Case Report

Sporadic Creutzfeldt–Jakob disease with glial PrP\textsuperscript{Res} nuclear and perinuclear immunoreactivity

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Proteinase K-resistant prion protein (PrP\textsuperscript{Res}) nuclear and perinuclear immunoreactivity in oligodendrocytes of the frontal cortex is found in one case of otherwise typical sporadic Creutzfeldt-Jakob disease (sCJD) type VV2a. The PrP nature of the inclusions is validated with several anti-PrP antibodies directed to amino acids 130–160 (12F10), 109–112 (3F4), 97–102 (8G8) and the octarepeat region (amino acids 59–89: SAF32). Cellular identification and subcellular localization were evaluated with double- and triple-labeling immunofluorescence and confocal microscopy using antibodies against PrP, glial markers, and histone H3. Based on review of the literature and our own experience, this is a very odd situation that deserves further validation in other cases.

Key words: astrocytes, Creutzfeldt-Jakob disease, oligodendrocytes, prion.

INTRODUCTION

Prion diseases are a group of transmissible encephalopathies linked to cellular prion protein (PrP\textsuperscript{C}) which is converted into an abnormally conformed proteinase K-resistant prion protein (PrP\textsuperscript{Res}). The human prion diseases are sporadic, iatrogenic or genetic Creutzfeldt–Jakob disease (sCJD, iCJD and gCJD, respectively), variant CJD, Gerstmann-Sträussler-Scheinker disease (GSS), and familial insomnia (FFI); gCJD, GSS, and FFI are due to mutations in the PPNP gene (PPNP)\textsuperscript{1–5}.

sCJD is characterized clinically by rapid dementia accompanied by other neurological symptoms, and neuropathologically by neuronal loss, spongiform change, astrocytosis, microgliosis, and PrP\textsuperscript{Res} deposition.\textsuperscript{4,5} Several PrP types (types I and II, and variants) are recognized depending on the pattern of mono-glycosylated, di-glycosylated, and non-glycosylated prion.\textsuperscript{6,7} In addition, codon 129 (methionine or valine) in PRNP is largely contributory to clinical and neuropathological phenotypes.\textsuperscript{4,5} Moreover, PrP types correlate with characteristic PrP\textsuperscript{Res} deposits in tissue sections.\textsuperscript{8}

PrP\textsuperscript{Res} deposits have particular patterns including diffuse/synaptic, patchy/perivacuolar, perineuronal, plaque-like, and kuru-like plaques, largely depending on the sCJD subtype.\textsuperscript{4,5,9} In addition, punctuate intracytoplasmic neuronal deposits, rows of PrP\textsuperscript{Res} deposits along myelinated fibers, and rare intracytoplasmic PrP\textsuperscript{Res}-positive granules in astrocytes and microglia are reported in CJD and other prionopathies.\textsuperscript{5,9–13}

The present report describes unique abundant intracytoplasmic PrP\textsuperscript{Res} nuclear and perinuclear immunoreactivity in astrocytes and oligodendrocytes in one case of sCJD VV2.

CASE PRESENTATION

A 66-year-old man had suffered from gait ataxia, cognitive impairment, sleeping difficulties, and myoclonus for the previous two months. There was no family history of neurological disease. Cerebrospinal fluid test was positive for 14-3-3 protein. EEG showed slow waves with periodic
synchronous discharges. Extensive cortical ribbon-like, basal ganglia, and cerebellum hyper-signals were noted on magnetic resonance imaging (MRI) examination. Progression of the disease was to a vegetative state. The patient died of bronchopneumonia four and a half months after clinical onset.

**PATHOLOGICAL EXAMINATION**

The brain weight was 1400 g. The left cerebral hemisphere was rapidly frozen and stored at −80°C for biochemical studies. Gross examination of the right cerebral hemisphere after 4% buffered formalin fixation revealed severe atrophy of the cerebellum, mainly involving the vermis, and the presence of a lacunar infarct in the internal capsule. Antemortem factors including agonal state, hypoxia, acidosis, fever, seizures, and medication were ruled out. Post-mortem factors were within normal limits. Post-mortem delay (interval between death and sample processing) was around 5 h and the duration of fixation was up to 3 weeks.

