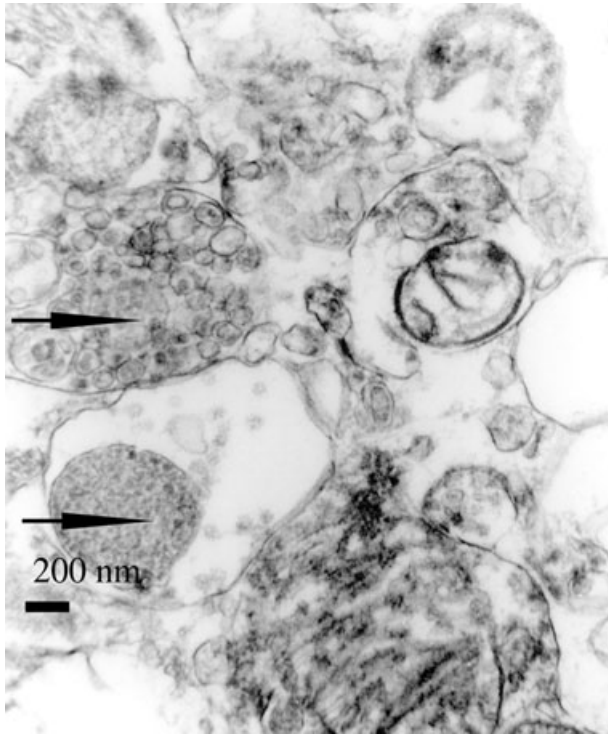


Figure 7. Vacuoles around a plaque contain multivesicular body (arrows) in variant Creutzfeldt–Jakob disease. F, amyloid fibrils.

While the search for tubulovesicular structures (TVSs), the only disease-specific particles found in all TSEs at the level of electron microscopy [38], was not the aim of this study, we readily found them in all GSS specimens [39], but not in autopsy vCJD specimens. In contrast, TVSs were previously readily found in a brain biopsy vCJD specimen [40].

Immunohistochemistry for glial fibrillary acidic protein (GFAP) showed astrocytosis in all cases in both cerebral and cerebellar cortex. In the cerebellum, astrogliosis was most pronounced in the kuru case. The GFAP-positive cell processes were observed around the plaques and in the peripheral part of the plaques in kuru and, to a lesser degree, in sCJD. In vCJD and GSS, the astrocytic processes surrounded the plaques, but did not penetrate into the plaques. Immunohistochemistry for human leucocyte antigen DR (HLA-DR) revealed the presence of HLA-DR-positive microglial cells around the plaques in all cases. In GSS and vCJD, there was also significant HLA-DR immunoreactivity in the central parts of the plaques. Immunohistochemistry for amyloid precursor protein (APP) showed high numbers of APP-positive structures corresponding to DNAs around the plaques in vCJD and GSS. In

kuru and sCJD, we also observed some APP-positive distended processes, but they were dispersed throughout cortex and not gathered around the plaques. Immunohistochemistry for ubiquitin also revealed DNAs at the plaque margin as well as some dispersed abnormal neurites in vCJD and in GSS. In kuru and sCJD, the number of ubiquitin-positive DNAs was much lower and the intensity of staining for ubiquitin around the plaques was less intense (Figure 8). Similar results were obtained following immunohistochemistry for phosphorylated neurofilament proteins (NFP), which also demonstrated some axonal swellings in the cerebellar cortex in both vCJD and GSS. These structures were distributed randomly, not around the plaques. An interesting observation was made on immunohistochemistry for hyperphosphorylated tau with the AT8 anti-tau antibody. In vCJD, there was significant immunoreactivity in DNAs around the plaques and some AT8-positive structures were also dispersed in the cerebral and, to a lesser degree, the cerebellar cortex (Figure 9a). In sCJD, GSS and kuru, immunoreactivity for tau was much less intense and was restricted to the periphery of the plaques, but not in the neuropil (Figure 9b–d).

