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BRIEF COMMUNICATION

Variably protease-sensitive prionopathy presenting within ALS/FTD spectrum

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Abstract

We report clinico-pathological features of a 65-year-old woman and a 56-year-old man with a 5-year clinical history who had clinical and neuropathological characteristics of upper and lower motor neuron disease consistent with amyotrophic lateral sclerosis, and a frontotemporal atrophy pattern in case 2 without TDP-43 pathology. Instead, spongiform change and pathological prion protein deposits were observed in several brain regions. No prion protein gene mutations were found. Western blot analysis showed a five-band profile compatible with variably protease-sensitive prionopathy. We conclude that this disease can display prolonged disease duration and clinico-pathological features within the ALS/FTLD spectrum.

Introduction

Creutzfeldt–Jakob disease (CJD) is a fatal neurodegenerative disorder caused by a conformational alteration of the prion-protein (PrP^{sc}). Typical clinical features include rapidly progressive dementia, cerebellar, extrapyramidal, pyramidal, and visual signs. In 2008, a novel sporadic variably protease-sensitive prionopathy (VPSPr) was described.[1] VPSPr differs from classical CJD in its clinical presentation with prominent aphasia, ataxia, and parkinsonian signs; a longer disease duration of up to 45 months; and by a unique ladder-like electrophoretic profile of proteinase K (PK)-resistant PrP^{sc} fragments.[1] Despite the frequent finding of pyramidal signs in CJD,[2–4] we are not aware of reports of VPSPr presenting simultaneous upper and lower motor neuron (MN) dysfunction, as in ALS, and as we present here.

Patients and Methods

The first patient was a 65-year-old woman, visited at the Neurology Department for gait difficulties since 1 year. Neurological examination disclosed "scissor" gait, spasticity, and hypertonia of lower limbs, brisk reflexes with patellar and ankle clonus reflexes, and Hoffmann′s and Babinski signs. She had symmetrical atrophy of the shoulder girdle and the thenar eminence. Neither cerebellar nor sensory alterations were detected. Cervical-brain MRI revealed slight ventricular enlargement. Dopamine-transporter SPECT was unremarkable. Electromyography confirmed the alteration of the pyramidal tract in the four limbs with denervation signs in the upper limbs and indemnity of the somatosensory pathway. Neuropsychology revealed mild, fronto-subcortical cognitive impairment. Lumbar puncture yielded normal cell count and routine biochemical parameters. 14-3-3 protein determination was negative. No mutations of *C9orf72* or *TARDBP* were detected.

The disease progressed and signs of lower MN were clearly appreciable in three body regions with weakness and atrophy of several muscular groups meeting the revised El Escorial Criteria for definite ALS.[5] The patient died in a long-term care facility at age 70, in a state of complete anartria, dysphagia requiring feeding by gastrostomy, and tetraplegia, after a total disease duration of 5 years.

The second patient was a 56-year-old man, visited at the Neurology Department for cognitive and behavioral problems since 1 year. His character became more irritable, with inappropriate comments and childish attitude. Neurological examination disclosed apraxia, apathy, and anosognosia and he scored 15/30 on the Minimental State Examination. No muscular weakness or pyramidal signs were observed, but fasciculations. Electromyography showed mild generalized denervation with alteration of the pyramidal tract and indemnity of the somatosensory pathway. CSF showed mildly increased total protein concentration with no cells, normal $A\beta 42$ (891 pg/mL), increased total tau (1200 pg/mL), and phospho-tau (95.54 pg/mL). There was no *C9orf72* expansion mutation and APOE genotype was e3/3. At follow up, he developed gait problems and cognitive and motor problems worsened rapidly, becoming mute and wheel chair dependent 3 years after disease onset. He required percutaneous endoscopic gastrostomy and he died at age 59 due to respiratory complications, 52 months after symptoms onset.

Preparation of brain homogenate, PK digestion, and PrP^{sc} deglycosylation

Fresh frozen brain tissue was homogenized (10% w/v) in lysis buffer (100 mmol/L NaCl, 10 mmol/L EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 100 mmol/L Tris) at pH 6.9 and digested with proteinase K (PK) (Roche Diagnostics) at a final concentration of 2 U/mL for 1 h at 37°C. After blocking PK activity with phenylmethylsulfonyl fluoride (PMSF, final concentration 3.6 mmol/L), samples were boiled in sample buffer (final concentration: 3% SDS, 4% β-mercaptoethanol, 10% glycerol, 2 mmol/L EDTA, 62.5 mmol/L Tris) for 6 min at 100°C. N-Linked glycans were removed by using a peptide-N-glycosidase F kit (New England Biolabs) according to the manufacturer's instructions.

