Creutzfeldt–Jakob Disease and Inclusion Body Myositis: Abundant Disease-Associated Prion Protein in Muscle

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Pathological prion protein (PrP^{Sc}) is the hallmark of prion diseases affecting primarily the central nervous system. Using immunohistochemistry, paraffin-embedded tissue blot, and Western blot, we demonstrated abundant PrP^{Sc} in the muscle of a patient with sporadic Creutzfeldt–Jakob disease and inclusion body myositis. Extraneural PrP^{C}-PrP^{Sc} conversion in Creutzfeldt–Jakob disease appears to become prominent when PrP^{C} is abundantly available as substrate, as in inclusion body myositis muscle.

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Presence of the normal cellular prion protein (PrP^{C}) is essential in the formation of a protease-resistant pathological conformer termed *PrP^{Sc}*, a hallmark of prion diseases or transmissible spongiform encephalopathies.[1,2] PrP^{C}–PrP^{Sc} conversion may be initiated by external PrP^{Sc}, as in iatrogenic or variant Creuzfeldt–Jakob disease (vCJD). The basis of sporadic CJD (sCJD) is currently undetermined, whereas genetic cases are suggested to result from structural instability of a mutated PrP gene (*PRNP*) product.[2]

PrP^{C} is expressed, although in lower amounts, in nonneural tissue including skeletal muscle. PrP^{Sc} was recently demonstrated in muscle of mice and hamster infected with hamster- or mouse-adapted laboratory scrapie strains.[3,4] However, transmissible spongiform encephalopathies affect primarily the central nervous system.[2,5] Pathology of the CJD brain includes spongiform change, neuronal loss, gliosis, and deposition of PrP^{Sc} detectable by immunohistochemistry (IHC), paraffin-embedded tissue blotting (PET blot), or Western blotting.[5,6] Fibers with rimmed vacuoles characterize hereditary inclusion body myopathy (hIBM) and sporadic inclusion body myositis (sIBM) featuring also inflammatory infiltrates.[7] Although extensive studies by Askanas and Engel demonstrated increased expression of PrP in hIBM and sIBM, it was only recently biochemically characterized as PrP^{C} in sIBM.[7,8] Here, we demonstrate prominent PrP^{Sc} deposition in sIBM muscle tissue of a patient with concomitant CJD.

Case Report

A Brazilian man, aged 68 years at death in 2001, who was previously healthy until 1988 started to have gait difficulties with weakness and myalgia in the legs and arms. He became tetraparetic. IBM was diagnosed after a muscle biopsy. Since December 1997, progressive dysphasia was noted. In 1998, suspicion of CJD was raised; however, cerebrospinal fluid examination including 14-3-3 protein and cranial magnetic resonance imaging were normal; electroencephalogram showed paroxysmal, nonperiodic, triphasic sharp waves of frontal origin. He deteriorated in motor and cognitive functions and became disoriented, apraxic, incontinent, dysphagic, and finally bedridden. Rigidity, cerebellar ataxia, and myoclonus also appeared. At this stage, the disorder was classified as possible CJD.[9] He was in coma for 1 year and died from bronchopneumonia and sepsis. Total duration of the cerebral disease was 3 years and 11 months.

Neuropathology

We examined formalin-fixed, paraffin-embedded biceps muscle, spleen, lung, cerebellum, and cerebral cortex of this case and muscle tissue from three sIBM biopsies and one autopsy case without muscle disease.

In addition to routine stains, we immunostained sections with monoclonal anti–PrP antibodies 3F4 (1:500; Senetek PLC, Maryland Heights, MD), 6H4 (1:500; Prionics AG, Schlieren, Switzerland), and 12F10 (1:1000, CEA, Saclay, France) after a three-tiered tissue pretreatment.[10] Negative controls included omission or substitution of primary antibodies by nonspecific, isotype-matched antibodies.

Paraffin-Embedded Tissue Blot

This was performed on cerebellum, frontal cortex, and biceps muscle of this case. Controls included cerebellar

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cortex from a case without neurological disease and negative anti–PrP IHC, and one sCJD case with positive anti–PrP IHC. In addition, muscle tissue from three pathologically characterized sIBM biopsies and one autopsy case without neurological disease was examined. We used antibody 3F4 following published protocol,[6] with the alkaline phosphatase–coupled rabbit anti–mouse antibody replaced by an avidin-biotin system. A negative control, in which the primary antibody was omitted, was included for each section examined.

