Objective: To describe a novel molecular and pathological phenotype of Creutzfeldt-Jakob disease.

Patient: A 69-year-old woman with behavioral and personality changes followed by rapidly evolving dementia.

Results: Postmortem examination of the brain showed intracellular prion protein deposition and axonal swellings filled with amyloid fibrils. Biochemical analysis of the pathological prion protein disclosed a previously unrecognized PrPSc tertiary structure lacking diglycosylated species. Genetic analysis revealed a wild-type prion protein gene. The prion agent responsible for this atypical phenotype was successfully passaged to bank voles.

Conclusion: To our knowledge, our results define a new human prion disorder characterized by intracellular accumulation of a novel type of pathological prion protein.

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Human prion diseases include Creutzfeldt-Jakob disease (CJD), variant CJD, kuru, Gerstmann-Straussler-Scheinker disease, and sporadic and familial fatal insomnia. The causative agent of these conditions is an insoluble, protease-resistant abnormal isoform of the cellular prion protein (PrPc) called PrPSc. Distinct PrPSc conformers and glycotypes have been recognized as epigenetic determinants of disease phenotype in different prion disorders. In human prion diseases, 2 main PrPSc conformers with deglycosylated protease-resistant fragments of 21 kDa (type 1 PrP27-30) and 19 kDa (type 2 PrP27-30) have been identified. Both PrPSc types are generated by multiple protease K cleavage sites within the PrPSc74-102 region (primary cleavages at Gly82 for type 1 PrPSc and Ser97 for type 2 PrPSc).

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REPORT OF A CASE

A 69-year-old woman was admitted because of depression, behavioral disturbances, and severe, rapidly evolving dementia. The patient had a history of bilateral cerebellar vascular lesions at age 46 years with residual deafness and, thereafter, reactive depression treated for several years with seasonal cycles of hypothalamic phospholipids extracted from bovine brains. In April 2002, a brain magnetic resonance image mainly showed old ischemic lesions in the cerebellum and left basal ganglia with cortical atrophy. By December 2002, the patient manifested occupational and social neglect, with ensuing disorientation and obsessive-compulsive behavior. In February 2003, hallucinations and delusions were reported. Electroencephalographic recordings disclosed generalized slowing, more evident in the right frontotemporal regions. Treatment with neuroleptics was started after an attempted suicide. In July 2003, the patient was anorexic, disoriented, and dysarthric and had generalized hypertonnia and hyporeflexia. On admission in October 2003, she was severely demented, restless, anarthric, and dysphagic. Electroencephalographic tracings revealed generalized slowing and nonperiodic sharp-and-slow wave complexes. Cerebrospinal fluid examination showed increased tau protein levels (1388 ng/mL) and a positive 14-3-3 protein assay. Repeat brain magnetic resonance imaging results were unchanged. The patient developed akinetic mutism and died in January 2004.

On autopsy, the left half of the brain was fixed in formalin for neuropathological ex-
amination, whereas the right half was frozen. Neuro-pathological and immunohistochemical studies, including immunoelectron microscopy with SA65 antibody, were performed as previously described in detail.7 Immunoblot and PrP gene (PRNP) sequencing were performed by standard techniques.8 Purified PrP27-30 was inactivated and treated with cyanogen bromide. One aliquot was further digested with trypsin. Each peptide mixture was investigated in triplicate by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.9

Nine bank voles (Clethrionomys glareolus) were intracerebrally inoculated with 20 µL of a 10% brain suspension from the index case. Immunoblot and pathological studies were done as described.10

RESULTS

PRNP GENOTYPE

Sequencing of the entire open reading frame of PRNP showed no disease-associated mutations. In addition, this investigation showed Met/Val heterozygosity at codon 129.

NEUROPATHOLOGICAL FINDINGS

Sectioning of the left brain revealed diffuse cortical atrophy, a 10-mm cavitory thalamic lesion, and vascular lesions in the territories of the anterior inferior and posterior inferior cerebellar arteries. Microscopical examination showed astrocytosis and spongiosis in neocortical areas and in the entorhinal cortex. Marked vacuolation was seen in basal ganglia, thalamic nuclei, and the amygdala. Mild spongiosis was observed in the cerebellar molecular layer. The neocortex showed synaptic-type and intraneuronal deposition of PrP (Figure 1A-C). Intracellular PrP was also detected in the entorhinal cortex, basal ganglia, thalamus, and brainstem nuclei. Prion protein–positive axonal swellings were observed, especially in the basal ganglia and thalamus (Figure 1D and E). The cerebellar cortex showed diffuse punctuate and granular PrP positivity in the molecular layer and intracellular PrP deposition in Purkinje cells (Figure 1F and G). By ultrastructural examination, axonal swellings were filled with coiled 7- to 10-nm fibrils, positive for anti-PrP antibody SA65 (Figure 1H and I).
REGIONAL DISTRIBUTION, GLYCOTYPE, AND CONFORMATION OF PrPSc

Immunoblot with 3F4 antibody showed the highest levels of PrPSc in entorhinal and prepiriform cortices (Figure 2A). Densitometric analysis of proteinase K-resistant PrPSc disclosed a predominant unglycosylated PrP form (approximately 75%), low amounts of monoglycosylated PrP, and virtually undetectable highly glycosylated species (Figure 2B); PrP27-30 glycoforms were endoglycosidase H resistant, suggesting a post-Golgi conversion. This unglycosylated dominant pattern differs from glycoforms encountered in sporadic CJD (sCJD) and variant CJD (Figure 2C), whereas it is similar to that encountered in PRNP Thr183Ala and Val180Ile mutations that result in allelic removal of the first consensus glycosylation site. Differently from these mutations, the index case showed a normal representation of diglycosylated, monoglycosylated, and unglycosylated PrP isoforms (Figure 3A-D), ruling out a constitutive glycosylation defect. This suggests that this PrPSc pattern is encoded by the prion strain.