Microscopic examination revealed widespread microvacuoles (spongiform changes), mainly in the frontal and temporal cortex, with a laminar pattern involving the deep layers, hippocampus, striatum and thalamus, and the molecular layer of the cerebellum. Neuronal loss was found in all these regions and it was very marked in the cerebellum, affecting Purkinje cells and neurons of the granular layer; scattered axonal torpedoes were also observed in the granular layer. This was accompanied by marked astrocytosis and severe microgliosis. The cerebral white matter was normal. Neuronal loss and astrogliosis occurred in the midbrain, and spongiform change was also observed in the pons. Immunohistochemistry with the 3F4 anti-PrP mouse monoclonal antibody (1:200; Millipore, Billerica, MA, USA) following proteinase K incubation (100 μg/mL; 37°C; 1 h) and 10% formic acid for 1 h showed synaptically-like and frequent small granular plaque-like PrPRes deposits, and perineuronal deposits in the neocortex and basal ganglia. Plaque-like PrPRes deposits were abundant in the cerebellar cortex and white matter; synaptic-like PrPRes immunoreactivity was present in the molecular layer as well. Small PrPRes immunoreactive dots (between 0.2 and 1.0 μm) were present in the perikaryon of several neurons. In addition, PrPRes nuclear (perinuclear) immunoreactivity was noted, apparently in the vicinity of, or within, glial nuclei in the frontal, parietal and temporal cortex. For PrPRes identification of glial deposits, immunohistochemistry was performed using specific antibodies recognizing different PrP epitopes, conducted on 4-μm-thick paraffin sections following a protocol reported elsewhere. To summarize, sections were first exposed to a combined pretreatment including hydrated autoclaving with 3 mM HCL acid (Merck, Kenilworth, NJ, USA) in distilled water incubated for 2 min after boiling, followed by 5 min incubation in 96% formic acid (Merck, Madrid, Spain) at room temperature. After washing, sections were incubated with serial proteinase K (PK) concentrations: 5, 10 and 20 μg/mL for 15 min at 37°C, and were further blocked with Dako Real Peroxidase blocking solution (S2023, Dako, Barcelona, Spain). Sections were incubated overnight at 4°C with the primary antibodies, diluted in Dako Real Antibody Diluent (S2022, Dako) (Table 1). Afterwards, sections were incubated with MultiLink biotinylated antibody followed by horseradish peroxidase-conjugated streptavidin (QP009L-E BioGenex detection kit, Fremont, CA, USA). Immunodetection was obtained after incubation with 3,3'-diaminobenzidine tetrahydrochloride (DAB) (D6737; Merck) and H2O2. Sections were examined with a Nikon Eclipse E800 microscope with ProgRes® CapturePro 2.7.7 software (JENOPTIK, Jena, Germany). The results obtained using two additional anti-PrP antibodies, clones 12F10 and scrapie-associated fibril 32 (SAF32), were similar in the two assays (Fig. 1). Nuclear (perinuclear) immunoreactivity was not stained with anti-ubiquitin antibodies. No deposits of amyloid-β, hyperphosphorylated tau, α-synuclein, or transactivation response DNA-binding protein 43 kDa were observed.

Genetic studies revealed no mutation in PRNP and valine homozygosity at codon 129. Western blotting with the antibody 3F4 (Dako) at a dilution of 1:3000 revealed a type II PrPRes pattern; no bands of low molecular weight were present with this antibody.

In order to identify the characteristics and localization of PrPRes glial nuclear (perinuclear) immunoreactivity,
double- and triple-labeling immunofluorescence using specific neuronal and glial cell antibodies, and antibodies recognizing different PrP epitopes, was carried out in 4-μm-thick de-waxed paraffin sections of the frontal cortex. Sections were first subjected to combined pretreatment for PrP staining and then boiled in 10 mmol/L citrate buffer for 20 min. Sections were subsequently stained with a saturated solution of Sudan black B (Merck) for 15 min to block autofluorescence of lipofuscin granules, and then rinsed in 70% ethanol and washed in distilled water. Afterwards, sections were blocked with 10% fetal bovine serum (FBS) for 1 h at room temperature, and incubated overnight at 4°C with appropriate diluted primary antibodies (Table 1). After washing, sections were incubated with Alexa-Fluor 488, 555, or 647 conjugated secondary antibodies (Molecular Probes, Eugene, OR, Life Technologies, Carlsbad, CA, Thermo Fisher Scientific Inc., Waltham, MA, USA, respectively). The sections were mounted in Immuno-Fluore mounting medium (ICN Biomedicals, Irvine, CA, USA), sealed, and dried overnight.
Sections were examined with a Leica TCS-SL confocal microscope and analyzed for co-localization with Image J 1.8.0_112 using JACOP plugin (for Pearson’s coefficient) and double-staining co-localization macros.