PRNP Analysis

Genomic DNA was isolated from frozen brain using the Qiagen DNA isolation kit. *PRNP* exon 3 was analyzed by direct sequencing of two overlapping polymerase chain reaction (PCR) products The primer pairs used for PCR of genomic DNA were as follows: 1U (5′-GGGACTCTGACGTTCTCCTCTT-3′) and 2D (5′-CATGGCACTTCCCAGCATGTAG-3′); 3U (5′-GCTGCAGCAGCTGGGGCAGTG-3′) and 4D (5′-GCTGGAAAAAGATTAGAAAGATGG-3′). Primer pairs were designed with alternate forward and reverse M13 tails to facilitate dye-primer sequencing of both DNA strands. PCR conditions were as follows: 94°C for 4 minutes; 10 cycles of 94°C for 30 seconds, 60°C (decrease 1°C each cycle) for 30 seconds, and 72°C for 90 seconds; 25 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 90 seconds; 72°C for 6 minutes. For the identification of the methionine (M)/valine (V) coding alternative at polymorphic codon 129, a PCR product was generated using the primers 5′-GTGGCCACATGGAGTGAC-3′ and 5′-AACGGTGCATGTTTTCAC-3′, respectively, and the PCR conditions described above, followed by digestion with the restriction endonuclease *Nsp*I.

Western Blot

All procedures were performed in a microbiological containment level-three facility. Brain was homogenized in nine volumes of lysis buffer according to Parchi and colleagus.[11] Aliquots before and after proteinase K (PK) digestion were resolved on 16% polyacrylamide gels and immunoblotted. The monoclonal antibody 3F4 (1:10,000) was used as primary antibody. The blots were developed using the enhanced chemiluminescence detection system (Amersham, Buckinghamshire, UK).

Muscle tissue processing was done with and without phosphotungstic acid (PTA) precipitation.[12] PK was added to a final concentration of 20μg/ml and incubated for 30 minutes at 37°C. Total protein (approximately 100–200μ g) was electrophoresed through 12% sodium dodecyl sulfate polyacrylamide gels, transferred to nitrocellulose membranes (BA 85, 0.45 μ m pore size; Schleicher & Schnell, Keene, NH), probed with antibody ICSM18 (1:20.000),[13] and visualized with a chemiluminescent substrate (CDP-star; Amersham) on a Bio-Rad VersaDoc 5000 digital imager (Bio-Rad, Hempstead, UK). Because the available small frozen brain sample of the propositus was completely consumed by Western blotting, a standard curve and the correlation coefficient for linear regression (0.99) were calculated on serial CJD brain dilutions of frontal cortex from another case (sCJD methionine/valine [MV] type 1 according to Parchi and colleagues,[11] or MV type 2 according to Hill and colleagues[14]) and used for the quantification of muscular PrP^{Sc} content on one blot, without PTA pretreatment of both brain and muscle.

Results

Neuropathology and Paraffin-Embedded Tissue Blot

The propositus' brain had nearly complete loss of neurons in cerebral and cerebellar cortex with severe astrogliosis and confluent spongiform change (Fig 1A, C). Immunostaining for PrP showed diffuse synaptic type deposits in the cerebral cortex and stained cerebellar kuru type amyloid plaques (see Fig 1B, D). Walls of intracerebral vessels and tissues of spleen and lung lacked immunoreactivity. In cross-sections and longitudinal sections of muscle, we noted lymphocytic infiltration and rimmed vacuoles (see Fig 1E). IHC demonstrated strong diffuse granular PrP deposits within muscle cells surrounding vacuoles (see Fig 1F–H). This was not seen in formalin-fixed muscle of other sIBM cases. PET blot showed focal PrP^{Sc} deposits in muscle of the case, but not in controls (see Fig 1I).

PRNP and Western Blot Analysis

No *PRNP* mutation was detected. The polymorphic codon 129 was MV heterozygous.

Immunoblotted brain tissue of the propositus contained high amounts of PrP^{Sc} in a pattern of type 2A according to Parchi and colleagues,[11] or type 3 according to recent classification by Hill and colleagues[14] (Fig 2A). Blotting for PrP^{Sc} in muscle gave a strong signal after PTA treatment (data not shown) and was detectable by conventional Western blotting (see Fig 2B), showing a pattern similar to type 2B (or type 4).[11,14]

By performing serial dilutions of sCJD diseased brain tissue, we found the relative PrP^{Sc} content in muscle to be 30% of that found in another sCJD diseased brain.