Discussion

We describe herein the spectrum of amyloid plaques in vCJD. The most characteristic plaques of vCJD are florid plaques – stellate structures surrounded by vacuoles, visible in haematoxylin and eosin sections in light microscopy. Those plaques differ from unicentric kuru plaques of sCJD and multicentric plaques of GSS. Florid plaques are also unicentric, but they were of a less compact structure than kuru plaques, with constituent amyloid fibrils being thicker than those of sCJD/GSS. The diameter of the single fibril of a florid plaque measured *in situ* is much higher than in the amyloid plaques of GSS or AD. Merz *et al.* [41] showed that fibrils isolated from AD brain were 4–8 nm in diameter and fibrils from GSS brain were 7–9 nm. Those results also showed that AD fibrils are thinner in comparison with GSS. The differences between our numbers and those showed in Merz *et al.*'s [41] paper are most probably evoked by the different methodology of experiments (isolation and negative staining vs. fixed tissues and positive staining).

As in the plaques of GSS, but unlike most plaques of sCJD, florid plaques are neuritic – they contain DNAs accumulating electron-dense lysosomal bodies and vesicles,

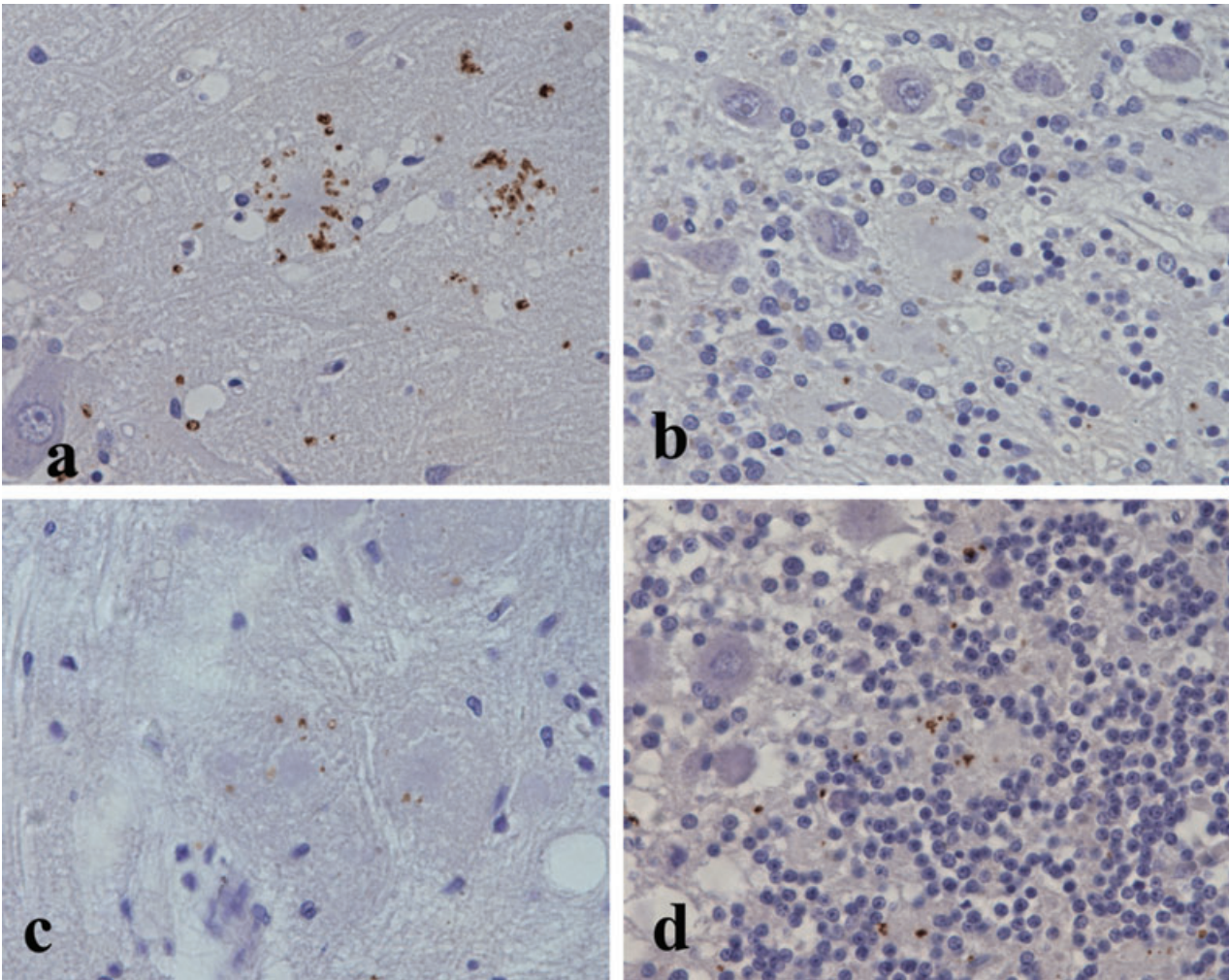


Figure 8. Hyperphosphorylated tau immunoreactivity around amyloid plaques in human TSEs in structures that appear to be dystrophic neurites (AT8 anti-tau antibody with haematoxylin counterstain, $\times 600$). (a) Variant Creutzfeldt–Jakob disease; (b) sporadic Creutzfeldt–Jakob disease; (c) Gerstmann–Sträussler–Scheinker disease; (d) kuru.