Double-labeling immunofluorescence to glial fibrillary acidic protein (GFAP) and PrP (3F4 antibody) showed significant PrP immunoreactivity in the vicinity of GFAP fibrils (Fig. 2A, upper row). Yet double-labeling with anti-oligodendrocyte transcription factor 2 antibody (Olig2) and SAF32 clearly showed PrP localization in the perinuclear region and within the nucleus of oligodendrocytes (Fig. 2A, middle row). No relationship was found between microglia identified with antibody to ionized calcium-binding adaptor molecule 1 (Iba1) and PrP deposits (Fig. 2A, lower row). Quantification of the percentage of cell markers with the PrP marker showed significant increase only in the %Olig2 that co-localized with %SAF32 (Fig. 2B). Moreover, Pearson’s coefficient disclosed only a strong positive correlation between Olig2 and SAF32 (Table 2).

Similar PrP immunostaining was obtained with different anti-PrP antibodies directed to amino acids 130–160 (12F10), 109–112 (3F4), 97–102 (8G8), and the octarepeat region (amino acids 59–89: SAF32) as seen by double-labeling immunofluorescence to GFAP and PrP (Fig. 3).

Triple-labeling with distinct cellular markers, PrP antibodies, and anti-histone H3 revealed that most PrP immunoreactivity was localized within the nucleus or in the perinuclear region of oligodendrocytes, and rarely in astrocytes, including subpopulations of YKL40-immunoreactive astrocytes (Fig. 4). Pearson’s coefficient showed a strong positive correlation of 12F10 with YKL40, Olig2, and cellular nuclei (Table 2).

### Table 2  Pearson’s coefficient values for prion protein (PrP) and cell markers

| Staining                        | PrP marker | Cell marker | Pearson’s co-efficient |
|---------------------------------|------------|-------------|------------------------|
| Double SAF32 +αIba1             | SAF32      | αIba1       | 0.001                  |
| Double SAF32 +αGFAP             | SAF32      | αGFAP       | −0.031                 |
| Triple 12F10+αH3 +αGFAP         | 12F10      | αGFAP       | 0.023                  |
| Triple 12F10+αH3 +αYKL40        | 12F10      | αH3         | 0.261                  |
| Triple 12F10+αH3 +αYKL40        | 12F10      | αH3         | 0.215                  |
| Double SAF32 +Olig2             | SAF32      | Olig2       | 0.287                  |
| Triple 12F10+αH3 +Olig2         | 12F10      | Olig2       | 0.305                  |
| Triple 12F10+αH3 +Olig2         | 12F10      | αH3         | 0.240                  |

**DISCUSSION**

Clinical symptoms, neuroimaging, genetics, neuropathological findings, and Western blot pattern of PrP<sub>Res</sub> are typical of sCJD VV2a.⁴⁻⁵,⁸ Regarding PrP<sub>Res</sub> deposition, synaptic-like and small granular plaque-like PrP<sub>Res</sub> deposits, and perineuronal deposits in the neocortex and basal ganglia, together with plaque-like PrP<sub>Res</sub> deposits in the cerebellar cortex and white matter, and synaptic-like PrP<sub>Res</sub> immunoreactivity in the molecular layer in the present case, are consistent with sCJD VV2.⁵ In addition, punctate intraneuronal PrP immunoreactivity, here described as small PrP<sub>Res</sub>-immunoreactive dots in the perikaryon, has been observed in scrapie, in experimental models of prion disease, and rarely, in sCJD.¹²⁻¹⁵

Rare intra-cytoplasmic PrP<sub>Res</sub> granules in human and animal prion diseases have been reported in microglia and astrocytes, suggesting that both cells may play a role in the processing, degradation and removal of PrP<sub>Res</sub>.¹⁶,¹⁷ PrP<sub>Res</sub> microsphere formation in the neuropil has been described in a rare case of familial CJD.¹⁸ However, nuclear and
perinuclear immunoreactivity in cortical astrocytes and oligodendrocytes as observed here have not been previously reported to our knowledge.

The PrP nature of these deposits is supported by the recognition of similar staining using different anti-PrP antibodies directed against different epitopes of the prion protein, including 12F10 (amino acids 130–160), 3F4 (amino acids 109–112), 8G8 (amino acids 97–102), and SAF32 (amino acids 59–89).

