

# Neuropathology of prion diseases

## Herbert Budka

*Austrian Reference Centre for Human Prion Diseases (ÖRPE) and Institute of Neurology,  
University of Vienna, Austria*

In prion diseases, neuropathology has remained the most important tool to give a definite diagnosis, and neuropathological research has contributed significantly to our current pathogenetic understanding. Immunohistochemistry for the disease-associated prion protein (PrP<sup>Sc</sup>) is indispensable for the neuropathological confirmation of prion diseases. The amount and distribution of PrP<sup>Sc</sup> deposits do not always correlate with type and severity of local tissue damage. PrP<sup>Sc</sup> deposition occurs only where neuronal parenchyma is present; in scarred infarctions with prominent gliosis, PrP<sup>Sc</sup> does not accumulate. Early, severe and selective loss affects a subset of inhibitory GABAergic neurons both in human and experimental prion diseases. The central pathogenetic cascade includes oxidative stress to neurons and their apoptosis. New patterns of PrP<sup>Sc</sup> immunoreactivity include granular ganglionic and tiny adaxonal PrP<sup>Sc</sup> deposits in peripheral nervous tissue, and dendritic cells and macrophages in vessel walls, suggesting that mobile haematogenous cells may be involved in spread of prions.

Neuropathology has a major role in surveillance of, and research on, prion diseases. For surveillance, it contributes diagnostic confirmation as well as potential identification of new disease (sub)types. This is important in view of the wide and steadily growing spectrum of clinical and pathological phenotypes and prion protein (PrP) gene (*PRNP*) genotypes. For research, it contributes to our pathogenetic understanding of prion diseases. The present brief review focuses on recently emerging points to consider in the neuropathology of human prion diseases (Table 1).

## Macroscopy of human prion diseases

*Correspondence to:  
Prof. Herbert Budka,  
Institute of Neurology,  
University of Vienna,  
AKH 4J, Währinger  
Gürtel 18–20, A-1097  
Wien, Austria*

Gross inspection of the brain in sporadic Creutzfeldt-Jakob disease (CJD), the paramount human prion disease, may not reveal obvious abnormalities. More commonly, however, there is some degree of cerebral atrophy, which can be diffuse [Plate X(A)] or have focal accentuations. Based on preferential involvement of specific regions, occipital<sup>1</sup>, striatal, thalamic and cerebellar<sup>2</sup> varieties have been

**Table 1** Important points to consider and newly recognised features in the neuropathology of human prion diseases

Diagnosis	Classical histopathological triad – spongiform change, neuronal loss, and gliosis (astro- and microglia) Recognition of disease (sub)types Immunocytochemistry for PrP <sup>Sc</sup> – use and significance, technique and pitfalls
Pathogenetic research	Function of the normal cellular PrP <sup>C</sup> Development, patterns and distribution of PrP <sup>Sc</sup> deposition Correlation of PrP <sup>Sc</sup> deposition with disease (sub)type Correlation of PrP <sup>Sc</sup> deposition with histopathology Pathogenetic models – neurotoxicity <i>versus</i> loss of function Selective neuronal vulnerability Pathways to neuronal death – oxidative stress and apoptosis New patterns of PrP <sup>Sc</sup> deposition in the PNS and vessel walls

described<sup>3</sup>. However, these subtypes are part of a spectrum of lesioning of the brain<sup>4</sup>. The hippocampal formation is usually well preserved even in cases of severe brain atrophy, at variance with other degenerative dementias including Alzheimer's disease. Gerstmann-Sträussler-Scheinker disease (GSS) with the classical ataxic clinical phenotype features prominent cerebellar atrophy and degeneration of spinal tracts<sup>5</sup>.

## Histopathology of human prion diseases

Histopathological features of human Prion diseases have been extensively described (*e.g.* Budka<sup>6</sup>) and will not be fully elaborated here. The classical triad of spongiform change, neuronal loss, and gliosis (astro- and microglia) is the neuropathological hallmark of prion diseases. Since neuronal loss and gliosis accompany many other conditions of the CNS, it is the spongiform change that is mostly specific to prion diseases. This spongiform change may be mild, moderate or severe [Plate X(B)] and is characterised by diffuse or focally clustered, small, round or oval vacuoles in the neuropil of the deep cortical layers, cerebellar cortex or subcortical grey matter, which might become confluent. Ultrastructurally, the spongiform changes correspond to enlarged cell processes (mainly neurites) containing curled membrane fragments and amorphous material<sup>7</sup>. Spongiform change should not be confused with non-specific spongiosis. This includes status spongiosus ('spongiform state'), comprising irregular cavities in gliotic neuropil following extensive neuronal loss (including also lesions of 'burnt-out' CJD), 'spongy' changes in brain oedema and metabolic encephalopathies, and artefacts such as superficial cortical, perineuronal, or perivascular vacuolation. Focal changes indistinguishable from spongiform change may occur in some cases of Alzheimer's and diffuse