but many of these abnormal structures are mixed with amyloid fibrils. Thus, a subpopulation of DNs are situated not at the periphery of the plaque, but more often within the centre. The latter situation is not unusual in AD, where in a reconstruction of plaques using confocal laser microscopy, Masliah *et al.* [42] elegantly demonstrated that DNs are located at the plaque centre and their spurious location at the periphery reflects the plane of the section, not the real plaque structure. The plaque pathology in vCJD may, however, reflect the latter situation, as there are many more plaques in vCJD than in sCJD or even GSS, and thus there are more planes at which these plaques are sectioned for electron microscopy. In any case, the problem of whether small DNs within the plaque centre is an intrinsic characteristic of the florid plaque of

vCJD, as already suggested in a case report from France [25], or an artefact of sectioning, requires computer-based image analysis for final elucidation. The ultrastructure of florid plaques of vCJD is reminiscent of neuritic plaques in AD, in spite of the obvious difference that a proportion of DNs in AD contain PHFs. It must be stressed, however, that a high proportion of DNs in AD do not contain PHFs [43].

The presence of neuritic dystrophy and occasional Hirano bodies in processes around the florid plaques of vCJD suggests that they might represent a neuronal reaction to the abnormal accumulation of amyloid. However, these changes are not present around other forms of PrP amyloid plaques, suggesting that differences in the conformation and arrangement of the amyloid fibres in different

