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Short Communication

MM1+2C Sporadic Creutzfeldt-Jakob Disease Presenting as Rapidly Progressive Nonfluent Aphasia

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Abstract. We report a 77-year-old man, presenting with progressive aphasia as an initial symptom, who developed severe dementia over the course of 20 months. Frontal cortex PrP^{Sc} western blot was type 2 and codon 129 was MM; brain neuropathology showed cortical vacuoles with perivacuolar PrP immunostaining characteristic of MM2C. Cerebellum showed focal coarse, patchy staining in different sections of the molecular layer, diffuse fine punctuate and coarse PrP immunopositive deposits in the granule cell layer, and focal synaptic immunostaining in the molecular layer, suggestive of MM1+2C by histotyping. This clinical presentation has not yet been described in an MM1+2C subtype by histotyping.

Keywords: Aphasia, MM1+2C subtype, MM2 cortical subtype, prion, sporadic Creutzfeldt-Jakob disease

INTRODUCTION

Human transmissible spongiform encephalopathies are characterized by deposition of a pathological, insoluble, and protease-resistant disease-specific protein (PrP^{Sc}). PrP^{Sc} designates the pathologic isoforms of the normal cellular prion protein (PrP^{c}) [1]. Sporadic Creutzfeldt-Jakob disease (sCJD) is the most frequent human transmissible spongiform encephalopathy [2]. sCJD presents clinical and pathological heterogeneity and has been classified into six different pure subtypes, namely, MM1 or MV1; VV2; MV2; MM2-thalamic; MM2-cortical; and VV1, based on the physicochemical properties of PrP^{Sc} and the genotype of the prion protein gene (*PRNP)* at codon 129, each with different phenotypes from the clinical, molecular, and neuropathological point of view; 5% of the 300 cases examined in Parchi's series had both types 1 and 2 PrP^{Sc} (mixed types). MM2-C subtype corresponded to 2% of all sCJD cases [3]. Clinical symptoms reported for MM2 cortical subtype include rapidly progressive dementia without cerebellar or visual impairment.

Primary progressive aphasia was originally described by Mesulam [4]. Although language disorders are common in neurodegenerative dementias,

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they have particularly been described as part of sCJD [5].

We report a case of MM2C sCJD presenting with progressive nonfluent aphasia as the debut symptom, in which cerebellar immunostaining was consistent with MM1+2C sCJD by histotyping [6].

METHODS

Biochemical and molecular genetic studies

PRNP codon 129 analysis

DNA was extracted from blood (PROMEGA kit) following manufacturer's instructions. A specific internal fragment was amplified and analyzed by RFLP using a published protocol with minor modifications.

PRNP gene sequencing was performed in the Center for Human Genetics of University Hospitals of Cleveland.

CSF western blot analysis

Cerebrospinal fluid (CSF) sample aliquots (50 μl, case and controls) were cracked, separated using SDS-PAGE, and nitrocellulose transferred. Membranes were incubated following a published protocol with minor modifications; antibody used was Sc629-G, Santa Cruz.

PrP^{Sc} western blot analysis

A frontal lobe sample of brain-tissue homogenate (10% w/v) was prepared in homogenization buffer, digested with Proteinase K, separated by SDS-PAGE, and nitrocellulose transferred. Membrane was incubated following a published protocol with minor modifications [7]; monoclonal antibody used was 3F4 against PrP residues 108–111, provided by Dr. Kascsak.

Neuropathology

Brain autopsy

Brain tissue was fixed in 10% formaldehyde, with the exception of the left frontal lobe, which was stored at −80°C for biochemical analysis. Tissue samples from the neocortex (frontal, parietal, temporal, and occipital), caudate nucleus, putamen, hippocampus, thalamus (anterior and mediodorsal nuclei), midbrain, pons, medulla, and cerebellum were immersed in 98% formic acid for an hour, postfixed in 10% formaldehyde for another 48 hours, and embedded in paraffin. Sections were stained with hematoxylin-eosin and Luxol-fast blue-periodic acid Schiff.