Lewy body diseases<sup>8</sup>. In contrast to prion diseases of animals, the presence of vacuoles in nerve cell bodies is uncommon in CJD. Ballooning of neurons observed in some instances is related to accumulation of neurofilament proteins. Spongiform changes and astocytosis may also involve the white matter<sup>9,10</sup>. Extensive white matter degeneration distinguishes the ‘panencephalopathic’ form of CJD, which is particularly frequent in Japan<sup>11</sup>.

Presence and distribution of spongiform change vary greatly between cases and disease subtypes. An almost constant location is the head of the caudate nucleus<sup>12</sup>. By contrast, spongiform changes are rarely present in the brainstem and spinal cord, although PrP accumulation can be demonstrated at these sites. Normally, extensive sampling from various brain areas (including frontal, temporal, and occipital lobes, basal ganglia, and cerebellum) is mandatory in every suspected case. However, one block of tissue with typical histological changes and/or unambiguous PrP<sup>Sc</sup> immunoreactivity is sufficient for a definite diagnosis. Brain biopsy has been found to be diagnostic in 95% of CJD cases in which the disease has been confirmed at autopsy or by experimental transmission<sup>13</sup>. However, this procedure should be restricted to rare instances where a treatable alternative diagnosis is suggested by clinical or laboratory findings.

In sporadic CJD, the regional distribution of spongiform change in distinct patterns was shown to depend upon PrP<sup>res</sup> fragment sizes and

**Table 2** Neuropathological criteria for CJD and other human prion diseases

**Creutzfeldt-Jakob disease (CJD)**

- A Sporadic, iatrogenic (recognised risk) or familial (same disease in first degree relative or disease-associated PrP gene mutation)
- Spongiform encephalopathy in cerebral and/or cerebellar cortex and/or subcortical grey matter; and/or
  - Encephalopathy with prion protein (PrP) immunoreactivity (plaque and/or diffuse synaptic and/or patchy/perivacuolar types)
- B New variant CJD (vCJD)
- Spongiform encephalopathy with abundant PrP deposition, in particular multiple fibrillary PrP plaques surrounded by a halo of spongiform vacuoles (‘florid’ plaques, ‘daisy-like’ plaques) and other PrP plaques, and amorphous pericellular and perivascular PrP deposits especially prominent in the cerebellar molecular layer

**Gerstmann-Sträussler-Scheinker disease (GSS)** (in family with dominantly inherited progressive ataxia and/or dementia and one of a variety of PrP gene mutations):

- Encephalo(myelo)pathy with multicentric PrP plaques

**Familial fatal insomnia (FFI)** (in family with PRNP<sup>178</sup> mutation):

- Thalamic degeneration, variably spongiform change in cerebrum.

**Kuru:** spongiform encephalopathy in the Fore population of Papua-New Guinea.

Updated and modified from Budka *et al.*<sup>8</sup> as currently used by the WHO<sup>18</sup>.

glycotypes, and codon 129 genotype in the PrP gene, *PRNP*<sup>14,15</sup>. However, some prion diseases have equivocal, little, or no spongiform change, such as fatal familial insomnia (FFI)<sup>16</sup> that is specifically characterised by prominent thalamic atrophy with profound astrogliosis. Then immunohistochemistry for PrP and *PRNP* genotyping have a decisive diagnostic role<sup>17</sup>. Current neuropathological criteria for human prion diseases<sup>18</sup>, including the specific diagnostic features of variant CJD (vCJD), are listed in Table 2.

CJD brains may also show age-related Alzheimer-type amyloid deposits immunoreactive for the  $\beta$ /A4-peptide, with or without PrP<sup>Sc</sup> co-localisation<sup>19</sup>. Neuro-axonal dystrophy may be widespread in some CJD brains<sup>20</sup>.

## Immunohistochemistry for the prion protein (PrP)

### *Use and significance*

The function of the normal cellular protein (PrP<sup>C</sup>), the molecular prerequisite for the manifestation of any prion disease, has not been clarified. However, immunohistochemistry and other methods found it predominantly expressed in neural tissue, including neurons<sup>21</sup> and glial cells<sup>22</sup>; other organs (*e.g.* uterus, placenta, thymus, heart, lung, muscle, gastrointestinal tract) also contain considerable amounts<sup>23</sup>. Up-regulation of PrP<sup>C</sup> seems to be important in inflammatory conditions of muscle<sup>24</sup>, skin<sup>25</sup> and liver<sup>26</sup>, as well as in neurodegenerative disorders including Alzheimer and prion diseases<sup>27</sup>.