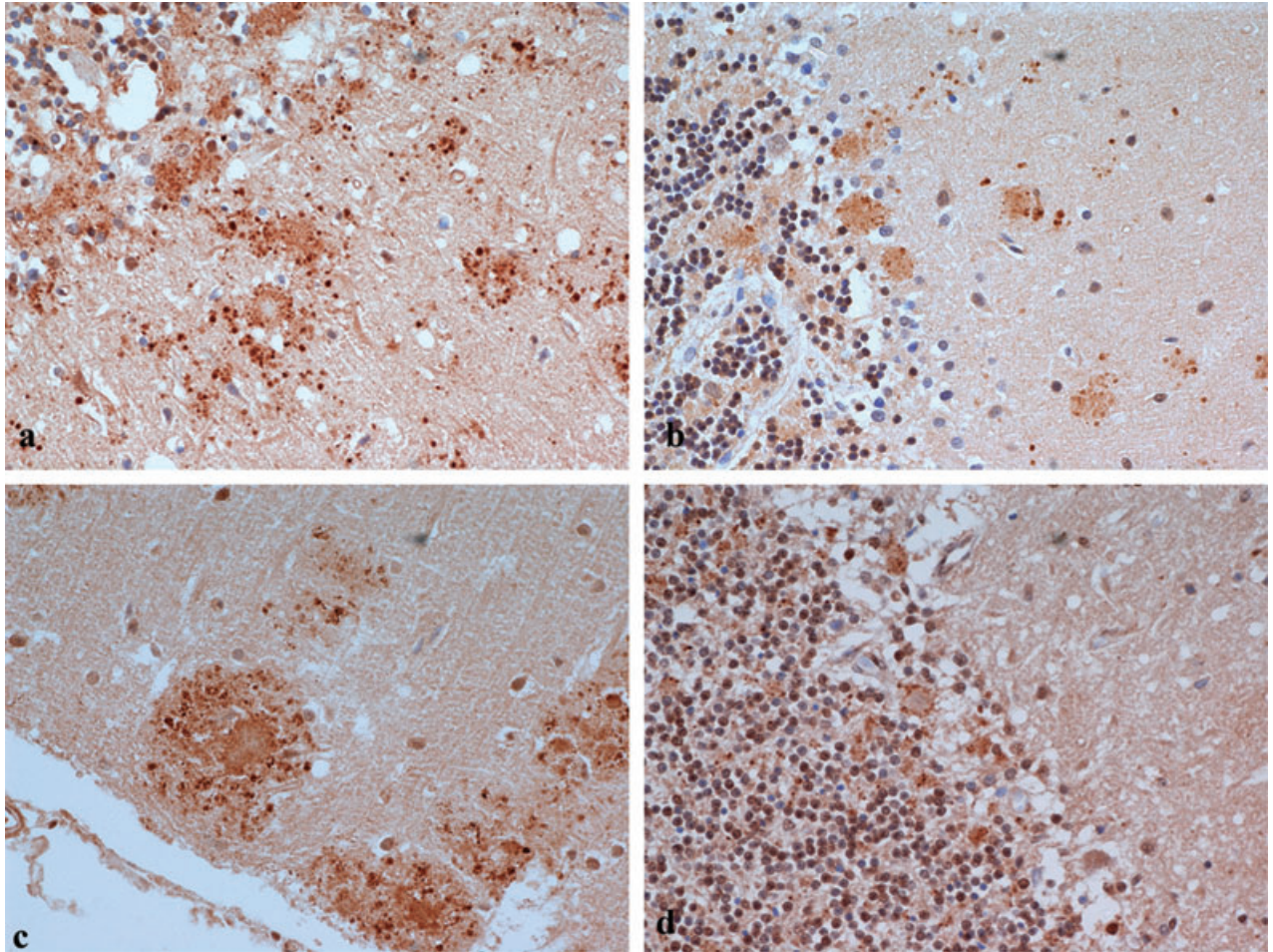


Figure 9. Differing patterns of ubiquitin immunoreactivity around amyloid plaques in neurites and in thread-like structures in the surrounding neuropil (anti-ubiquitin antibody with haematoxylin counterstain, $\times 600$). (a) Variant Creutzfeldt–Jakob disease; (b) sporadic Creutzfeldt–Jakob disease; (c) Gerstmann–Sträussler–Scheinker disease; (d) kuru.

forms of human prion disease may influence these reactions, or that there are other plaque constituents in vCJD that potentiate the neuronal reaction. Previous studies have suggested that tau accumulates within neuritic processes around plaques in some human prion diseases, including vCJD [44] and GSS [45]; on the other hand, the DNs in sCJD have been previously reported to accumulate NFP rather than tau [46].

Our immunohistochemical studies confirmed the presence of microglial cells, astrocytic processes and dystrophic neurites at the periphery of the plaques. Immunoreactivity with the AT8 antibody revealed hyperphosphorylated tau at periphery of the plaques in vCJD, in a distribution that is consistent with DNs. This finding is also reminiscent of AD plaques but, in contrast to AD, no PHF were observed within DNs in vCJD on electron micros-

copy. In contrast to a previous report [44], tau immunoreactivity in or around the plaques in kuru, GSS and sCJD was also observed. This difference is probably due to the lack of plaques in sCJD cases studied by Giaccone *et al.* [44]. To our knowledge, this is the first report on hyperphosphorylated tau immunoreactivity in sCJD and kuru.

