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The first case of protease-sensitive prionopathy (PSPr) in The Netherlands: a patient with an unusual GSS-like clinical phenotype

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ABSTRACT

An atypical case of prion disease is described in a 54-year-old Dutch man, homozygous for valine at codon 129 of the prion protein gene (*PRNP*). The clinical phenotype was characterised by progressive dementia, spastic paraplegia and sensorimotor polyneuropathy. The disease duration was 20 months. Genetic analysis of *PRNP* did not reveal any abnormalities.

Neuropathologically, only mild spongiform change and a coarse granular immunohistochemical staining for the abnormal prion protein, PrP^{Sc}, was observed, with poorly formed plaques in the molecular layer of the cerebellar cortex. However, Western blotting showed low but detectable levels of proteinase K(PK)-resistant PrP^{Sc} occurring in an unusual ladder-like profile. These features define a phenotype that corresponds to the recently described protease-sensitive prionopathy (PSPr). Our report on the first Dutch patient with PSPr further expands the spectrum of prionopathies and exemplifies the need to re-evaluate cases of atypical prion disease.

Prion diseases are unique neurodegenerative disorders that occur in sporadic, genetic and acquired forms.¹ According to the prion hypothesis, the causative agent consists of an abnormally folded isoform of a host-encoded prion protein (PrP^C) termed PrP^{Sc}.² In contrast to PrP^C, the pathological isoform PrP^{Sc} has a higher content of β -sheet and is partially resistant to degradation by proteinase K (PK).³ Based on the size of the protease-resistant core fragment, several isoforms of PrP^{Sc} can be recognised by immunoblotting,⁴ most commonly a type 1 (21 kDa) or a type 2 isoform (19 kDa). The currently accepted classification for sporadic Creutzfeldt-Jakob disease (sCJD) combines the PrP^{Sc} isotype with the presence of either methionine or valine at codon 129 of the prion protein to define six molecular subtypes that correspond to distinct clinical and neuropathological phenotypes.⁵ A smaller fragment of protease-resistant PrP^{Sc} (7–8 kDa) is found in patients with Gerstmann-Sträussler-Scheinker disease (GSS), a genetic form of prion disease.⁶

Recently, Gambetti *et al* reported 11 patients with a prionopathy characterised by the presence of an abnormal PrP species that was largely sensitive to protease digestion.⁷

The PrP^{Sc} present in these patients occurred in an unusual immunohistochemical staining pattern and generated a ladder-like profile that was hard to detect by conventional Western blotting. Since the relative PK sensitivity of the PrP^{Sc} present was

considered to be a defining feature, the authors referred to this condition as protease-sensitive prionopathy (PSPr).

We report the first Dutch patient with a neuropathological and biochemical phenotype consistent with PSPr.

CLINICAL HISTORY

The patient presented with walking difficulties at the age of 54 years. He had stiffness and slowness of voluntary movements. His medical history was otherwise unremarkable. There was no known history of prion exposure. He had a family history of neurodegenerative disease: his mother was reported to have a neurodegenerative disorder with amyotrophic lateral sclerosis (ALS)-like phenotype, although no medical records from that time period could be retrieved.

On examination, 1 year after the first symptoms, he was slightly dysarthric and showed reduced facial expression. He had word-finding difficulties. Atrophy and fasciculations were noted in the extremities. He had increased tone of the leg musculature and demonstrated dysidiadochokinesia. The plantar responses were extensor.

MRI of the brain showed cerebral cortical atrophy with normal basal ganglia. Western blot assay for the 14-3-3 protein in cerebrospinal fluid was weakly positive. An EEG did not show characteristic abnormalities suggestive of CJD. Genetic analysis showed no abnormalities of the *Huntingtin* gene or of the genes causing spinocerebellar ataxia and hereditary spastic paraplegia. An electromyogram revealed sensorimotor polyneuropathy of the axonal type. On the basis of dementia, spastic paraplegia and sensorimotor polyneuropathy, a clinical diagnosis of atypical Alzheimer's disease or GSS was considered. The patient died at the age of 57 years, 20 months after the onset of symptoms.