Immunohistochemistry

For immunohistochemistry evaluation, tissue sections of selected areas were immunostained using monoclonal antibodies against glial fibrillary acidic protein (GFAP) (Dako,1:100); monoclonal antibody anti-neurofilament (Invitrogen, 1:150); monoclonal antibody CD68 (Dako:1/100), and prion protein (PrP) monoclonal antibody 3F4 with epitope at PrP residues 108–111 (1:400) provided by Dr. Kascsak.

RESULTS

Clinical summary

A 77-year-old-man was first assessed in December 2006 after six months of progressive mild dysarthria. In April 2007, dysarthria had progressed, and the patient presented difficulty finding words, mild loss of speech comprehension, bradypsychia, and apathy. Four months later, the patient was referred to our memory clinic (RFA).

Neuropsychological assessment showed nonfluent aphasia and dysexecutive syndrome. Presence of associated dysexecutive syndrome does not exclude a progressive aphasia diagnosis because inclusion criteria describe that the "most prominent but nonexclusive clinical feature is difficulty in language" [8]. Laboratory studies (including thyroid hormones and antibodies, collagenogram, vitamin B12, folic acid, anti-Hu antibodies, HIV, and VDRL) were normal. Brain CT scan showed no relevant data, and EEG did not show abnormalities. In October 2007, the patient developed severe nonfluent aphasia and memory impairment, becoming disoriented in time and space. On neurological examination, right-sided rigidity, gait apraxia, and primitive reflexes (suction and grasping-prehension) were observed. Brain MRI showed bright cortical signal on FLAIR sequences, mainly in the left frontotemporal areas (Fig. 1A). Brain SPECT showed left frontotemporal hypoperfusion. CSF analysis revealed acellular fluid with low protein levels (4 mg/dl), and CSF western blot was positive for 14.3.3 protein. In December 2007, the patient worsened, developing global aphasia with severe dementia, predominant right myoclonus, and generalized rigidity with resting tremor. Follow-up brain MRI showed findings similar to earlier studies as well as abnormal signal in the basal ganglia region (Fig. 1B). EEG showed nonperiodic synchronous discharges. Clinical condition continued to worsen until the patient died in February 2008 as a result of severe infection. Brain autopsy was performed.