All our GSS and vCJD cases were homozygous for methionine at codon 129 of the *PRNP* gene. Thus, we cannot conclude that the differences in plaque ultrastructure result from differences in *PRNP* codon 129 polymorphism as suggested by Cervenakova *et al.* [47]. All described kuru cases with plaques were homozygous for methionine or for valine [47,48]. However, the number of studied kuru cases was small, and the influence of the presence of valine at codon 129 on the clinical course and pathology of prion diseases require further investigation.

In conclusion, we can say that there are three types of amyloid plaques in vCJD observed by light microscopy: florid plaques, small kuru plaques (cluster plaques) and amorphous plaques. Ultrastructurally, all consist of thick amyloid fibrils, different from the amyloid fibrils seen in other human prion diseases. The florid plaques are not only surrounded by vacuoles, but also contain DNs, microglial cells and occasional Hirano bodies at their border; thus, the ultrastructural composition of florid plaques in vCJD is reminiscent of A β plaques in AD, but their fibrils are significantly thicker. The DNs around the plaques in vCJD contain APP, ubiquitin, hyperphosphorylated tau and phosphorylated NFP; the number of tau-positive DNs around florid plaques was higher than any of the amyloid plaques in GSS, kuru and sCJD.

The reasons why florid plaques differ in structure from kuru plaques and multicentric plaques observed in sCJD, kuru and GSS are open to speculation. The strain of TSE agent that causes vCJD is different from those of sCJD and kuru, and closely resembles the BSE agent [49–51]. It has been shown for decades that quantitative TSE neuropathology is under strict control of both the host *PRNP* genotype and the strain of the agent, and the amount of amyloid in the form of plaques is one of those characteristics [52]. However, the ultrastructural pathology is the same ‘kuru plaque’ irrespective of the experimental model selected [53]. Thus, the strain difference influences quantitative characteristics of amyloid plaques, but not necessarily their ultrastructural appearance. In sCJD, kuru plaques are characteristically confined to cases that are heterozygous at codon 129 in the *PRNP* gene (as in our cases) [18]. Furthermore, the agent that causes vCJD is also the cause of BSE in cattle, which is characterized by a virtual absence of amyloid plaques in the brain [43], further highlighting the importance of host factors in the pathological phenotype of this group of disorders.

Acknowledgments

This work was prepared within the EC ‘NeuroPrion’ project, Medical University of Lodz Grant No. 502-11-260, and the project of the Polish Ministry of Science and Higher Education No. K129/P05/2005. We are grateful to Ms Gerda Ricken, Ms Leokadia Romańska and Mr Kazimierz Smoktunowicz for expert technical assistance. PPL and JWI are grateful for support from the British Council, Poland.