The conformationally abnormal, disease-associated isoform (PrP<sup>res</sup>, derived from proteinase-resistant, or PrP<sup>Sc</sup>, the latter term derived from scrapie) accumulates in the CNS in the whole group of prion diseases and has become the most important diagnostic marker. Routine detection of PrP<sup>Sc</sup> for diagnostic purposes uses methods such as immunohistochemistry, immunoblotting or ELISA assays performed on diseased tissue samples from patients obtained at autopsy, or from slaughtered animals as is done with current EU-wide testing of cattle for BSE and sheep for scrapie. Immunohistochemistry for PrP<sup>Sc</sup> has emerged as an indispensable adjunct to the neuropathological confirmation of prion diseases, especially in cases with equivocal histopathological changes<sup>8,28</sup>. It is suitable on routinely formol-fixed and paraffin-embedded tissues, although the technique may prove capricious, and pitfalls need to be considered (see below). It is noteworthy that, as for the spongiform change, PrP deposition may be focal and, in rare instances, the detection of PrP immunoreactivity may require staining of several blocks. Unfortunately, routine immunohistochemistry for PrP<sup>Sc</sup>

might yield a negative result in exceptional cases, especially in FFI<sup>16</sup>. More recently developed techniques such as the paraffin-embedded tissue blot<sup>29</sup> or the use of Carnoy's fixative<sup>30</sup> are promising alternatives to increase sensitivity for the detection of PrP<sup>Sc</sup> in tissues. However, in our European neuropathological study of human prion diseases<sup>31</sup> that now encompasses tissues from almost 1000 patients, we have seen only 2 FFI brains negative with immunohistochemistry for PrP among tissue specimens fulfilling criteria for a human prion disease.

Given the long incubation periods that make experimental transmission impractical, immunohistochemistry for PrP has also been used as a surrogate marker for infectivity in peripheral tissues that are important for considerations of infectivity risks, such as the lymphoid system<sup>32</sup> or the peripheral nervous system<sup>33,34</sup>. Moreover, PrP is also an important marker for development, spread and distribution of pathology. However, the amount and distribution of PrP deposits do not always correlate with type and severity of local tissue damage<sup>16,35</sup>. In a sequential experimental study on the time course and intensity of tissue lesioning and immunohistochemistry for PrP in mice inoculated with a human CJD agent, PrP accumulation does not precede, but follows spongiform change in some brain regions<sup>36</sup>. Local PrP<sup>Sc</sup> deposition requires the local presence of neuronal, but not glial, elements: in pre-existing brain lesions such as infarctions in which neuronal elements had been focally destroyed and replaced by a gliotic scar, PrP deposition is absent [Plate X(D)]<sup>4</sup>.

PrP<sup>Sc</sup> and infectivity are not uniformly distributed in an individual or animal affected with a prion disease. Two distinct groups can be distinguished: in the first, PrP<sup>Sc</sup> and infectivity have been detected in a distribution mainly limited to the central nervous system (brain, spinal cord, parts of the eye, trigeminal and spinal ganglia). This pattern is typical of sporadic and iatrogenic CJD, genetic human prion diseases and BSE of cattle. In the second, PrP<sup>Sc</sup> and infectivity involve also peripheral tissues, in particular the lymphoid system<sup>37,38</sup>, and this distribution is characteristic of vCJD, natural and experimental scrapie, experimental BSE in sheep, and CWD. In all prion diseases, however, most PrP<sup>Sc</sup> and infectivity reside in the CNS during clinical disease or late in the incubation period. This differential distribution of infectivity according to species and disease phenotype is one important factor when considering risks for transmission.

### *Technique and pitfalls*

Since all anti-PrP antibodies that are currently used in immunohistochemistry do not distinguish between PrP<sup>C</sup> and PrP<sup>Sc</sup>, specific pre-treatment

of tissue sections<sup>39</sup> is required for a prion disease diagnosis to abolish simultaneous reactivity with PrP<sup>C</sup>, just as tissue extracts have to undergo proteinase K digestion before detection of PrP<sup>res</sup> by immunoblotting. In our hands as well as those of others, a protocol using formic acid, guanidine thiocyanate, and hydrated autoclaving<sup>40</sup> gave the strongest and most consistent signals for formol-fixed and paraffin-embedded brain tissue. Minor modifications have been recommended<sup>41</sup>, but are not necessary for optimal immunostaining<sup>39</sup>. It should be noted that the possibility of pitfalls requires extensive experience in technique and interpretation. Sometimes unspecific labelling of diffuse neuronal somata, dystrophic neurites,  $\beta$ /A4 amyloid, and neurofibrillary tangles may be seen, probably representing incomplete abolishment of PrP<sup>C</sup> immunoreactivity. Thus, diagnostic interpretation of positive labelling has to be made by experienced observers and must consider the morphology of obtained signals. Antibodies such as 6H4 and 12F10 failed to give this type of labelling and are, therefore, less likely to recognise non-pathological PrP material in immunohistochemistry<sup>39</sup>.