MATERIALS AND METHODS

PRNP analysis

DNA was extracted from frozen brain tissue, and the codon 129 (methionine/valine) polymorphism of the prion protein gene (*PRNP*) was determined by restriction fragment length polymorphism analysis, with full sequence analysis of *PRNP* using established methods.⁸

Histopathology and immunohistochemistry

Neuropathological examination was performed on 5-micrometer-thick sections of formalin-fixed and

Figure 1 Microscopic findings (A, C, E and F) and immunohistochemistry for prion protein (B, D, G and H) in this patient with protease-sensitive prionopathy. Sections are stained with H&E (A, C and E), combined Luxol–periodic acid-Schiff stain (H) and the 3F4 anti-PrP antibody, original magnification $\times 20$ (A–E and G–H) and $\times 40$ (F). (A) Mild spongiform change in the frontal cortex. (B and D) Multiple rounded clusters of granules with larger granules at the centre and smaller granules at the periphery in a target-like pattern in the frontal cortex and putamen. (C) Moderate spongiform change in the putamen. (E) Mild spongiform change in the molecular layer of the cerebellum. (F) Focal periodic acid-Schiff-positive plaques in the molecular layer (arrow). (G–H) Intense staining of granules in the molecular layer.

paraffin-embedded brain tissue blocks. Sections were stained with H&E and a combined Luxol–periodic acid-Schiff stain. Monoclonal antibody (mAb) 3F4 (1:400; Signet Laboratories, Dedham, Massachusetts, USA) was used for immunohistochemical detection of PrP^{Sc} following pretreatment with PK according to standard procedures. Other immunohistochemical stains included mAb Ubiquitin (1/1000; Dako, Glostrup, Denmark) and mAb 2E2-D3 against TARD-BP (1/1600; Abnova, Walnut, California, USA).

Western blotting

Frozen CJD brain tissue was stored at -80°C and investigated for the presence of PrP^{Sc} by immunoblotting. Frozen tissues from the occipital and temporal cortices were homogenised, and Western blot analysis of PrP^{Sc} was performed using an established method with minor modifications.⁹ Homogenates, both with and without PK digestion, were analysed. The homogenates were immunoblotted with mAb 3F4 (1:1000; Dako) and with mAb 1E4 (1.022 $\mu\text{g}/\text{ml}$) to human PrP residues 109–112 and 97–102, respectively.

RESULTS

PRNP analysis

The patient was homozygous for valine (VV) at codon 129 of *PRNP*. No mutations, insertions or silent polymorphisms were found.

Histology and immunohistochemistry

Gross examination of the brain (weight 1520 g) was unremarkable. The substantia nigra and locus coeruleus were normally pigmented. Histological evaluation showed only mild spongiform change in the cerebral and cerebellar cortices (figure 1A,E), with a mixture of small and intermediate-size vacuoles (mean diameter 7.2 (2.5) μ m). Spongiform changes were more pronounced in the putamen and caudate nucleus (figure 1C; mean diameter 8.1 (2.7) μ m). The hippocampal pyramidal layer was relatively spared. In the molecular layer of the cerebellum, several small plaque-like structures were noted in the Luxol–periodic acid-Schiff-stained sections (figure 1F). These were occasionally clustered, giving the appearance of multicentric plaques. Histological examination of the other brain regions was unremarkable.

Immunohistochemical staining for PrP^{Sc} with mAb 3F4 in the cerebral cortex, basal ganglia and thalamus showed aggregates of coarse granules with relatively large granules located in the centre of these formations and smaller ones in the periphery, resembling a target-like pattern (figure 1B,D). Immunoreactivity in the cerebellum was limited to rounded structures in the molecular layer (figure 1G,H). This pattern of PrP^{Sc} immunostaining could be pathognomonic for PSPr, as described in the original cases by Gambetti *et al.*⁷ Immunohistochemical staining with TDP-43 and Ubiquitin did not show neuronal inclusions or neuropil threads.

Western blotting

On immunoblotting using the mAb 3F4, PK-resistant PrP^{Sc} bands were less readily detectable when compared to sCJD type 1 and variant CJD type 2B control subjects. Furthermore, this PK-resistant material constituted a relatively small fraction of the immunopositive material present in PK-undigested brain tissue of the same regions, and it formed a ladder of differently sized fragments, ranging from the most abundant low molecular weight band to those with a similar electrophoretic mobility to the 20–30 kDa bands commonly seen in cases of CJD (figure 2). The ladder-like electrophoretic mobility of these PrP^{Sc} fragments did not match those associated with the recognised subtypes of sCJD (20–30 kDa) or with those found in variant CJD. Immunoblotting using the 1E4 mAb resulted in qualitatively similar results, with only minor differences in the sensitivity of detection of individual bands within the PrP^{Sc} ladder-like profile (figure 2).