PrP immunoreactivity in the inclusions is mostly PK-resistant; PrP immunoreactivity is maintained at PK concentrations of 20 μg/mL. Double- and triple-labeling of PrP with different neuronal and glial cell markers, and histone H3, clearly demonstrates the glial localization of PrP immunoreactivity mainly in the nucleus and perinuclear region in oligodendrocytes.

PrPC is normally expressed in neurons, astrocytes, and oligodendrocytes during development and adulthood. Functions of PrPC during development include modulation of the differentiation of human stem cells into neurons, astrocytes, and oligodendrocytes. Therefore, the PrP deposits here observed in glial cells are probably produced in the same cells. How the conversion of PrPC into PrPRes is produced in oligodendrocytes is not known, as oligodendrocytes are apparently resistant to PrPRes infectivity.

The nuclear localization of glial PrP immunoreactivity is curious. However, nuclear localization of normal and abnormal PrP has been reported in various settings. Abnormal transport of C-terminally truncated and abnormally glycosylated mutant PrP to the nucleus has been reported in cell models of familial prion disorders associated with a stop codon mutation at residues 145 or 160 of the PrP. The N-terminal region of human and ovine PrP has been described as harboring nucleic acid binding and chaperoning properties. When acting together, two independent nuclear localization signals in the N-terminal domain of PrP complement each other in transporting the N-terminal fragment of PrP to the nucleus of transfected cells, where it accumulates.

In addition to truncated forms, PrPC can localize in the nucleus of different cell types including neural cells, and it interacts with structural chromatin components, principally histone H3; the interaction of PrPC with histone H3 suggests that PrP is involved in transcriptional regulation in the nucleus. Nuclear PrP interacts with distinct proteins involved in cell proliferation and cell junction in several cell types, and PrP participates in the process of DNA repair after genotoxic stress.

It may be argued that the PrP immunostaining here observed is not simply up-regulation of PrP for any reason. However, we have spent many years studying hundreds of brains with CJD using similar methodological procedures and the same routine protocols, and have never before observed those intriguing inclusions. The present findings in a single case must be corroborated in additional cases to prove that, although rarely, PrPRes can be localized in the cytoplasm and nucleus of glial cells, mainly oligodendrocytes, in CJD.

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DISCLOSURE

The authors declare no conflicts of interest for this article.

REFERENCES

1. Prusiner SB. An introduction to prion biology and diseases. In: Prusiner SB, (ed). Prion Biology and Diseases, 2nd edn. New York: Cold Spring Harbor Laboratory, 2004; 1–87.

2. Aguzzi A. Prion diseases of humans and farm animals: Epidemiology, genetics, and pathogenesis. J Neurochem 2006; 97: 1726–1739.

3. Gambetti P, Cali I, Notari S, Kong Q, Zou WQ, Surewicz WK. Molecular biology and pathology of prion strains in sporadic human prion diseases. Acta Neuropathol 2011; 121: 79–90.

4. Budka H, Head MW, Ironside JW, Gambetti P, Parchi P, Tagliavini F. Sporadic Creutzfeldt-Jakob disease. In: Dickson DW, Weller RO, (eds). Neurodegeneration: The Molecular Pathology of Dementia and Movement Disorders, 2nd edn. Chichester: Willey-Blackwell, 2011; 322–335.

5. Head MW, Ironside JW, Ghetti B, Jeffrey M, Piccardo P, Will RG. Prion diseases. In: Love S, Piccardo P, Will RG. Prion diseases. In: Love S, Piccardo P, Wil (eds). Blackwell, 2011; 322–335.

6. Parchi P, Giese A, Capellari S et al. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 1999; 46: 224–233.

7. Parchi P, Notari S, Weber P et al. Inter-laboratory assessment of PrPSc typing in Creutzfeldt-Jakob disease: A western blot study within the NeuroPrion Consortium. Brain Pathol 2009; 19: 384–391.

8. Parchi P, de Boni L, Saveraion D et al. Consensus classification of human prion disease histotypes allows reliable identification of molecular subtypes: An inter-rater study among surveillance centres in Europe and USA. Acta Neuropathol 2012; 124: 517–529.

9. Kovacs GG, Head MW, Hegyi I et al. Immunohistochemistry for the prion protein: Comparison of different monoclonal antibodies in human prion disease subtypes. Brain Pathol 2002; 12: 1–11.

