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Concurrent variably protease-sensitive prionopathy and amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) and prionopathies, such as Creutzfeldt-Jakob disease (CJD), are devastating neurodegenerative diseases with a relatively early age of onset and a rapid disease course. Prior to 1983, slowly progressing dementia with motor neuron signs was often considered part of the CJD spectrum, termed the “amyotrophic form” of CJD [4]. The landmark study by Salazar and colleagues demonstrated that “amyotrophic CJD” was not a transmissible form of CJD, but was more akin to classic ALS [7], with the spongiosis noted in such cases now recognized as a nonspecific feature of frontotemporal degenerations [1]. While 27 cases with amyotrophy and prion disease have been reported, only 4 have had immunohistochemical proof of prion disease, while 7 had anterior horn cell pathology without mention of evidence of upper motor neuron pathology [8]. Moreover, none had immunohistochemistry with the molecular pathologic marker of ALS, TAR DNA-binding protein of 43 kDa (TDP-43), which labels neuronal and glial inclusions in ALS [5]. In 2008, a novel human prion disease was discovered by Gambetti and colleagues characterized by variable sensitivity of prion protein to protease digestion [2]. Named “variably protease-sensitive prionopathy” (VPSPr), the clinical presentation, age of onset, and disease duration as well as the histopathology of this disorder differed between *PRNP* genetic variants (129V/V, 129M/V, 129M/M) and from CJD [3, 9].

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We report a 74-year-old woman followed longitudinally as part of prospective studies of dementia with Lewy bodies at Mayo Clinic who had a one-year history of gradually progressive dementia, cognitive fluctuations, excessive daytime sleepiness, visual hallucinations, and probable rapid eye movement sleep behavior disorder. General motor exam revealed normal strength and muscle tone. Magnetic resonance imaging indicated mild generalized cortical atrophy. By age 75, she developed agitation, behavioral disturbances, incontinence, and struggles with activities-of-daily-living. A year later, she had dysarthria, and difficulty ambulating, with stooped posture, shuffling, and unsteady gait. By age 77, she had dysphagia and was wheelchair bound and unresponsive. She died at the age of 78.

Macroscopic examination of the fixed left hemibrain revealed diffuse cortical atrophy, enlargement of lateral ventricles, and thinning of the cortical ribbon. The hippocampus and amygdala were atrophic, but the basal ganglia and thalamus were unremarkable. There was visible pigmentation in the substantia nigra, but pallor of the locus ceruleus.

Microscopic examination showed spongiform change and gliosis throughout the neocortex with ballooned neurons (**Fig. 1a**). Spongiform change was also noted in the hippocampus, basal ganglia (**Fig. 1b**), and minimally in the thalamus. Purkinje cell loss and Bergmann gliosis was seen in the cerebellum, and was associated with dense-cored plaques with thioflavin S fluorescent microscopy. In addition, skein-like inclusions, hyaline inclusions, and Bunina bodies were observed (**Fig. 1c**). Prion protein (PrP) immunohistochemistry (3F4 antibody; 1:50,000) revealed many plaque-like lesions and coarse granular immunoreactivity in the neocortex (**Fig. 1d**), dentate fascia, amygdala, and basal ganglia, with small dense plaques in the cerebellum (**Fig. 1e**). PrP pathology and spongiform change was uniform in severity across the neocortex. Immunohistochemistry for TDP-43 (MC2085 antibody; 1:2,500), a neuropathologic hallmark of ALS, revealed neuronal cytoplasmic inclusions in residual Betz cells, as well as skein-like and Lewy-like hyaline inclusions in the hypoglossal nucleus (**Fig. 1g**). No additional white matter TDP-43 pathology or extramotor TDP-43 pathology was detected. Marked neuronal cell loss of anterior horn cells was observed in the cervical spinal cord in addition to the aforementioned TDP-43 immunoreactive lesions (**Fig. 1h**). There was mild demyelination and microglial activation in the pyramidal tract with Luxol fast blue for myelin and IBA-1 immunohistochemistry (1:3000) for microglia (data not shown). Together, these findings were consistent with lower motor neuron predominant ALS. No Lewy bodies or Lewy neurites were detected by α -synuclein immunohistochemistry. There were no neurofibrillary tangles or amyloid angiopathy on thioflavin-S fluorescent microscopy. Many diffuse, non-neuritic senile plaques were seen throughout the neocortex on thioflavin-S microscopy as well as in the hippocampus with A β immunohistochemistry (33.1.1 antibody; 1:1,000). C9RANT immunohistochemistry (Rb5823 antibody; 1:5,000), a specific marker of the C9ORF72 hexanucleotide repeat expansion, was negative. Ultrastructural analysis confirmed spongiform change and PrP-positive plaques in the hippocampus (**Fig. 1f**) as well as skein-like inclusions in motor neurons composed of TDP-43-positive ~10-nm diameter granule-coated filaments (**Fig. 1i**).