### *Patterns and distribution of PrP deposition*

Characteristic patterns of PrP deposition are synaptic, that is the most difficult to reveal, patchy/perivacuolar, and plaque types [Plate X(C)] and which may overlap in the individual brain<sup>8</sup>; sometimes prominent perineuronal deposits surround neuronal somata and processes. Frequencies of these patterns differ between cerebral and cerebellar cortex<sup>35</sup>. Synaptic-type deposits and unicentric PrP plaques occur both in CJD and GSS, while abundant multicentric plaques are peculiar to GSS<sup>5</sup>. Plaque-like deposits are the only type of PrP deposits extending to the subcortical white matter<sup>35</sup> and are more frequent than true compact Kuru-type plaques with fringed outline that are clearly visible without immunohistochemistry [Plate X(E)]. They also stain with periodic acid-Schiff, alcian blue, Congo Red (staining disappears after formic acid treatment) and thioflavine S. Kuru plaques decorate a minority of sporadic CJD brains and are most frequent in the cerebellar cortex where they are usually confined to the granular layer. While very rarely 'florid' or 'daisy-like' plaques may be observed in other prion diseases<sup>35</sup>, their prominence is restricted to vCJD [Plate X(F)]. As with spongiform change, also type and distribution of PrP deposition in sporadic CJD were shown to depend upon PrP<sup>res</sup> fragment size and *PRNP* codon 129 genotype<sup>14,15</sup>.

### *New patterns of PrP deposition in the PNS and vessel walls*

Recently, granular ganglionic and tiny axonal PrP deposits were described in spinal and vegetative ganglia, spinal roots and peripheral nerves in rare cases of human prion disease<sup>34</sup> and experimental scrapie<sup>33</sup>.

It remains to be established by sequential studies whether this involvement of the peripheral nervous system reflects centripetal or centrifugal spread of PrP deposition and follows the pathways of travel by the infectious agent. In sporadic and variant CJD, we also found PrP<sup>Sc</sup> deposits in intracranial vessel walls by immunohistochemistry and paraffin-embedded tissue blotting. Using double immunofluorescence, these deposits co-localise with HLA-DR and S-100 immunoreactive cells in the intima, which are components of the vascular-associated dendritic cell network, as well as with HLA-DR and CD-68 immunopositive macrophages of the intima and media. Thus, mobile haematogenous cells in vessel walls may be involved in the spread of disease-associated prion protein and possibly also of infectivity<sup>42</sup>.

## Pathogenetic contribution

One enigma of prion diseases remains the pathogenesis of brain tissue damage, in particular of neuronal drop-out and subsequent tissue loss that is usually much more striking in human than in animal and experimental prion diseases. In principle, models involving a neurotoxic gain of function (most likely for aggregated PrP<sup>Sc</sup>)<sup>43</sup> or loss of function (of PrP<sup>C</sup>) are conceivable and might even co-operate to manifest disease. A variety of studies were interpreted to support either model, or even both<sup>16,44-48</sup>.

Both in human and experimental prion diseases, oxidative stress was identified as an important pathogenetic event<sup>49,50</sup>. Neuronal loss appears to follow an apoptotic pathway that is apparently independent of local deposition of PrP<sup>Sc</sup> but correlates with astrogliosis, microglial activation and axonal damage<sup>51</sup>. Specific vulnerability of a peculiar, parvalbumin-expressing subset of inhibitory GABAergic neurons was found both in human<sup>52,53</sup> and experimental prion diseases<sup>54</sup>. In fact, this vulnerability was detectable early in the incubation period and thus represents the earliest changes ever described after experimental inoculation<sup>54</sup>. However, FFI differs in such vulnerability<sup>55</sup> from all other human prion diseases. Other vulnerabilities include that of the granular layer of the cerebellum that is frequently depleted in sporadic CJD, and variable involvement of the basal nucleus of Meynert, either primarily or secondarily to cortical neuronal loss<sup>56,57</sup>. The molecular basis for such selective neuronal vulnerabilities is still obscure.

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