References

- Gajdusek DC, Gibbs CJ, Alpers MP. Experimental transmission of a kuru-like syndrome to chimpanzees. *Nature* 1966; **209**: 794–6
- Gibbs CJ Jr, Gajdusek DC, Asher DM, Alpers MP, Beck E, Daniel PM, Matthews WB. Creutzfeldt-Jakob disease (spongiform encephalopathy): transmission to chimpanzee. *Science* 1968; **161**: 388–9
- Masters CL, Gajdusek DC, Gibbs CJ Jr. Creutzfeldt-Jakob disease virus isolations from the Gerstmann-Sträussler syndrome. With an analysis of the various forms of amyloid plaque deposition in the virus induced spongiform encephalopathies. *Brain* 1981; **104**: 559–88
- Hainfellner J, Brantner-Inhaller S, Cervenakova L, Brown P, Kitamoto T, Tateishi J, Diringer H, Liberski PP, Regele H, Feucht M, Mayr N, Wessely K, Summer K, Seitelberger F, Budka H. The original Gerstmann-Sträussler-Scheinker family of Austria: divergent clinicopathological phenotypes but constant PrP genotype. *Brain Pathol* 1995; **5**: 201–13
- Collinge J, Palmer MS, Sidle KCL, Gowland I, Medori R, Ironside J, Lantos P. Transmission of fatal familial insomnia to laboratory animals. *Lancet* 1995; **346**: 569–70
- Dickinson AG. Scrapie in sheep and goats. In *Slow Virus Diseases of Animals and Man*. Ed. RH Kimberlin. Amsterdam: North Holland Publ Comp, 1976; 209–41
- Kimberlin RH, Marsh RE. Comparison of scrapie and transmissible mink encephalopathy in hamsters. I. Biochemical studies of brain during development of disease. *J Infect Dis* 1975; **131**: 97–103
- Williams ES, Young S. Chronic wasting disease of captive mule deer: a spongiform encephalopathy. *J Wildlife Dis* 1980; **16**: 89–98
- Williams ES, Young S. Spongiform encephalopathy of Rocky Mountain elk. *J Wildlife Dis* 1982; **18**: 465–71
- Liberski PP, Guiryo DC, Williams ES, Walis A, Budka H. Deposition patterns of disease-associated prion protein in captive mule deer brains with chronic wasting disease. *Acta Neuropathol (Berl)* 2001; **102**: 496–500.
- Wells GAH, Scott AC, Johnson CT, Gunning RF, Hancock RD, Jeffrey M, Dawson M, Bradley R. A novel progressive spongiform encephalopathy in cattle. *Vet Rec* 1987; **121**: 419–20
- Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RL, Beyreuther K. Neuronal origin of a celebrated amyloid: neurofibrillary tangles of Alzheimer’s disease contain the same protein as the amyloid of plaque cores and blood vessels. *EMBO J* 1985; **4**: 2757–63
- Ghiso J, Vidal R, Rostagno A, Miravalle L, Holton JL, Mead S, Revesz T, Plant G, Frangione B. Amyloidogenesis in familial British dementia is associated with a genetic defect on chromosome 13. *Ann N Y Acad Sci* 2000; **920**: 84–92