Brain homogenates were prepared in LB100 lysis buffer, treated with 2 U/ml of proteinase K for 1 hour at 37°C, and run on 15% Tris/glycine SDS-polyacrylamide gels [6]. Western

blot examination (with 3F4 and 1E4 antibodies) demonstrated a PrP five band ladder profile consistent with VPSPr compared to the three band profile of sporadic CJD (**Fig. 2**) [2, 9]. Sequencing of *PRNP* failed to detect pathogenic mutations and demonstrated methionine/valine heterozygosity at codon 129 [9]. These results led to the final neuropathologic diagnosis of coincident VPSPr and ALS.

The comorbidity of prion disease and ALS is exceedingly rare, but may be more likely with VPSPr given the later disease-onset and longer disease duration compared to typical CJD. Initial clinical features consistent with dementia with Lewy bodies in this patient were likely related to VPSPr since there was no extramotor TDP-43 pathology. Later gait disturbances and dysphagia were probably due to ALS involvement of motor tracts and motor neurons in spinal cord and brainstem. In summary, this is the first report of a patient with both VPSPr and ALS, confirmed with PrP biochemistry, as well as light and electron microscopic TDP-43 immunohistochemistry.

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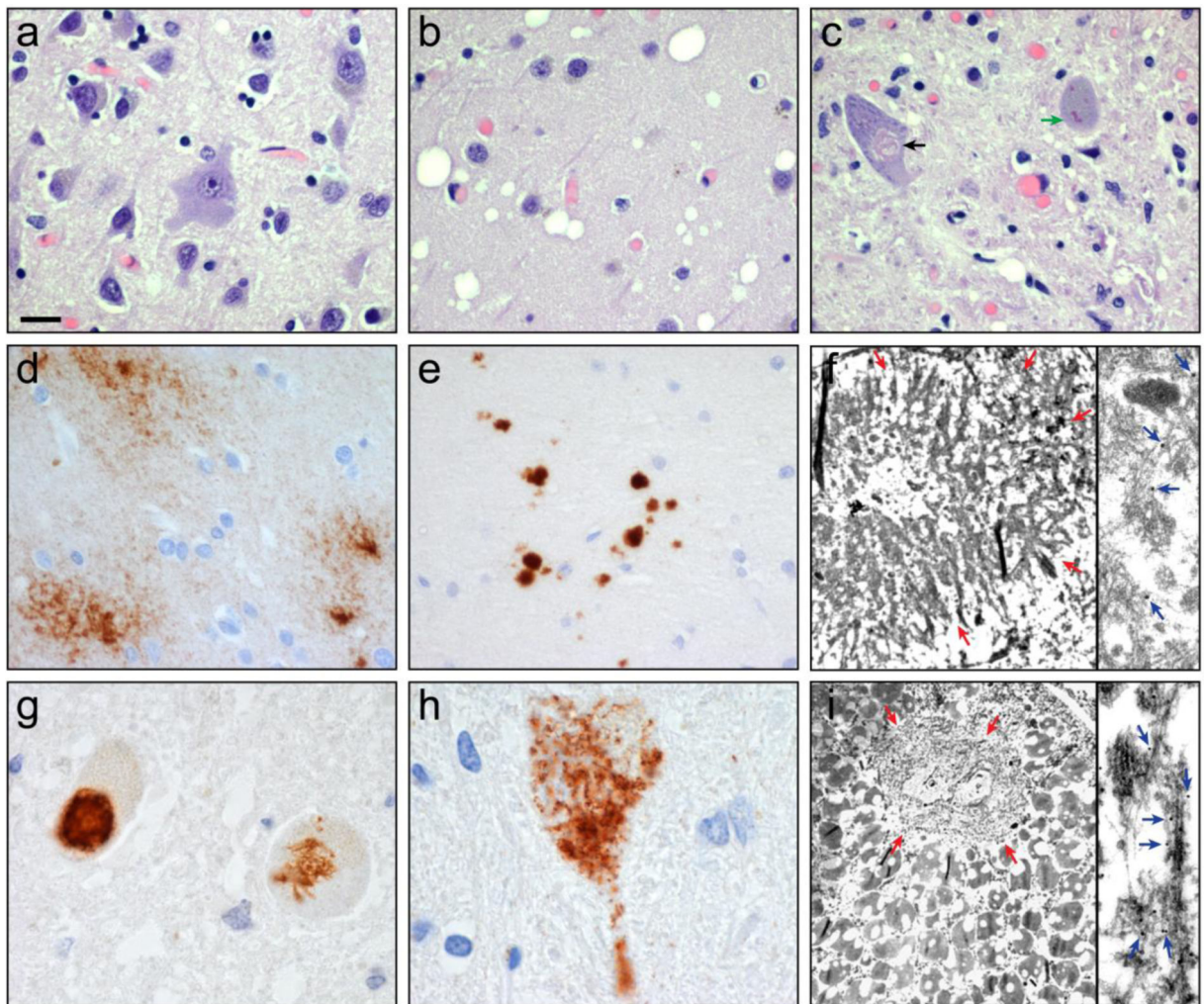


Fig. 1.