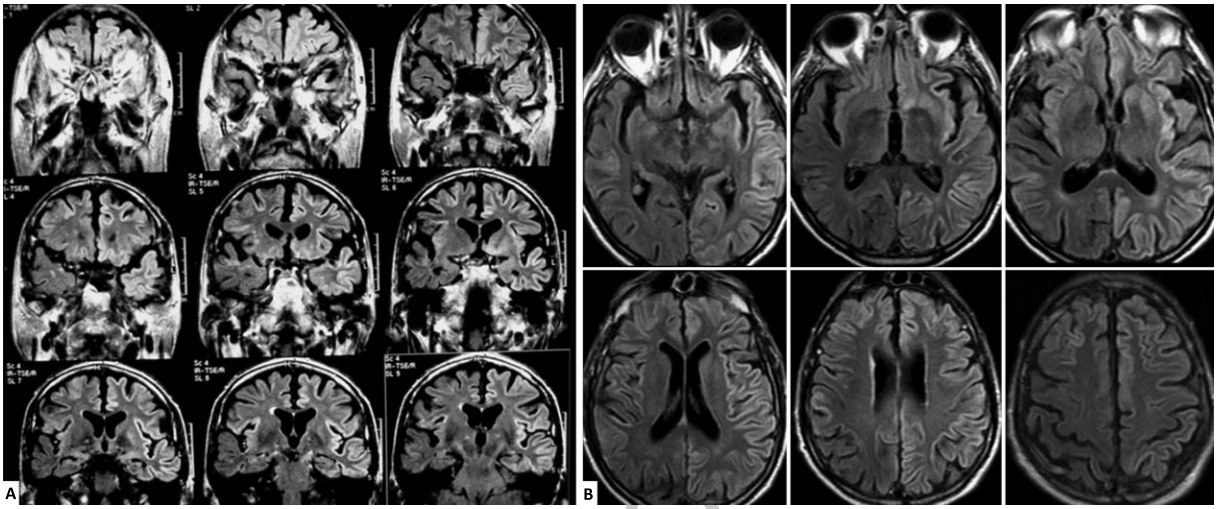

Fig. 1. a) Initial brain MRI, on fluid-attenuated inversion recovery (FLAIR) acquisition, showing increased signal in the left cortical ribbon. Abnormal bright signal in the cortical ribbon was also observed in some areas of the right frontal, temporal, and parietal lobes. b) Follow-up brain MRI on FLAIR performed two months later. Abnormal bilateral signal brightness was found in the caudate and putamen as well as findings similar to baseline MRI.

Neuropathology studies

Total brain weighed 1250 grams. Mild diffuse atrophy was observed predominantly in both frontal lobes and slight ventricular dilatation on coronal sections. Basal ganglia, thalamus, Ammon's horn, cerebellum, brain stem structures, and white matter were preserved.

Microscopy findings

Spongiform changes with clusters of large confluent vacuoles, perivacuolar PrP immunostaining, and astrocytic hyperplasia were prominent in the frontal cortex and cingulate gyrus (Fig. 2); superior and medium temporal gyri; enthorinal cortex; parietal, insular, and calcarine cortices; and basal ganglia. Large confluent vacuoles, although more prominent on the left frontal and temporal cortex, were also observed on the right side. PrP immunoreactivity, gliosis, or microglial activation did not differ between the left and right frontal or temporal cortex. The hippocampus and the thalamic nuclei showed milder focal changes. No spongiform changes were observed in the cerebellum, pons, or medulla, and only focal ones were present in the midbrain. Different cerebellar sections showed focal synaptic patterns or patchy/coarse focal PrP staining in the molecular layer of the cerebellum and extensive fine punctate and coarse PrP positive immunoreactivity in the granule cell layer (Fig. 2). Fine small vacuoles were not observed, except in the calcarine cortex where they present together with synaptic immunostaining close to large confluent vacuoles with coarse PrP immunostaining. Abundant perivascular and periventricular white matter corpora amylacea were present, while myelin was preserved.

Biochemical and molecular genetic studies

PRNP sequencing showed methionine homozygosity at codon 129, ruling out presence of pathogenic mutation in the coding region. Western blot analysis for 14-3-3 protein in CSF was positive, and PrP^{sc} western blot with 3F4 antibody at frontal cortex was type 2 (19 kDa, nonglycosylated isoform).

DISCUSSION

A patient, who developed rapidly progressive aphasia, worsening over a 20-month period, was diagnosed as presenting MM2C sCJD, based on frontal cortex PrP^{Sc} western blot type 2 and codon 129 polymorphism. Extensive cortical spongiform changes with clusters of large confluent vacuoles and perivacuolar and coarse PrP immunostaining were characteristic of this subtype [3].

Cerebellar immunostaining in different sections showing focal synaptic pattern of PrP deposition, or patchy and coarse focal staining in the molecular layer, and extensive, diffuse fine punctate and coarse PrP