10. El Hachimi KH, Chaunu MP, Brown P, Foncin JF. Modifications of oligodendroglial cells in spongiform encephalopathies. Exp Neurol 1998; 154: 23–30.

11. Kordek R, Hainfellner JA, Liberski P, Budka H. Deposition of the prion protein (PrP) during the evolution of experimental Creutzfeldt-Jakob disease. Acta Neuropathol 1999; 98: 597–602.

12. Kovacs GG, Head MW, Bunn T, Laszlo L, Will RG, Ironside JW. Clinicopathological phenotype of codon 129 valine homozygote sporadic Creutzfeldt-Jakob disease. Neuropathol Appl Neurobiol 2000; 26: 463–472.

13. Gonzalez L, Martin S, Jeffrey M. Distinct profiles of PrP(d) immureactivity in the brain of scrapie- and BSE-infected sheep: Implications for differential cell targeting and PrP processing. J Gen Virol 2003; 84: 1339–1350.

14. Jeffrey M, McGovern G, Siso S, Gonzalez L. Cellular and sub-cellular pathology of animal prion diseases: Relationship between morphological changes, accumulation of abnormal prion protein and clinical disease. Acta Neuropathol 2011; 121: 113–134.

15. Kovacs GG, Molnar K, Keller E, Botond G, Budka H, Laszlo L. Intraneuronal immunoreactivity for the prion protein distinguishes a subset of E200K genetic from sporadic Creutzfeldt-Jakob disease. J Neuropathol Exp Neurol 2012; 71: 223–232.

16. Jeffrey M, Goodsr CM, Bruce ME, McBride PA, Farquhar C. Morphogenesis of amyloid plaques in 87V murine scrapie. Neuropathol Appl Neurobiol 1994; 20: 535–542.

17. Kovacs GG, Preusser M, Strohshneider M, Budka H. Subcellular localization of disease-associated prion protein in the human brain. Am J Pathol 2005; 166: 287–294.

18. Honda H, Ishii R, Hamano A et al. Microsphere formation in a subtype of Creutzfeldt-Jakob disease with a V180I mutation and codon 129 MM polymorphism. Neuropathol Appl Neurobiol 2013; 39: 844–848.

19. Moser M, Colello RJ, Pott U, Oesch B. Developmental expression of the prion protein gene in glial cells. Neuron 1995; 14: 509–517.

20. Bribián A, Fontana X, Llorens F et al. Role of the cellular prion protein in oligodendrocyte precursor cell proliferation and differentiation in the developing and adult mouse CNS. PLoS One 2012; 7: e33872.

21. Leey YJ, Baskakov IV. The cellular form of the prion protein guides the differentiation of human embryonic stem cells into neuron-, oligodendrocyte-, and astrocyte-committed lineages. Prion 2014; 8: 266–275.

22. Prinz M, Montrasio F, Furukawa H et al. Intrinsinc resistance of oligodendrocytes to prion infection. J Neurosci 2004; 24: 5974–5981.

23. Lorenz H, Windl O, Kretzschmar HA. Cellular phenotyping of secretory and nuclear prion proteins associated with inherited prion diseases. J Biol Chem 2002; 277: 8508–8516.

24. Gabus C, Derrington E, Leblanc P et al. The prion protein has RNA binding and chaperoning properties characteristic of nucleocapside protein NCP7 of HIV-1. J Biol Chem 2001; 276: 19301–19309.

25. Gu Y, Hinnerwisch J, Fredricks R, Kalepu S, Mishra RS, Singh N. Identification of cryptic nuclear
localization signals in the prion protein. *Neurobiol Dis* 2003; **12**: 133–149.

26. Strom A, Wang GS, Picketts DJ, Reimer R, Stuke AW, Scott FW. Cellular prion protein localizes to the nucleus of endocrine and neuronal cells and interacts with structural chromatin components. *Eur J Cell Biol* 2011; **90**: 414–419.

27. Cai H, Xie Y, Hu L, Fan J, Li R. Prion protein (PrP (c)) interacts with histone H3 confirmed by affinity chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 2013; **929**: 40–44.

28. Rousset M, Leturque A, Thenet S. The nucleo-junctional interplay of the cellular prion protein: A new partner in cancer-related signaling pathways? *Prion* 2016; **10**: 143–152.

29. Bravard A, Auvré F, Fantini D et al. The prion protein is critical for DNA repair and cell survival after genotoxic stress. *Nucleic Acids Res* 2015; **43**: 904–916.