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Variably Protease-Sensitive Prionopathy in An Apparent Cognitively Normal 93 Year Old

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Introduction

Variably protease-sensitive prionopathy (VPSPr) is a recently described novel prion disease biochemically characterized by abnormal prion protein (PrP) which has various degrees of sensitivity to proteases and generates a distinct profile on Western blots ¹. VPSPr is the second most common sporadic prion protein disease after CJD ². All reported cases to date have exhibited cognitive, behavioral, and psychiatric deficits with an age at onset range of 48-81 years and disease duration range of 7-72 months ¹⁻⁴. Initial clinical diagnoses included normal pressure hydrocephalus (NPH), dementia with Lewy bodies (DLB), or frontotemporal dementia (FTD) ^{1,5}, but Creutzfeldt-Jakob disease (CJD) often was considered clinically after rapid decline became apparent ¹.

All VPSPr cases evaluated have lacked mutations in the prion protein gene (*PRNP*) ^{1,2,5}. Initial reports indicated all cases were homozygous for valine at codon 129 (VV) in *PRNP* ¹. However, more recent reports have found VPSPr in all three codon 129 genotypes ^{2,5-7}.

Here we report a case of VPSPr in which there were no known clinical manifestations of prion disease during life and yet pathognomonic findings were revealed at autopsy.

Methods

The individual described herein was enrolled in an ancillary study of cognitively normal individuals at the Knight Alzheimer's Disease Research Center at Washington University, St Louis, MO. She had 2 in-person assessments (1996 and 1999), although limited objective

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testing (Mini-Mental Status Exam (MMSE) ⁸) was obtained only at initial assessment. At death, her family was administered a postmortem dementia interview to ascertain any cognitive or neurological changes that may have occurred since her last in-person assessment ⁹. Pre-terminal hospitalization records were also reviewed. Genomic DNA had been extracted from blood during life for *APOE* genotyping by Knight Alzheimer's Disease Research Center Genetics Core ¹⁰.

After death, the brain was examined macroscopically. Paraffin sections from formalin-fixed blocks taken from 12 areas were obtained. These were processed using standard neuropathologic methods including hematoxylin and eosin (H&E) and a modified Bielschowsky silver impregnation. Spongiform changes were noted and prompted further detailed evaluation in conjunction with the National Prion Disease Pathology Surveillance Center (NPDPS), Case Western University, Cleveland, OH, USA. Monoclonal antibodies used for immunohistochemistry were as follows: phosphorylated tau (PHF-1, a gift of Dr. P. Davies, Albert Einstein College of Medicine, New York, NY, USA), beta-amyloid (10D5, Eli Lilly, Indianapolis, IN, USA), alpha-synuclein (LB509, Zymed Laboratories, Inc., San Francisco, CA, USA), and PrP (3F4, EMD Millipore Corporation, Billerica, MA, USA) ¹⁰.

DNA was extracted from frozen brain tissue for *PRNP* sequence analysis by the NPDPS. The *PRNP* coding region was sequenced to determine the codon 129 genotype and the absence of pathogenic mutations ². Frozen brain tissue was obtained and sent to the NPDPS for prion protein characterization by proteinase K (PK) digestion and Western blot analysis ^{2,10}. The brain tissue from the frontal cortex was used for Western blot analysis. Other brain regions were examined including occipital cortex, cerebellum, temporal cortex, striatum, midbrain, thalamus, and substantia nigra, in which much less PK-resistant protein was detected compared to the frontal cortex.

Results

Clinical Assessment

This individual was determined to be cognitively intact during her initial visit at age 87 with a MMSE 29 (only error was that she missed the date by 4 days) ⁸. Furthermore, she was determined to be cognitively intact at her death at age 93 with a Clinical Dementia Rating (CDR) score of 0, ¹¹ as assessed by the retrospective collateral dementia interview (RCDI) ⁹. The RCDI is a structured telephone interview conducted by a clinician experienced in dementia evaluation with a knowledgeable collateral source. In particular, questions about memory and orientation are asked to elicit the onset, course, and nature of any problems (i.e., recent memory, orientation, and activities of daily living). Because the participant was asymptomatic, no additional clinical studies such as EEG, CSF (14-3-3) or imaging were obtained to evaluate the possibility of prion disease. Specifically, no neurologic abnormalities associated with prion disease were reported ².