Results

Neuropathological findings

The brain of both brain donors was removed for diagnostic and research purposes after obtaining written informed consent by the next of kin.

Case 1

Unfixed brain weight was 935 g. Gross examination revealed prominent global atrophy (Fig. 1, upper left) accentuated at the precentral gyrus, pontine base, bulbar pyramids, and spinal cord. Histology revealed prominent loss of MN of the spinal cord, and severe degeneration of lateral and anterior corticospinal tracts (Fig. 1G1–H1). There were no Bunina bodies and no other inclusion bodies. Neuronal loss, gliosis, and spongiform change was found in the neuropil of all neocortical regions and basal ganglia (Fig. 1A1). Severe loss of Betz cells and intense microglial reaction was seen in the primary motor cortex.

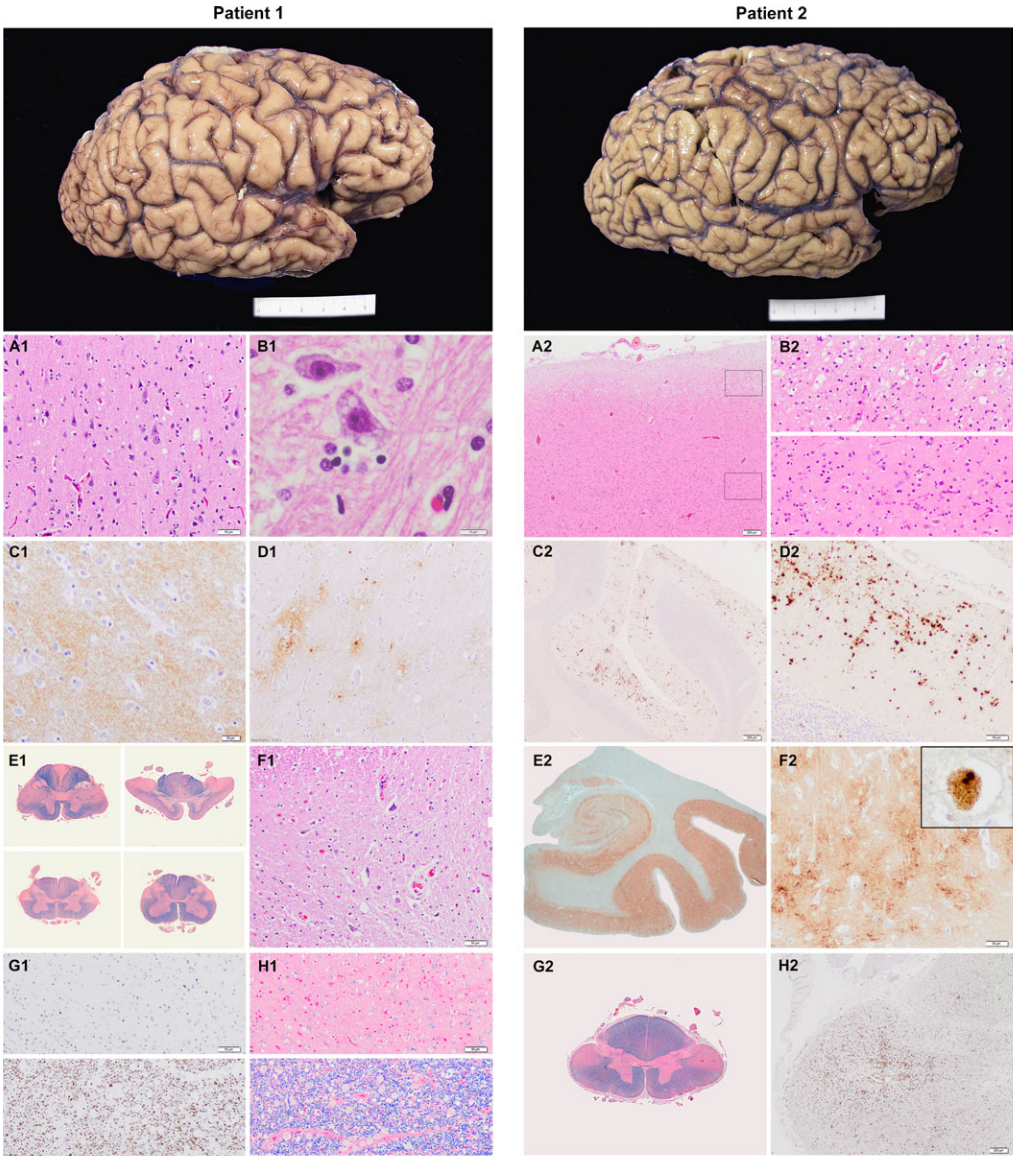

Figure 1. Neuropathological findings. A1–H1: Representative images of patient 1 A2–H2: Representative images of patient 2 Upper panel: Gross aspect of the right brain hemisphere of each patient shows a frontally accentuated brain atrophy in patient 1 (left) and a more generalized atrophy pattern in patient 2 (right). A1–B1: Haematoxylin–eosin-stained sections show mild spongiform change in frontal cortex (A1) and intraneuronal vacuoles in neurons from the basis pontis (B1); cerebellum was well preserved and showed only isolated torpedoes in granular layer, without obvious spongiform change in molecular layer (not shown). C1–D1: Immunohistochemistry for PrP (antibody 12F10) reveals abnormal diffuse synaptic PrP^{sc} deposits in grey matter in frontal cortex and, hippocampus with focal atypical deposits in subiculum (D1) and diffuse patches and occasional "microplaques" in cerebellar molecular layer (not shown). E1–H1: Luxol-fast blue stain (E1, H1) and immunohistochemistry for neurofilaments (RT97) (G1) showing prominent atrophy of spinal cord with degeneration of lateral and anterior corticospinal tracts (E1), with loss of axonal profiles (G1: upper panel shows reduced density of axonal profiles, in comparison with lower panel, which shows normal density) and myelin sheaths (H1: upper panel shows reduced density of myelin sheaths in comparison with lower panel, which shows normal density) associated with intense macrophage activity (not shown). Haematoxylin–eosin staining reveals loss of motor neurons in anterior horns, with shrunken residual neurons (F1). A2–B2: H&E-stained section of the frontal cortex reveals a characteristic superficial spongiosis as seen in FTLD. Superficial cortical layers show perineuronal, irregular vacuoles (B2 upper panel), while deeper cortical