Discussion

In addition to neural tissue, PrP^{Sc} in humans has been demonstrated only in lymphoreticular tissue of vCJD and in vessels walls of sCJD and vCJD cases.[15,16] Muscle tissue in experimental prion infected mice contains

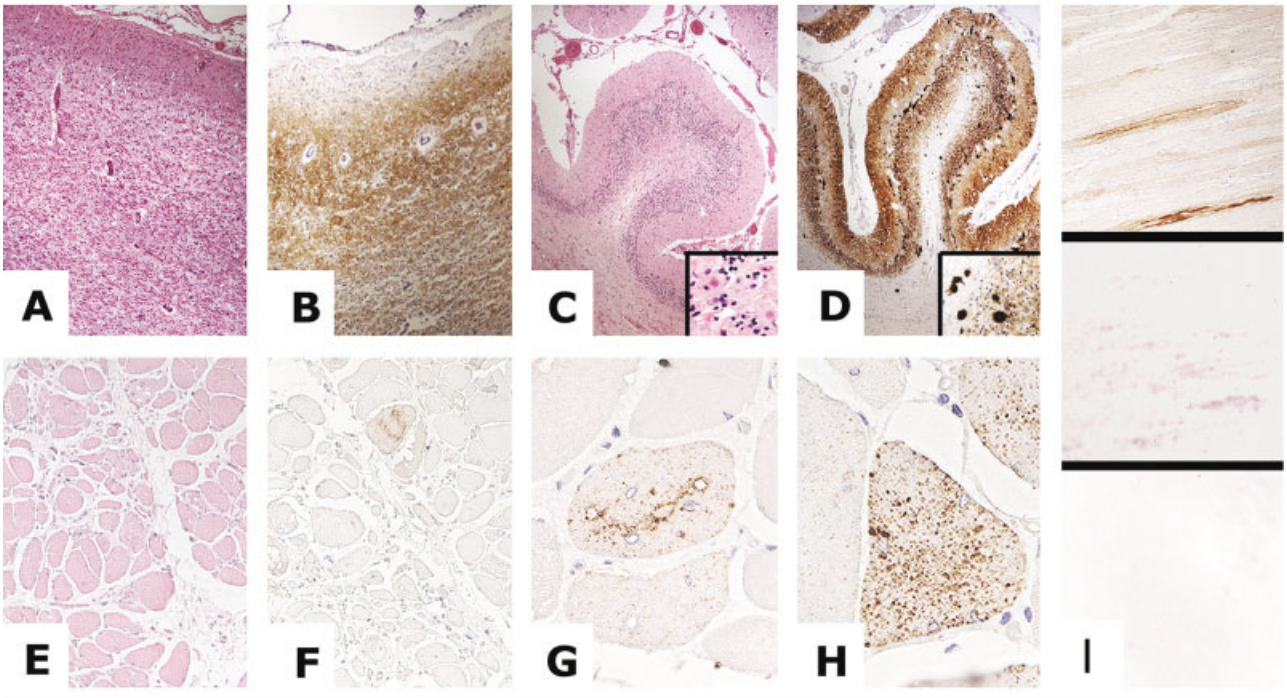

Fig 1. (A) Severe spongiform change, neuronal loss, and astrogliosis in frontal cortex of the propositus (×20; H and E). (B) Diffuse/synaptic PrP immunoreactivity in the same area shown in panel A (×20; immunostaining with 3F4). (C) Loss of granular cell layer and spongiform change of molecular layer in the cerebellum of the propositus (×10; H and E). Note the kuru type plaques enlarged in the lower right corner. (D) Diffuse/synaptic and plaque (enlarged in lower right corner) type PrP immunoreactivity in the same area shown in panel C. (×10; immunostaining with 3F4). (E) Vacuoles in the muscle fibers and variation in caliber size in the propositus (×10; H and E). (F) Coarse granular PrP immunoreactivity in some muscle fibbers (×10; immunostaining with 3F4). (G, H) Patterns of PrP immunoreactivity around vacuoles and within muscle fibers (both ×20; immunostaining with 3F4). (I) Top panel shows longitudinal section of paraffin-embedded biceps muscle tissue from the propositus immunostained with 3F4 (×20). Middle panel indicates longitudinal section of paraffin-embedded tissue (PET) blot of biceps muscle tissue immunostained with 3F4 from the propositus (×20). Bottom panel shows longitudinal section of PET blot of muscle immunostained with 3F4 from a control case without muscle disease (×20).