Chart review revealed no known risk factors for prion exposure. There was no family history of dementia. The RCDI was conducted with both her surviving spouse and their daughter, and neither had concerns about her memory and thinking abilities. She was oriented to her home and hometown. She continued to manage the household finances and paid the bills.

She maintained her appointments but was less engaged in activities at her retirement home due to physical frailty. She continued to cook and work crossword puzzles. She maintained her activities of daily living and was only occasionally incontinent due to diuretics and physical limitations. She may have been depressed according to family. There was no history of seizures, strokes, unusual movements, tremors, visuospatial deficits, hallucinations, aphasia. This individual transitioned from assisted living to hospice care one month prior to expiration due to end-stage congestive heart failure. Her cause of death was due to inanition and congestive heart failure.

Genetic Findings

Genetic analysis of this individual was performed by the NPDPS and confirmed both the absence of a mutation in the coding region of *PRNP* and the codon 129 genotype as being homozygous for methionine (MM). Her *APOE* genotype was $\epsilon 3:\epsilon 3$.

Neuropathology

The fresh, unfixed brain weighed 1,180 g. There was mild atrophy of the frontal lobes and mild to moderate dilatation of the lateral ventricles. The amygdala and hippocampus were small in size. The basal ganglia and cerebellum were unremarkable (Figure A). Microscopic examination revealed pathognomonic changes for prion disease: neuronal loss and astrocytosis with spongiform changes in the deeper cortical layers of the frontal, parietal, and temporal neocortices were noted – in areas where initial pathological changes may precede the detection of PrP deposition by immunohistochemistry in CJD¹²⁻¹⁴ (Figure B-E, Table). Furthermore, the current case exhibited a distinct and decreased vacuolation (“spongiform change”) profile as compared to sporadic CJD MM and previously described VPSPr MM cases (Figure G)². The cerebellum was unremarkable with a well-preserved complement of Purkinje and granule cells and neurons in the dentate nucleus. Weak PrP immunostaining revealed PrP deposits in the neocortex and none in the cerebellum (Figure D, Table). Mild Alzheimer's disease (AD) pathology was noted in the parahippocampal gyrus (Figure E, Table). Western blot analysis of frozen brain tissue from the frontal lobe of this case demonstrated the presence of a five band ladder-like electrophoretic profile of PrP that is fairly resistant to proteinase K (PK) digestion as expected in VPSPr-129MM, and that is distinct from sporadic CJD² (Figure F). As no beta-amyloid plaques were present, the neuropathologic diagnosis of AD was excluded¹⁵. However, some neurofibrillary tangles (Braak neurofibrillary tangle stage III) were present in the medial temporal lobe. In conclusion, sufficient neuropathologic and biochemical evidence were present to support the diagnoses of VPSPr.

Discussion

We describe herein the first report of histopathologically diagnosed VPSPr in a 93-year-old individual lacking clinically recognized cognitive, behavioral, or neurologic deficits at the time of death, as based on the RCDI and pre-terminal hospitalization records. Although it is possible that she may have had undetected neurological signs and symptoms, they were not appreciated by her family or her medical team. While other VPSPr cases of long duration have been reported, they were all symptomatic with findings consistent with Parkinson's

disease or an underlying psychiatric illness, or else they exhibited myoclonus, ataxia, or aphasia^{1,2,4,5,16-18}. Our asymptomatic case had no prion disease exposures and had no family history of dementing illnesses.

One possibility is that the autopsy-confirmed VPSPr in our cognitively normal case may have led to symptomatic disease had she continued to live. Pathologically, VPSPr MM typically has medium-sized vacuoles and microplaques in the cerebellum⁴. In our case, the characteristic vacuolation pattern found in VPSPr was notably reduced, which suggests overall low pathological burden. PrP grains and plaques were found in the temporal lobe while the cerebellum was normal. There is a VPSPr MV case report with normal cerebellum but that case was also symptomatic with cognitive and visuospatial deficits in the context of coexisting tau, beta-amyloid, and alpha-synuclein lesions¹⁹. It is possible that longer duration was needed in this case to increase vacuole and PrP burden to a threshold at which point symptoms would manifest. In transgenic mouse models an age-dependent accumulation of prions and their infectivity has been shown to be inversely proportional to incubation time²⁰. Presymptomatic Tg2866 mice sacrificed at 56 days conferred no infectivity to Tg196 mice, but presymptomatic mice sacrificed at 112 days were able to induce disease in the Tg196 mice and after a shorter incubation time than if sacrificed at 84 days²⁰.