- 14 Brown P, Goldfarb LG, Gajdusek DC. The new biology of spongiform encephalopathy: infectious amyloidoses with a genetic twist. *Lancet* 1991; **337**: 1019–22
- 15 Liberski PP, Brown P. Prion diseases: from transmission experiments to structural biology – still searching for the cause. *Folia Neuropathol* 2004; **42** (Suppl. A): 15–32
- 16 Brown P, Brandel JP, Preece M, Sato T. Iatrogenic Creutzfeldt-Jakob disease: the waning era. *Neurology* 2006; **67**: 389–93
- 17 Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchari M, Hofman A, Smith PG. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996; **347**: 921–5
- 18 Parchi P, Capellari S, Brown P, Schulz-Schaeffer W, Windl O, Zerr I, Budka H, Kopp N, Piccardo P, Poser S, Rojiani A, Streichenberger N, Julien J, Vital C, Ghetti B, Gambetti P, Kretzschmar H. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Ann Neurol* 1999; **46**: 224–33
- 19 Klatzo I, Gajdusek DC. Pathology of kuru. *Lab Invest* 1959; **8**: 799–847
- 20 Hainfellner JA, Liberski PP, Guiroy DC, Cervenakova L, Brown P, Gajdusek DC, Budka H. Pathology and immunocytochemistry of a kuru brain. *Brain Pathol* 1997; **7**: 547–54
- 21 Ironside JW, Bell JB. Florid plaques and new variant Creutzfeldt-Jakob disease. *Lancet* 1997; **350**: 1475
- 22 Liberski PP, Budka H. Ultrastructural pathology of Gerstmann-Sträussler-Scheinker disease. *Ultrastruct Pathol* 1995; **19**: 23–36
- 23 Fraser H. The pathology of a natural and experimental scrapie. *Front Biol* 1976; **44**: 267–305
- 24 Casalone C, Zanusso G, Acutis P, Ferrari S, Capucci L, Tagliavini F, Monaco S, Caramelli M. Identification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. *Proc Natl Acad Sci USA* 2004; **101**: 3065–70
- 25 Fournier FG, Kopp N, Streichenberger N, Escaig-Haye F, Langeveld J, Brown P. Electron microscopy of brain amyloid plaques from a patient with new variant Creutzfeldt-Jakob disease. *Acta Neuropathol (Berl)* 2000; **99**: 637–42
- 26 Boellaard JW, Doerr-Schott J, Schlote W. Miniplaques and shapeless cerebral amyloid deposits in a case of Gerstmann-Sträussler-Scheinker disease. *Acta Neuropathol (Berl)* 1993; **86**: 532–5
- 27 Brown P, Goldfarb LG, Brown WT, Goldgaber D, Rubenstein R, Kascak RJ, Guiroy DC, Piccardo P, Boellaard JW, Gajdusek DC. Clinical and molecular genetic study of a large German kindred with Gerstmann-Sträussler-Scheinker syndrome. *Neurology* 1991; **41**: 375–9
- 28 Boellaard JW, Brown P, Tateishi J. Gerstmann-Sträussler-Scheinker disease – the dilemma of molecular and clinical correlations. *Clin Neuropathol* 1999; **18**: 271–85
- 29 Budka H, Aguzzi A, Brown P, Brucher JM, Bugiani O, Gullotta F, Haltia M, Hauw J-J, Ironside JW, Jellinger K, Kretzschmar HA, Lantos PL, Masullo C, Schlote W, Tateishi J, Weller RO. Consensus report. Neuropathological diagnostic criteria for Creutzfeldt-Jakob disease (CJD) and other human spongiform encephalopathies (prion diseases). *Brain Pathol* 1995; **4**: 459–66
- 30 Alwasiak J, Mirecka B, Wozniak L, Liberski PP. Neuroblastic differentiation of metastases of medulloblastoma to extracranial lymph node: an ultrastructural study. *Ultrastruct Pathol* 1991; **15**: 647–54
- 31 Liberski PP. Spongiform change – an electron microscopic view. *Folia Neuropathol* 2004; **42** (Suppl. B): 59–70
- 32 Field EJ, Mathews JD, Raine CS. Electron microscopy observations on the cerebellar cortex in kuru. *J Neurol Sci* 1968; **8**: 209–24
- 33 Barcikowska M, Liberski PP, Boellaard J, Brown P, Gajdusek DC, Budka H. Microglia is a component of the prion protein amyloid plaque in the Gerstmann-Sträussler-Scheinker syndrome. *Acta Neuropathol (Berl)* 1993; **85**: 623–7
- 34 Liberski PP, Sikorska B, Bratosiewicz-Wasik J, Gajdusek DC, Brown P. Neuronal cell death in transmissible spongiform encephalopathies (prion diseases) revisited: from apoptosis to autophagy. *Int J Biochem Cell Biol* 2004; **36**: 2473–90
- 35 Sikorska B, Liberski PP, Giraud P, Kopp N, Brown P. Autophagy is a part of ultrastructural synaptic pathology in Creutzfeldt-Jakob disease: a brain biopsy study. *Int J Biochem Cell Biol* 2004; **36**: 2563–73
- 36 Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, Cuervo AM. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. *J Neuropathol Exp Neurol* 2005; **64**: 113–22
- 37 Wisniewski HM, Terry RD. Re-examination of the pathogenesis of the senile plaques. In *Progress in Neuropathology Vol. 2*. Ed. HM Zimmerman. New York: Grune & Stratton, 1973; 1–26
- 38 Liberski PP, Brown P. Disease-specific particles without prion protein in prion diseases – phenomenon or epiphenomenon? *Neuropathol Appl Neurobiol* 2007; **33**: 395–7
- 39 Liberski PP, Sikorska B, Hauw J-J, Kopp N, Streichenberger N, Giraud P, Budka H, Boellaard JW, Brown P. Tubulovesicular structures are a consistent (and unexplained) finding in the brains of human prion diseases. *Virus Res* 2008; **136**: 226–8
- 40 Liberski PP, Streichenberger N, Giraud P, Soutrenont M, Meyeronnet M, Sikorska B, Kopp N. Ultrastructural pathology of prion diseases revisited: brain biopsy studies. *Neuropathol Appl Neurobiol* 2005; **31**: 88–96
- 41 Merz P, Wisniewski HM, Somerville RA, Bobin SA, Masters CL, Iqbal K. Ultrastructural morphology of amyloid fibrils from neuritic and amyloid plaques. *Acta Neuropathol (Berl)* 1983; **60**: 113–24