layers show mild spongiform change (B2 lower panel). C2–F2: Immunohistochemistry for PrP (antibody 12F10) reveals intense diffuse synaptic deposits with some fleecy areas in CA1 sector of the hippocampus (C2, D2), and diffuse, patchy and microplaques in molecular layer of the cerebellum (E2, F2). Occasional intraneuronal granular and coarser immunoreactivity was observed in cortical neurons (F2, inset). G2–H2: Signs of corticospinal tract degeneration at the level of thoracic spinal cord with prominent microglial activation in grey matter and macrophage activity in degenerated tracts (H2, anti-HLA-DR).

Neuronal loss was also observed in the pontine nuclei, with discrete spongiform change and intraneuronal vacuoles (Fig. 1B1). Immunohistochemistry (anti-PrP 12F10, Bertin Pharma, France) after appropriate tissue pretreatment showed scarce synaptic PrP^{sc} deposits in cortical areas (Fig. 1C), basal ganglia, thalamus, pons, and spinal cord. Atypical patchy PrP^{sc} aggregates and pseudoplaques were identified in the hippocampus, primary visual area (Fig. 1D1), and cerebellum. Isolated tau+ (AT8, Thermo-Scientific, Rockford, IL) neurofibrillary pathology was identified in the entorhinal cortex and occasional alpha-synuclein (KM51, Novocastra, Newcastle, UK) aggregates in the olfactory bulb. No additional abnormal protein aggregates (beta-amyloid (6F/3D, DAKO, Glostrup, Denmark), TDP-43 (Abnova, Taiwan), FUS (Sigma Aldrich, St. Louis, MO, USA; or Lifespan Biosciences, Seattle, WA), ubiquitin (DAKO), or p-62 (Transduction Laboratories TM)) were identified.

Case 2

Unfixed brain weight was 1015 g. Gross examination revealed prominent global atrophy, preferentially involving frontal, temporal and parietal regions (Fig. 1, upper right), and caudate nuclei. Histology revealed moderate neuronal loss and gliosis in cortical areas, basal ganglia, and anterior horn of the spinal cord at all levels, with severe degeneration of lateral and anterior corticospinal tracts (Fig. 1G2, H2). Besides focal superficial spongiosis in frontal (Fig. 1A2, B2) and temporal cortex, spongiform change was evident in cortical areas (Fig. 1B2, lower panel), limbic system, basal ganglia, and cerebellum. No abnormal ubiquitin, p62, TDP-43, FUS tau, beta-amyloid, alpha-synuclein, or alpha-internexin aggregates were detected. In contrast, diffuse abnormal PrP^{sc} deposits (12F10 antibody) were detected in all gray matter regions, combining a diffuse synaptic pattern with focal fleecy aspect (Fig. 1E2, F2), some intraneuronal deposits (Fig. 1F2, inset), and frequent patches and microplaque-like deposits in cerebellum (Fig. 1C2–D2).