PrP^{Sc} at a level approximately 5 to 10% of that in brain, but PrP^{Sc} accumulation varies between muscle groups.[3] Using IHC, PET blot, and Western blot, we demonstrated PrP^{Sc} in biceps muscle in a patient with CJD and sIBM. Remarkably, very high amounts of PrP^{Sc} (30% of that observed in a sCJD brain) were present in the muscle. Apparently, sIBM in the muscle has greatly amplified the amount of PrP^{Sc} that might be present in CJD muscle.

On one hand, a protracted course of disease might result in PrP^{Sc} reaching a higher level in a tissue such as muscle, with normally little conversion to PrP^{Sc}, which then becomes observable even by IHC. On the other hand, the unique co-occurrence of sIBM, known to be related with a high expression of PrP^{C} in muscle, and CJD offers an increased chance for extraneural PrP^{C}–PrP^{Sc} conversion as a consequence of a yet uncharacterized pathogenic event. Whether other tissues also show increased expression of PrP^{C} in sIBM is unknown. PrP^{Sc} was restricted to brain and muscle in our case.

MV at *PRNP* codon 129 accompanied by type 2A or type 3 PrP^{Sc} in the Western blot represents a subgroup of sCJD with amyloid plaques and prolonged duration of illness.[11,14] However, this case is unusual for several reasons: the protracted course of brain disease; the initial progressive dysphasia; end-stage degree of neuronal loss; and the prolonged muscle disease in form of progressive tetraparesis. The role of *PRNP* polymorphism at codon 129 in sIBM is debated.[17,18] Presence of muscle disease in CJD subtypes merits further systematic study. We show that distinct glycotypes of PrP^{Sc} may exist in muscle and brain in accordance with the different PrP^{C} glycoform profile.[8] Interestingly, the glycotype of muscle in this case resembles that observed in vCJD brain.

Knowledge of potentially infectious tissues has implications for safety and public health. As PrP^{Sc} is considered as surrogate for infectivity, its presence in muscle might indicate a new iatrogenic transmission risk.