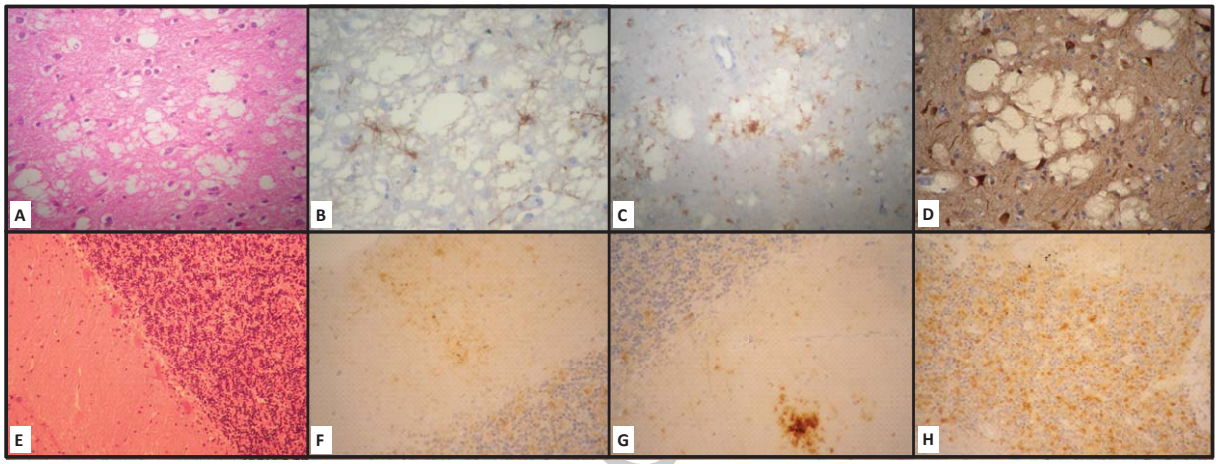

Fig. 2. a) Spongiform changes with large confluent vacuoles. Left frontal cortex, H and E, 200x. b) Astrocytic hyperplasia. Left frontal cortex, GFAP immunostaining, 400x. c) Coarse-like PrP immunostaining. Left frontal cortex, PrP immunostaining, 400x. d) Spongiform changes and neuronal loss. Left frontal cortex, NF immunostaining, 400x. e) No evidence of spongiform changes. Cerebellum, H and E, 200x. f) Focal synaptic pattern of PrP deposition in molecular layer. Cerebellum, PrP immunostaining, 400x. g) Focal patchy/coarse PrP staining in the molecular layer. Cerebellum, PrP immunostaining, 400x. h) Extensive/diffuse fine punctuate and coarse PrP positive immunoreactivity in the granule cell layer. Cerebellum, PrP immunostaining, 400x.

positive immunoreactivity in the granule cell layer (Fig. 2B) were more characteristic of MM1+2C by histotyping [6]. Because the clinical presentation was atypical in this case (prominent progressive nonfluent aphasia), the patient did not initially meet the diagnostic criteria for possible or probable sCJD. Eight months passed before global aphasia and dementia developed. Only after characteristic cortical and basal ganglia hyperintensity were observed on DWI MRI was this diagnosis suspected. In a series on MM2C subtype published by Krasniansky et al. [9], basal ganglia hyperintensity was reported to be 13% on T2 (1/8 cases) and 100% on DWI (2/2 cases). Interestingly in this case, initial signal hyperintensity findings were limited to the left frontoparietal region, and SPECT results coincided with progressive aphasia. Rapid progression to global aphasia with dementia and presence of extrapyramidal signs with myoclonus led us to consider possible sCJD. Eighteen months after onset, cortical FLAIR hyperintensity became more extensive. Increased signal intensity of the left striatum was also evidenced in a subsequent MRI. CSF 14-3-3 protein was detected by western blotting, further increasing likelihood of the diagnosis, whereas EEG findings remained negative throughout. In a MM2C subtype series, CSF 14-3-3 protein was present in up to 91%, although periodic sharp wave complexes were observed in 5 of 12 patients [9]. This case was PrP^{Sc} type 2, although only frontal cortex was available for western blot. In the sCJD classification proposed by Parchi et al., 5% of the 300 cases examined had both type 1 and type 2 PrP^{Sc} (mixed types) [3]. The classification has since been updated as more mixed type sCJD cases are being reported [10, 11]. Parchi et al. [6] stressed the importance of CJD "histotyping," especially when $PrPS^{c}$ typing cannot be performed in cases lacking enough fresh or frozen brain tissue (as in this case in which only one frontal lobe sample was available). Since the classification consensus, histopathology examination has become the most sensitive method to recognize mixed phenotypes in subjects carrying MM or MV at codon 129.

In conclusion, we describe a sCJD with early aphasia, subsequently progressing over a 20-month period to global aphasia with dementia and extrapyramidal signs, classified originally as MM2C and updated by histotyping to MM1+2C. This form of presentation beginning with progressive nonfluent aphasia has been previously described by Johnson et al. in an MV2C subtype [5], but to our knowledge, it is the first in an MM2C subtype, MM1+2C by histotyping [12].

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