Neuropathologic profile of VPSPr-129M/V and ALS case. H&E shows balloon neurons in the superior temporal gyrus (a) and spongiform change in the caudate (b). H&E also revealed skein-like inclusions (black arrow) and Bunina bodies (green arrow) in the hypoglossal nucleus (c). PrP immunohistochemistry in the occipitotemporal gyrus reveals many prion plaques in the superficial cortical layers (d) and small dense plaques in the cerebellum (e). Ultrastructural examination of a prion plaque in the hippocampus (red arrows) reveals PrP immunopositive fibrils (f; blue arrows of gold particles). TDP-43 immunohistochemistry in the medulla exhibits skein-like and Lewy-like inclusions in motor neurons of the hypoglossal nucleus (g) as well as pre-inclusions in the anterior horn cells (h). Ultrastructural examination of a skein-like cytoplasmic inclusions (red arrows) in the hypoglossal nucleus reveals TDP-43 immunopositive filaments coated with a granular material (f; blue arrows of gold particles). [bar = 20 μ m in a-e; 10 μ m in g; 8 μ m in h; 2.5 μ m in i (large); 1 μ m in f (large); 0.2 μ m in f and i (small)]

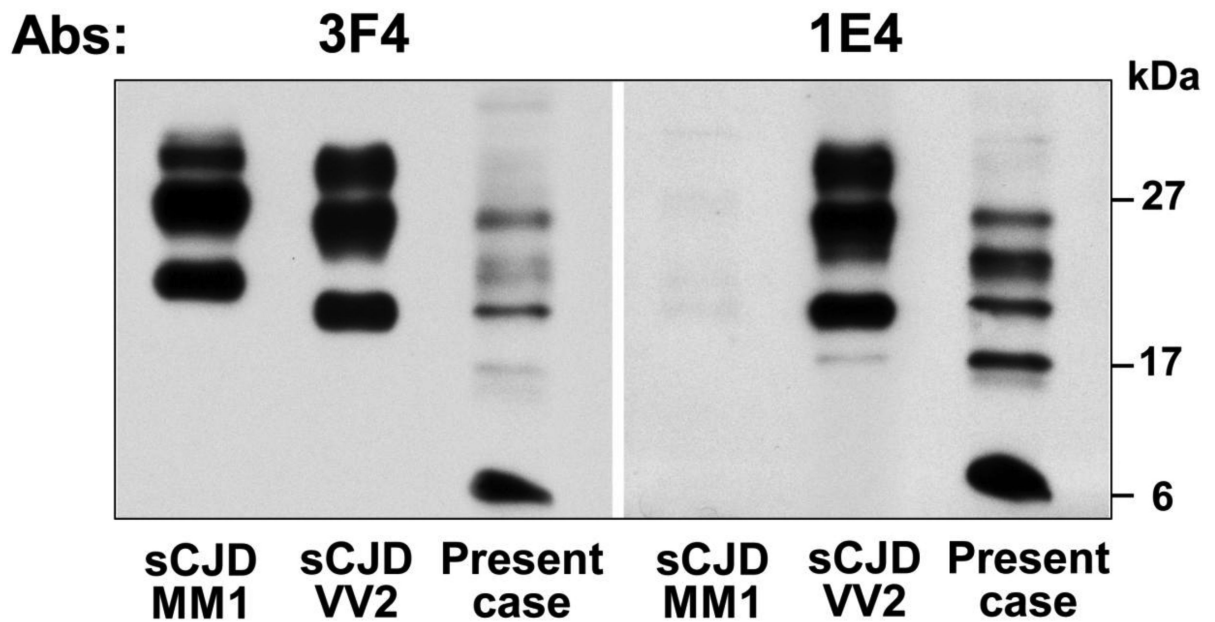


Fig. 2. Detection and characterization of PrP. Immunoblot of total homogenates, treated with proteinase K, obtained from the frontal cortex of the present case and of the most common sporadic CJD subtypes, sCJD-MM1 and sCJD-VV2. Membranes were probed with the anti-PrP monoclonal antibodies 3F4 (residues 109-112) and 1E4 (residues 97-108). PrP of the present case, detected by both Abs, showed the typical electrophoretic pattern of VPSPr-PrP. As expected in VPSPr, the most efficient immunoreactivity signal was obtained by 1E4, an antibody also known for its weak immunoreactivity to PrP-type1.