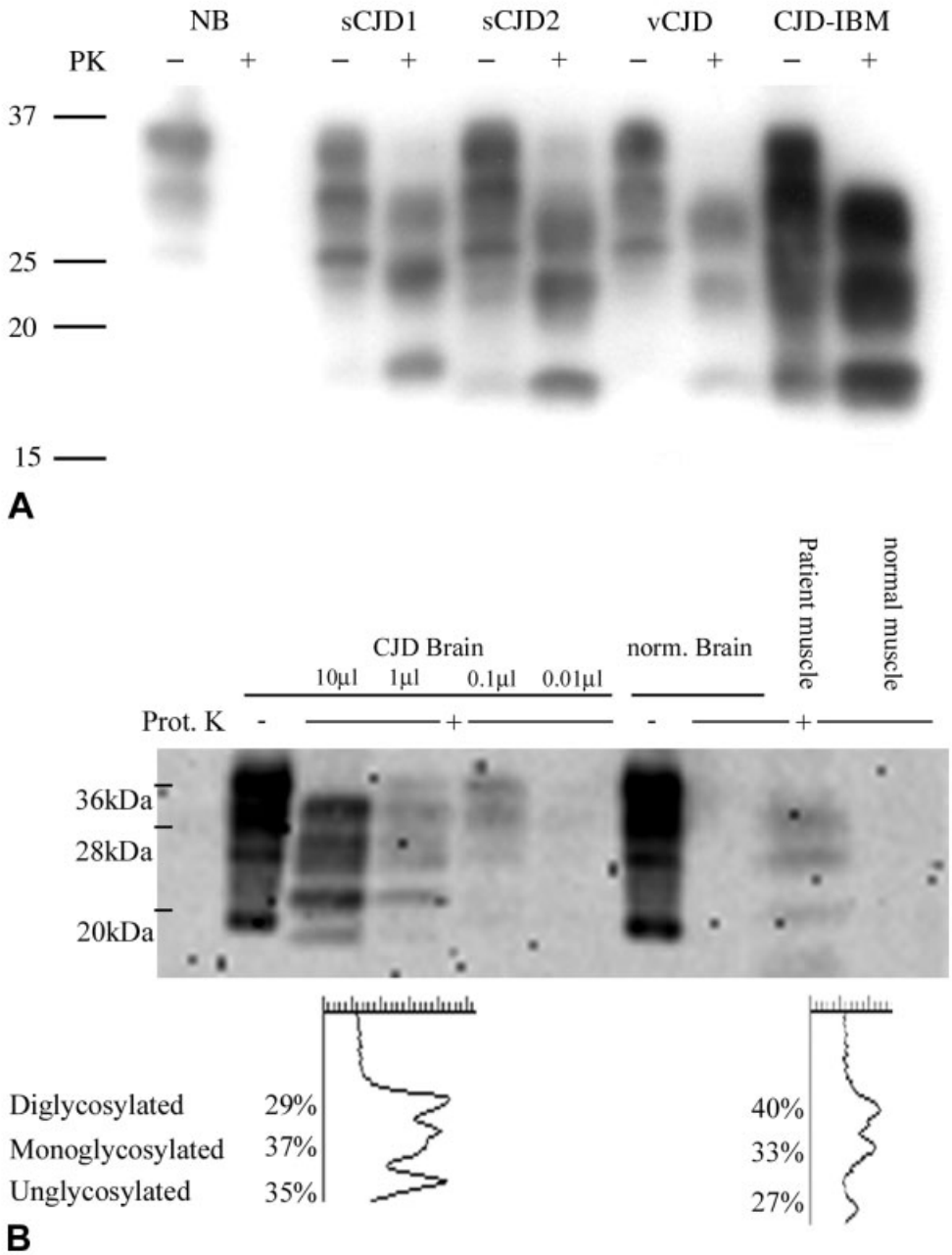

Fig 2. (A) Western blot analysis of brain. The positions of molecular markers are indicated in kilodaltons. Controls include normal brain (NB), sCJD brain of types 1 (sCJD1) and 2 (sCJD2) according to Parchi and colleagues,[11] and vCJD. PrP^{Sc} is prominently detectable in the patient's brain (CJD-IBM) in a type 2 pattern according to Parchi and colleagues,[11] or type 3 according to Hill and colleagues.[14] Proteinase K (PK) digest is indicated above lanes. (B) Western blot analysis of muscle including densitometric quantification. The positions of molecular markers are indicated. Controls include normal brain, normal muscle, and serial dilutions of a sCJD brain of type 1 according to Parchi and colleagues or type 2 according to Hill and colleagues.[11,14] PrP^{Sc} is well detectable in the patient's muscle similar to type 2B according to Parchi and colleagues[11] or type 4 according to Hill and colleagues.[14] Proteinase K (Prot. K) digest is indicated above lanes. The relative abundance of diglycosylated, monoglycosylated, and unglycosylated PrP^{Sc} are indicated below respective lanes.

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Brain Dopamine-Stimulated Adenylyl Cyclase Activity in Parkinson's Disease, Multiple System Atrophy, and Progressive Supranuclear Palsy

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The dopamine D_1 receptor is considered to participate in levodopa's antiparkinsonian action and levodopa-induced dyskinesias. We examined the functional status of the D_1 receptor in brain of patients with Parkinson's disease (PD), multiple system atrophy (MSA), and progressive supranuclear palsy (PSP). Dopamine-stimulated adenylyl cyclase activity was significantly increased in putamen (+43%) and frontal cortex (+52%) in PD, normal in PSP, but decreased by 47% in putamen in MSA. The supersensitive dopamine D_1 receptors in both striatum and cerebral cortex in PD might compensate for dopamine deficiency, but could also contribute to long-term complications of levodopa therapy.

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The dopamine D_1 receptor (D_1 receptor), originally classified by its ability to stimulate adenylyl cyclase (AC, EC4.6.1.1), recently was confirmed to contribute to the antiparkinsonian function of L-dopa in Parkinson's disease (PD) and also to the long-term complications of the therapy, because the selective D_1 receptor agonist ABT-431 possesses full antiparkinsonian effect in PD patients and induces dyskinesias.[1] On the other hand, the D_1 receptor is the predominant subtype of dopamine receptors in human cerebral cortex and plays a critical role in aspects of cognition, for example,

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