The VPSPr MM subtype also appears to be less prevalent and less severe in disease presentation (less severe histopathology and less prominent PrP immunostaining) when considering that 25 of 28 VPSPr cases in one series had at least one valine (V) allele^{2,5}. VPSPr MM is characteristically more resistant to PK such that a ladder of fragments persists even after relatively high concentration PK exposure as is seen in our case^{2,4-6}. Although the brain tissue in this case was not evaluated for infectivity, it is theoretically possible based on animal data²⁰ that even minimal pathology could be transmissible. To our knowledge, this is the first case report of VPSPr in a cognitively and neurologically intact person and adds to the growing spectrum of prion disease phenotypes.

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Author Disclosure Information

N. Ghoshal

- Role: design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript

- Dr. Ghoshal had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
- Neither Dr. Ghoshal nor her family owns stock or has equity interest (outside of mutual funds or other externally directed accounts) in any pharmaceutical or biotechnology company. Dr. Ghoshal has participated or is currently participating in clinical trials of antedementia drugs sponsored by: Elan/Janssen, Eli Lilly and Company, Wyeth, Pfizer, Novartis, and Bristol-Myers Squibb, Other financial or material support. None.

A. Perry –Per CTA

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- Ferring Pharmaceuticals, Grants/Research Support; Ferring Pharmaceuticals, Consultant; Member of the TSE Advisory Board, Board Member/Officer;

J.C. Morris:

- Role: design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript
- Neither Dr. Morris nor his family owns stock or has equity interest (outside of mutual funds or other externally directed accounts) in any pharmaceutical or biotechnology company. Dr. Morris has participated or is currently participating in clinical trials of antideementia drugs sponsored by: Janssen Alzheimer Immunotherapy Program, Pfizer, and Eli Lilly/Avid Radiopharmaceuticals. Dr. Morris as served as a consultant or has received speaking honoraria from: Lilly USA. Other financial or material support. None.