DISCUSSION

Gambetti *et al* recently described a new prion disease phenotype in 11 patients, based on clinical, pathological and biochemical findings⁷: First, a typical clinical disease course, characterised by slow cognitive decline, gait impairment and incontinence. Second, valine homozygosity at codon 129 of *PRNP* with no pathogenetic mutations or insertions but with neuropathological features distinct from VV1 and VV2 sCJD. Third, the presence of PrP^{Sc} that was more sensitive to PK than that usually found in prion diseases and which exhibited a characteristic ladder-like electrophoretic profile after PK digestion. Fourth, an atypical immunohistochemical profile with poorly formed plaques in the molecular layer of the cerebellum.

Figure 2 Western blot analysis of the PSPr type. Standard samples of type 1 PrP^{Sc} from sporadic CJD MM1 (1) and type 2B PrP^{Sc} from variant CJD (2B) were included for reference, and the molecular weights of the marker protein (M) are shown in kDa. The tissues available for analysis were taken from the temporal cortex (TC; lanes 2 and 4) and the occipital cortex (OC; lanes 6 and 8). When probed with the monoclonal antibody 3F4, proteinase K (PK)-resistant PrP^{Sc} is prominent in both the sporadic CJD and variant CJD control subjects. Smaller amounts of PK-resistant PrP^{Sc} are present in the index case, forming a ladder-like profile of small fragments (lanes 4 and 8). Undigested brain homogenates from the temporal and occipital cortices of the index case are included (lanes 2 and 6), showing that the protease-resistant PrP is only a small fraction of the total amount of the PrP present. A qualitatively similar result is obtained when the 3F4 mAb is replaced with mAb 1E4.

We describe the first Dutch patient with a similar phenotype. The disease course was characterised by dementia, spastic paraplegia and sensorimotor polyneuropathy, raising a clinical suspicion of an atypical form of Alzheimer's disease or GSS.

Immunohistochemical staining for PrP^{Sc} revealed several poorly formed, sometimes multicentric plaques in the cerebellar cortex, but genetic analysis did not show any abnormalities of *PRNP*, ruling out GSS.

On re-evaluation 3 years later, histological examination was largely comparable to the cases described by Gambetti *et al.*⁷ and a sensitive and optimised immunoblotting technique did indeed reveal the presence of fragments of PK-resistant PrP^{Sc} in a ladder-like profile.

Although, in general, PrP^{Sc} can be distinguished from PrP^C by its resistance to treatment with PK, a protease-sensitive but disease-associated form has been described before.¹⁰ Furthermore, accumulation of PrP^{Sc} that is relatively protease sensitive has been described in patients affected by GSS.^{11 12} Interestingly, immunoblots of these cases also show multiple smaller fragments of PK-resistant PrP^{Sc},⁶ sometimes in combination with conventional

PrP^{Sc} but differing from the profile seen in patients with PSPr. Gambetti *et al*⁷ considered the preferential staining of these fragments with mAb 1E4 and *not* with 3F4, an additional argument against GSS. In our hands, however, these fragments were detectable by *both* antibodies, and the mechanism by which two antibodies with such closely adjacent epitopes might produce different results remains speculative.

PSPr is probably not an extremely rare disease entity, occurring in approximately 3% of sCJD patients in the North American cohort reported by Gambetti *et al*,⁷ although we were only able to find one patient in our database of 135 sCJD patients. This, however, corresponds to 9% of all VV homozygotes in our patient population, which is comparable with the number mentioned by Gambetti *et al*⁷.

In the past, PSPr patients probably escaped postmortem examination because clinical diagnoses other than prion disease were made. Clinical recognition of the possibility of atypical prion disease in these patients seems to be the key to a diagnosis of PSPr, and this will, in part, depend on familiarity with these admittedly rare diseases.

Our patient's case indicates that PSPr is not associated with exclusively North American risk factors and exemplifies the benefits of re-evaluating cases of "atypical dementia". It also underlines the importance of considering PSPr in patients with a GSS-like phenotype but no mutations in *PRNP*.

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