Molecular studies

Western blot analysis of striatum and temporal cortex (3F4 anti-PrP antibody) revealed in both patients a pattern of five relatively weak bands migrating at 25, 22, 19, 17, and 8 kDa (Fig. 2). Deglycosylation, although not fully complete especially in one sample (lane 5), showed an enrichment or the lack of modification of the 19, 17, and 8 kDa bands, representing unglycosylated PrP^{Sc} fragments.

Analysis of *PRNP* revealed methionine homozygosity at codon 129 in case 1 and valine homozygosity in case 2, without mutations or insertions.

RT-QuIC analysis of CSF of patient 2 (obtained 10 days after the initial visit) was performed retrospectively (as described previously[6]) after obtaining the neuropathological result and was negative.

Discussion

We present two patients with neuropathologically and biochemically confirmed VPSPr manifesting symptoms within the ALS/FTD clinical spectrum. The patient who presented primary with ALS carried MM at *PRNP* codon 129 while the patient with FTD-ALS was VV. In VPSPr, the most frequent genotype is VV (65%) and 60% of patients may have symptoms mimicking FTD.[1] In contrast, among less frequent genotypes, MV (23%) and MM (12%), motor signs are more common.

Upper MN signs are a common and prominent finding in CJD and have been included in clinical criteria.[7] Although the term "amyotrophic form of CJD" has been used in the past for CJD patients in whom amyotrophy was a prominent feature[8,9] this entity has been questioned as amyotrophy may be common at end stages of disease.[9] Only few sCJD patients with amyotrophy as an early and prominent feature, with short disease duration (1–8 months)[2–4] have been reported.

Recently, a patient with concomitant VPSPr and ALS-TDP43 has been reported.[10] This was a woman with a 4-year history of progressive dementia and features suggestive of Lewy body disease. Neuropathology showed a spongiform encephalopathy, a five-band ladder profile of abnormal PrP consistent with VPSPr, and TDP-43 neuronal inclusions in motor neurons.

Recently, Yaguchi et al. also reported a CJD patient with clinical features of ALS/FTD, but prion diagnosis relied on RT-QuiC, but not on postmortem examination.[11]

Two clinical features differentiate our patients from most previous CJD cases: the simultaneous development of upper and lower MN dysfunction, fulfilling the revised El Escorial Criteria for definite ALS,[5] and the atypically prolonged disease duration. Neuropathological findings were consistent with ALS in case 1. However, and in contrast to the case reported by Cannon et al.[10] we did not find ubiquitin-, neurofilament, TDP43-, or FUS-positive neuronal inclusions or Bunina bodies. Instead, we found spongiform change and PrP^{Sc} deposition widely distributed throughout the brain and spinal cord, which together with absent *PRNP* mutations, the western blot pattern and the atypical immunohistochemical features are consistent with VPSPr. The same neuropathological features were observed in patient 2, who had additional FTLD. We could not find an alternative explanation for