The deglycosylated PrP27-30 fragment migrated in a 22- to 20.5-kDa zone, as opposed to the migration of type 1 PrPSc at 21 kDa. After 2-dimensional immunoblot separation, the PrPSc core fragment of Met/Val sCJD cases with type 1 PrPSc migrates as 2 to 4 spots at the isoelectric point of 6.6 to 7.2 (Figure 3E), likely matching N-terminal Gly78-Gly82-Gly90-Ser97 variants. Here, 2-dimensional immunoblot with SP-214 antibody showed 2 additional spots of the PrPSc core fragment (isolectric points, 7.5 and 7.8), consistent with novel N-terminal species (Figure 3E and F). Two-dimensional maps confirmed the absence of highly glycosylated PrPSc species and disclosed an acidic shift of monoglycosylated PrP27-30 charge isomers in keeping with exclusive glycosylation at Asn197, which contains acidic glycans in higher amounts than Asn181.

MASS SPECTROMETRY ANALYSIS OF PrP27-30

The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis of digested PrP27-30 showed the presence of mass-charge ratio signals indicative of PrP peptides 67-104, 71-101, and 90-106, consistent with PrPSc diglycosylation, monoglycosylation, and unglycosylation of PrPSc isoforms (Figure 3A-D), ruling out a constitutive glycosylation defect. This suggests that this PrPSc pattern is encoded by the prion strain.

TRANSMISSION STUDIES

All of the bank voles developed clinical disease but only 2 were euthanized (+21 days after inoculation). Incubation times were different from those observed in animals inoculated with brain homogenates from Met/Met

Figure 2. Intracerebral distribution of PrPSc. A, Immunoblot with 3F4 antibody shows the highest amounts of PrPSc in the temporal, entorhinal, and frontal cortices. Every lane was loaded with 300 µg of proteinase K-digested homogenate; values are reported as the relative percentage of the highest value obtained. B, Relative proportions of the 3 PrPSc isoforms, Relative %

Figure 4. Distribution of PrPSc in entorhinal and prepiriform cortices. Every lane was loaded with 300 µg of proteinase K-digested homogenate; values are reported as the relative percentage of the highest value obtained. C, Relative proportions of the 3 PrPSc isoforms, Relative %

n = 16; mean ± SD incubation time, 188 ± 22 days) or Met/Val (n = 20; mean ± SD incubation time, 179 ± 10 days) sCJD cases with type 1 PrPSc. Immunoblots with 6H4 antibody showed an unglycosylated PrP27-30 fragment of 22 kDa (Figure 4A), different from fragments of 21 kDa and 19 kDa obtained in animals inoculated with brain homogenates from sCJD cases with type 1 or type 2 PrPSc (Figure 4B).
Neuropathological studies disclosed spongiform changes, gliosis, and PrP deposition (Figure 4C-E).

COMMENT

We have identified a PrPSc type with a novel conformation and a distinctive glycotype in a patient with CJD. These results increase the number of possible conformational changes of the pathological PrP and define a new prion strain that imparts its structure to bank vole PrP.

In mammalian prion diseases, differences in PrPSc conformation and glycosylation account for molecular diversities of prion strains and contribute to distinct disease phenotypes.\(^3^,4\) Pathologically, typical features of prion diseases were found in this subject. However, PrP showed unusual patterns of accumulation in neuronal perikarya and along their extensions. This suggests major trafficking abnormalities and impaired cellular sorting of the pathological PrP as a likely consequence of its structure or glycosylation state. Strikingly, similar patterns of intraneuronal PrP deposition have been observed in Thr183Ala and Val180Ile mutations, 2 distinct genetic conditions that affect PrP glycosylation at the first consensus site.

Human prion diseases exhibit sporadic, inherited, or infectious presentation. Here, in the absence of PRNP mutations, arguments to entertain a diagnosis of iatrogenic CJD were suggested by the protracted treatment with bovine-derived hypothalamic phospholipids. However, the protein-free nature of liposome preparations as well as the PrPSc structure of this case, which is different from bovine PrPSc types characterized so far,\(^11\) do not support an iatrogenic cause. Therefore, the index case fits best with sCJD. Establishing the full range of PrPSc conformers and the spectrum of disease phenotypes in sCJD has important implications for epidemiological studies.\(^12\)

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**Figure 3.** Characterization of detergent-soluble and proteinase K (PK)-resistant prion protein. The detergent-soluble prion protein fraction obtained from the frontal cortex of the index case after 1- and 2-dimensional immunoblot with 3F4 antibody (A and B) and SP-214 antibody (C and D) shows a normal glycoforms profile. The PK-resistant type 1 PrP\(^{Sc}\) from a sporadic Creutzfeldt-Jakob disease case with Met/Val heterozygosity at codon 129 (E) and the index case (F) are shown following 2-dimensional immunoblot with SP-214 antibody. H indicates highly glycosylated prion protein isomers; M, monoglycosylated prion protein isomers; and U, unglycosylated prion protein isomers. F, Highly glycosylated prion protein isomers and basic monoglycosylated (M) species (arrowheads) are undetectable; additional spots (arrows) of the unglycosylated (U) PrP27-30 are shown. pl indicates isoelectric point.
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