N.J. Cairns, None

- Role: design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript

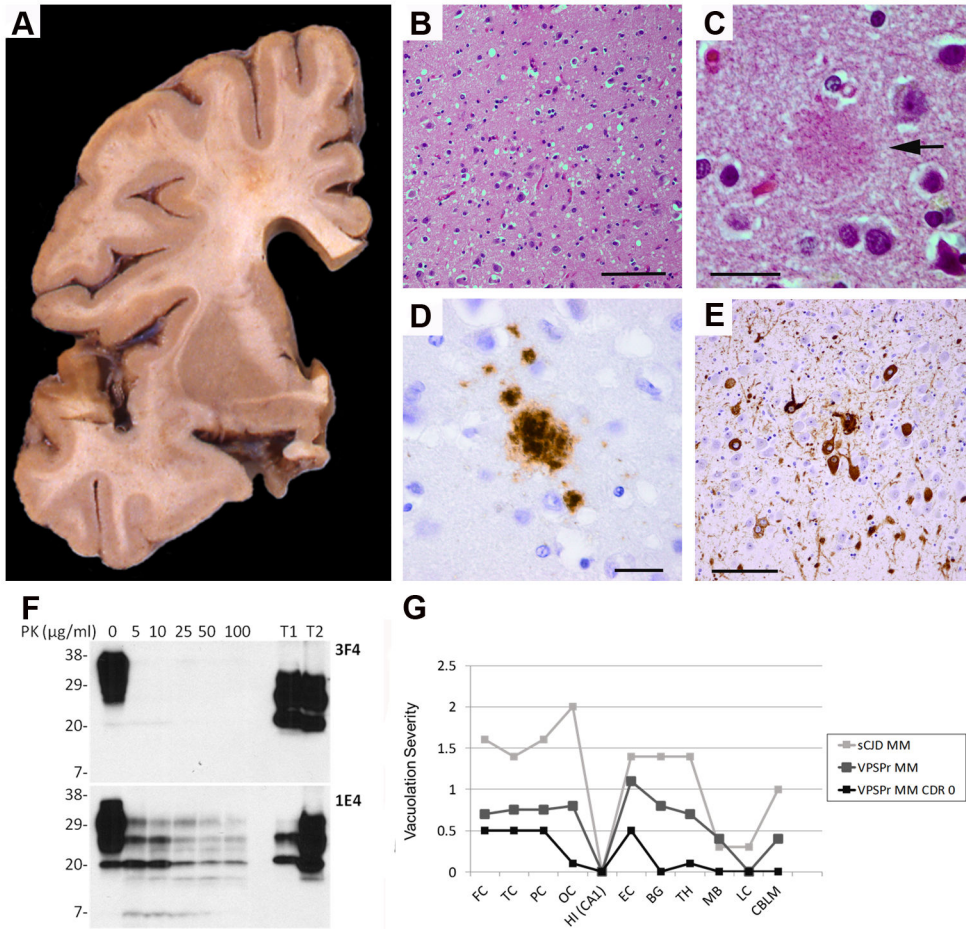


Figure.

A: There is mild dilatation of the lateral ventricle and some increase in space in the Sylvian fissure. The basal ganglia are unremarkable. **B:** There is mild and widespread moderate spongiform degeneration with vacuoles of intermediate size throughout the cortical ribbon of the frontal lobe. **C:** A rounded eosinophilic structure (arrow) indicates a possible prion plaque in the temporal cortex. **D:** Granular PrP plaques-like deposits in the temporal lobe. **E:** Neurofibrillary tangles in the parahippocampal gyrus. B and C, hematoxylin and eosin; D, PrP (3F4) immunohistochemistry; E, phosphorylated tau (PHF1) immunohistochemistry. Scale bars: B and E, 100µm; C and D, 20µm. **F:** Detection of prion protein by Western blotting. Brain homogenates from the frontal cortex of this case were subjected to treatment with different amounts of proteinase K (PK) prior to Western blotting with 3F4² or 1E4²¹. T1 and T2: Disease causing PrP isoform (PrP^{Sc}) type 1 and type 2 from sporadic CJD positive controls. 3 µl of brain homogenate (~120 µg protein) was loaded per lane. Numbers on the left sides of the blots refer to the molecular weights of standards used. **G:** Vacuolation profiles. The current VPSPr MM CDR 0 case exhibits a distinct and decreased vacuolation profile as compared to sporadic CJD MM and previously described VPSPr MM cases². Semi-quantitative assessment of vacuolation scored on the y-axis on a 0 to 4 scale (0 = not detectable; 1 = mild; 2 = moderate; 3 = severe; 4 = confluent) 1. X-axis abbreviations: FC –

Frontal cortex; TC – Temporal cortex; PC – Parietal cortex; OC – Occipital cortex; HIP (CA1)– Hippocampus CA1 subfield; EC – Entorhinal cortex; BG – Basal ganglia; TH – Thalamus; MB – Midbrain; LC – Locus coeruleus; CBLM – Cerebellum.

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Table

Neuron loss, gliosis, and prion protein (PrP) deposits

Area	Neuron loss/gliosis	Vacuolation	PrP	NFT
FC	+	++	+	0
TC	+	++	+	0
PC	+	++	+	0
OC	+/-	+/-	0	0
HIP	+/-	0	0	+
EC	+	++	+	++
BG	0	0	0	0
TH	+	+	+	0
SN	+/-	0	0	0
LC	0	0	0	0
MED	0	0	0	0
CBLM	0	0	0	0

Abbreviations: FC – Frontal cortex; TC – Temporal cortex; PC – Parietal cortex; OC – Occipital cortex; HIP – Hippocampus; EC – Entorhinal cortex; BG – Basal ganglia; TH – Thalamus; SN – Substantia nigra; LC – Locus coeruleus; MED – Medulla; CBLM – Cerebellum.