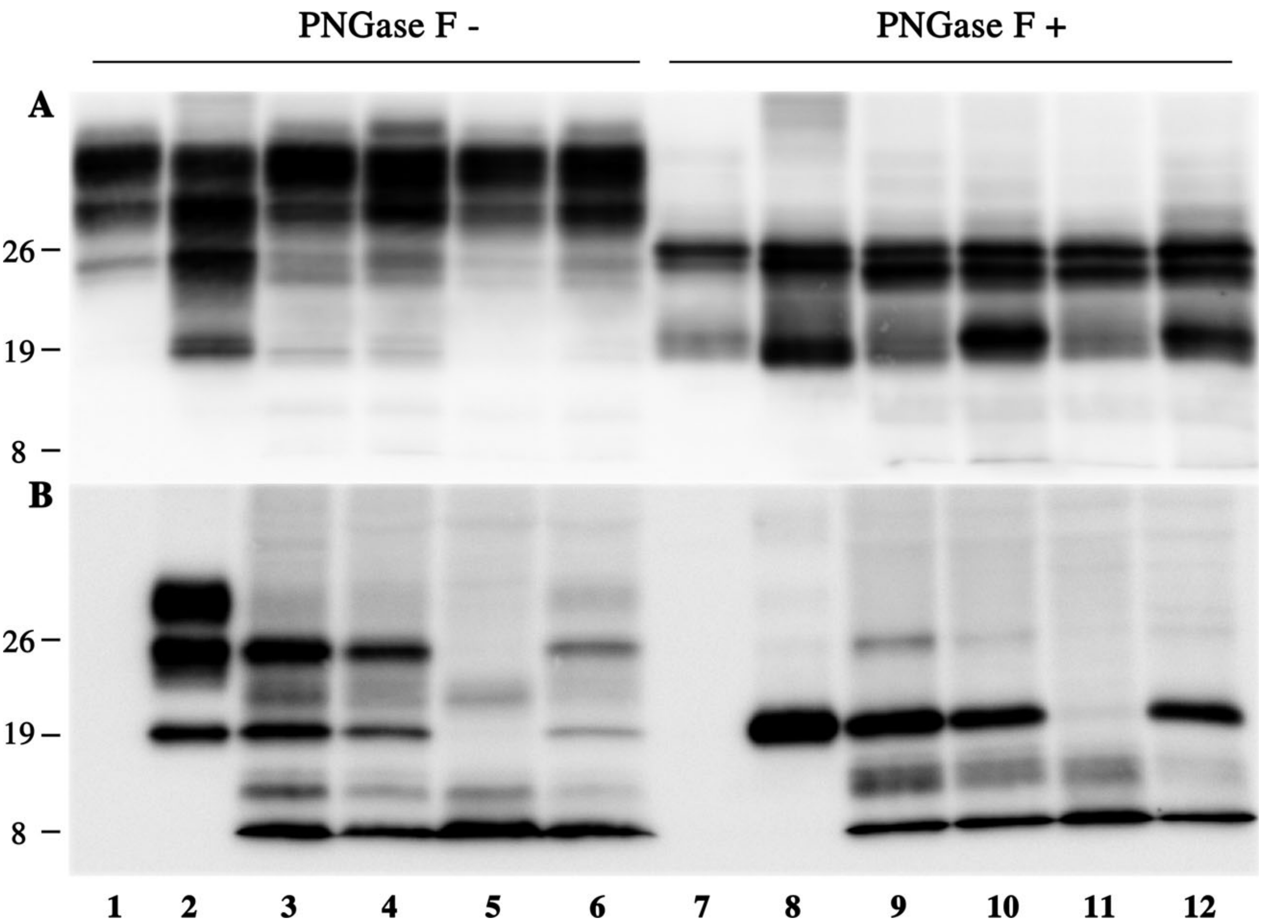

Figure 2. Western blot. Western blot analysis of PrP isoforms before (A) and after (B) PK digestion in normal human brain, sCJDVV2, and VPSPr. Membranes were incubated with the primary antibody 3F4. Relative molecular masses are expressed in kDa. Samples were either not treated (lanes 1–6) or treated by PNGase F (lanes 7–12) to remove N-Linked glycans. Lanes 1 and 7, normal human brain (negative control), lanes 2 and 8 sCJDVV2 (positive control); lanes 3 and 9: patient 1, temporal cortex; lane 4 and 10: patient 1 striatum; lanes 5 and 11: patient 2, temporal cortex; lanes 6 and 12: patient 2 striatum. At variance with sCJD, the PrP^{sc} profile in VPSPr cases comprises five major bands migrating at 25, 22, 19, 17, and 8 kDa. After deglycosylation only a single 19-kDa band is recognized in sCJDVV2, while three major unglycosylated bands (of 19, 17, and 8 kDa) are detected in VPSPr. Moreover, the PrP^{sc} profile in VPSPr varies from case to case and from one brain area to the other, depending on both glycosylation and relative amount of the three fragments. Most significantly, the 19-kDa fragment and its monoglycosylated form (25 kDa band) is almost undetectable in the temporal cortical sample of cases 2, while an additional faint diglycosylated fragment, corresponding to the diglycosylated band of the 19 kDa fragment of sCJDVV2, is seen in the striatum of case 2.

this clinico-pathological phenotype. The possibility of the co-occurrence of two rare conditions, VPSPr and an atypical form of ALS/FTLD lacking abnormal protein inclusions seems unlikely. The MM genotype of our first patient with ALS, which is the less frequent genotype in VPSPr, could be another explanation for the atypical clinical course, while valine homozygosity of the second patient could be more concordant with dementia and psychiatric symptoms.

In conclusion, we describe ALS/FTD-ALS mimics with underlying VPSPr, that expands the phenotypic spectrum of human prion disease, specifically of VPSPr.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

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