- 42 Masliah E, Mallory M, Deerinck T, DeTeresa R, Lamont S, Miller A, Terry RD, Carragher B, Ellisman M. Re-evaluation of the structural organization of neuritic plaques in Alzheimer's disease. *J Neuropathol Exp Neurol* 1993; **52**: 619–32
- 43 Liberski PP, Kloszewska I, Boellaard I, Papierz W, Omulecka A, Budka H. Dystrophic neurites of Alzheimer's disease and Gerstmann-Sträussler-Scheinker's disease dissociate from the formation of paired helical filaments. *Alzheimers Res* 1995; **1**: 89–93
- 44 Giaccone G, Mangieri M, Cpobianco R, Limido L, Hauw JJ, Haik S, Fociani P, Bugiani O, Tagliavini F. Tauopathy in human and experimental variant Creutzfeldt-Jakob disease. *Neurobiol Aging* 2007 (Epub ahead of print)
- 45 Ishizawa K, Komori T, Shimazu T, Yamamoto T, Kitamoto T, Shimazu K, Hirose T. Hyperphosphorylated tau deposition parallels prion protein burden in a case of Gerstmann Sträussler Scheinker syndrome P102L mutation complicated with dementia. *Acta Neuropathol* 2002; **104**: 342–50
- 46 Liberski PP, Budka H. Neuroaxonal pathology in Creutzfeldt Jakob disease. *Acta Neuropathol* 1999; **97**: 329–34
- 47 Cervenakova L, Goldafrb LG, Garruto R, Lee HS, Gajdusek DC, Brown P. Phenotype –genotype studies in kuru: Implications for new variant Creutzfeldt Jakob disease. *Proc Natl Acad Sci* 1998; **96**: 13239–41
- 48 McLean CA, Ironside JW, Alpers MP, Brown P, Cervenakova L, Anderson RMcd, Masters CL. Comparative neuropathology of kuru with the new variant of Creutzfeldt Jakob disease: evidence for strain of agent predominating over genotype of host. *Brain Pathol* 1998; **8**: 429–37
- 49 Hill AF, Desbruslais M, Joiner S, Sidle KCL, Gowland I, Collinge J, Doey LJ, Lantos P. The same prion strain causes vCJD and BSE. *Nature* 1997; **389**: 448–50
- 50 Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCardle L, Chree A, Hope J, Birkett C, Cousens S, Fraser H, Bostock C. Transmission of mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* 1997; **389**: 498–501
- 51 Scott MR, Will R, Ironside J, Nguyen HO, Tremblay P, DeArmond SJ, Prusiner SB. Compelling transgenic evidence for transmission of bovine spongiform encephalopathy prions to humans. *Proc Natl Acad Sci USA* 1999; **96**: 15137–42
- 52 Fraser H. Neuropathology of scrapie: the precision of the lesions and their significance. In *Slow Transmissible Diseases of the Nervous System* Vol. 1. Eds SB Prusiner, WJ Hadlow. New York: Academic Press, 1979; 387–406
- 53 Jeffrey M, Goodsir CM, Bruce ME, McBride PA, Farquhar C. Morphogenesis of amyloid plaques in 87V murine scrapie. *Neuropathol Appl Neurobiol* 1994; **20**: 535–42

Received 25 February 2008

Accepted 29 February 2008